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# Monitoring Atrazine residue in *Clarias gariepinus* tissues

**Venees, F. Yassa[*]; Dalia, E. El-Hefny[**] and Nashwa, S. Elias[***]**

*Biochemistry, Nutritional Deficiency Diseases and Toxicology Dept.;
***Fish Diseases Dept. Animal Health Res. Inst.;
**Pesticide Residues and Environmental Pollution Department, Central Agricultural Pesticide Laboratory, Agricultural Research Center, Dokki, Giza, Egypt

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**Abstract**

The aim of the present study was to determine ($LC_{50}$) of Atrazine herbicide on *Clarias gariepinus* female fish. Fish were exposed to ½ $LC_{50}$ (6.5 ppm) for 3, 9 and 15 days. The effect of Atrazine was investigated on some biochemical parameters as serum total protein, albumin, globulin, glucose, cholesterol, calcium, ALT, AST and creatinine of *Clarias gariepinus* fish. Atrazine showed significant decrease in biochemical parameters as total protein, albumin, cholesterol, glucose as a stress indicator which decreased then increased. Serum creatinine showed significant increase.

Assay of the Atrazine residue in the muscles, livers, kidneys, skins and gills of exposed fish revealed detectable Atrazine residue in all examined tissues at the 3rd, 9th and the 15th days of exposure. Atrazine residues increased in muscles, livers and kidneys by increasing the time of Atrazine exposure. Gills recorded the highest Atrazine residues in fish exposed to Atrazine for 3days. Livers of fish exposed to Atrazine for 9 days contained the highest level of Atrazine residue. While kidneys recorded the highest level of Atrazine residues at the 15th day of Atrazine exposure. The residues level of Atrazine in the muscles, livers and kidneys was more remarkable at the 15th day of exposure in contrast to gills and skins. Therefore, the use of herbicide Atrazine must be restricted in agricultural fields with maximum protection of aquatic life.

***Key words:*** *Atrazine, Clarias gariepinus, $LC_{50}$, Biochemical parameters, residues.*

## Introduction

Fish can serve as bio-indicators of environmental pollution and can play significant roles in assessing potential risk associated with contamination in aquatic environment since they are directly exposed to chemicals resulting from agricultural production via surface run-off or indirectly through food chain of ecosystem **Lakra and Nagpure (2009).** Using herbicides to control weeds is a part of agricultural practices in the world. Unfortunately, the indiscriminate use of these herbicides to improve agricultural production and yield may have effects on non-target organisms, especially aquatic life forms and their environment. **(Sudhasaravanan and Binukumari (2014).**

Atrazine (2-chloro4- ethylamino-6-isopropylamino-s-triazine) a member of s-triazine group of herbicides. It is one of the most commonly used herbicides found in the rural environments. Its extensive use to control of annual and perennial grassy or broad leaf weeds in major crops is due to its low cost and high effectiveness **(Shahitha, 2012).** It is widely used on corn, sorghum, sugarcane, pineapples, and to some extent on landscape vegetation. Atrazine rated as moderately toxic to aquatic species (**Sudhasaravanan and. Binukumari, 2014).** Its utilization is controversial worldwide, Atrazine was banned from use in the EU member states starting from 2004 (**European**

**Commission decision 2004)** but not in the U.S. and other countries **(Khan *et al.,* 2016b).**

Atrazine is non-volatile and its half-life is about 200 days but it varies from 21 days to 1 year depending on the environmental factors such as pH of the soil, type of soil, moisture content, temperature and the microbial communities **(Zhang *et al.*, 2012).** Due to the excessive use and the high persistence of Atrazine, it is moved to water bodies such as rivers, lakes and drinking water supplies and it had also been found in ground waters **(Spalding *et al.*, 1994).** The potential contamination of water resources and soil with Atrazine may cause pollution to the environment and bring enormous harm to human and other animals as it can be concentrated by plants and transferred to the food chain **(Topp *et al.*, 2000 and Reyad *et al*., 2017)**

