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Full Length Research Paper

Acute and chronic effects of organophosphate pesticides (Basudin) to amphibian tadpoles (*Ptychadena bibroni*)

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Toxicity of basudin (an organophosphate pesticide) on the larval stages of the dominant amphibian; *Ptychadena bibroni* of the Niger Delta ecological zone of Nigeria was assessed using acute and chronic toxicity in the laboratory. Mortality and body glycogen levels were used as ecological endpoints. The American society of testing and material (ASTM) recommended semi-static renewal bioassay was employed and LC_{50} was measured at 96 h. The amphibian larval stages were exposed to basudin concentrations of 0.1, 1.0, 10.0 and 25 ug/l. Derived 96 h LC_{50} values decreased with increased exposure duration. Estimated 96 h LC_{50} was 0.860 ug/l. Substantial mortality and incidence of abnormal avoidance response occurred more at higher concentrations. Body glycogen levels in all test concentrations were significantly different between the test and the control experiments at P<0.05. The decrease in glycogen level varied negatively with mortality, exposure duration and concentrations. The amphibian assay described in this study can be used to assess the toxicity of basudin pesticides in the course of regulatory surveillance and monitoring of the waters of the Niger Delta ecological zones of Nigeria.

Key words: Acute toxicity, basudin, glycogen, amphibian tadpoles.

INTRODUCTION

The decline of world populations of amphibians is a major environmental issue (Vertucci and Corn, (1996). Agricultural practices affect natural habitats in several ways, such as through land conservation, increased fragmentation and agrochemical contamination (Hecnar, 1985; Hayes et al., 2002; Davidson et al., 2002). Much of the interest on amphibian declines is currently focused on the role of pesticides on the observed global declines (Johansson et al., 2006). Agricultural sites where pesticides are often used have lower amphibian species richness and abundance than adjacent non-agricultural sites (Bonin et al., 1997).In some cases it results in the disappearance of amphibians from agricultural landscapes. Although pesticides are used on a local scale, they are ubiquitous and spread regionally and globally. They have been found in bodies of frogs from areas where pesticide use has not occurred historically or in the past 25 years (Datta et al., 1998; Russell et al., 1995, 1997).

Amphibians are especially at risk from agricultural contaminants because they have permeable skin and eggs that readily absorb chemicals from the environment. Moreover, many species complete their life cycle in ponds and streams adjacent to agricultural fields where pesticides are applied and these applications often coincide with breeding and larval development (Verucci and Corn, 1996; Hayes et al., 2003).

Pesticide concentrations found in the environment have been shown to have negative effects on amphibian development. Amphibians living with pesticides in their habitats exhibit high mortality and physiological defects from these pesticides. Recent studies provide evidence that pesticide reduce hatching success, decrease size at metamorphosis, causes physiological stress, liver and kidney degeneration, teratogenic effects and paralysis, to mention a few (Smith, 1987; Kamrin, 1997; Power et al., 1989). Also, they have been shown to have negative effects on growth, development, immune responses and

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behaviour of tadpoles (Bridges, 2000; Christin et al., 2003; Broomhall, 2005).

The frog species, *Ptychadena bibroni* is the most dominant and most widely spread anuran species in the Nigeria Delta regions of southern Nigeria (Akani and Luiselli, 2002). Because of its abundance, it is an important species in the Niger Delta ecological communities. The permeable skin of amphibians makes it potentially a good bioindicator of chemical pollution (Venturmo et al., 2003). Tadpoles can be exposed to pesticides through contaminated surface water after heavy rains or pesticide spray drift into the breeding ponds adjacent to farms. Thus, an exposure study during the aquatic phase is a highly relevant approach.

This study attempts to determine the acute (survival) and sub lethal (glycogen level and behaviour) effects of basudin (an organophosphate, used mainly for the control of insect pests in most rice, yam, and vegetable farms in Nigeria) pesticide on larval stages of the dominant frog species *P. bibroni* of Niger Delta of Nigeria.

