Effects of organophosphate, diazinon on some haematological and biochemical changes in *Rutilus frisii kutum* (Kamensky, 1901) male brood stocks

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Abstract

The acute toxicity and effects of diazinon on some haematological parameters of kutum (*Rutilus frisii kutum*, Kamensky, 1901) weighing 613.33 ± 157.06 g were studied under static water quality conditions at $15\ °C \pm 2\ °C$. These experiments were carried out based on the standard TRC, 1984 method over 4 days, and controlled for the effective water physicochemical factors having pH rangesof 7-8.2, total hardness 300 mgl^{-1} ($caco_3$), dissolved oxygen 7 mgl^{-1}. The results showed that the 96h LC_{50} value of diazinon was 0.4 mgl^{-1} and that the maximum allowable concentration (MAC) value of this toxin was 0.04 mgl^{-1}. The second stage of experiments consisted of four treatments: LC_0: 0 as blank, treatment A with a concentration of LC_1: 0.107 mgl^{-1}, treatment B with a concentration of LC_5: 0.157 mgl^{-1}, treatment C with a concentration of MAC value: 0.04 mgl^{-1}. Male brood stocks of *R. frisii* were treated with these concentrations for 45 days. The results showed that long-term exposure to diazinon causes a decrease in the erythrocyte count (RBC), haemoglobin (Hb), haematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), leucocyte count (WBC), lymphocyte, testosterone, iron (Fe), sodium (Na), lactate dehydrogenase (LDH) and cholinesterase (CHeS) ($P<0.05$). Diazinon also causes an increase in prolymphocyte, aspartate aminotransferase (AST), cholesterol, alkaline phosphatase (ALP) and adrenaline ($P<0.05$). There are no significant effects on monocyte, eosinophil, magnesium (Mg), chloride (Cl), glucose (BS), urea (BUN), uric acid (U.A), triglyceride (TG), calcium (Ca), albumin (Alb), total protein (TP), cortisol, noradrenaline and high density lipoprotein (HDL) levels in *R. frisii* male brood stocks ($P>0.05$). The results showed that long-term exposure to low concentrations of diazinon causes changes in some haematological and biochemical parameters of *R. frisii* male brood stocks.

Keywords: Diazinon, Haematological, Biochemical, *Rutilus frisii kutum*

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Introduction

Pesticide use causes serious environmental problems, especially in the dry season when the dilution capacity of the water systems is low, increasing the risk of high concentrations of toxic chemicals. Moreover, the dry season is often the critical period for many animals, especially fish and birds. Fish stocks suffer from natural mortality and high fishing pressure at the end of the dry season (Adedeji et al., 2009). Direct or indirect contamination of water by pesticides can lead to fish kills, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissue, which can affect the health of humans eating these fish (Adedeji et al., 2000). Organophosphate compounds are useful as pesticides due to their ability to inhibit acetylcholinesterase, an enzyme responsible for the inactivation of the neurotransmitter acetylcholine (Ecobichon and Joy, 1994; Pesando et al., 2003). The organophosphate insecticide diazinon (*O,O*-diethyl *O*-[6-methyl-2-(1-methylethyl)- 4-pyrimidinyl] phosphorothioate) has agricultural and commercial uses and it is used to control a variety of insects, primarily aphids, beetles, scales and pill bugs, in the household environment (Cox, 1992). Trade names for diazinon include Knox-out, Dianon and Basudin. Diazinon is highly toxic to fish (EPA[1], 2004). Blood analysis is crucial in many fields of ichthyological research and fish farming and in toxicology and environmental monitoring, as an indicator of physiological or pathological changes in fishery management and disease investigation (Adedeji et al., 2000). Haematological indices are important parameters for the evaluation of fish physiological status, the changes in which depend on fish species, age, the sexual maturity of spawners and disease (Zhiteneva et al., 1989; Golovina, 1996; Luskova, 1997). *Rutilus frisii kutum* (Kamensky, 1901) of *Cyprinidae* is one of the most important and valuable commercial bony fish in the Caspian Sea. This species only exists in the Caspian Sea, particularly near Iran's north shores (Holchik, 1995). Kutum enters the rivers of Iran from December till April. Post-spawners migrate downstream to the sea. Fry migrate in July-August and move away from the coast to the deep-water area; later grown fish approach the coasts. Another major point of concern is that most of the rivers in which these fish migrate, spawn, and produce larvae are located near farms that use diazinon as a pesticide. According to the above subjects in this study the effects of some factors on the toxin diazinon hematological, biochemical and hormonal *R. frisii* male brood stocks was studied in reproductive migration time.