Some studies had reported Atrazine as one of the endocrine disruptors **(Moore and Waring, 1998)** and as a probable human carcinogen **(Luciane *et al.,* 2010).** Moreover, Atrazine was found to be the reason for low sperm levels in men, the premature birth, miscarriage and various birth defects in humans **(Ackerman, 2007; Pathak and Dikshit, 2011).**

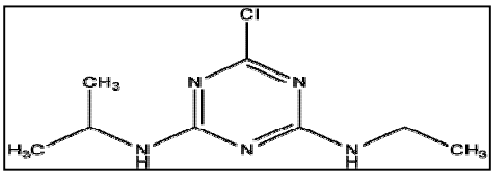
This work aimed to determine Atrazine residue in *C. gariepinus* fish and its effect on serum protein, glucose, cholesterol, liver and kidney function.

## Materials and Methods

**Fish sampling:** A 108 female catfish, *C. gariepinus* of average body weight 300 ±25 g were obtained from private fish farm. Fish were transferred to the Animal Health Research Institute, in well aerated containers. The fish were kept at Fish Diseases Department - Wet lab in identical glass aquaria measuring (100 x 50 x 50 cm) aerated with air pumps supplied with dechlorinated tap water and left for two weeks for acclimatization. Fish were examined clinically to assure the absence of any abnormalities or external lesions.

**Herbicide:**

**Atrazine:** 2–chloro-4-ethylamino-6- isopropylamino-S-triazine**,** product of Syngenta




**Determination of half lethal concentration ($LC_{50}$)/ 96 hours of Atrazine:**

The half lethal concentration ($LC_{50}$) was determined for Atrazine on female *C. gariepinus* weighting (300 ± 25g). A minimum of five concentrations; namely: 2.5, 5, 10, 20 and 40 mg/l, plus the control group where eight fish were used for each concentration.

Estimation of the concentration of Atrazine which causes 50% mortality as ($LC_{50}$) for 96 hours exposure was calculated using the method of **Litchfield and Wilcoxon (1949).**

**Experimental design:**

Sixty fish were divided into four groups. The 1st group is the control group. The three other groups were exposed to 1/2 $LC_{50}$ (6.5 mg/l) of Atrazine for 2 weeks. Blood samples were obtained after the 3rd, 9th and 15thday of Atrazine exposure.

**Clinical investigation**

The exposed fish were kept under proper observation during the period of experiment for any clinical abnormalities and deaths according to the method described by **Amlacher (1970).**

**Mortality rate:** was calculated in control and Atrazine groups.

**Collection of blood samples:**

Under the effect of benzocaine (50mg/l), fish were anesthetized for 5 minutes according to **Post (1989).** Blood samples were collected from the caudal vein using plastic syringes in dry sterilized vials. Blood samples were allowed to clot at room temperature and centri-

fuged at 4000 r.p.m. for 10 minutes for serum separation.

**Biochemical examination:**
The separated serum samples were used for determination of Total protein according to the method described by **Bradford (1976).** Serum albumin was measured as described by **Dumas** and **Biggs (1972)**. Globulin was calculated according to the method described by **Coles (1986)**. Glucose and cholesterol were determined according to the method described by **Trinder (1959)** and **Richmound (1973)**, respectively. Serum calcium was carried out according to **Glinder and King (1972)**. Serum alanine amino transferase activity (ALT) and aspartate amino transferase activity (AST) were carried out according to the method of **Reitman and Frankel (1957).** Kinetic determination of serum creatinine was performed according to the method described by **Henry (1974).**

**Determination of Atrazine residues:**
**Chemicals and Reagents:**
Analytical standard of Atrazine (99.37% purity) was supplied by Dr. Ehrenstorfer, Germany.
Acetonitrile (HPLC-grade) were supplied by SDS (France).
Acetic acid purchased from El Nasr pharmaceutical chemicals Co., (Egypt).

