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Determination of Acute Toxicity of Atrazine Herbicide in Caspian Kutum, *Rutilus frisii kutum*, Larvae

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***Abstract*—**Pesticides and drugs used in agriculture and veterinary medicine may end up in aquatic environments and bioaccumulate in the food chain, thus causing serious problems for fauna and human health. For determination of the toxic effects of atrazine herbicide on Caspian kutum, *Rutilus frisii kutum* larvae, the 96-h LC50 of atrazine was measured for newly hatched larvae as 18.53 ppm. Toxicity of atrazine herbicide on Caspian kutum larvae was investigated using concentrations: 9.25ppm, 4.62 ppm and 2.31 ppm for 7 days. Comparison of the length, weight and condition factor showed that no significant differences between atrazine exposed and control groups. The concentration of Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- in whole body of larvae in control and atrazine exposure groups were measured and the results showed that concentrations of all these ions is higher in atrazine exposure group than control group. It is obvious from this study that atrazine negatively affects osmoregulation process and changes ion compositions of the body even at sublethal concentration and acute exposure but have no effects on growth parameters of the body.

***Keywords*—**Atrazine, Caspian Kutum, Acute Toxicity, Body Ions, LC50.

I. Introduction

ATRAZINE (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a pre-emergent herbicide first approved for use in the US in 1958, where it is used primarily on corn, sorghum and sugar cane [1]. Atrazine inhibits electron transport in Photosystem II, which results in a disruption of photosynthesis and in turn leads to death from starvation in broad-leaf plants [2].

Herbicide application generally occurs in the spring or early summer, a time that coincides with the breeding periods of many fish species, some of which breed in aquatic habitats that are often subject to runoff from agricultural fields. Atrazine has low volatility, but its moderate water solubility (33 mg/L at 25◦C) makes it relatively mobile in soil and aquatic environments, where it tends to partition into the water column rather than sorbing to sediments [2].

Several recent laboratory studies have shown that environmentally realistic concentrations of atrazine cause significant toxic effects to fish. For example, low concentrations of atrazine (1 µg/L) altered olfactory-mediated endocrine function in male Atlantic salmon (*Salmo salar*) [3]. At 100 µg/L atrazine altered Na^+, K^+-ATPase activity in common carp (*Cyprinus carpio*) held in fresh water, indicating osmoregulatory disturbances [4]. In addition, in vitro studies in fish have shown that atrazine may affect the secretion of cortisol, involved in osmoregulation and stress response [5].

Early developmental stages are considered to be one of the most sensitive stages in the fish life cycle to the toxic effects of chemical contaminants [6]. Short-term sublethal effects on growth, behavior or osmotic control may affect the survival of these critical stages and impact recruitment [7]-[9]. For example, loss of osmotic control altering water content may influence larval density and buoyancy. The vertical position of larvae in the water column affects their patterns of drift and their interactions with preys or predators. Thus, a temporary loss of osmotic control in fish larvae may increase their susceptibility to predation or impair their feeding abilities [8]. Disruption of normal cortisol secretion in early life stages may also affect their survival by reducing the ability to cope with acute stressful situations and by inducing adverse secondary effects on osmoregulation, growth, development and immune function [10]-[12].

Caspian kutum is an important commercial fish species in the Caspian Sea in Iran. The sharp decline in its annual catch observed in 1970s and early 1980s due to declining natural population and other factors [13] had prompted the Iranian government to launch its restocking project in 1984.

This study is a first step to understand the acute effects of atrazine on one species of cyprinids (an economical and ecological important family), Caspian kutum, *Rutilus frisii kutum*. The aim of the present study was to assess the toxicity of a commercial formulation of the herbicide atrazine and the impact on some biochemical indices, like body ions, in *R. frisii kutum*. The information obtained may be useful for management and monitoring of atrazine contamination in the environment.

