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Lethal and Sublethal Effects of Atrazine to Amphibian Larvae

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Abstract

The effects of Atrazine contamination on amphibian larval stages were assessed, using acute and chronic toxicity in the laboratory. Tadpoles of *Ptychadena bibroni* at varying post-hatch developmental stages (1, 2, 3, and 4 weeks) were exposed to environmental relevant treatment concentrations of 200, 400, 600µg/L and 3, 30, 100µg/L for acute and chronic toxicity tests respectively. The effects were assessed by comparing mortality, glycogen levels and behavioral response of a control group and a group exposed to the pesticide. The American Society for Testing and Material (ASTM) recommended semi-static renewal bioassay method to be employed, and LC_{50} was measured at 96 hours. Percentage of mortality increased with increase in concentration and exposure duration; but decreased as the tadpoles matured. Mean percentage mortality of tadpoles were significantly affected by concentrations and developmental stages . Derived 96 hours LC_{50} values decreased with increase in exposure duration but increased with each successive developmental stage. Estimated 96 hours LC_{50} ranged from 230.06 – 431.32µg/L. Glycogen levels varied negatively with concentrations, but it increased with each successive developmental stage. Mean glycogen level of tadpoles, exposed to Atrazine, were significantly different in the developmental stages but showed no significant difference with concentrations (F= 1.493, P>0.05). The above results of acute and chronic exposure to Atrazine indicate marked behavioral and physiological effect of Atrazine on *Ptychadena* tadpoles. Results obtained from this study would serve as a fundamental platform for development of Atrazine safety limits for monitoring the waters of the Niger Delta ecological zones of Nigeria.

الملخص

تم تقييم أثار التلوث بالمبيد الحشري أترازين على يرقات الضفادع باستخدام السميةالحادة والسمية المزمنة ف المختبر. حيث تم تعريض يرقات الضفدع *Ptychadena bibroni* البالغ عمرها 1 و2 و3 و4 اسابيع للتراكيز 200و 400و 600 ميكروجرام في اللتر و كذلك3و 30و 100 ميكروجرام في اللتر. وتم التقييم بمقارنة نسب الوفيات ومستوى الجلايكوجين والاستجابات السلوكية باليرقات التي لم تتعرض للتسمم. وتمت المقارنة حسب طريقة الجمعية الاميركية(ASTM) للفحوصات. وتم حساب LD_{50} بعد 96 ساعة. لقد وجد ان نسبة الوفيات تزداد بازدياد التركيز ومدة التعرض ولكنه يتناقص كلما زاد عمر اليرقة. لقد وجد ان حساب LD_{50} يتناقص بزيادة التركيز والمدة ويرتفع بزيادة عمر اليرقة وتراوحت $_{LD50}$ من 23.06 الى 431.32 مايكروجرام في اللتر. تناقصت مستويات الجلايكوجين مع التركيز وازدادت مع عمر اليرقة. وتدل النتائج السابقة على وجود تأثير سلوكي وفسيولوجي للمبيد الحشري أترازين على يرقات الضفدع *Ptychadena*. وتعتبر هذة النتائج أساسا لوضع الحدود اللازمة لتراكيز الاترازين في المناطق البيئية لمياه دلتا النيجر.

Keywords: Amphibian; Atrazine; Life-Stage; LC_{50}; Glycogen Level

1. Introduction

Using Pesticides has increased worldwide over the years to secure food supply for the teaming global population. In tropical regions, Nigeria in particular, an intensive practice has led to higher pesticides usage (Osibanjo and Jensen, 1980). Although it is undisputed that pesticides are essential in modern agriculture, there is a growing concern about possible environmental contamination from agrochemicals. The ecological effects of pesticides on amphibian populations are a growing concern (Bishop 1992, Hall and Henry 1992, Philips 1994). Human activities have led to the release of

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pollutants, for instance, pesticides into the natural environment. This often results in habitat distortion and extinction of local amphibian populations. While pesticides have the potential to affect many aquatic taxa, the impacts on amphibians are of particular concern in the past decade because of the apparent global decline of many species (Blaustein and Wake 1990, Alford and Richards 1999, Houlihan *et al*. 2001, Kiesecker *et al*. 2001). The lists of possible causes of amphibian declines are numerous, and pesticides have been implicated in at least some of these declines.

