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Research Article

Comparative Evaluation of Acute Toxicity of Butaforce and Termex Pesticides on *Bufo regularis* Tadpole

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Abstract

Water contamination is a current environmental problem in different parts of the world and a huge variety of pesticides are involved in this process. Butaforce is a major herbicide used in agriculture for the control of grass and other broad leaves while termex is an insecticide used for the control of termites and other insects in homes and agricultural fields. The present study evaluated the toxicity of these two widely used agricultural pesticides, butaforce and termex on *Bufo regularis* tadpole. The 96h LC_{50} of butaforce and termex on *B. regularis* were 0.42mg/L and 1.13mg/L respectively. The safe levels ranged from 4.20×10^{-2}mg/L to 4.20×10 mg/L in butaforce and from 1.13×10^{-1} to 1.13×10^{-5} in termex. The results also indicate concentration and duration dependent increase in mortality rates of *C. gariepinus* exposed to the pesticides. There were significant differences ($p<0.05$) in LC(10-90), no observe effect concentration (NOEC) and lowest observed effect concentration (LOEC) for different times of exposures. While the maximum allowable concentration (MAC) of butaforce was 4.2mg/L in *B. regularis* tadpole the value increased to 11.30mg/L in termex showing that butaforce was more toxic to *B. regularis* than termex. The preset findings suggest that both pesticides were toxic to *B. regularis* and should be used with utmost care in our environment.

Keywords: Pesticides; Lethal concentrations; Amphibians; Nigeria

Introduction

The introduction of pesticides in pest management by agriculturalists is very vital for increase food production. However, this benefit is not without negative impact on the ecological system especially aquatic habitat as studies have shown that 99% of applied pesticides go into the air, water and soil and only 1% reach the target organism [1]. Butaforce and termex are widely used pesticides in Nigeria and other developing countries for the control of weeds and termites respectively. Butaforce (2-chloro-N-2, 6-diethylphenyl acetamide) is commercial herbicide formulations used for the control of grasses in rice fields and many broad leaf weeds whereas termex is a trade name for termiticide emulsiphable concentrate formulation of imidacloprid used for pre and post protection of building against termite. The Amphibian eggs and tadpoles can be exposed to pesticides while feeding in contaminated water or sediments [2]. Studies have shown decline in amphibian population due to pollution, disease outbreak and climate change [3] Pesticides from agricultural run-offs impair the normal balance of the flora and fauna of the aquatic ecosystem [4]. When run-off containing pesticides enter the aquatic ecosystem, the concentrations are rapidly diluted and are partitioned among various components of hydrosphere [5].

Bufo regularis tadpoles play an important role in aquatic ecosystem. They act both as consumers and prey to other species thus, contribute to energy transfer in the energy budget. Tadpoles are mostly herbivores feeding on algae, detritus and some plants, although they also eat other animals in small amount [6]. However the loss of tadpoles could affect the ecosystem of freshwater streams as tadpoles play a significant role in maintaining the structure and function of streams [7]. The presence of pesticides in surface water systems is a concern because of their potential adverse effect on human and animal health, and aquatic ecosystems. Tadpoles are more susceptible to pollution than birds or mammals because of permeable skin. Therefore, if aquatic environment is altered continuously by chemical contaminants it creates danger to aquatic biota leading to decline in *Bufo regularis* population. Despite the huge use of agricultural pesticides in Nigeria, no published information has been reported to the best our knowledge, on the toxicity of butaforce and termex on *Bufo regularis* tadpoles. There is thus, the need to investigate the effects of butaforce and termex pesticides on aquatic organisms such as *Bufo regularis* tadpoles. The tadpoles are wide spread in most wet agricultural fields, can acclimatize easily under laboratory conditions and can thus be used as good sentinel organism for toxicological studies. The present study was desinged to determine the cumulative mortality, median lethal concentration (LC_{50}), safe level, no observe effect concentration (NOEC), lowest observed effect concentration (LOEC) and the maximum allowable concentration (MAC) of butaforce and termex pesticides on *Bufo regularis* tadpoles.