II. Materials and Methods

A. Fish and Sampling

Caspian kutum, *Rutilus frisii kutum*, larvae, were obtained from Shahid Ansari Fish Proliferation and Culture Facility, Rasht, Iran, in July 2011. Total length (cm) and Body weight (g) were measured and based on the length and weight, the Condition Factor (CF) was calculated using Williams [14] method: $K= (100\times w) L^3$.

B. Determination of LC50 and Sublethal Concentration

Acute toxicity was conducted to determine the 96 h LC50 value of atrazine with definitive test in semi-static system in laboratory as per standard methods [15]. The range finding

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test was carried out prior to the definitive test to determine the concentration of the test solution. For the test, the atrazine was dissolved in distilled water, and added to the aquarium following the method of Pluta [16]. In the definitive test, a set of 10 fish specimens were randomly exposed to each of the atrazine concentrations (viz. 14, 15, 16, 17, 18, 19, 20, 21, 22 and 23 mgL^{-1}) and the experiment was set in triplicate to obtain the LC50 value of the herbicide for the species. The LC50 value of test chemical in *R. frisii kutum* was determined by Probit analysis method [17] for 12, 24, 48, 72 and 96 h.

C. Experimental Design

After the determination of the LC50, three sublethal concentrations of atrazine were determined as ½ LC50, ¼ LC50 and 1/8 LC50 [18]. Three aquaria each contain 50 kutum larvae were exposed to each sublethal concentration for 7 days, and the same number of aquaria and larvae in clean water (no atrazine) were held as control group. Sampling was begun after 24hrs of exposure and continued every 24 h until the end of the experiment. During the experiment water factors: pH, temperature and dissolved oxygen (DO) were measured using Eutech instruments, pcd650.

D. Body Ions

For measuring the concentration of the ions, fish samples were freeze using liquid nitrogen at the time of sampling. The concentration of Na^+, K^+, Ca^{2+} and Mg^{2+} determined by Atomic Absorption Spectrometry (Flame atomic absorption spectrometry GBS Avanta PM), and to determination of the Cl^- concentration, flame spectrophotometry (UV-Vis HACH DR 5000) was conducted.

E. Statistical Analysis

All the data were subjected to one-way ANOVA using statistical software SPSS version 15.0. Indipendent sample *t*-tests was used to determine the differences among treatment means at $p<0.05$.

III. Results

A. Physico-Chemical Parameters of the Test Water

The physico-chemical characteristics of the test water are presented in Table I. The water temperature varied from 17.9 to 19.1˚C and the pH ranged from 7.7 to 7.9. The dissolved oxygen concentration ranged from 7.11 to 8.01 $mg{\cdot}L^{-1}$.

B. Toxic Stress and Poisoning Symptoms in Fish

Fish subjected to atrazine herbicide displayed uncoordinated behavior. At the initial exposure, fish were alert, stopped swimming and remained static in position in response to the sudden changes in the surrounding environment. After some time they tried to avoid the toxic water with fast swimming. Faster opercula activity was observed as surfacing and gulping for air. In aquaria with higher concentrations of test herbicide, the fish swam erratically. They secreted copious amounts of mucus from whole body continuously and soon a thick layer of mucus was found deposited in the buccal cavity and gills. Ultimately fish lost their balance, consciousness, engage in rolling movement and became exhausted and lethargic. Lastly, they remained in vertical position for a few minutes with anterior side or terminal mouth up near the surface of the water, trying to gulp air and tail in a downward direction. Soon they settled at the bottom of the aquaria, and after some time their bellies turned upward and the fish died.

C. Median Lethal Concentration (LC50)

Median lethal concentration (LC50) is the most widely accepted basis for acute toxicity test and it is the concentration of a test chemical which kills 50% of the test organisms after a particular length of exposure, usually 96 h. Generally in toxicity tests, death is a decisive criterion because it is easy to determine and has obvious biological and ecological significance. The LC50 values (with 95% confidence limits) of different concentrations of atrazine following Finney's (1971) method and using SPSS (version 15) showed in Table II.