The decline of world populations of amphibians is a major environmental issue (Vertucci and Corn 1996). Amphibians are an integral part of their ecosystems; affecting nutrient cycling and also serving as high quality prey for many species (deMaynadier and Hunter 1995). In the last 15 years, scientists have accumulated evidence supporting a global decline in amphibians. As the quantitative evidence grows, it is difficult to deny the validity of this global trend (Houlahan *et al.* 2000, Stuart *et al,* 2004). Amphibians are especially at risk from agricultural contamination because they have permeable skin and eggs that readily absorb chemicals from the environment. Many species are vulnerable to aquatic contamination because they experience aquatic and terrestrial stressors, and play vital roles in communities and are sensitive to contaminants. Most amphibians complete their life cycles near fields, where pesticides are applied and have vulnerable embryo and larval stages whose development coincides with pesticides application (Blaustein and Kiesecker 2002, Hayes *et al*. 2003).

The larvae of frog species, *Ptychadena bibroni*, was chosen as test organism for this study because it is the most dominant and widely spread in the Niger Delta regions of Southern Nigeria (Akani and Luiselli 2003) where Atrazine use is substantial.

Atrazine (2 – Chloro – 4 ethylamino -6-isopropylamino-S-trazine) is a selective, pre and post–emergence herbicide used on a variety of terrestrial food crops, non-food crops, forests, residential turf, golf course turf, recreational areas, and rangeland. In Nigeria, it is commonly used for the control of weeds in most farms. Although used to control broadleaf and many other weeds on a range of agricultural and non-agricultural sites, the herbicide's largest use is on corn, sorghum, and sugarcane (Solomon *et al*. 1996). Despite its widespread, intensive use of Atrazine is considered safe because of its short half-life and negligible bioaccumulation and biomagnifications (Solomon *et al*. 1996). The present study investigates the hypothesis that Atrazine may interfere with survival of tadpoles (*P .bibroni*) at ecologically relevant low doses.

Until recently the adverse effects of pesticides on non-target organisms have not seriously been considered when compared with research in the parent fields of experimental Ecology and Toxicology (Sparling *et al. 2000*). Pesticide use is known to cause serious environmental problems, especially in the dry season when the dilution capacity of water systems is low; thus increasing the risk of high concentration of toxic chemicals. Studies on the effectiveness of many commonly used pesticides on target organisms have been carried out extensively in virtually all agro – ecozones globally. However, the side effects of these pesticides on non-target organisms remain largely unknown.

This study simultaneously evaluates the lethal (survival) and sublethal (glycogen level and behaviour) effects of Atrazine on larval stages of the dominant amphibian species: *Ptychadena bibroni* of the Niger Delta of Nigeria. The results of this study would provide a fundamental platform for establishing regulatory limits for Atrazine load in Nigerian Niger Delta waters.

2. Materials and Methods

2.1. Collection of Test Organisms

Eggs of the amphibian species were collected from an inlet of Ikpoba River, an inland River in Southern Nigeria. Egg clutches of the frog were identified in the field by a dichotomous field guide (Amphibian Web 2003, Gosner 1960, and Roedel 2000).

Hatching of eggs, rearing of tadpoles, and testing were done in the post-graduate ecotoxicological research laboratory at the Department of Animal and Environmental Biology, University of Benin, Nigeria. After hatching, emerging larval tadpoles were distributed into six (2.2 x 2.2cm) plastic tanks each containing 1 liter of dechlorinated tap water. They were allowed to acclimatize for seven days in the holding tanks prior to the bioassay (ASTM, 1985). Tadpoles were fed with ad–libititum daily with ground maize powder. Larvae were reared on a 10:14h light: dark cycle (dark from 6 p.m. to 8 a.m.) to minic natural , condition, and room temperature were maintained at 30± 2^0C throughout the duration of the experiment. The water in each holding tank was change every three days.

