Bioconcentration and Antioxidant Status Responses in Zebrafish (Danio Rerio) Under Atrazine Exposure

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***Abstract*—The present study was planned designed to investigate the chronic impacts of herbicide atrazine exposure on stress biomarkers acetylcholinesterase activity (AChE) and oxidative stress responses in brain of zebrafish (Danio rerio), and determine the bioconcentration of atrazine in whole body of fish. Chronic exposure to atrazine unveiled a markedly discourage in the activity of AChE. However, significant increase in the activities of catalase (CAT) and superoxide dismutase (SOD) and both influenced by atraizine, and CAT was over sensitivity to atrazine compared with SOD. The highest bioconcentration factor (BCF) of atrazine in the fish treated was (12.8543×10^4 and 13.5891×10^4) after 24h exposure to (0.957 and 1.913 mg L^{-1}) and 13.238×10^4, after 25 d exposure to 0.638 mg L^{-1} of atrazine respectively. This study showed that the zebrafish have ability to bioaccumulate of the herbicide atrazine rapidly. atrazine accumulation in zebrafish has a private importance due to the implication of atrazine in chronically health problems, as well as the information contained in our study is useful for understand the mechanism of atrazine induced oxidative stress in fish.**

***Index Terms*—Atrazine, stress biomarkers, bioconcentration factor, zebrafish.**

I. Introduction

The presence of pesticides in the environment has caused significant social and scientific development anxiety worldwide, as their all over the world extensive usage can create potential risks to the environment and human health, as they can easily pollute bodies of water, resulting in extensive damage to non-target species, including fish [1]. Fish representing as bio-indicators of environmental contamination and may play an important role in the evaluation of the potential risk of pollution in aquatic environment, from they may directly exposed to chemicals caused by agricultural output through runoff or indirectly by food chain of ecosystem, this may reflect the biological influences of environmental contamination in water [2]. The zebrafish (*D. rerio*) is a small equatorial freshwater fish that has a great tolerance for a wide range of breeding circumstances has been employed as an experimental species in the intensive studies of many other scientists, from the 1980s. Particularly in recent years, numerous studies have shown the identified benefits of hiring of zebrafish in environmental toxicological studies [3]. The use of herbicides to control weeds has been admitted, and its frequent applications as a part of farming practices worldwide. Sadly, the random use of these herbicides to improve agricultural output, careless handling, accidental spillage or discharge of untreated effluents into natural waterways may have adverse impacts on non-target organisms, private fish and other aquatic objects and may contribute to long term impacts in the environment [4]. Atrazine is one of the more widely used herbicides found in the rural environments. It is widely used on corn, sorghum, sugarcane, pineapples, and fairly on landscape vegetation, As well as to control aquatic weeds has implementation in fish management where they are used in aquatic habitats particularly rice fields and some fish farms. It is a commonly used that prevents electron transfer mechanisms of photosystem II in objective plants [2]-[5]. Atrazine also been classified as moderately toxic to aquatic species, mobile in the environment, it is from more pesticides observed in creeks, rivers, ponds, reservoirs and ground waters [6]. It contains a comparatively high proportion of water-soluble (32 mg L^{-1}), which assists in its penetration of ground water because of its high mobility through soil [5]. In contrast, WHO [7] noted that atrazine may be degraded in surface water by photolysis and microorganisms and the half-life of 20 - 50 days at 20 - 25 ℃ was found in vitro circumstances and increasing at lower temperatures. The properties of atrazine are summarized in Table 1. Sublethal impacts with biochemical changes of fish tissues may occur with long term exposure to levels of lowest from 2 mg L^{-1} of atrazine [4]. Several writers have mentioned the effect of atrazine on the physiology, metabolism, growth and reproduction of aquatic organisms, special on fishes [8] and also can cause impaired embryonic growth [3]. Hence, it is necessary to identify certain parameters used in the assessment of environmental hazards of pesticides, as the degree of accumulation and stress biomarkers of atrazine in aquatic objects. The study on the environmental hazards allows strategies to be development towards secure fishing managing. The implementation of these strategies makes possible the survival of sanitary fish living on aquatic regions close to the crops where atrazine is used [9]. Therefore, impacts of contaminants bioaccumulation on fish are assessed by chronic toxicity test, and because the stress response of fish is similar in several ways to that which happening in higher vertebrates [10]. The aim of this study was to investigate the bioconcentration level of herbicide atrazine in fish body during sublethal exposure .Then, to evaluate the impact on some biochemical indices in zebrafish (*Danio rerio*). In order to obtain a better understanding of the potential environmental implications of atrazine and to enable

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appropriate instructions to be drawn up for the safe application of this herbicide in the field.