A dose dependent increase and time dependent decrease were observed in mortality rate, such that as the exposure time increases from 12 to 96 h, the median concentration was reduced. It was observed that as the concentration of the herbicide increased fish mortality also increased that indicates a direct proportional relationship between mortality and concentration of atrazine herbicide. No mortality was observed in the control during the experimental period.

D. Length, Weight and Condition Factor

The mean body weight (BW) and mean total length (TL) of larvae were: 0.26±0.01 g and 3.5±0.02 cm, respectively. Measuring the length, weight and condition factor of the control and atrazine exposure groups showed no significant differences ($p>0.05$) between these two experimental groups (Tables III-V) (Fig. 1).

E. Body Ions

Measurement of the total body ions showed that the highest concentration is belong to the Ca^{2+} in atrazine exposure group and the lowest is belong to the Mg^{2+} in control group. Result showed that all ions in both experimental groups were increased during the experiment; also, all the ions showed higher concentrations in atrazine exposed group compare to control group, except for the Cl^-, that has higher concentration in control group. It seems that, exposure to atrazine is increased the cations and decreased the anions. On the other side, the concentration of the ions in control group is as following order: $Ca^{2+}>K^+>Na^+>Cl^->Mg^{2+}$, but this order is different in atrazine exposed group: $Ca^{2+}>Na^+>K^+>Mg^{2+}>Cl^-$, this showed that atrazine besides increasing and decreasing the concentration of the ions, is affected the ion composition of the body. Statistical analysis showed that, there is no significant difference between the concentrations of the K^+, Ca^{2+}, Mg^{2+}, Cl^- ions in control and atrazine exposure groups, but the concentration of the Na^+ is significantly ($p<0.05$) higher in atrazine exposed group.

IV. Discussion and Conclusion

Fish are often used as sentinel organisms for ecotoxicological studies because they play number of roles in the trophic web, accumulate toxic substances and respond to low concentration of mutagens [19] therefore, the use of fish biomarkers as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems [20]-[21]. Acute toxicity data has been used to derive water quality guidelines for regulatory measures [22]. The result of the LC50 (median lethal concentration) for atrazine in the present study at 96 h was 18.53 $mg.L^{-1}$. The results showed that the toxicity of atrazine for *R. frisii kutum* is both time and concentration dependent, thus, accounting for differences in LC values obtained at different concentrations and time of exposure. However, some other researchers have shown that exposure time is not significant in LC50 determinations for fish [23]. The LC50 value obtained for *R. frisii kutum* in this study is higher than that reported by Bathe et al. [24], Neskovic et al. [25], and Hussein et al. [26], who reported LC50 values of 16.0, 18.8 and 9.37 mgl^{-1} for *Lepomis macrochirus* (Bluegill sunfish), *Cyprinus carpio* and *Oreochromis niloticus*, respectively, exposed to atrazine. Toxicity of chemicals to aquatic organisms has been shown to be affected by age, size and health of the species [27].

Physiological parameters like quality, temperature, pH, dissolved oxygen and turbidity of water, amount and kind of aquatic vegetation, concentration and formulation of chemical and its exposure also greatly influence such studies [28]. Fish exposed to atrazine were stressed progressively with time before death. The respiratory impairment due to the toxic effect of atrazine on the gills of *R. frisii kutum* is similar to the reports of Abdul-Farah et al. [27]; De Mel and Pathiratne [29]; Tilak et al. [30] and Ayoola [31] that pesticides impair respiratory organs. Death could have, therefore, occurred either by direct poisoning or indirectly by making the medium unconducive for the fish or even by both. The abnormal behavior observed during the exposure period like restlessness and surface to bottom movement were similar to the observations of Hussein et al. [26]; Pandey et al. [32] and Chandra [33].

The length-weight relationship of fish is an important fishery management tool. Its importance is pronounced in estimating the average weight at a given length group [34] and in assessing the relative well being of a fish population [35]. Consequently, length-weight studies on fish are extensive. Notable among these are the reports Shenouda et al. [36], for *Chrysichthys* spp. from the Southernmost part of the River Nile (Egypt), Alfred-Ockiya and Njoku [37] for mullet in New Calabar River, Ahmed and Saha [38] for carps in Lake Kapital, Bangladash, King [39] for Nigeria fresh water fishes, Hart [40] for *Mugil cephalus* in Bonny Estuary; Diri [41] *Tilapia guineensis* in Elechi creek.