2.2. Test Chemicals

The pesticide, used for the 96-hour acute toxicity and chronic toxicity tests, was the organochlorine, Atrazine (Atraforce, 80% Top Atazine). The pesticide is commonly used on farms in Nigeria for the control of weeds.

2.2.1. Test Water

Water for toxicity testes was dechlorinated tap water. The water was dechlorinaated by allowing it to stand exposed for 36 hours (Ezemonye and Enuneku, 2005). This water was used for acclimatization, control tests, and for making the various concentrations of the test chemical.

2.2.2. Test Solutions

Stock solutions of the required concentrations were prepared for both pesticides. 1g of 80% pure commercially available Atrazine was dissolved in 1 litre of dechlorinated tap water. The solution was mixed thoroughly until all granules dissolved. One milliliter of this solution was added to 999 ml of dechlorinataed tap water to make a stock solution of 1mg/L. The stock was than diluted into environmental relevant treatment concentrations of 0, 200, 400, 600 µg/L and 0, 3, 30, 100µg/L for acute and chronic tests respectively (Freeman and Rayburn, 2004, Storrs and Kiesecker, 2004).

2.2.3. Acute Toxicity Tests

Acute toxicity tests were conducted according to standard procedures (ASTM, 1996). Fourty amphibian

larvae (two replicates of 20 each) were exposed for 96 hours to each selected concentration of pesticide solution. The semi-static renewal bioassay procedure started with a range finding test (ASTM, 1985, ASTM, 1996). This was used to determine the range that would produce the desired LC_{50} effect for the different life stages. Amphibian larvae of Gosner stages 20, 27, 35 and 43 (Gosner, 1960), which were 1, 2, 3, and 4 weeks old respectively, were used for the test. Exposures lasted for approximately 28 days. Twenty (20) tadpoles were assigned to individual experimental units containing one of the treatments of Atrazine (0, 200, 400 and 600µg/L).

A new stock solution for Atrazine was made up every 3 days immediately before each water change since it has a minimum half-life of 48hours in water (Solomon *et al*. 1996).

2.2.4. Mortality

Mortality was recorded at an interval of 24 hours over a period of 4 days (96-hours) for each post-hatch maturation stage. Tadpoles were taken dead when they turned upside down and sank to the bottom of the tank or when their tail showed no form of movement even when prodded with a glass rod (Mgbaeruhu, 2002).

2.2.5. Chronic Toxicity Tests

The Chronic toxicity test was carried out in a similar manner as the acute test, however, for chronic toxicity tests; very low, and sublethal concentrations of the pesticides were used. Amphibian larvae were exposed to concentrations of 0, 3, 30, and 100µg/L. The lowest concentration 3µg/L of Atrazine was based on the drinking water standard of Atrazine, as set by the U.S. Environmental Protection Agency, (U.S. EPA, 2002). Exposures lasted for approximately 28 days, and every week tadpoles were collected to assess their glycogen levels.

2.2.6. Behavioural Response

Larval behavioral response was monitored in this Behavioral response was assessed in-situ by observing the swimming activity of tadpoles. This was achieved by gently prodding all individual larvae and gauging their response as normal when larvae swim away immediately or as abnormal when there is a delay, or no response, or impaired swimming ability.

2.2.7. Glycogen level Bioassay

The glycogen levels of tadpoles were estimated using digestion, based on glucose oxidase method of Trinder (1969). Reagents used for the glucose oxidase method were Reagent 1(R_1), which contain phosphate buffer (PH7), phenol, and sodium azide. Reagent 2(R_2) contains glucose oxidase, peroxide, 4-aminophenozone, a standard glucose solution, and a color reagent. 1m l of the color reagent was pipetted into dry test tube and, 0.01 ml of sample was added. After color development, 2.0 ml of distilled water was added. The test tubes were thoroughly shaken and incubated at 37^{o}C for 10 minutes. A standard glucose solution was also similarly treated. They were subsequently analyzed, using spectrophotometer; and absorbance was read at 500ml against the reagent blank. Glycogen levels in the samples were extrapolated from a graph of glucose concentration vs. absorbance (Cicik and Engin, 2005).