Materials and Methods

Experimental animal and chemical

A total of three hundred (300) freshwater *Bufo regularis* tadpoles (Family: Bufonidae; Order: Anura) were collected from the stagnant pool in the Biological Garden, Department of Biology, Federal College of Education Eha-Amufu Enugu state, Nigeria using hand net. The tadpoles were transported in Four 100L plastic aquaria containing the habitat water collected from the same pool to the Fisheries Wet Laboratory, University of Nigeria Nsukka, Nigeria. They were acclimatized for ten days in the same plastic aquaria prior to the experiment. Water was changed daily and the aquaria cleaned thoroughly to remove faecal matter and other waste materials. The tadpoles were fed with algae three times daily. Any dead tadpoles were removed instantly with forceps to maintain good quality of the test water. The feeding of the experimental organism was stopped 24h before the commencement of lethal toxicity test to prevent fouling of the test water [8,9]. The institutional ethical Committee on Experimental Animal Care (UNN-EGACC-01618) was obtained and followed carefully. The herbicide (butarforce) and termiticide (termex) were purchased from agrochemical shop in the local market. The butachlor (trade name butaforce, China Agro Crop Care Co. Ltd China, containing 50% butachlor as an active ingredient) and termex (trade name termex, Rallis India Limited Mumbai, containing imidacloprid 30.5% SC) were used as the stock solutions.

Acute toxicity test

The acute toxicity of pesticides to aquatic organisms like toad, frog and fish are evaluated by the measurement of LC_{50} value that is, concentration of pesticide that results in 50% mortality of the test organism during exposure period (12 - 96 hours). Five concentrations of butaforce (0.35, 0.45, 0.55, 0.65, 0.75 mg/L) and five termex concentrations (0.72, 0.96, 1.2, 1.44, 1.68 mg/L) along with their controls were selected for exposures after a series of range finding tests. A set of 10 *Bufo regularis* tadpoles were randomly exposed to each of the selected concentrations in 40-L glass aquaria (60×30×30cm size) and were set in triplicates. Another set of 10 tadpoles was set up as control in triplicates under the same laboratory condition but contained only tap water in place of any pesticide. The experimental set up were renewed every alternate days to maintain the concentration of the chemicals. The temperature, pH, dissolved oxygen, conductivity and water hardness of the test water were determined according to [10] method. The mortality and survival values were recorded from 24 to 96h exposure duration. Any dead fish was sorted and removed from the tank to prevent fouling of the set up. The median lethal concentration (LC_{50}) which is the concentration at which 50% mortality occurred was determined by probit analysis using SPSS-version 17. The NOEC, LOEC and MAC were determined for each pesticide at the end of acute toxicity test [11]. The safe levels of the two pesticides were estimated by multiplying the 96 h LC50 with different application factors (AF) according to [12-17].

Statistical analysis

The data obtained were analyzed using the statistical package SPSS 17 computer program (SPSS Inc. Chicago, IL, USA) The data was subjected to one-way analysis of variance while Duncan's multiple range tests were used to determine significance difference at 5% probability. Results were expressed as means ± standard error