Condition factor compares the wellbeing of a fish and is based on the hypothesis that heavier fish of a given length are in better condition [42]. Condition factor has been used as an index of growth and feeding intensity [43]. Condition factor decrease with increase in length [43]; and also influences the reproductive cycle in fish [44]. Condition factors of different species of cichlid fishes have been reported by Siddique [45], Fagade [43], [46], [47], Dadzie and Wangila [48], Arawomo [49] and Oni et al. [50]. Some condition factors reported for other fish species include; Alfred- Ockiya [51], *Chana chana* in fresh water swamps of Niger Delta and Hart [40], *Mugil cephalus* in Bonny estuary, Abowei and Hart [52], ten fish species from the lower Nun River, and Abowei and Davies [53], *Clarotes lateceps* from the fresh water reaches of the lower Nun river. In present study no significant differences were found in atrazine exposed and control group, this result showed that all experimental fish were in the same growth condition and atrazine at sublethal concentration and acute condition have no effects on growth or condition factor of the *R. frisii kutum* fry.

Several studies have tested the effects of atrazine on survival and various measures of iono-regulatory performance in different fishes [3], [54], [55]. The present study differs from many in the literature in that the results did not reveal any significant effects of atrazine on survival, body weight, condition factor or ionoregulatory performance in *Rutilus frisii kutum* fry.

Plasma and whole body electrolyte levels, Na^+/K^+-ATPase activity and muscle water content are commonly measured as indicators of iono-regulatory performance in fishes.

In the present study, atrazine elevated whole body Na^+ levels significantly, while not affecting other ions. These results are similar to those of Waring and Moore [54] and that atrazine elevated plasma Na^+ and had no effects on plasma Cl^- levels. Cassano et al. [56] demonstrated that doses as low as 2μg l^{-1} atrazine can stimulate the short-circuit current of the ventral skin of frog (*Rana esculenta*), resulting in stimulated Na^+ absorption. Increases of plasma Na^+ at intermediate doses of atrazine may be a compensatory response to moderate damage of ion regulatory tissue. In some previous studies, exposure to different levels of atrazine caused elevation in plasma Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- levels [55].

The high mortality induced by atrazine reported in Waring and Moore [54] and Moore et al. [3] is all the more unexpected given the acute toxicity values for atrazine exposure in freshwater fish. These range from a 96-h LC50 of 4300 μg/L for the guppy (*Poecilia reticulata*) to a 96-h LC50 of N100,000 μg/L for the carp (*Carassius carassius*) [2]. In the case of salmonids, the 96-h LC50 was 13,000 μg/L for rainbow trout (*Oncorhynchus mykiss*), 12,000 μg/L for coho salmon (*O. kisutch*) and 18,500μg/L for Chinook salmon (*O. tshawytscha*). Both short term (4-day; present study) and longer term (21 day [55]) exposures to atrazine had no effects on body weight.

Results of the present study showed that sublethal concentration of atrazine even in acute and short term exposure can alter the biochemical composition of the fish body and affects some behavioral responses that could lead to failure of the surviving skills of the fish fry.

TABLE I
PHYSICOCHEMICAL PROPERTIES OF THE TEST WATER

Characteristics	Unit	Mean	Range
Air Temperature	˚C	23.4	22.8-24.3
Water Temperature	˚C	18.1	17.9-19.1
Dissolved Oxygen	$mg{\cdot}L^{-1}$	7.20	7.11-8.01
pH	-	7.8	7.7-7.9

Note: Lethal concentration values in rows with different letters significantly differ at $p < 0.05$.