II. Materials and Methods

A. Animals and Experimental Design

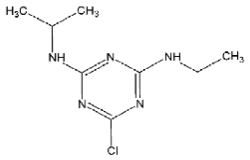
Zebrafish (3.1 -4.6 cm in length, 0.295 to 0.847 g in weight) used as object for sublethal test was purchased from the Institute of hydrobiology, Chinese academy of Science, Wuhan, P. R. China. They were fed twice a day with commercial fish food. The atrazine (2-chloro -4-(ethylamino) -6- (isopropylamino) - S-triazine); 97% purity was obtained from Xingyinhe Chemical Engineering Co. The 96 h LC50 of atrazine was (9.567 mg L^{-1}) by use of Finney's Probit Analysis according to [13] and $1/15_{th}$, $1/10_{th}$ and $1/5_{th}$ of the LC_{50} values were 0.638, 0.957 and 1.913 mg L^{-1} respectively, taken as sublethal concentrations for this study. Fish samples were divided into four groups, each containing 100 fishes in an aquarium of 40-liter capacity. Group I was held in tap water as control and other groups were exposed to sublethal concentration for 5, 10, 15, 20 and 25 days. At the end of each exposure period, the fish were removed from each tank to dissect.

B. Bioconcentration Test

Bioconcentration of attrazine in the whole body tissue of zebrafish was measured by determining the herbicide atrazine in water. After filtrating it using 0.22 μm filtration membrane and stored at 4℃, the samples were analyzed using high-performance liquid chromatography (HPLC). Pesticide were extracted from fish tissue according to the method described by [14], using whole body of zebrafish except its fins, after cut in small portions with a stainless steel scissors and crushed 2g wet weight of sample which that used to extract the pesticides with a quantity of acetonitrile. The sample was homogenized for 1.5 and 1 min, respectively, in vortex mixer before and after 2.00 g of $MgSO_4$ and 0.50 g of NaOAc was added. The sample was centrifuged at 4000 rpm for 5 min. A quantity of 1.0 mL of supernatant was transferred into a 2 mL plastic centrifuge tube containing 50.00 mg of PSA and 100 mg of $MgSO_4$, after blended the sample by a vortex, and centrifuged at 8000 rpm for 5 min. Then, the supernatant was transferred into a 2 mL plastic tube containing 30.00 mg PSA, after blended for 1.5 min and then centrifuged at 8000 rpm for 5 min, finaly the extract was filtered with a 0.22 μm filter and analysed by HPLC. The system SSI 2300-525 HPLC contained: Detector: Variable dual wavelength 525 UV detector; column: Apollo C18 column (250 mm × 4.6 mm, 5 μm) ALLTECH company; the Series III pump Cschrom Plus chromatography workstation; column temperature: 30 ℃. atrazine detections were made in the UV region (λ = 254 nm). The composition of mobile phase was acetonitrile (35%) plus 0.025 M dipotassium hydrogen phosphate (pH 3.0 with acetic acid) (65%). The flow rate and injection volume for herbicid atrazine was 1.0 mL min^{-1} and 20 μL, respectively. The retention times for it were 11.08 min. Under the chromatographic conditions, the quantitative determination of pesticides was performed by using external standards. A 1000 mg L^{-1} stock standard solution of atrazine was prepared by dissolved in (80% aceton) and serial dilutions were made and the standard calibration curve was set up as $y = 32444x - 4283.2$, $R^2 = 0.9933$.

Therefore, a calibration curve for atrazine was plotted between peak base area from the chromatogram and different concentrations.

TABLE I: Nomenclature and Physicochemical Characteristics of the Herbicide Atrazine [11]

Commune Name	Atrazine
Trade names	Malermais, X-Siprim, Vegfru Solaro
IUPAC name	2-chloro-4-ethylamino-6-isopropyl amino- 1,3,5-triazine
Class	Triazine, Herbicide
Physical state:	crystalline solid, white, colorless
Molecular weight:	215.7
Chemical formula:	C8H14ClN5
Chemical structure	 Atrazine
Density:	1.87 g/cm3 at 20oC
Solubility in water:	30 mg/ L
Vapor pressure:	3 x 10-7 mm Hg @ 20o C
Field half life:	60 days
aEPA limit (μg/L):	0.3

[a] Kulluru [12]

C. Determination of AChE and Antioxidant Activities

The brain tissues were collected for biochemical analysis, after homogenized to 1/10 (w/v) ratio of cold physiological saline solution NaCl (0.86%) using a mortar and pestle, and then, centrifuged at 8000 r/min for10 min in 4 ℃, the supernatant was used for biochemical analysis.