Materials and methods

Fish and chemical supply

The *R. frisii* male brood stocks weighing 613.33 ± 157.06 g and a total length of 42.83 ± 4.72 cm after fishing from peninsula Miankaleh and Gorganrod river were delivered to Hall of Aquaculture, University of Natural Resources and Agricultural Sciences, Gorgan (winter and spring of 2010), with a tank equipped with an oxygen capsule. They were brought to the laboratory and acclimated to laboratory

conditions for 7 days. The mean quality physic - chemical parameters of water were: pH 7-8.2, total hardness 300 mgl^{-1} ($caco_3$), dissolved oxygen 7 mgl^{-1} and temperature 15 ± 2 °C.

Acute toxicity

The first experiment was primarily to determine the effects of acute toxicity (LC5096 h) of the agricultural toxicant diazinon (60 EM). For this purpose, 4 treatments were used to test toxicity; each treatment was 3 replicated with 3 fish per tank with 180 litres water capacity. After obtaining the final results, the information was analysed statistically with Probit program version 1.5 (USEPA, 1985), and Mortality was assessed at 24, 48, 72, and 96 h after the start and dead fishes were removed immediately. The LC_{10}, LC_{50} and LC_{90} values at 24 48, 72 and 96 h; the maximum allowable concentration (MAC) value (96h LC50 divided by 10) (TRC, 1984); and the degree of toxicity were determined. The second stage of experiments consists of four treatments: LC_0: 0 as blank, treatment A with a concentration of LC_1: 0.107 mgl^{-1}, treatment B with a concentration of LC_5: 0.157 mgl^{-1}, treatment C with a concentration of MAC value: 0.04 mgl^{-1}. Male brood stocks of *R. frisii* were treated with these concentrations for 45 days. Experiments were carried out under static conditions based on the standard TRC, (1984) method over 45 days.

Haematology

After the biometric test period, brood stocks were anesthetized, blood samples were obtained through tail vein puncture and blood factors were measured using different experimental techniques in the laboratory. The blood samples were transferred to heparinized and non-heparinized tubes. At the time of blood sampling, the appropriate smears were prepared for Giemsa staining. The smears were air-dried, fixed in 96% ethanol for 30 minutes and stained with Giemsa staining for 30 minutes. The smears were examined for leucocyte differential count under a compound microscope (Klont, 1994). The haematological parameters examined were erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), leucocyte count (WBC) and differential leucocyte count (Klont, 1994).

Blood sampling and Haematology assay

Brood stocks were anesthetized, blood samples were obtained through tail vein puncture and blood factors were measured using different experimental techniques in the laboratory. The blood samples were transferred to heparinized and non-heparinized tubes. At the time of blood sampling, the appropriate smears were prepared for Giemsa staining. The smears were air-dried, fixed in 96% ethanol for 30 minutes and stained with Giemsa staining for 30 minutes. The smears were examined for leucocyte differential count under a compound microscope (Klont, 1994). The haematological parameters examined were erythrocyte count (RBC, $\times 10^6/mm^3$), haematocrit (Hct, %), haemoglobin (Hb, g/dL), mean corpuscular haemoglobin

(MCH= (Hb in gr / RBC in millions) × 10pg), mean corpuscular volume (MCV= (packed cell volume as percentage/RBC in millions) × $10\mu^3$, fl), mean corpuscular haemoglobin concentration (MCHC= (Hb in g/packed cell volume) × 100 g per 100 mL, %), leucocyte count (WBC$\times 10^4$/mm^3 and differential leucocyte count (Klont, 1994).