TABLE II
LETHAL CONCENTRATIONS (LC) OF ATRAZINE DEPENDING ON EXPOSURE TIME (12-96 H) FOR *R. FRISII KUTUM*

Point	Concentrations ($mg{\cdot}L^{-1}$) at various exposure times. (95% confidence intervals)				
	12h	24h	48h	72h	96h
LC_1	13.34^a (15.48-10.01)	11.84^b (14.16-9.18)	10.71^c (13.91-9.11)	10.62^c (13.88-8.98)	11.23^d (12.98-8.03)
LC_{10}	17.31^a (18.13-16.08)	16.16^b (17.24-15.41)	15.36^c (16.28-14.18)	14.59^d (15.87-13.53)	14.48^d (15.56-13.11)
LC_{50}	23.12^a (24.83-22.12)	22.43^b (24.52-22.02)	21.78^c (23.14-20.23)	19.77^d (19.11-18.98)	18.53^e (18.64-17.09)
LC_{90}	25.54^a (26.48-24.71)	25.18^a (26.33-24.48)	24.06^b (25.14-23.16)	23.15^c (24.40-22.21)	21.23^d (23.18-20.12)

Note: Lethal concentration values in rows with different letters significantly differ at $p < 0.05$.

TABLE III
MEAN VALUES OF LENGTH (MM) IN CASPIAN KUTUM LARVAE IN EXPERIMENTAL GROUPS

Day	Control	Concentration I (9.25 ppm)	Concentration II (4.62 ppm)	Concentration III (2.31 ppm)
1	6.27±0.09	6.52±0.06	6.12±0.02	6.27±0.02
2	6.59±0.05	6.78±0.05	6.72±0.04	6.58±0.02
3	6.85±0.04	6.98±0.01	6.88±0.02	6.88±0.02
4	7.11±0.03	7.09±0.02	6.95±0.00	7.03±0.01
5	7.21±0.04	7.22±0.03	6.09±0.01	7.12±0.02
6	7.30±0.03	7.30±0.04	7.23±0.02	7.14±0.03
7	7.35±0.04	7.32±0.04	7.39±0.04	7.43±0.04

TABLE IV
MEAN VALUES OF WEIGHT (MG) IN CASPIAN KUTUM LARVAE IN EXPERIMENTAL GROUPS

Day	Control	Concentration I (9.25 ppm)	Concentration II (4.62 ppm)	Concentration III (2.31 ppm)
1	2.00±0.00	2.50±0.28	2.00±0.00	2.00±0.00
2	2.50±0.28	3.00±0.00	3.00±0.00	2.70±0.25
3	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
4	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
5	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
6	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
7	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00

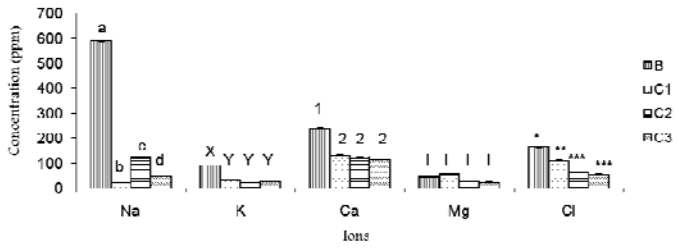

Fig. 1 Mean Concentration of body ions in caspian kutum in experimental groups. Different letters and symbols represented significant differences

TABLE V
MEAN VALUES OF CONDITION FACTOR IN CASPIAN KUTUM LARVAE IN EXPERIMENTAL GROUPS

Day	Control	Concentration I (9.25 ppm)	Concentration II (4.62 ppm)	Concentration III (2.31 ppm)
1	0.77±0.05	0.79±0.06	0.79±0.04	0.81±0.02
2	0.80±0.01	0.84±0.03	0.84±0.03	0.82±0.02
3	0.81±0.02	0.88±0.04	0.87±0.04	0.83±0.02
4	0.83±0.02	0.90±0.08	0.89±0.05	0.86±0.04
5	0.87±0.04	0.96±0.10	0.92±0.10	0.92±0.01
6	0.93±0.11	1.01±0.10	0.98±0.02	0.94±0.06
7	1.00±0.01	1.02±0.10	0.99±0.02	0.97±0.01

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