Results and Discussion

The physico-chemical parameters of the test water indicate that the water temperature varied from 24.40 °C to 26.40 °C while the pH ranged from 7.2 to 7.6. The dissolved oxygen varied from 6.4 to 7.2mg/L, conductivity from 250 to 280µM/cm and the water hardness from 180 to 240mg/L. The results of acute toxicity study indicate concentration and duration dependent mortality of *Bufo regularis* tadpoles exposed to butaforce and termex pesticides respectively. After 96h exposure to 0.35 and 0.75mg/L concentrations of butaforce, 33% and 100% mortality of *Bufo regularis* tadpoles were recorded while 14% and 100% mortality were observed in tadpoles exposed to 0.7 and 1.68mg/L concentrations of Termex (Table 1). The control group recorded 100% survival for both experiments. The results indicate that butaforce (herbicide) was more toxic compared to termex (insecticide). Similar results, have been reported in tadpoles and larvae of amphibian species exposed to related pesticides [3,4,6,18-21]. The response curve of *Bufo regularis* tadpole after 96h acute exposure to butaforce and termex (Figures 1 & 2) generally indicate that mortality of the tadpole is dependent on concentrations of the pesticides.The safe levels of the pesticides were determined by multiplying the 96 h LC_{50} with different application factors (AF) varied from 4.20 x 10^{-2}mg/L to 4.20x10^{6} mg/L in butaforce and from 1.13 x10^{-1} to 1.13 x 10^{-5}mg/ L in termex (Table 2). The safe levels recorded for the two pesticides indicated some variations but in view of the controversy arising from the extrapolation of laboratory data to field it has been difficult in accepting safe levels based on laboratory experiment [22,23]. In line with our study [20] recorded safe levels of 0.073 mg/L and 0.044mg/L for endosulfan and diazinon respectively on adult Amphibians (*Bufo regularis*). The 96h LC_{50} of 0.42mg/L and 1.13mg/L, obtained for butaforce and termex respectively showed that both pesticides were toxic to *Bufo regularis* tadpoles. Similar to our results, [20] obtained LC_{50} of 0.73 and 0.44mg/L in adult *Bufo regularis* exposed to endosulfan and diazinon respectively. While [6] obtained LC_{50} of 0.074mg/L for *Rana Dalmatina* tadpoles exposed to endosulfan, [21] obtained LC_{50} of 5.38mg/L for *Physalaemus albonotatus* exposed to glyphosate. [24] also obtained LC_{50} value of 20.81mg/L in *Osteopilus septentrionalis* (Family Hylidae) exposed to glifosan pesticide. The variations in the LC_{50} in the different pesticides and groups of amphibians may depend on the types of pesticides, the speies of organisms, life stages, time, duration of exposure and the prevailing environmental conditions [25].

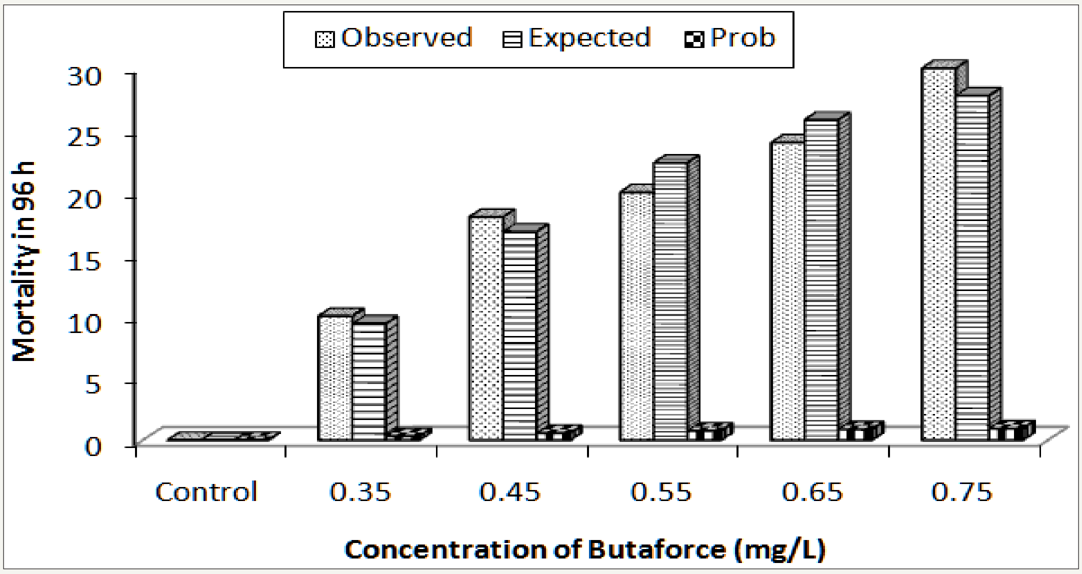

Figure 1: Response curve of *Bufo regularis* tadpole after 96h acute exposure to Butaforce.