Serum biochemistry

The non-heparinized blood samples were centrifuged for 5 minutes at 3000 *g,* and separated Serum were used to determine with an automatic analyser machine (Persige 24 I, Anthos 2020, Stat fax) the levels of the following factors: aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), testosterone, cholinesterase (CHeS), aspartate aminotransferase (AST), cholesterol, alkaline phosphatase (ALP), adrenaline, noradrenaline, cortisol**,** iron (Fe), sodium (Na), magnesium (Mg), calcium (Ca), chloride (Cl), glucose (BS), urea (BUN)**,** uric acid (U.A)**,** triglyceride (TG), albumin (Alb), total protein (TP) and high density lipoprotein (HDL).

Statistical Analysis

For analysis of all data SPSS version 13 and a software program for drawing graphs of Excel 2003 was used. Biochemical data were analyzed with SPSS 11.5 for Windows using one-way analyses of variance (ANOVA) and significant means were subjected to a multiple comparison test (Duncan) at P<0.05. When the normality of data did not present, the nonparametric test Kruskal-Wallis to compare treatments and test Mann - Whitney for paired comparison between treatments were used.

Results

Acute toxicity

The acute toxicity results at different time intervals are shown in Table 1. The obtained LC10, 50, 90 values after 96 h of diazinon toxicant were calculated on *R. frisii* male brood stocks as 0.193 mgl^{-1}, 0.4 mgl^{-1} and 0.831 mgl^{-1}, respectively. Thus, the MAC value of this toxin is 0.04 mgl^{-1}. In this study, the behaviours and reactions of the fish were evaluated in response to different concentrations of the toxicant. The results revealed that *R. frisii* male brood stocks immediately reacted to high concentrations of this toxicant, and they constantly moved fast until they got tired and fell to the bottom of breeding tank. When placed in low concentration, the fish did not react immediately in a significant way. The most important effect of the toxicant was alterations of the nervous and brain systems, which were obvious with lack of equilibrium and spiral swimming pattern of fish; other effects include curvature of spinal column, exophthalmia and bleeding in the gill and abdomen area. The fish mortality during the period (45 days) was 2 of the fish in treatment 3.

Haematology

There were significant differences between the various treatments at erythrocyte profile. The result showed that long-term exposure to diazinon causes a decrease in RBC, Hb, PCV, MCV, MCH and MCHC values (p<0.05) (Table. 2). The long-term exposure to diazinon causes a decrease in WBC and lymphocyte values and an

increase in prolymphocyte (p<0.05), but there were no significant differences in monocytes and eosinophils (p>0.05), (Table. 3).

Table 1: Acute toxicity of diazinon on *Rutilus frisii kutum* male brood stocks

	Concentration (mgl⁻¹)	24	48	72	96
	LC10	0.599	0.533	0.446	0.193
Diazinon	LC50	1.232	0.847	0.783	0.4
	LC90	2.713	1.346	1.375	0.831

Table 2: Erythrocyte profile of *Rutilus frisii kutum* following exposure to different diazinon treatments

Indices	Units	A	B	C	Control	P_value
RBC	$\times 10^6 mm^3$	1.6 ± 0.19 **a**	1.47 ± 0.07 **b**	1.32 ± 0.1 **a**	1.6 ± 0.05 **b**	**0.000**
Hb	)g/dl(	16.5 ± 0.93 **bc**	15.34 ± 1.81 **ab**	14.55 ± 1.67 **a**	17.56 ± 1.5 **c**	**0.003**
PCV	%	56.67 ± 7.28 **bc**	49.89 ± 2.93 **a**	51.33 ± 3.33 **ab**	59.89 ± 5.49 **c**	**0.001**
MCV	fl	385.7 ± 39.5 **b**	340.8 ± 22.5 **a**	315.7 ± 46.4 **a**	410.6 ± 35.4 **b**	**0.000**
MCH	pg	129.7 ± 12.9 **b**	113.8 ± 7.5 **a**	104.3 ± 16.3 **a**	136.2 ± 13.4 **b**	**0.000**
MCHC	%	33.13 ± 0.6 **a**	32.89 ± 0.76 **a**	32.82 ± 0.41 **a**	33.55 ± 0.17 **b**	**0.000**