2.2.8. Statistical Analysis

The susceptibility of the tadpoles to both pesticides was determined by using the Probit (Probit software) method of analysis (Finney, 1971), for median lethal concentration at 96 hours. Safe concentrations at 96 hours for each developmental stage were obtained by multiplying the lethal concentration by a factor of 0.1 (EIFAC, 1998). Computation of confidence internal of mortality rate was also obtained from the Probit analysis used to determine the LC_{50}. The two-factor ANOVA (analysis of variance) in Microsoft Excel was used to test the variable at $P< 0.05$ level of significance. Multiple bar graphs and line graphs were also generated in this study for the pictorial representation of assessment endpoints.

3. Results

The results of the acute and chronic toxicity of tadpoles of *P. bibroni,* exposed to varying concentrations of Atrazine pesticides, are presented in Tables 1 and 2 and further illustrated in Figures 1-3.

3.1. Control

No mortality or morphological changes were observed in the controls for the 96-hour acute toxicity test at the different developmental stages. Tadpoles in the control experiment for both acute and chronic toxicity tests appeared active and healthy throughout the test period. The proportion of abnormal behavioral response in the control was less than 10%.

3.2. Acute Toxicity

The tadpoles of *P.bibroni,* exposed to varying Atrazine concentrations, recorded mortality in all the concentrations. The mean percentage mortality was increased with increase in concentration and exposure duration for each developmental stage (Table I). This indicated that mortality was concentration-dependent. However, mortality was decreased with increase in the developmental stages (Table 1). One hundred percent (100%) mortality was observed in one (1) week old tadpoles at 96 hours. Successive developmental stages of two (2), three (3), and four (4) weeks showed a decrease in percentage mortality at 96 hours of 90%, 80% and 75% respectively (Figure).

Derived 96-hour LC_{50} values for the different developmental stages ranged between 230.058 – 431.323µg/L. Estimated 96-hours LC_{50} values were increased with increase in developmental stages (Table II), which is indicative of a decrease in mortality as the tadpoles mature (Figures I). The Probit analysis also showed that 96-hours LC_{50} values were also decreased with increase in concentration. This indicates an increase in toxicity with increase in concentrations and exposure duration.

LC_{50} values for 96-hours toxicity test at one (1) post hatch could not be determined by using the Probit analysis since the maximum allowable difference of four (4)

Table I. Mean Percentage Mortality of Tadpoles Exposed To Different Concentrations of Atrazine Pesticide at Successive Developmental Stages.

Treatment Time (Hours)	Conc. (µg/L)	Percentage (%) Mortality			
		1 week	2 weeks	3 weeks	4 weeks
24	0	0	0	0	0
	200	18	10	5	5
	400	20	15	13	8
	600	45	30	25	18
48	0	0	0	0	0
	200	38	35	23	15
	400	65	55	33	23
	600	95	68	45	38
72	0	0	0	0	0
	200	65	40	30	20
	400	98	70	50	35
	600	100	83	63	58
96	0	0	0	0	0
	200	95	43	38	28
	400	100	80	63	45
	600	100	90	80	75

Table 2. Relative Acute Toxicity of Atrazine Pesticides to *P. Bibroni* Tadpoles at 96 Hours.

Pesticide	Developmental stages (Weeks)	LC_{50}(95% CL) µg/L	Safe concentrations at 96 hours(µg/L)	Probit line equation
Atrazine	1	-	-	-
	2	230.058 (129.296-297.353)	23.0058	-2.878+3.336XLog(Conc.)
	3	305.897(115.277-461.692)	30.5897	-2.37+2.07XLog(Conc.)
	4	431.323(329.763-633.268)	43.1323	-3.241+3.128XLog(Conc.)