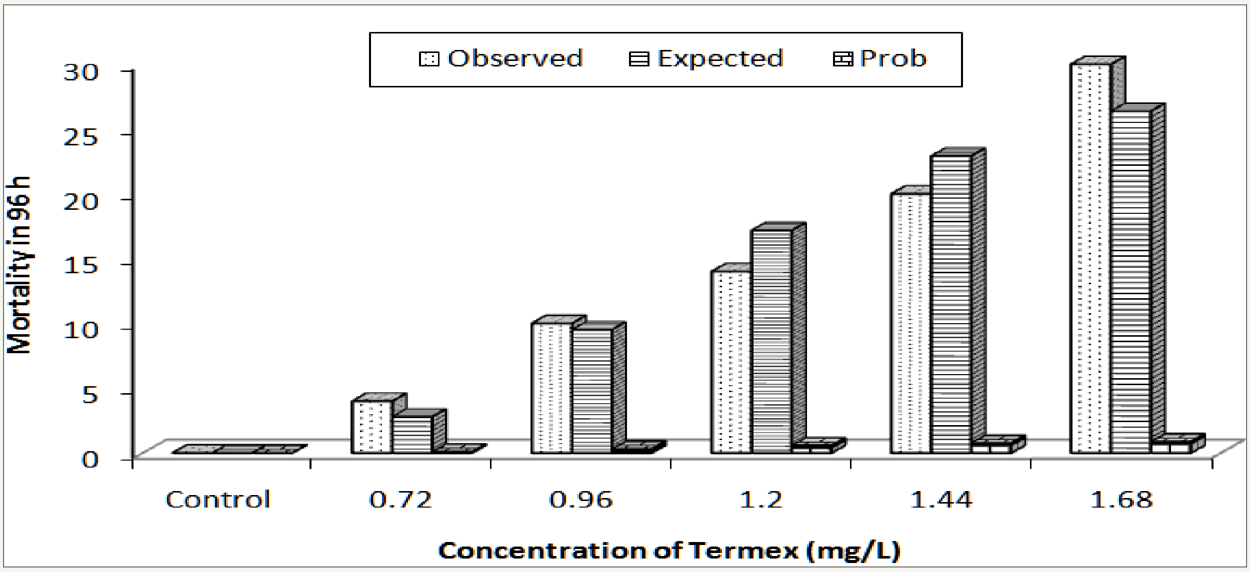

Figure 2: Response curve of *Bufo regularis* tadpole after 96h acute exposure to Termex.

Table 1: Cumulative mortality of *Bufo regularis* exposed to various concentrations of butaforce and Termex.

			Cumulative Mortality					
Pesticide	**Concentration (mg/L)**	**Number exposed**	**24-h**	**48-h**	**72-h**	**96-h**	**Mortality (%)**	**Survival (%)**
Butaforce	Control	30	0	0	0	0	0	100
	0.35	30	2	5	7	10	33	67
	0.45	30	2	7	13	18	60	40
	0.55	30	3	7	13	20	67	33
	0.65	30	4	10	18	24	80	20
	0.75	30	6	14	24	30	100	10

Termex	Control	30	0	0	0	0	0	100
	0.7	30	0	1	2	4	14	86
	0.96	30	1	3	6	10	34	66
	1.2	30	2	4	8	14	47	37
	1.44	30	3	8	13	20	67	33
	1.68	30	5	11	20	30	100	0

Table 2: Estimate of safe levels of Butaforce and Termex at 96h exposure duration to *Bufo regularis.*