❋The small Latin letters show that there are significant differences among different concentrations

Table 3: Leucocyte profile of *Rutilus frisii kutum* following exposure to different diazinon treatments (A: LC₁= 0.107 mgl⁻¹, B: LC₅= 0.157 mgl⁻¹, C: MAC value= 0.04 mgl⁻¹)

Indices	Units	A	B	C	Control	p-value
WBCs	mm³	33811.1 ± 9901.6 b	18722.2 ± 6317.8 a	12733.3 ± 1954.1 a	32611.1 ± 4825.8 b	**0.000**
Prolymphocyte	%	8.11 ± 4.05 **a**	11 ± 5.59 **ab**	14.83 ± 1.6 **b**	8.22 ± 2.44 **a**	**0.01**
Lymphocytes	%	91.89 ± 3.69 **b**	88.89 ± 5.26 **b**	83.33 ± 1.97 **a**	90 ± 3.54 **b**	**0.001**
Monocytes	%	1.0 ± 0.87 **a**	1.22 ± 1.2 **a**	1.67 ± 1.2 **a**	0.78 ± 0.83 **a**	**0.418**
Eosinophils	%	0.11 ± 0.33 **a**	0.22 ± 0.44 **a**	0.5 ± 0.55 **a**	0.11 ± 0.33 **a**	**0.266**

❋ The small Latin letters show that there are significant differences among different concentrations

Serum biochemistry

There were significant differences between the various treatments at biochemical factors. The result showed that long-term exposure to diazinon causes a decrease in testosterone, iron, sodium, LDH and cholinesterase. Long-term exposure to diazinon causes an increase in AST, cholesterol, ALP and adrenaline ($p < 0.05$), but there was no significant differences in magnesium, chloride, glucose (BS), urea (BUN), uric acid (U.A), triglyceride (TG), calcium (Ca), albumin (Alb), total protein (TP), cortisol, noradrenaline and HDL values of *R. frisii* male brood stocks ($p > 0.05$), (Table. 4).

Table 4: **The effect of diazinon on plasma biochemistry at *Rutilus frisii kutum* male brood stocks** (A: LC_1= 0.107 mgl^{-1}, B: LC_5= 0.157 mgl^{-1}, C: MAC value= 0.04 mgl^{-1})