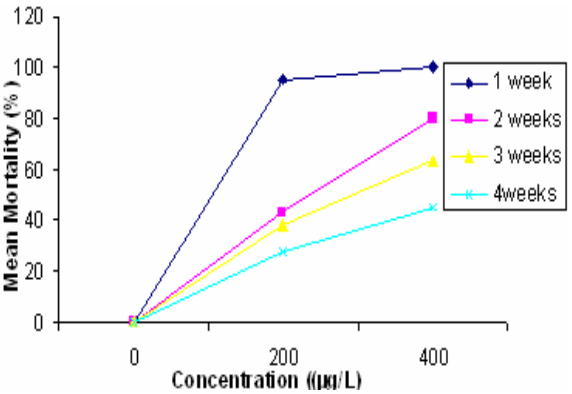

Figure 1. Mean Percentage Mortality of Tadpoles Exposed to Different Concentrations of Atrazine Pesticide at 96 Hours for the Different Developmental Stages.

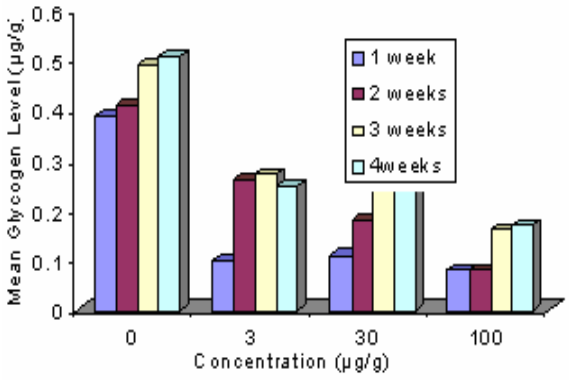

Figure 2. Mean Glycogen Level (µg/g) Of Tadpoles Exposed to Different Sublethal Concentrations of Atrazine Pesticide at Successive Developmental Stages.

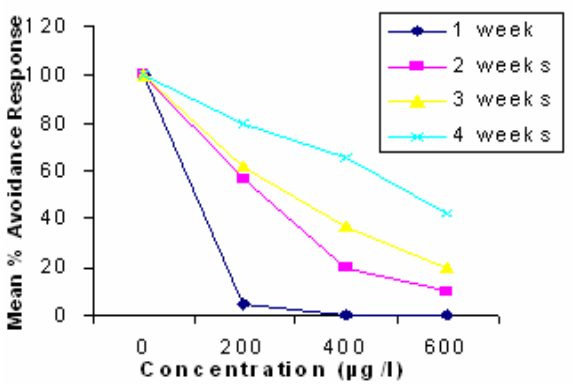

Figure 3. Mean Percentage Behavioural Response of Tadpoles Exposed to Different Concentrations of Atrazine Pesticide at 96 Hours for the Different Developmental Stages.

between successive mortalities was exceeded, and the very high mortality was observed.

Safe concentrations at 96 hours for 2, 3, and 4 weeks old tadpoles ranged between 23.0058 - 43.1323µg/L (Table II).

Mean % mortality of tadpoles was significantly affected by concentrations ($F= 5.120$, $df = 9$) at $P<0.05$ and developmental stages ($F= 29.407$, $df = 9$) at $P<0.05$.

3.3. Chronic Toxicity

Glycogen levels were used as an assessment endpoint in the chronic toxicity test. The result showed that glycogen levels of the amphibian tadpoles varied with concentrations of the test chemical and with successive developmental stages. The values obtained decreased with increase in concentration (Figure 2). However, glycogen values were observed to increase with each successive developmental stage. The levels of glycogen observed in the control experiments were higher than the test experiments. This is indicative of the possible effect of the pesticide on the glycogen levels of the tadpoles.

Mean glycogen level of tadpoles for Atrazine pesticides was significantly different in the developmental ($F= 13.460$), but showed no significant difference in concentrations ($F= 1.493$).