AChE activity was determined at 412 nm wavelength by method of [15]. Acetylcholine iodide and dithiobis nitrobenzoic acid were used as substrates. SOD and CAT activities were measured by method of [16]. The specific activity was defined as units of activity per milligram of protein. Total protein content was quantified by the procedure of [17] at 595 nm and using bovine serum albumin as standard. And the standard calibration curve was set as y=0.0051x-0.0013 (R^2=0.9993).

Statistical analysis

The data obtained was analyzed statistically by two-way Analysis of variance (ANOVA). The differences between the control and the exposed groups were well checked. The criterion for significance was set at $p < 0.05$. Statistical analysis was performed by GraphPad software. The bioconcentration factors (BCF) of atrazine in zebrafish were estimated using the following equation: BCF = Cf/Cw

where Cf is the concentration of toxicant in fish and Cw is the

concentration of atrazine in the exposure solution

III. Results

A. Bioconcentration of Atrazine in Zebrafish

Based on the results of toxicity, zebrafish were exposed to 0.638, 0.957 and 1.913 mg L^{-1} of atrazine, and the bioconcentrations of atrazine in fish were detected during different periods (Fig. 1).

The results showed that, for (0.957 and 1.913 mg L^{-1}) treatments, the BCF of atrazine in zebrafish increased rapidly shortly after exposure to the chemical, which reached a high level after 24 h (12.8543×10^4 and 13.5891×10^4) and then declined quickly to a low level after 15 and 25 days. In contrast, the highest BCF of atrazine in fish increased after 25 days in fish treated with 0.638 mg L^{-1} of the chemical was 13.238×10^4.

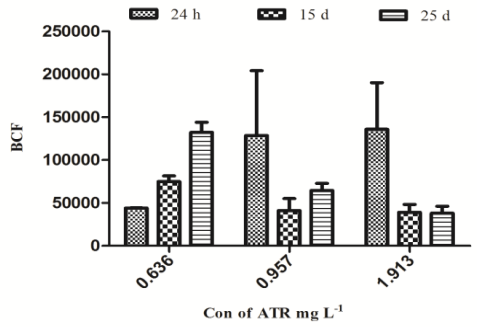


Fig. 1. Bioconcentration factor (BCF) of atrazine exposure to different sublethal concentrations to whole body of zebrafish during 24 h, 15 and 25 days. Values are presented as a mean ± SD.

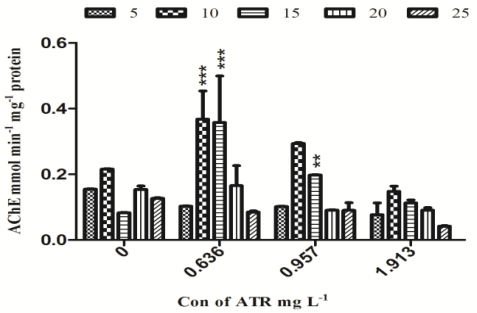


Fig. 2. AChE activities in brain tissues of zebrafish after exposure to different atrazine concentration for 5, 10, 15, 20 and 25 days. Values are presented as a mean ± SD. The asterisk represents a statistically significant difference when compared with the controls; * at $p<0.05$, ** at $p<0.01$ and *** at $p<0.001$ levels.

B. Effect of Atrazine on AChE and Antioxidants Activity in Brain Zebrafish

In brain initially at 5 days (atrazine 0.638 and 0.957 mg L^{-1}) doses the AChE level was decreased 66.452 and 65.806 % respectively. But showed sudden slip at 0.638 mg L^{-1} dose 66.929% after 25 days. whereas the level of AChE was a gradual decline at (atrazine 0.957 and 1.913 mg L^{-1}) doses 59.091 and 51.969% after 20 and 25 days when compared with control fish Fig. 2 ($p<0.05$).

As shown in Fig. 3, during the progress of the experiment, antioxidant enzymes CAT increased ($p<0.01$) in a dose and time-dependent manner in brain tissues after 5, 10, 15, 20, 25 days at all instances of atrazine treatment 162.252, 243.115, 187.608, 217.459 and 255.116 %, respectively, was higher than that observed in the controls, in zebrafish. the antioxidant SOD activity in brain tissue showed marked increase ($p < 0.01$) after 10, 15 days of atrazine treatment at 0.638 and 0.957 mg L^{-1} concentrations by (226.121 and 216.309 %). Whereas, at 1.913 mg L^{-1}, the enzyme activity increased by 110.602, 193.645, 151.763 and 139.423%, respectively, after 5, 10, 15 and 20 days, over control values Fig. 4.