MATERIALS AND METHOD

Collection of test organisms and acclimatization

Eggs of the amphibian species, *P. bibroni*, were colleted from an inlet of Ikpoba River, an inland River in southern Nigeria. Hatching of eggs, rearing of tadpoles and testing were done in the Postgraduate ecotoxicological research laboratory, at the Department of Animal and Environmental Biology, University of Benin, Nigeria. After hatching, emerging larvae tadpoles were distributed into six (22 × 22 cm) plastic tanks each containing 1 litre of dechlorinated tap water and were allowed to acclimatize for seven days. Tadpoles were fed *ad-libitum* daily with ground maize powder. Larvae were reared on a 10:14 h light: dark cycle to mimic natural condition and room temperature were maintained at 30 ± 2°C throughout the duration of the experiment. Larvae were subjected to concentrations 0, 0.1, 1, 10 and 25 µg/l of basudin pesticide. Each treatment concentration contained 20 tadpoles each per container. Acute toxicity was done in replicate tanks.

Pesticides

The pesticide used for the 96 h acute toxicity test was the organophosphate, basudin. The pesticide is commonly used on farms in Nigeria for the control of soil insects.

Bioassay procedure

Stock solutions of the required concentrations were prepared for the pesticide. Serial dilutions of 0.1, 1, 10 and 25 µg/l (Harris et al., 1998) were made. The semi-static renewal bioassay procedure started with a range finding test (ASTM, 1985). This was used to determine the range of concentrations to be tested and approximate the range that would produce the desired LC_{50} effect for the different life stages.

Mortality rate

Mortality was recorded at an interval of 24 h over a period of 4 days (96 h). 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).

Avoidance response

Larval avoidance response was monitored in this study. Avoidance response was assessed *in-situ* by gently prodding all individual larvae and gauging their response as normal when larvae swims away immediately or abnormal when there is a delayed, no response or impaired swimming ability. The avoidance response of larvae 24 h after herbicide treatment was used for statistical analysis (Wojtaszek, 2004).

Glycogen analysis

Glycogen analysis was adapted from Good et al. (1933). The results were expressed as a percentage of the wet weight of tissue.

Statistical analysis

The susceptibility of the tadpoles to both pesticides was determined using the probit method of analysis (Finney, 1971) for median lethal concentration LC_{50} at 96 h. Computations of confidence interval of mortality rate were also obtained from the probit analyses used to determine the LC_{50} (Probit software). The two-factor ANOVA (analysis of variance) in Microsoft Excel was used to test the variables at $P < 0.05$ level of significance. Multiple bar graphs were also used in this study for the pictorial representation of assessment endpoint.

RESULTS

Acute toxicity

No mortality or morphological changes were observed in the control experiment for the 96 h acute toxicity test. Tadpoles in the control experiment appeared active and healthy throughout the test period. The proportion of abnormal avoidance response in the control was less than 10%. The test organisms exposed to varying basudin concentrations to 96 h recorded mortality in all the concentrations. The mean % mortality for the 2 weeks old tadpoles of *P. bibroni* exposed to the pesticide were 35, 58, 68 and 75% for 0.1, 1.0, 10.0 and 25 µg/l concentrations respectively. This indicated that mortality was concentration dependent (Table 1 and Figure 1).

Chronic toxicity

The concentrations also had varying degrees of behavioural alternations in the surviving tadpoles as observed in the avoidance response (Table 2 and Figure 2). The avoidance responses were also concentration dependent. In the highest treatment concentrations, many tadpoles displayed abnormal avoidance responses at approximately 3 h post-treatment. Derived 96 h LC_{50} basudin was 0.860 µg/l. (Table 3).

Glycogen levels of amphibian tadpoles varied with the test chemical concentrations. The values obtained decre-

Table 1. Mean mortality of tadpoles exposed to different concentrations of basudin pesticide (96 h).