The concentrations also had varying degrees of behavioral alternations in surviving tadpoles as observed in the behavioral response for the pesticide. The behavioral response was also concentration dependent (Figure 3). In the highest treatment concentration for the different developmental stages of the test organism, tadpoles displayed abnormal avoidance response at approximately three (3) hours post-treatment and some died in subsequent days.

4. Discussion

Until recently, the adverse effects of pesticides on non-target organisms have not been seriously considered in Nigeria, and toxicological studies with amphibians are relatively limited in number (Sparling *et al.,* 2000, Ezemonye and Enunekwu 2005). Consequently, only limited data on the toxicity of Atrazine to amphibian larvae of *P. bibroni* are available for comparison with the results of this study. The results of the present study further demonstrated that Atrazine could have adverse direct (mortality) and indirect (physiological and biochemical) effects on amphibian tadpoles with special reference to *P.bibroni*.

4.1. Acute Toxicity

4.1.1. Variations in Percentage Mortality of Tadpoles with Concentrations

In this study, no observable mortality was reported in all the control tests while varying degrees of mortality were reported in the tests concentrations. This is also a clear expression of the effects of the pesticides as possible source of death of test organisms. The results from this study clearly indicate that Atrazine varied greatly in their effects on survival of *P. bibroni.* The highest mortality was found at the highest concentrations, suggesting dose-dependent survival and concentration graded lethality.

Atrazine did not affect larval survival in gray frogs (Diana *et al.* 2000), Northern leopard frogs (Allran and Karasov 2000), and American toads (Berrill *et al.* 1994). However, significant lower survival was reported in streamside salamander (*Amoystoma barbouri*) and spring peepers (*Pseudacros crucifer, Bufo americanus, Rana clamitans* and *Rana sylvatica*) at low concentrations of 3ppb and 4ppb respectively (Storrs and Kiesecker 2004, Rohr *et al.* 2003). Atrazine produced mortality on *Xenopus laevis* as reported by Freeman and Rayburn 2004; and is consistent with this study.

The mortality of larvae could be explained by bioconcentration of this agrochemical or by the vulnerability of amphibian larval stages. Atrazine has been reported to bioconcentrate in amphibian tadpoles (Allarn and Karasov 2004, Naqvi and Vaishnqvi 1993, Saglio and Trijasse 1998). This is an issue of serious ecological consequence because this pesticide is retained in the amphibian's body tissues, which when fed on by a predator can lead to concentration of the chemical from one trophic level to the next (ASTM, 1998, Suter, 1993). Larval mortality occurred most rapidly in the higher concentrations of Atrazine than the controls, suggesting that death may have been influenced by pesticide concentrations.

The data from the present study suggests that exposure of early developmental stages of tadpoles of *P. bibroni* to Atrazine may have permanent effects on these amphibians. They may not have any recovery from their exposure as earlier reported by Rohr *et al.* (2003).

4.2. Stage Dependent Variation in Percentage Mortality of Tadpoles of P. Bibroni

Pesticide toxicity and accumulation studies with freshwater organisms of different trophic levels indicated that uptake and toxicity of pesticides were stage-dependent (Harris *et al.,* 1998). Hall and Henry (1992), Holcomb *et al.* (1987), also stated that many effects of pesticide toxicity seem to be species or life-stage specific. Berrill *et al.* (1998) describing the lethal and sublethal effects of endosulphan pesticide, an organochlorine on the development of embryos and tadpoles of *R. sylvaltica, R. clamitans* and *B.. americanus* reported that different developmental stages of these amphibians displayed obvious differences in susceptibility. Two weeks-old tadpoles of all species tested were sensitive, displaying paralysis as the primary effects. This observation is consistent with the results of this study, the percentage

mortality values decreased with increase in developmental stages for the amphibian species of *P. bibroni.* The varying degree of mortality reported in this study is consistent with the report of Sparling *et al.* (2001), who reported that differences in an organism's biological adjustment and behavioral responses to changes in water chemistry and osmotic conditions depend on the stages of development. The implication of this observation is that the high level contamination of the aquatic environment with pesticides would adversely affect early developmental larval stages of amphibian species.