Indices	Units	A	B	C	Control	p-value
Na	meq/l	141 ± 3.35 **b**	142.22 ± 2.39 **b**	134.83 ± 2.32 **a**	136.89 ± 2.93 **a**	**0.000**
Fe	mg/dl	84.11 ± 18 **b**	72.22 ± 15.23 **b**	36 ± 15.32 **a**	170.67 ± 60.63 **c**	**0.000**
Ca	mg/dl	11.94±0.53 **a**	12.17±0.83 **a**	12.35± 0.62 **a**	12.2 ± 0.51 **a**	**0.665**
Mg	mg/dl	4.74 ± 0.56 **a**	5.12 ± 0.69 **a**	5.22 ± 0.94 **a**	4.62 ± 0.39 **a**	**0.209**
Cl	meq/l	115.33 ± 3 **ab**	113.44 ± 2.35 **a**	117.5 ± 3.62 **b**	115 ± 3.32 **ab**	**0.116**
FBS	mg/dl	125.9 ± 35.8 **a**	109 ± 34.4 **a**	127.2 ± 38.6 **a**	92.3 ± 21.5 **a**	**0.123**
BUN	mg/dl	25.67 ± 4.92 **a**	27.2 ± 2.84 **a**	22.94 ± 8.98 **a**	22.73 ± 3.27 **a**	**0.229**
U.A	mg/dl	0.93 ± 0.2 **a**	0.76 ± 0.08 **a**	0.998 ± 0.34 **a**	0.78 ± 0.27 **a**	**0.146**
TG	mg/dl	167 ± 45.67 **a**	154.33 ±47.42 **a**	163.5 ± 24.68 **a**	168.89 ± 38.43 **a**	**0.882**
Cholesterol	mg/dl	318.56 ± 63.38 **ab**	340 ± 69.03 **b**	350.33 ± 95.24 **ab**	267.44 ± 64.04 **a**	**0.065**
TP	mg/dl	3.91 ± 0.52 **a**	4.31 ± 0.41 **a**	3.72 ± 1.27 **a**	4.2 ± 0.3 **a**	**0.306**
Alb	gr/dl	1.18 ± 0.15 **a**	1.29 ± 0.29 **a**	1.32 ± 0.17 **a**	1.38 ± 0.26 **a**	**0.204**
HDL	mg/dl	71.22 ± 10.13 **a**	74.89 ± 13.2 **a**	76.17 ± 9.56 **a**	72.3 ± 10.5 **a**	**0.809**
CHeS	Iu/l	1358.2 ± 233.1 **a**	587.6 ± 117.2 **a**	457.5 ± 180.9 **a**	4616.7 ± 2533.8 **b**	**0.000**
AST	Iu/l	98.6 ± 43.64**b**	111.67 ± 59.29 **b**	181.83 ± 36.54 **c**	49.11 ± 26.52 **a**	**0.000**
ALP	Iu/l	203.3 ± 50.5**a**	225.78 ± 44.43 **a**	280.5 ± 41.61 **b**	181.33 ± 55.28 **a**	**0.005**
LDH	Iu/l	1835.4 ± 241.4**b**	1564.11 ± 275.46 **b**	1175.33 ± 187.34 **a**	1879.6 ± 441.1 **b**	**0.001**
Testosterone	ng/ml	1.11 ± 0.11**a**	0.93 ± 0.25 **a**	0.84 ± 0.16 **a**	1.62 ± 0.43 **b**	**0.000**
Cortisol	ng/ml	52.3 ± 26.3**a**	49.71 ± 23.71 **a**	33.17±28.17 **a**	35.08 ± 20.16 **a**	**0.292**
Adrenaline	pg/ml	12.2 ± 6.3**ab**	12.95 ± 3.59 **ab**	13.37 ± 3.68 **b**	8.29 ± 2.97 **a**	**0.091**
Noradrenaline	pg/ml	34.1 ± 6.2**a**	13.89 ± 8.43 **a**	31.62 ± 12.66 **a**	24.9 ± 6.37 **a**	**0.13**

❈ The small Latin letters show that there are significant differences among different concentrations

Discussion

In other studies, the LC_{50} 96h values for diazinon were determined for the following species: *Anguilla anguilla*, 0.08 mgl^{-1}, the diazinon Granol 5% LC50 at 48 hours for *Channa punctatus* is 14 mgl^{-1}, and the LC50 value at 96 hours in Blue gill is 0.46 mgl^{-1}, in Fathead minnows is 7.8 mgl^{-1} and zebrafish (*Brachydanio rerio*) is 2.12 mgl^{-1} (Ansari et al., 1987); silver carp and *Abramis brama,* 1.9 mgl^{-1} and 8.1 mgl^{-1} (Nasri Tajan, 1996) *Acipenser persicus,* 4.38 mgl^{-1} (Pajand, 1999; *Acipenser nudiventris*, 4.6 mgl^{-1} (Khoshbavar-Rostami and Soltani, 2002); *Acipenser gueldenstadtii*, 6.09 mgl^{-1} (Soltani and Khoshbavar-Rostami, 2002); *Huso huso* (14 g ± 2 g), 4.99 mgl^{-1} (Khoshbavar-Rostami et al., 2004); *Acipenser stellatus* (7 g ± 1 g), 4.98 mgl^{-1} (Khoshbavar-Rostami et al., 2005); grass carp (5 g ± 1.0 g), 15.13 mgl^{-1} (Pourgholam et al., 2006); fingerling European cat fish (*Silurus glanis*), 4.142 mgl^{-1} (Köprücü et al., 2006); African catfish (*Clarias gariepinus*) (350 g ± 15 g), 6.6 mgl^{-1} (Adedeji et al., 2009) and *Bufo regularis,* 0.44 mgl^{-1} (Lawrence and Isioma, 2010). Also, the results of this study and its comparison to the literature finally show the range of sensitivity to diazinon toxicant, as shown below:

Anguilla anguilla > Rutilus frisii kutum and *Bufo regularis > Cyprinus carpio > Lepomis macrochirus > Hypophthalmichthys molitrix > Danio rerio* (common name: zebrafish) > *A. nudiventris > A. persicus > A. stellatus > A. gueldenstaedtii > Clarias gariepinus > Pimephales promelas* (common name: fathead minnow) > *Abramis brama > Rutilus rutilus caspicus > Channa punctatus > Ctenopharyngodon idell*a

Goodman et al. (1979) showed that diazinon was not found in sea water but it was observed in polluted rivers from agricultural and urban sewage. These authors also proved that the decline in the generation lock of enzyme activity in bony fish is the effect of long-term exposure to diazinon toxicant; in the present study, the decrease was observed in the value of *R. frisii* male brood stocks. Montez (1983) showed that the toxicity of diazinon was different in various species of fish and that it depends on age, gender, size, weather conditions, pesticide formula, chemical characteristics of the environment and other factors. Alison and Hermantuz (1987) recognised that the worst known diazinon-induced abnormality is a change in the spinal column (lordosis and scoliosis) and curvature of the spinal column. These changes were observed in *Pimephales promelas* after 19 weeks of exposure to 3.2 mgl^{-1} of diazinon, similar to the changes we observed in *R. frisii* male brood stocks. Iqbal and Mufti (1992) showed that just 30-50% of *Salmo salar* eggs hatched when exposed to a certain concentration of diazinon for 30 days, and decreased growth and breeding are negative effects of diazinon. Keizer et al. (1993) determined that the degree of sensitivity to diazinon is different even among fish that are in the same family and genus. Dutta et al. (1997) showed that fresh water fish that were exposed directly to a low concentration of diazinon over a long period have a higher mortality rate than fish exposed to a high concentration. Rahmi and Koprücü (2005) studied the acute toxicity of diazinon on common carp *(Cyprinus carpio)* embryos and larvae and showed that low levels (0.25 mgl^{-1}) of diazinon in the aquatic environment could have a significant effect on the reproduction and development of carp. With reference to the toxicity of various pesticides in the pesticide dictionary (Piri Zirkoohi and Orfog, 1997), diazinon is highly toxic for *R. frisii*. In this study, the behaviours and reactions of fish were considered in response to different concentrations of the toxicant during the

experiment. Our results revealed that *R. frisii* male brood stocks immediately reacted to the high concentration of this toxicant, and they moved fast continually until they got tired and fell to the bottom of breeding tank; however, when exposed to low concentrations, the fish did not react significantly upon the initial exposure. The most important effect of toxicant was disorder of the nervous and brain systems, which were obvious based on the lack of equilibrium and the spiral swimming patterns. Other apparent toxicity effects include curvature of the spinal column and exophthalmia bleeding in the gill and abdomen area. Similar results have been reported by Barak (1990) Mance (1990), Zamini (1996), Alinezhad (2004) and Mirzaie (2004).

The results of this study show that long-term exposure to diazinon causes a decrease in RBC, Hb, PCV, MCV, MCH, MCHC, WBC and lymphocyte values. In addition, it causes an increase in prolymphocyte levels. Conversely, there is no significant effect on monocyte and eosinophil values of *R. frisii* male brood stocks. In other studies, Svoboda et al. (2001) showed that diazinon exposure in carp led to a decrease in RBC, Hb, PCV and WBC, whereas the neutrophil and granulocyte levels increased. Pourgholam et al. (2001) studied the effect of different sub-lethal concentrations of diazinon (1 mgl^{-1}, 2 mgl^{-1} and 4 mgl^{-1}) on grass carp weighing 850 g $\pm$ 155 g after 45 days exposure to the toxicant. Their results showed that diazinon causes a decrease in RBC, Hb, PCV and WBC in grass carp. Soltani and Khoshbavar-Rostami (2002) investigated the effects of diazinon on *Acipenser gueldenstaedtii,* and they came to the conclusion that the indices of MCV and MCH were lower, whereas the values of neutrophils and monocyte were higher, in the experimental group than in the control group, which is similar to results of the present study. However, there was not a significant difference in neutrophil and

