

ACUTE TOXICITY OF AGROCHEMICALS AND DETERGENT ON TADPOLES OF THE COMMON AFRICAN TOAD, *Amietophrynus Regularis*

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ABSTRACT

Changing urban ecology has increasingly put amphibians living in swampy portions of coastal city centres in constant threat of mortality resulting from acute toxicity effects. In this study, commonly used agrochemicals (Antrazine, Carbofuran, Cypermethrin and Pirimiphos methyl) in urban gardening and farms in Lagos and a linear alkyl benzene sulphonate (LABS) detergent commonly used in household washing were exposed to tadpoles (external gill stage) of the African toad, *Amietophrynus regularis* in 96 hrs acute toxicity assays. All test substances were found to be acutely toxic to the tadpoles from 48 hrs following exposure. The pesticide Carbofuran exhibited the highest level of toxicity (48 hrs LC_{50} - 4.27 mg/L, 72 hrs LC_{50} - 1.48 mg/L, 96 hrs LC_{50} - 0.97 mg/L), in most cases, the herbicide, Antrazine had the least toxicity to the tadpoles (48 hrs LC_{50} - 156.62 mg/L, 72hrs LC_{50} - 135.23 mg/L, 96 hrs LC_{50} - 60.83 mg/L). Despite the level of development and technological advancement in urban centres, there remains the need for cautious use of synthetic products because they pose threats to sensitive and ecologically important organisms living at the fringes of the remaining natural ecosystems.

Keywords: Aquatic Pollution, Anurans, Waste Management, Urbanization

INTRODUCTION

Amphibians worldwide are on the decline and the reason for their significant decline remains contentions and divergent (Houlahan *et al.*, 2000 and Kiesecker *et al.*, 2001). Some investigators have related this trend to global warming which has resulted in inter annual variability in precipitation, UV-B exposure, pathogenic and disease outbreaks caused by agents such as oomycete, *Saprolegnia ferax* (Kiesecker, 2001) and chytrid fungus (*Batrachochytrium dendrobatidis*) (Pounds *et al.*, 2006). Acute toxicity of endosulfan and diazone pesticides on adults amphibians (*Bufo regularis*) also been reported by Ezemonye and Tongo (2010).

Despite the apparent high fecundity, their evolutionary selection to water as reproductive sites makes them vulnerable to pollution. Water pollution is one of the major issues in urban centres. Idowu *et al.* (2014) reported widespread distribution of heavy metals in water, sediments and toads within the Lagos metropolis. The permeable skin of amphibians also makes them particularly vulnerable to chemical pollution (Venturmo *et al.*, 2003).

Pesticide use is indispensable in contemporary agriculture and gardening practices in keeping off both economic and noxious pests. They are beneficial by providing reliable, persistent and relatively efficient control measures against harmful pests with less cost and effort. They have, no doubt, increased crop yields by killing different types of pests,

which are known to cause substantial or total crop damage. About 90% of the pesticides applied never reach the intended targets (Sparling *et al.*, 2001) and as a result, many other non-target organisms sharing the same environment with pests are accidentally poisoned (Amaeze *et al.*, 2011). One of the non-target biological groups mostly affected by pesticides is amphibians (Berrill *et al.*, 1994; Sparling *et al.*, 2001). Agrochemicals particularly pesticides have been linked with reduced hatching success, decrease size at metamorphosis, physiological stress, liver and kidney degeneration, teratogenic effects and paralysis and impairment of growth (Kamrin, 1997; Power and Felcker, 1998).

Detergents have also been associated with aquatic pollution particularly eutrophication. Despite the exclusion of phosphates in modern detergents (Scholten *et al.*, 1994) the quest to improve wash quality has also led to the increased use of potentially toxic compounds. Detergents contain surfactants, builders, bleaching agents, enzymes and a few other components which help in improving washing efficiency and overall quality of wash.

This study therefore is aimed at providing an update on the acute toxicity of the agrochemicals and detergent commonly used in Lagos on sensitive species occupying these urban ecosystems so as to justify the need to minimize their use and increase awareness of their impacts in the environment.

MATERIALS AND METHODS

Test Animals, Description, Source and Acclimatization

The selection of organisms used was based on the vertebrate species common to ponds which are associated with agro-ecosystems as well as gutters and ditches in most urban and sub urban areas of the Lagos metropolis. Tadpoles of the common African toad, *Amietophrynus regualaris* were collected from an undisturbed temporary pond in University of Lagos, Campus. Hand nets (diameter = 4 cm) was used to collect the tadpoles into plastic bowls (13.5 X 8 X 7 cm) containing their natural pond water. They were then transported to the laboratory and kept in plastic tanks (75 L capacity) containing 25 L of their natural pond/habitat water mixed with dechlorinated tap water. The tadpoles were fed with pieces of lettuce during the acclimatization period. Water in the acclimatization tanks were aerated with a 220 V air pump (Cosmo aquarium air pump- double type 12000) so as to oxygenate the tanks (58 X 37 X 38 cm) and then changed every 48 hrs to prevent accumulation of toxic wastes. The tadpoles were left to acclimatize to laboratory condition for one week before using them for laboratory bioassays. Only batches with less than 1% mortality during acclimatization were used in the bioassays.