Conc. (µg/l)	Number Tested	Number dead		Mean mortality (%)	Mean Probit value
		X1	X2		
Control	20	0	0	0	0
0.1	20	7	7	35	4.63
1.0	20	12	11	58	5.03
10.0	20	13	14	68	5.42
25	20	15	15	78	5.58

Table 2. Abnormal avoidance response for basudin.

Conc. (µg/l)	Number tested	Abnormal avoidance response		Mean abnormal avoidance response (%)
		X1	X2	
Control	20	0	0	0
0.1	20	7	8	38
1.0	20	12	16	70
10.0	20	13	17	75
25.0	20	15	19	85

Table 3. Mean glycogen level (ug/g) of basudin exposure at the end of 96 h.

Conc. (µg/l)	Number Tested	Mean glycogen level		Mean glycogen level (%)
		X1	X2	
Control	20	3.30	3.30	16.50
0.1	20	2.70	2.70	13.50
1.0	20	1.20	1.18	5.95
10.0	20	0.80	0.80	4.00
25.0	20	0.60	0.60	3.00

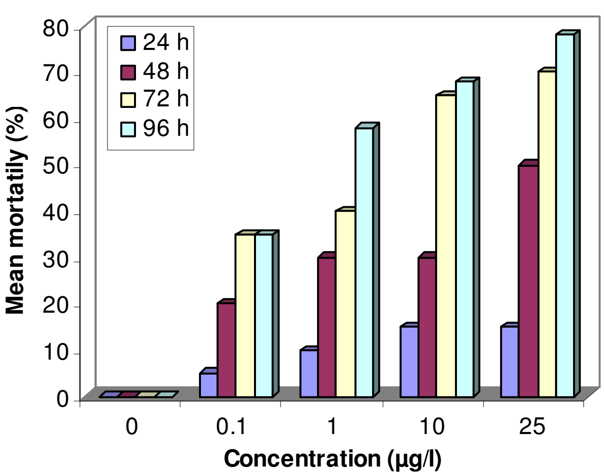

Figure 1. Mean mortality of tadpoles exposed to different concentrations of basudin pesticide.

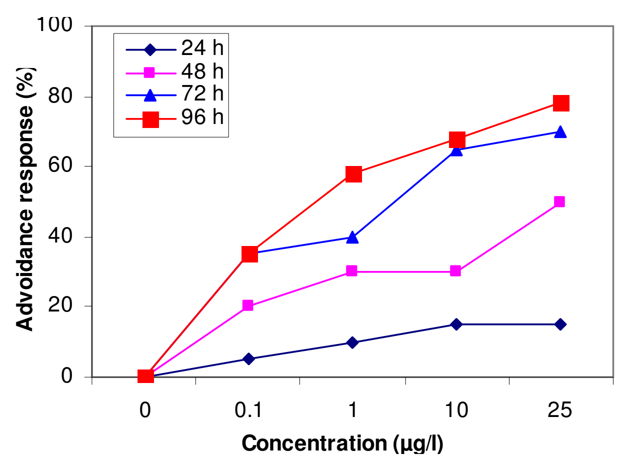

Figure 2. Mean abnormal avoidance response of tadpoles exposed to different concentrations of basudin pesticide.

ased with increase in concentration of chemical and exposure duration (Figure 3). The levels of glycolgen 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 tadpoles.