monocyte values. Khoshbavar-Rostami and Soltani (2002) showed that the effects of diazinon on *Acipenser nudiventris* caused lower non-specific immunity, and their results were similar to results of this study. Khoshbavar-Rostami et al. (2004) studied the effect of diazinon on *Huso huso* weighing 14 g $\pm$ 2 g at diazinon concentrations of 5.89 mgl^{-1}, 5.65 mgl^{-1}, 5.60 mgl^{-1} and 4.99 mgl^{-1}. After 96 hours of exposing the fish to the LC50 concentration of the toxicant (5.6 mgl^{-1}), the RBC, PCV, Hb, MCHC, MCH, WBC, lymphocyte and eosinophil counts were significantly lower than the control group, while there was a significant increase in neutrophil and leucocyte values and the MCV of the test group compared to the control group. Khoshbavar-Rostami et al. (2005) exposed *Acipenser stellatus* (7 g $\pm$ 1 g) to diazinon at concentrations of 3 mgl^{-1}, 4 mgl^{-1}, 5 mgl^{-1}, 6 mgl^{-1}and 7 mgl^{-1}. They reported that diazinon causes a decrease in RBC, Hb, MCHC, WBC and lymphocytes, and the results are identical to the present study. They also reported an increase in values of MCH, MCV, PCV, neutrophils, leucocytes, eosinophils and monocytes, which differs from the present study. Pourgholam et al. (2006) studied the toxicity of diazinon in grass carp. They expressed levels of RBC, PCV, Hb, MCHC, WBC, lymphocytes and monocytes were lower in the exposed fish than in control fish. Köprücü et al. (2006) studied acute toxicity of diazinon on fingerling European cat fish at concentrations of 1 mgl^{-1}, 2 mgl^{-1}, 4 mgl^{-1}, 8 mgl^{-1}, 16 mgl^{-1}, 32 mgl^{-1} and 64 mgl^{-1}. With increasing diazinon concentration, more fish exposed for 1-96 h died. They observed a decrease in values of erythrocytes, leukocytes, Hb, PCV, MCV, MCH, and MCHC, and we observed similar changes in *R. frisii* male brood stocks. Banaee et al. (2008) studied the effects of sub-lethal diazinon concentration on blood plasma biochemistry of *Cyprinus carpio* and

expressed that diazinon doesn't affect haematology factors and their results are not identical to the present study. Adedeji et al. (2009) showed that exposure of African catfish (*Clarias gariepinus*) to diazinon reduced RBC, Hb and PCV levels, the leucocyte count and the relative and absolute lymphocyte count. It also increases both the relative and absolute count of developmental forms of neutrophil granulocytes: myelocytes and metamyelocytes. Relative and absolute counts of monocytes and both the banded and segmented neutrophil granulocytes were comparable in both groups during the study. Changes in both the erythrocyte and leucocyte profiles after exposure to the diazinon-based preparation can disrupt haematopoiesis and decrease the non-specific immunity of the fish.

The present study shows that long-term exposure to diazinon causes a decrease in values of testosterone, CHeS, LDH, Na and Fe and also causes an increase in values of AST, adrenaline, ALP and cholesterol. There were no significant effects on values of Mg, Cl, BS, BUN, U.A, TG, Ca, Alb, TP, cortisol, noradrenalin and HDL of *R. frisii* male brood stocks. In another study, Pourgholam et al. (2001) studied the effect of different sub-lethal concentrations of diazinon on grass carp after 45 days and found that levels of ALT, ALP and AST were lower than control. Similar changes were not observed in *R. frisii* male brood stocks. Luscova et al. (2002) examined the effect of diazinon on carp and showed that Na and K levels were higher and AST, ChES, LDH, TP, LDH, Ca, P and BS levels were lower in common carp after being exposed to diazinon. Their results are not identical to the present study. Khoshbavar-Rostami et al. (2004) found similar results in *Huso huso*. They determined that the levels of ALP, LDH and TP were lower in fish exposed to the diazinon while the AST level was higher