Test Compounds

The herbicides (Antrazine), pesticides (Carbofuran, Cypermethrine, Pirimiphos methyl) and a commonly used household detergent (active ingredient- Linear Akyl Benzene Sulfonate) were procured from the Lagos Island market and transported to the laboratory

where they were stored at 26-28°C to ensure that their quality is maintained throughout the course of the assay.

Bioassay Procedure

Preliminary tests were carried out to determine suitable range of concentration for the definitive assays for all test substances against the tadpoles for 96 hrs (i.e. 4 days). These volumes were carefully measured out with either syringes or micro pipettes depending on the required concentrations to make out the correct measurement in duplicates for each pesticides, herbicides and detergents. The definitive tests comprised 4 replicates each holding 4 tadpoles per 1L of water and respective concentrations in 1.5 L transparent plastic tanks. A total of 16 tadpoles were used per concentration. The tadpoles were subsequently exposed to the following concentrations:

Cypermethrin: 30 mg/L, 60 mg/L, 120 mg/L, 300 mg/L and untreated control (0 mg/L)
Pirimiphos: 0.6 mg/L, 1.2 mg/L, 6 mg/L, 12 mg/L and untreated control (0 mg/L)
Carbofuran: 0.3 mg/L, 0.6 mg/L, 1.2 mg/L, 6 mg/L and untreated control (0 mg/L)
Atrazine: 60 mg/L, 120 mg/L, 300 mg/L, 600 mg/L and untreated control (0 mg/L)
LAS Detergent: 11 mg/L, 16.5 mg/L, 22 mg/L, 33 mg/L and untreated control (0 mg/L)

Assessment of Quantal Response (Mortality)

Mortality assessment was carried out every 24 hrs over the 96 hrs test period. Tadpoles were presumed to be dead when there was no body movement even when prodded with a forceps or when their ventral side becomes turned up and they show no sign of voluntary movement.

Statistical Analysis

Toxicological dose-response data involving quantal response (mortality) were determined by probit analysis using SPSS Version 16 adapted from Finney, (1991) based on a computer program written by Ge Le Pattourel, Imperial College, London. The following indices of toxicity measurement at 95% confidence limits were derived:

- LC_{50} = Median lethal concentration that causes 50% response (mortality) of exposed organism
- LC_{95} = Lethal concentration that causes 95% response (mortality) of exposed organisms
- LC_5 = Sub-lethal concentration that causes 5% response (mortality) of exposed organisms all at 95% confidence limits (CL)
- TF = Toxicity factor of relative potency measurements e.g. 96 hrs LC_{50} of a compound / 96 hrs LC_{50} of the least toxic compound tested against same species.

RESULTS

The results indicate that the carbamate, carbofuran was the most toxic substance within the 96 hrs study (Tables 1-3). There was no substantial consistent dose-response data at 24 hrs following exposures across the test substances. Therefore the results presented consist of responses from 48 hrs to 96 hrs.

Specifically, on the basis of 48 hrs LC₅₀ value, carbofuran with LC₅₀ value of 4.27 mg/L was found to be most toxic being 1, 1.1, 2.4 and 17.2 times more toxic than pirimiphos methyl, the LAS detergent, cypermethrine and herbicide (atrazine) respectively (Table 1).

For the 72 hrs LC₅₀ value, carbofuran with LC₅₀ value of 1.48 mg/L was also the most toxic. This was followed by the organochloride, pirimiphos methyl, the LAS detergent and cypermethrin while atrazine with LC₅₀ value of 135.23 mg/L was the least toxic. As indicated by the calculated toxicity factor (TF), carbofuran was 1.9, 6.8 49.6 and 91.4 times more toxic than the respective other toxicants tested (Table 2).

Thirdly, 96 hrs LC₅₀ results indicated that carbofuran with LC₅₀ value of 0.97 ml/L remained the most toxic. This was followed by pirimiphos methyl with LC₅₀ value of 3.34 ml/L, LAS detergent with LC₅₀ value of 11.43 ml/L, atrazine with LC₅₀ value of 60.83 ml/L and cypermethrin with LC₅₀ value of 65.27 ml/L (Table 3).

Thus the calculated TF indicated therefore that carbofuran was 3.4 times more toxic than pirimiphos methyl, 11.8 times more toxic than LAS detergent, 62.7 times more toxic than the atrazine and 67.3 times more toxic than cypermethrine (Table 3).

Overall, increase in toxicity over the 96 hrs test period was observed in all but tadpoles exposed to detergents.