**Extraction and determination**
Samples from muscles, livers, kidneys, skins and gills of fish were used for determination of Atrazine residue at the 3rd, 9th and the 15th days of exposure. The homogenized samples were extracted with acetonitrile acidified with 1% (v/v) of acetic acid and the residues were determined by GC-μECD according to **Rocha *et al.* (2015).**

**Statistical Analysis:**
Results were expressed as means ± standard errors. Data obtained were analyzed using T-student test according to **Petrie and Watson (1999).**

**Results**
Half lethal concentration ($LC_{50}$)/96h of Atrazine in female *C. gariepinus* was 13.28 mg/l (table, 1). Protein profile revealed significant decrease in serum total protein and albumin levels of *C. gariepinus* exposed to ½ $LC_{50}$ Atrazine for 3, 9 and 15 days (table, 2). Significant decrease in serum glucose level observed after 3 days of ½$LC_{50}$ Atrazine exposure meanwhile, significant elevation of glucose recorded at 9 and 15 days. Serum cholesterol level showed significant decrease throughout the three exposure periods while serum creatinine showed significant increase (table, 3).
Assay of the Atrazine residue in the muscles, livers, kidneys, skins and gills of exposed fish revealed detectable Atrazine residue in all examined tissues at the 3rd, 9th and the 15th days of exposure. Atrazine residues increased in muscles, livers and kidneys by increasing the time of Atrazine exposure. Gills recorded the highest Atrazine residues in fish exposed to Atrazine for 3days. Livers of fish exposed to Atrazine for 9 days contained the highest level of Atrazine residue. While kidneys recorded the highest level of Atrazine residues at the 15th day of Atrazine exposure. The residues level of Atrazine in the muscles, livers and kidneys was more remarkable at the 15th day of exposure in contrast to gills and skins (table, 4).

**Table (1).** Half lethal concentration ($LC_{50}$) /96h of Atrazine in female *C. gariepinus*.

| Atrazine Conc. (mg/l) | No. of Fish in each group | No. of alive fish | No. of dead fish | a | b | ab |
|---|---|---|---|---|---|---|
| 0 | 8 | 8 | 0 | 0 | 0 | 0 |
| 2.5 | 8 | 7 | 1 | 2.5 | 0.5 | 1.25 |
| 5 | 8 | 6 | 1 | 2.5 | 1 | 2.5 |
| 10 | 8 | 3 | 5 | 5 | 3 | 15 |
| 20 | 8 | 2 | 6 | 10 | 5.5 | 55 |
| 40 | 8 | 0 | 8 | 20 | 7 | 140 |
| | **∑a x b =213.75** | | | | | |

Half lethal concentration of Atrazine = Highest conc. – ∑a x b /n

$LC_{50}$ = 40 - 213.75/8 $LC_{50}$ = 40 - 26.72= 13.28 mg/l

Where :

a: Constant factor of difference between groups.

b: Mean value of dead fish between each two successive groups.

n: Number of fish in each group.

**Clinical examination** revealed highly nervous manifestations such as jumping and gasping of air, abnormal swimming behavior (erratic swimming).

**Mortality rate:** Mortality rate in the three groups exposed to Atrazine was 20% (9 fish) while mortality rate of the control group was 0% (0 fish).

**Table (2).** Protein profile of control and Atrazine groups.

| Parameters | Control | 3 days | 9 days | 15 days |
|---|---|---|---|---|
| **Total protein (g/dl)** | 5.65 ±0.12 | 5.24* ±0.05 | 4.99** ±0.02 | 5.15* ±0.07 |
| **Albumin (g/dl)** | 2.67 ±0.05 | 2.31** ±0.03 | 2.36** ±0.01 | 2.43** ±0.03 |
| **Globulin (g/dl)** | 2.98 ±0.15 | 2.93 ±0.04 | 2.63 ±0.02 | 2.72 ±0.05 |

Values represent as means ± standard errors (n=10). * Significantly at P< 0.01 using t- student test

**Significantly at P< 0.001 using t- student test.

**Table (3).** Some serum biochemical parameters of control and Atrazine groups.

| Parameters | Control | 3 days | 9 days | 15 days |
|---|---|---|---|---|
| **Glucose (mg/dl)** | 96.20 ±1.21 | 75.69*** ±2.72 | 102.65*** ±0.97 | 102.27** ±1.52 |
| **Cholesterol (mg/dl)** | 161.12 ±3.76 | 139.51*** ±2.51 | 149.202** ±1.44 | 153.01* ±0.34 |
| **Ca (mg/dl)** | 7.73 ±0.21 | 7.90 ±0.22 | 8.05 ±0.22 | 8.35 ±0.28 |
| **ALT(u/ml)** | 37.38 ±0.66 | 35.34 ±0.97 | 36.85 ±0.62 | 36.26 ±0.66 |
| **AST(u/ml)** | 38.91 ±0.96 | 37.46 ±0.38 | 37.54 ±0.87 | 39.98 ±0.05 |
| **Creatinin (mg/dl)** | 0.52 ±0.05 | 1.03** ±0.14 | 1.33*** ±0.04 | 1.22*** ±0.13 |