in experimental fish. Similar results were observed in the present study. Khoshbavar-Rostami et al. (2005) found that values of TP and BS were lower than control juveniles of *Acipenser stellatus* (7 g ± 1 g) when exposed to diazinon. These results were not similar to this study, and we did not observe any significant difference between TP and BS. Pourgholam et al. (2006) showed that the levels of AST, ALP and LDH were lower in grass carp exposed to the diazinon while the levels of BS and TP were higher in experimental fish than control fish, and levels of TP and cholesterol decreased insignificantly in fish exposed to the diazinon. Their results show that although diazinon can be classified as a slight toxic chemical for grass carp, the toxicant negatively affects some immunophysiological functions of grass carp including immunocompetent cells. Similar changes were not observed in Kutum male brood stocks. Uner et al. (2006) studied the effects of diazinon on acetylcholinesterase (AChE: EC3.1.1.7) activity in the brain of a freshwater fish, *Oreochromis niloticus* for 1 day, 7 days, 15 days and 30 days. In the experimental group, AChE activity in the brain decreased (up to 93% of control), which was similar to the control group after 30 days of diazinon exposure. Similar changes were observed in Kutum male brood stocks. Ozcan Oruc and Usta (2007) evaluated the effect of sub-lethal concentration of diazinon (0.0036 ppb, 0.018 ppb and 0.036 ppb) in *Cyprinus carpio* for 5 days, 15 days and 30 days. Their study suggested that AChE (in gill and muscle tissues) activities decreased. Elnwishy et al. (2007) studied the effect of diazinon on Acetylcholinesterase in male tilapia (*Oreochromis niloticus*) uniform (40 g) and revealed that the 30 day in vivo chronic exposure of tilapia to sub-lethal concentrations of diazinon for caused a reduction in AChE activity. Similar

changes were observed in the present study. Van Cong et al. (2008) studied the brain cholinesterase response in the snakehead fish (*Channa striata*) after field exposure to diazinon. Their study showed the reactivation of the cholinesterase diazinon complex to within 80% of the control level. These experiments also showed that chemical ageing of the diazinon cholinesterase binding occurred, which may explain the long-term effects of this pesticide. Van Cong et al. (2009) studied the effects of repeated exposure of diazinon on cholinesterase activity and growth in snakehead fish (*Channa striata*). The snakehead fish were exposed twice to 4-day pulses of 0.016 mgl^{-1}, 0.079 mgl^{-1} or 0.35 mgl^{-1} of diazinon, separated by a 2-week interval to imitate the exposure conditions in the field. After the 4-day exposures to these environmentally realistic concentrations, the fish were moved to clean water for recovery (the experiment lasted for 2 months). The authors showed that diazinon caused long-term inhibition of brain ChES activity, which was still significantly depressed at the termination of the experiment, and also that the highest concentrations caused a significant 30% growth inhibition. Gaworecki et al. (2009) studied biochemical and behavioral effects of diazinon exposure in hybrid striped bass. Their results suggested that sublethal exposure to AChE-inhibiting substances may decrease the ecological fitness of hybrid striped bass by reducing their ability to capture prey. Machova et al. (2010) studied toxicity of diazinon 60 EC for embryos and larvae of tench, *Tinca tinca*. Their results showed that diazinon caused mortality and high incidence of malformations, a decrease in growth rate and ontogenetic development. Bannee et al. (2011) determined the acute toxicity and evaluated the effect of sub-lethal concentrations of diazinon on some biochemical parameters of blood in rainbow trout *(Oncorhynchus mykiss)* after

7, 14 and 28 days. Their study showed that acetylcholinesterase activity and the levels of total protein, albumin as well as globulin in plasma was significantly reduced. Lactate dehydrogenase activity was only decreased on the 7th day and aspartate aminotransferase, alanine aminotransferase activities and glucose levels in diazinon treated groups were significantly higher than the controlled group at experimental periods. In conclusion, long-term exposure to diazinon at sublethal concentrations induced biochemical alterations in rainbow trout, and offers a simple tool to evaluate toxicity-derived alterations. Finally, the results of this study show that long-term exposure to low concentrations of diazinon changes some haematological and biochemical parameters of *R. frisii* male brood stocks.

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