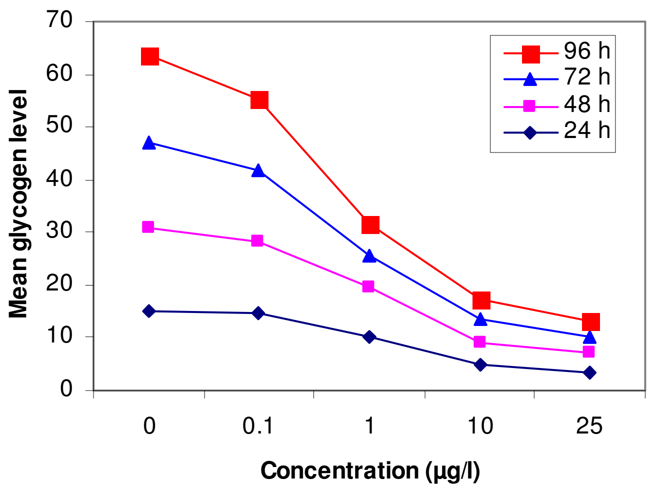

Figure 3. Mean glycogen level of tadpoles exposed to different concentrations of basudin pesticide.

DISCUSSION

Acute toxicity

Examination of the results of this study indicates that basudin pesticide can greatly affect the survival of *P. bibroni.* The acute toxicity treatments showed strong negative effects on survival as pesticide concentration increased. This suggests dose-dependent survival and concentration graded lethality. 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 behaviour response to change in water chemistry and osmotic conditions depend on the stage of development. The implication of this observation is that early life stages of amphibians are not only vulnerable to pesticide contamination but are usually adversely affected. Although limited data are available for comparison with the present results, mortality rates were comparable with the reports of Harris et al. (1998), who worked on the effects of pesticides on two species of amphibians, *Rana pipens* and *Rana clamitans* exposed to similar concentrations of basudin pesticide.

Deaths of tadpoles occurred most rapidly in the higher concentrations suggesting that death may have been influenced by pesticide concentrations. The concentrations could be detrimental. Basudin has been reported to bioconcentrate in tissues of amphibians (Smith 1987; Hill, 1995). The high mortality recorded could be explained by bioconcentration of these agrochemicals or by the vulnerability of the amphibian larval stages. This is an issue of serious ecological consequence because these pesticides are retained in the amphibian's body tissue which when fed on by a predator can lead to the concentration of the chemical from one trophic level to the next (ASTM, 1998; Suter, 1993). Most disorders in animals exposed to organophosphate compound like basudin have been linked to their toxic effects in the central nervous system (Berrill et al., 1998; Saglio et al., 1998). The pesticides have been found to concentrate in tissue of frogs with depressed cholinesterase activity (Sparling et al., 2001) and have induced hyperactivity in frogs followed by paralysis (Berrill et al., 1998).

Chronic and sub lethal effects

Sub lethal exposure of amphibians to pesticides may be more 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 (Peacor and Werner, 2001).

Avoidance response of tadpoles in the treatment concentration varied inversely with mortality; many tadpoles displaying abnormal avoidance responses perished in subsequent days. The avoidance response in the control treatment was normal. Alternation in avoidance response could and precede mortality (Brodie and Formanowicz, 1983, Cooke, 1997) indicating that behavioural alternations resulting from intoxication are more sensitive measures of toxicity than mortality. Moreso, tadpoles exhibiting abnormal avoidance response have greater susceptibility to predation (Kreutzweiser et al., 1994).

Glycogen level, an ecological endpoint of energy stress was assessed in this study. The glycogen level of tadpoles exposed to varying concentrations of basudin pesticide was observed to have an inverse relationship when compared with mortality. Glycogen level was highest in the control experiment. The inverse relationship observed in the tadpoles could probably be due to the fact that the higher the energy loss the lower the glycogen level. Reduced glycogen level (hypoglycoemia) could result in energy stress and could finally result in death of the organism (Smith, 1987).

Conclusion

The acute and sub lethal effects of basudin pesticide on tadpoles of *P bibroni* was reported. Effects of varying concentrations of basudin pesticide to amphibian tadpoles resulted in mortality, abnormal avoidance response and reduced glycogen level. The amphibian assay described in this study can be used to assess the toxicity of basudin pesticides in the course of regulatory surveillance and monitoring of the waters of the Niger Delta ecological zones of Nigeria.

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