Values represent as means ± standard errors (n=10). * Significantly at P< 0.05

* *Significantly at P< 0.01 * **Significantly at P< 0.001 using t- student test

**Table (4)**. Atrazine residues in different tissues of *C. gariepinus*.

| **Atrazine residues** (µg/g) | **3 days** | **9 days** | **15 days** |
|---|---|---|---|
| Muscles | 0.37±0.18 | 1.39±0.19 | 3.84±0.17 |
| Livers | 2.50±0.29 | 4.03±0.72 | 4.71±0.57 |
| Kidneys | 1.01±0.15 | 3.08±0.17 | 5.49±0.29 |
| Skins | 2.75±0.32 | 0.38±0.07 | 0.09±0.04 |
| Gills | 4.33±0.38 | 1.81±0.18 | 0.28±0.02 |

Values represent as means ± standard errors.

## Discussion

Pollution of water forms a pressing worldwide problem in aquatic environment. Moreover, fish have been widely documented as useful bioindicator for ecotoxicological studies because of their differential sensitivity to pollution.

Result of half lethal concentration ($LC_{50}$) /96h revealed that the ($LC_{50}$)/96h of Atrazine is 13.28 mg/l (Table 1). The result is nearly similar to those of **Marzouk *et al.* ( 2012 )** who mentioned that the $LC_{50}$ of Atrazine for *C. gariepinus* was 13.75 mg/l**. Tomlin (2000)** and **Brodeur *et al.* (2009)** recorded that $LC_{50}$ values of Atrazine for bluegill sunfish *Lepomis macrochirus a*nd toads *Rhinella arenarum* were 16 mg/l and 14.41 mg/l, respectively. This also appeared far beyond **Neskovic *et al.* (1993**) and **Nwani *et al*., (2010)** who found it 18.8 mg / l and 42.38 mg / l for *Cyprinus carpio* and *Channa punctatus* respectively and more than **Hussein *et al.* (1996)** and **Kreutz *et al.* ( 2008)** who recorded it 9.37 mg / l and 10.5 mg /l for *O. niloticus* and silver catfish respectively. In spite of being the same pesticide the difference in $LC_{50}$ was referred to species difference, age and environment.

Due to bioaccumulation of herbicides, the concentration of herbicides increase enough to induce toxic responses in fish **(Cavas, 2011).** The observed behavioral changes in *C. gariepinus* exposed to Atrazine in the present study, were highly nervous manifestations such as jumping and gasping of air, abnormal swimming behavior (erratic swimming) which indicated internal effects on body physiology, may be attributed to their neurotoxic effects. This abnormal behavior came in accordance with those mentioned by **Bekeh *et al*. (2016)** due to Atrazine pollution and was explained by **Odo *et al.* (2017)** to be a fish attempt to avoid breathing in poisoned water. These hypoxic conditions arose primarily due to gills damage which in turn hampers oxygen uptake.

Proteins are involved in the architecture and physiology of the cell and in cell metabolism. Blood serum proteins were defined by **Moustafa (1999)** to be a fairly biochemical system, precisely reflecting the condition of the organism and its physiology under the influence of internal and external changes. **Hanna and El-Maedawy (2007)** attributed total protein decrease due to protein catabolism and hepatocellular damage caused by pesticide. This was the comprehensible reason for the highly significant decrease in the total serum protein levels of *C. gariepinus* exposed to Atrazine throughout the three exposure periods (table, 2). The reduction in total protein come in agreement with **Owolabi and Omotosho (2017)** who reported significant decrease in blood protein of *C. gariepinus* exposed to different concentrations of Atrazine for 96 h (acute exposure) and 28 days (chronic exposure) and **Khan *et al* (2016 a, b)** in Atrazine exposed common carp, and grass carp fish, respectively. **Jenkins *et al.* (2003)** declared that exposure of fish for a long time to most toxicants (including herbicides) interferes with protein metabolism, and in their study they attributed the decrease in protein levels to stress-mediated immobilization of these compounds,