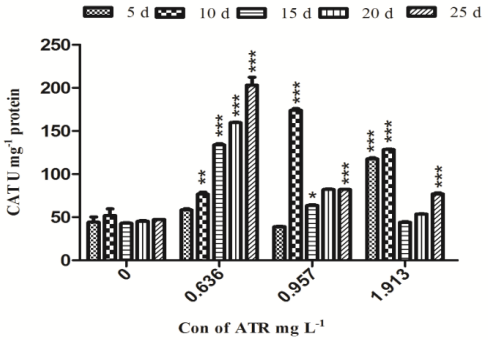


Fig. 3. CAT activities in brain tissues of zebrafish after exposure to different atrazine concentration for 5, 10, 15, 20 and 25 days. Values are presented as a mean ± SD. The asterisk represents a statistically significant difference when compared with the controls; * at $p<0.05$, ** at $p<0.01$ and *** at $p<0.001$ levels.

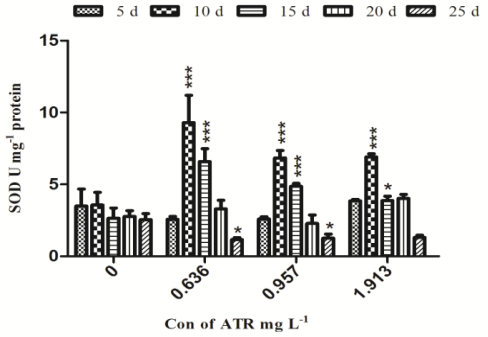


Fig. 4. SOD activities in brain tissues of zebrafish after exposure to different atrazine concentration for 5, 10, 15, 20 and 25 days. Values are presented as a mean ± SD. The asterisk represents a statistically significant difference when compared with the controls; * at $p<0.05$, ** at $p<0.01$ and *** at $p<0.001$ levels.

IV. Discussion

The bioaccumulation of chemical compounds in aquatic organisms has long been assessed by using indices called the 'bioconcentration factor (BCF) [18]. Aquatic organisms accumulate and maintain some of chemicals when exposed to these chemicals via water and their diet [19]. In this study, we select the BCF value for small fish that plays an important role in the aquatic ecosystem. The study showed that atrazine could be rapidly accumulated in fish shortly after their exposure to a sublethal concentration of atrazine, and the highest BCFs of atrazine in zebrafish were 12.8543×10^4 and 13.5891×10^4 (24 h) after exposure to 0.957 and 1.913 mg L^{-1} of atrazine respectively. Outcomes suggested that short periods of atrazine exposure are enough to check the bioaccumulation in fish or other aquatic organisms. our work

Similar to that reported by Zhao [14] indicated that pyrimorph could be rapidly accumulated in zebrafish shortly after their exposure to a sublethal concentration of pyrimorph, and found the highest BCFs of pyrimorph in fish were 1.07×10^2 (144 h) and 23.1 (96 h) after exposure to 2.00 and 0.25 mg L^{-1} of pyrimorph respectively. In turn, these concentrations a decrease of the BCF after 25 days can be noted, which may possibly alludes on promoted organizational processes, as for instance metabolisation and excretion, as suggested by Contardo-Jara [20]. In the same time, the larger BCF value was observed in the zebrafish treated at lower concentration (0.638 mg L^{-1}) of atrazine 13.237×10^4 after 25 days. Similarly, exposure to atrazine the larger BCF value was observed in the Carassius auratus with lower concentration of atrazine [21], as well as in Japanese medaka (*Oryzias latipes*) were exposed to different concentrations of pentachlorophenol (PCP) and 2, 4-dichlorophenol (2, 4-DCP) for 60 days using a continuous flow system, found that the BCF values for both PCP and 2, 4-DCP increased as their aqueous concentrations decreased [18].

The bioconcentration factors in the zebrafish tissues (BCF) in this study were much higher than bioconcentration factors calculated by du Preez and van Vuren, [22] (0.9 to 20.0, in the *Tilapia sparrmanii*) and Jacomini [23] (8.11 to14.85, in two bivalves species (*Anodontites trapesialis* and *Corbicula fluminea*) exposure to atrazine. May be, the smaller fish have highest metabolic rates (per gram of body tissue) and are thus capable to take up chemical material, by food and water, more quickly than larger fish, as proposed by Nussey [24] and rely heavily on species, exposure period, and aqueous concentration of the chemicals [18].