The 4 weeks old larval stage was found to be a better experimental material for ecotoxicological studies. This is attributable to the observed higher survival rates of 4 weeks old tadpoles of *P. bibroni,* exposed to Atrazine, and make them possible sentinel species for the pesticide. On the other hand, the sensitivity of early-stage amphibian larvae may be a more appropriate bioindicator for these pesticides. It is therefore imperative that a test organism's stage of development should be clearly specified if valid toxicologic comparisons are to be made.

4.3. Chronic and Sublethal Effects.

Sublethal exposure of amphibians to pesticides may be valuable in assessing sensitivity to contaminants than lethal effects (Little *et al.* 1990). This can have important impacts on amphibian communities, and can be more detrimental to amphibians than direct mortality.

4.4. Behavioural Response

Abnormal behavioral response of tadpoles in the treatment concentrations positively correlated with the concentration gradient, many tadpoles displaying abnormal behavioral responses died in subsequent days. However, behavioral response in the control treatment was normal. Again, behavioral response in tadpoles increased as the tadpoles mature. Alteration of normal behavioral response could increase susceptibility to predation (Brodie *et al*,.1983, Cooke 1997) and precede mortality (Kreutzweiser *et al*. 1994). This supports the view that abnormal behavioral alterations resulting from intoxication are more sensitive measures of toxicity than mortality (Brodie *et al*. 1983).

Tadpoles, displaying abnormal behavioral response, were sluggish with impaired locomotion and distorted. Similar effects could be problematic in natural environments by increasing susceptibility to larvae predation and reducing foraging capability. This could be especially detrimental given that pesticides particularly herbicides like Atrazine can also reduce or eliminate larval food supplies (Howe *et al*. 1998). deNoyelles *et al.* (1982) reported that Atrazine concentrations as low as 1- 4µg/L inhibited phytoplankton growth and reduced dissolved oxygen concentrations due to inhibition of photosynthesis. Low oxygen concentrations, which may cause additional stress and could only serve to magnify pesticide toxicity.

4.5. Glycogen Level

Glycogen level, an ecological endpoint of oxidative stress was assessed in this study. The interactions of chemicals in organisms are frequently associated with depletion in storage glycogen, which is evident in decreased energy production (Cicik and Engin, 2005). The glycogen levels of tadpoles exposed to varying concentrations of Atrazine were observed to vary negatively with concentrations. Glycogen level was highest in the control experiment. The depletion in the glycogen levels in organisms. exposed to chemicals and compared to the control experiment, is an indication of probable toxicological effect as observed in oxidative stress. The reduction in glycogen levels of tadpoles exposed to varying test concentrations could be the result of the pesticide affecting the activities of enzymes that work in glycogenolysis (Fournier *et al*. 2004). There was a general increase in glycogen levels at each successive developmental stage, indicative of reduced toxicity with development. The implication of this is that the glycogen level reserves of early larval stages of the amphibian species could be more adversely affected.

Some investigations have also showed that organic contaminants like pesticides could decrease the glycogen level of invertebrates and fish by affecting the activities of enzymes that play active role in the carbohydrate metabolism (Cicik and Engin, 2005). The loss of glycogen (a secondary stress response) could be regarded as a nonspecific response signifying stress, and this has been linked to changes in cortisol during exposures in various stressors (Wedemeyer *et al.* 1990).

5. Conclusion

The significant difference observed in the mortality between the controls and the test concentration showed that the pesticide may have impacted the death of the tadpoles. The study showed that accidental and intentional release of this pesticide into the aquatic environment could threaten amphibian survival. Chronic exposure to Atrazine resulted in reduced glycogen levels and, abnormal avoidance response in tadpoles of P. *bibroni*. The results obtained indicated the pesticide is toxic and could bioconcentrate along food chain; therefore, it is imperative that the use of Atrazine should be carefully monitored.

The amphibian assay described in this study can therefore be used to assess the toxicity of Atrazine in the course of regulatory surveillance and monitoring of the waters in the Niger Delta ecological zones of Nigeria.

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