as a result of an increase in energy demands by the fish to cope with environmental conditions caused by the toxicant. Moreover, **Das *et al* (2004)** mentioned that the increased energy demand might trigger protein catabolism, a process in which protein is converted into energy, and therefore the serum protein will be reduced. In the present study, the reduction of fish serum protein might be due to stress and the toxic effect of Atrazine on the kidney and liver **( Abdali *et al*., 2011)**.

Serum albumin plays an important role in maintaining the osmotic balance between the circulating blood and the tissue membrane (**Harper *et al.,* 1997)**. A significant decrease in serum albumin observed in the this study was in agreement with **Khan *et al.* (2016b)** and **Abdali *et al.* (2011)** in acute and chronic exposure of Atrazine in grass carp. The same observation reported by **Rani, and Gautam (2009)** in *Channa punctatus* exposed to sub-lethal concentrations of Nuvan toxicant. The decrease in serum albumin may be due to a transient inhibitory effect on the synthesis of albumin by the hepatic parenchyma as suggested by **Grimoldi *et al.*** (**1993**).

Blood glucose is a sensitive and reliable indicator of pollutants causing environmental stress in fish **(Mekkawy *et al.,* 2011).** The results of this research revealed that Atrazine was very toxic and exerting much stress which appeared in the highly significant decrease in glucose level with the start of exposure at 3 days which was explained by **Ramesh *et. al.* (2009)** as a result of hypoxic condition caused by Atrazine exposure in *Cyprinus carpio*. Hyperglycemia observed at the $9^{th}$ and $15^{th}$ days could be explained by the increased secretion of catecholamine from the adrenal medulla, which enhancement glycogen breakdown and increases the blood glucose level and corticosteroids as a stress response of fish exposed to herbicides **(Khan *et al*., 2016a)**. The present elevation in glucose concentration coincide with **Owolabi and Omotosho (2017)** who reported significant increase in glucose in the blood of *C. gariepinus* fish exposed to different concentrations of Atrazine for four days. Also**, Blahova *et al.* (2014) and Khan *et al.* (2016a)** recorded an elevation in plasma glucose level of the Freshwater Common Carp (*Cyprinus carpio*) exposed to Atrazine for four days.

Serum cholesterol level revealed significant reduction in cholesterol concentration observed after 3, 9 and 15 days of Atrazine exposure (table, 3). The present reduction in cholesterol may be related to the utilization of cholesterol in the manufacture of Cortisol as a result of stress induced by the Atrazine exposure (**Abdali *et al.,* 2011)**.

Environmental stress conditions caused a decline in concentrations of plasma glucose. Fish need energy to overcome stress, so for this purpose protein catabolism and then lipid contents catabolism occurred, causing cholesterol concentration to decline for the purpose of matching the needed energy during stressful conditions (**Binukumari and Vasanthi, 2013)**. The decrease in cholesterol concentration was in accordance with the findings of **Khan *et al*. (2016a**) and **Abdali *et al.* (2011)** in Atrazine exposed Common Carp and grass carp, respectively.

Serum creatinine level revealed significant increase in *C. gariepinus* fish in the $3^{rd}$, $9^{th}$ and $15^{th}$ day of Atrazine exposure compared to control group. Creatinine is an accurate marker of kidney function. Elevation of creatinine concentration underlying that many waste products in the fish bloodstream would not be cleared, indicating that the kidneys were not functioning properly **(Ajeniyi And Solomon, 2014**). Moreover, **Joythi, and Narayan, (2000)** mentioned that elevation of creatinine level reflects malfunction of kidneys under stress being functionally insignificant because of cellular damage as result of pesticide exposure. Similar result reported by **Amin and Hashem (2012)** who found significant elevation in serum creatinine in catfish exposed to 0.75 µg/l deltamethrin for 48 h. **El-Said (2007)** found marked increase in the creatinine concentration in *Oreochromis niloticus* fish exposed to abamectin.