Table 1: Comparative assessment of relative 48 hrs acute toxicity of pesticides (cypermethrin, pirimiphos methyl, carbofuran), herbicide (atrazine) and LAS detergents (mg/L) acting singly against tadpoles of *Amietophrynus regularis*.

Chemical	Concentrations (CL)			SE	DF	Probit line equation	TF
	LC ₅	LC ₅₀	LC ₉₅				
Cypermethrine	8.25 (0.00-23.75)	73.40 (28.58-202.76)	652.69 (222.78-1456974.51)	0.70	2	y= 1.29-2.50x	17.2
Pirimiphos methyl	0.19 (0.00-0.86)	4.76 (1.75-78.300)	111.44 (18.76-1.96)	0.48	2	y= 0.32-2.50x	1.1
Carbofuran	0.279 (0.00-0.77)	4.27 (1.72-992.46)	65.42 (10.18-2.29)	0.58	2	y= 0.29-3.06	1
Atrazine	14.85 (0.00-47.09)	156.62 (53.62-474.87)	1652.36 (516.39-1.59)	0.65	2	y= 1.44-2.44x	36.7
LAS Detergent	3.15 (0.00-6.98)	10.23 (0.08-14.84)	33.25 (20.62-786515.19)	1.52	3	y= 1.80-1.80x	2.4

Table 2: Comparative assessment of relative 72hrs acute toxicity of pesticides (cypermethrin, pirimiphos methyl, carbofuran), herbicide (atrazine) and LAS detergents (mg/L) acting singly against tadpoles of *Amietophrynus regularis*.

Chemical	Concentrations (CL)			SE	DF	Probit line equation	TF
	LC ₅	LC ₅₀	LC ₉₅				
Cypermethrine	8.25 (0.00-23.75)	73.40 (28.58-202.76)	652.69 (222.78-1456974.51)	0.70	2	y= 1.29-2.50x	49.6
Pirimiphos methyl	0.40 (0.00-0.46)	2.79 (0.14-1.59)	193.59 (18.40-4.23)	0.45	2	y= 0.28- 1.41x	1.9
Carbofuran	0.25 (0.03-0.59)	1.48 (0.77-3.44)	7.70 (3.19-149.81)	0.65	2	y= 0.27-1.11x	1
Atrazine	6.78 (0.00-35.29)	135.23 (0.52-843.69)	2697.52 (586.55-5.83)	0.62	2	y= 1.38-1.95x	91.4
LAS Detergent	3.95 (0.00-7.65)	10.13 (0.00-13.89)	25.94 (17.54-1.59)	2.03	3	y= 2.33-1.74x	6.8

Table 3: Comparative assessment of relative 96hrs acute toxicity of pesticides (cypermethrin, pirimiphos methyl, and carbofuran), herbicide (atrazine) and LAS detergent (mg/L) acting singly against tadpoles of *Amietophrynus regularis*.

Chemical	Concentrations (CL)			SE	DF	Probit line equation	TF
	LC ₅	LC ₅₀	LC ₉₅				
Cypermethrine	8.42 (0.18-23.05)	65.27 (25.04-145.54)	505.80 (193.83-163488.88)	0.70	2	y= 1.31-2.56x	67.3
Pirimiphos methyl	0.08 (0.00-0.47)	3.34 (0.83-129.20)	138.05 (17.81-8.42)	0.46	2	y= 0.29-1.83x	3.4
Carbofuran	0.10 (0.00-0.27)	0.97 (0.44-2.80)	9.32 (3.07-1580.26)	0.57	2	y= 0.25- 0.10x	1
Atrazine	6.39 (0.07-19.43)	60.83 (20.70-114.52)	578.85 (237.74-18699.88)	0.54	3	y= 1.07-2.80x	62.7
LAS Detergent	5.41 (0.00-8.69)	11.43 (2.63-4.88)	24.167 (17.41-1373.63)	2.26	3	y= 2.59-2.07x	11.8

DISCUSSION

The findings from this study showed that all test substances exhibited acute toxicity towards the tadpoles and therefore pose some level of threat to the species living in urban settings. The trend of toxic response by the tadpoles varied with the toxicant but for most, acute toxicity increased with time. The cause for concern is centred on the fact that the tadpoles are non target species for agrochemicals and many other wastes (Amaeze *et al.*,

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INTRODUCTION

Amphibians were the first tetrapods on land and are a diverse group represented today by the caecilians (order Gymnophiona), newts and salamanders (order Caudata) and frogs and toads (order Anura). The frogs and toads are commonly referred to as the anurans (Hickman et al. 2001).

Nigeria being a country in the tropical zone with conducive climatic conditions and vegetation has a significant diversity of human population. According to Bakari et al. (2004), from a biogeographic point of view, Nigeria is situated between West and Central Africa, and hence between the biodiversity hotspots of the Upper and Lower Guinea forest. Therefore the country is expected to harbor high biological diversity composed of West and Central African species alike.