In ecotoxicology, it is highly advised to use cellular and biochemical parameters to evaluate the toxic impacts of chemicals to animals. Because biomarkers can be used to measure the interaction among a biologicalsystem and an ecological agent, which may be chemical, physical, or biological. Discourage or incitement of biomarkers is a well environmental tool to evaluate the exposure and the possible impacts of xenobiotics on objects [25]. The present study attempted to refer to significant impacts of long-term exposure to sublethal concentrations of atrazine pesticide on AChE and antioxidant CAT and SOD activity in the brain of zebrafish.

Can Antioxidant enzymes, whose function is to dispose of oxygen free radicals and protect the organism could indirectly, reflect the alterations of oxygen free radical content in living cells. SOD and CAT protection biomolecules from ROS-mediated harm in *vivo*. SOD can stimulating $O_2^{\cdot-}$ to O_2 and H_2O_2 quickly and then H_2O_2 is disposed of the H_2O_2-scavenging enzyme CAT [26], [27]. Moreover, SOD and CAT activities rose in the brain, after atrazine exposure in different periods (Fig. 2, 3). Like other organisms, fish can combat the high levels of ROS in their systems with protective ROS-scavenging enzymes such as SOD and CAT, which convert superoxide anions ($O_2^{\cdot-}$) into H_2O_2 and then into H_2O and O_2. Thus, it is potential that any further in the activities of these enzymes contributes to the elimination of ROS from the cell resulting by atrazine exposure. Similar finding have also been reported in other species. For example, Jin [28] reported that the various concentrations (1, 10, 100 and 1000 μg L^{-1}) of atrazine exposure induced a significant dose-dependent increase in liver (SOD and CAT) activities in zebrafish (*Danio rerio*). The rise in the activity of SOD and CAT in all of the low-dose groups of atrazine and chlorpyrifos may reflect a countervailing response to increased oxidative stress, as proposed by Xing [29]. Consequently, Singh [30] reported that the activities of SOD and CAT in liver of adult male rats were a great increased following atrazine administration.

The activity of AChE is another biochemical biological marker commonly used to monitor aquatic environments mainly polluted by pesticides. This enzyme can be discouraged by various kinds of agrochemicals, causing excessive-stimulation of muscle fibers and leading to palsy and even death, of animals [27]. In the present study, atrazine exposure increased caused brain AChE activity decline (Fig. 2), similar to that reported by Roex [31], indicated that AChE discourage is extensively consider as a well biomarker of exposure to organophosphorus pesticides (OP). In a work conducted by Xing [32] juveniles of common carp (*Cyprinus carpio*) showed inhibited AChE activity in brain and muscle after 40 d exposure to various atrazine concentrations (4.28, 42.8 and 428 μg L^{-1}). This difference among the results obtained for zebrafish and *C. carpio* can be produced by the variations in exposure periods, the size and the type of the fish. Currently, reports on the toxicity of pesticides on AChE activity in aquatic species focused on acute toxicity, but there are few reports on sub-chronic toxicity of pesticides on AChE activity in aquatic species. Statements about the impact of atrazine on AChE in aquatic vertebrate species are sparse. This was because the discourage of AChE activity is connect directly with the mechanism of toxic action of OPs. Furthermore, AChE was often used as an indicator of OPs exposure and physiological impact in exposed animals [33].

V. Conclusions

SOD, CAT and AChE activities in brain of zebrafish were all affected by atrazine, until at low concentration, which proposed that atrazine could cause oxidative stress to fish brain. Moreover, CAT in fish brain was more sensitive to atrazine compared with SOD. The important variations of antioxidant enzyme and AChE activity may be considered as the sensitive and early biomarker to reveal the pollution impacts.

The zebrafish, *Danio rerio*, have the ability to bioaccumulate the herbicide atrazine in aquarium experiments. There was a rapid absorption of atrazine from water in fish tested, which means that atrazine could accumulate fast in fish. Therefore, should further attention to the residue of atrazine in fish, as well as refers from the public health point of consideration; atrazine accumulation in zebrafish has special importance because of the effects of atrazine in chronic health problems.

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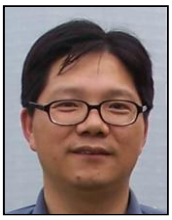

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