Assay of the Atrazine residue in the muscles, livers, kidneys, skins and gills of exposed fish shown in table (4). Results revealed detectable Atrazine residue in all examined tissues at the 3rd, 9th and the 15th days of exposure. Atrazine residues increased in muscles, livers and kidneys by increasing the time of Atrazine exposure. Gills recorded the highest residues of Atrazine in fish exposed for 3 days. Livers of fish exposed to Atrazine for 9 days contained the highest level of Atrazine residue. While kidneys recorded the highest level of Atrazine residues at the 15th day of Atrazine exposure. The residues level of Atrazine in the muscles, livers and kidneys was more remarkable at the 15th day of exposure in contrast to gills and skins.

The presence of pesticide residues in *Clarias gariepinus* and *Tilapia zilli* is an evidence of bioconcentration (from water via gills and epithelial tissues) and bioaccumilation (through water and food, leading to biomagnification in different organisms) of pesticides from the surrounding environment **(Ezemonye *et al.,* 2015).** Our results agreed with **Ezemonye *et al.* (2015)** who found Atrazine residues in *Clarias gariepinus* and *Tilapia zilli* obtained from Illushi, Owan and Ogbesse rivers in Edo State, Nigeria. While **Reindl *et al.* (2015)** reported the presence of Atrazine in whole Baltic herring and their livers. Atrazine was found in the muscles and livers of birds and mammals in addition to fish. They also, mentioned that Atrazine became accumulated in the liver of birds and mammals while magnification was determined in their muscles. **Kidwell *et al.* (1990) and Maurano *et al.* (1997)** equally adds that pesticide accumulation in fish was due to their lipid content, this implies that the high lipid content in *Clarias gariepinus*, allows more pesticide residues tend to be trapped in their lipid stores. Also, **Romanic *et al.,* (2014)** observed a positive correlation between the lipid content of fish muscles and the concentration of organochlorine pesticides.

The presence of Atrazine pesticide in fish is a major concern because pesticides have a number of adverse effects on the aquatic organism such as reproductive impairment and suppression of the immune system **(Aguilar *et al.,* 2002),** which can have long-term consequences for population viability. Furthermore, consumption of each fish species, especially species with more fat content (*Clarias gariepinus*), and from high trophic levels, may expose consumers to possible health hazard because the consumption of contaminated food (including fish) has been established as a major route of human exposure to pesticides and other contaminants **(Biego *et al.,* 2010; Ni *et al.,* 2012 and Barnhoorn *et al.,* 2015).**

Previous studies had reported Atrazine as one of the endocrine disruptors **(de la Casa-Resino *et al.,* 2012)** in the common quail and as a probable human carcinogen (**Luciane *et al.,* 2010).** Moreover, Atrazine was found to be the reason for low sperm levels in men, the premature birth, miscarriage and various birth defects in humans **(Ackerman, 2007; Pathak and Dikshit, 2011 and Reyad *et al.,* 2017).**
The primary target of Atrazine in humans and animals is the endocrine (hormonal) system. Effects reported in adults (human and experimental animals) include shortening of estrous cycle length, attenuation of the LH (luteinizing hormone) surge, decreases in pituitary hormone levels, ovarian histopathology (changes in ovarian tissue), and liver effects including increased serum lipids and liver enzymes and liver histopathology. Other effects on the central nervous system, immune system, and cardiovascular function have been reported in adults. Exposure to Atrazine may be associated with some types of non-Hodgkin's lymphoma in adult humans. Significantly increased risk of preterm delivery, intrauterine growth retardation, and decreased birth weight were associated with Atrazine concentrations in drinking water (**EPA, 2007**).

**Conclusion**

Atrazine has strong effect on the serum biochemical parameters of the fish. Therefore, these parameters can be used as effective guidelines for the toxic level indices for fish. Atrazine residues increased in muscles, liver and kidney by increasing the time of Atrazine exposure. Therefore, the use of the herbicide Atrazine must be restricted in agricultural fields with main protection for aquatic life.

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