Chemical	96h LC50 (mg/L)	Method	AF	Safe Level (Mg/L)
Butaforce	0.42	Hart et al. [12]	-	1.42×10^{-2}
		Sprague [13]	0.1	4.2×10^{-2}
		CWQC [14]	0.01	4.2×10^{-3}
		NAS/NAE [15]	0.1 - 0.00001	$4.2 \times 10^{-2} – 4.2 \times 10^{-6}$
		CCREM [16]	0.05	2.1×10^{-2}
		IJC [17]	5% LC_{50}	2.1×10^{-2}
Termex	1.13	Hart et al. [12]	-	3.30×10^{-2}
		Sprague [123]	0.1	1.13×10^{-1}
		CWQC [14]	0.01	1.13×10^{-2}
		NAS/NAE [15]	0.1 - 0.00001	1.13×10^{-1} - 1.13×10^{-5}
		CCREM [16]	0.05	5.65×10^{-2}
		IJC [17]	5% LC_{50}	5.65×10^{-2}
*C = 48h LC_{50} × 0.03/S2, where C = presumable harmless concentration and S = 24 h LC_{50}/48h LC_{50}				

Studies on acute toxicity in *Bufo regularis* tadpoles showed variations in lethal concentration (LC10-90) values which were dependent on the type of the pesticides, concentrations and duration of exposure (Table 3 & 4). The toxicity of chemicals to organisms has been reported to be dependent on concentration, pH, temperature, developmental stages, nature of toxicants and periods of exposures [26]. The significance differences in LC(10-90), NOEC and LOEC obtained at different times of exposures of the pesticides are in agreement with those reported in fishes exposed to other pesticides [23,26].The maximum allowable concentration (MAC) of butaforce was 4.2mg/L while the value was 11.30mg/L for termex in *B. regularis* tadpole thus indicating that butaforce is more toxic than termex. The comparative investigation of the 96h LC_{50} of other pesticides in other anurans indicate that butaforce and termex are very toxic to the amphibians (Table 5). The results of statistical end points of toxicity of butaforce and termex showed variations of NOEC, LOEC and LC_{50} with respect to concentrations and duration of exposures (Figure 3 & 4). The variations in these end points were minimal in tadpoles exposed to termex thus indicating that the extent of lethality was dependent on the type of pesticide [27-30].

Table 3: Lethal concentration (LC_{10-90}) of butaforce depending on exposure time (24-96h) for *Bufo regularis* tadpole.

Point	Concentrations (mg/L) At Various Exposure Times (95% Confidence Intervals)			
	24h	48h	72h	96h
LC_{10}	0.48	0.28	0.26	0.26
	(0.22-0.58)[a]	(0.03-0.39)[b]	(0.15-0.33)[b]	(0.17-0.31)[b]
LC_{20}	0.67	0.42	0.33	0.3
	(0.55-I.31)[a]	(0.16-0.52)[a]	(0.22-0.40)[a]	(0.23-0.35)[b]
LC_{30}	0.85	0.56	0.4	0.35
	(0.68-3.64)[a]	(0.43-0.79)[a]	(0.30-0.46)[b]	(0.27-0.39)[b]
LC_{40}	1.04	0.72	0.46	0.38
	(0.77-1.10)[a]	(0.59-1.88)[a]	(0.38-0.52)[b]	(0.32-0.43)[b]
LC_{50}	1.26	0.91	0.53	0.42
	(0.87-21.61)[a]	(0.70-4.80)[a]	(0.47-0.61)[b]	(0.37-0.47)[b]
LC_{60}	1.53	1.15	0.61	0.47
	(0.97-51.52)[a]	(0.81-12.52)[a]	(0.55-0.74)[b]	(0.42-0.51)[b]

LC70	1.87	1.48	0.71	0.52
	(1.09-130.78)[a]	(0.94-35.22)[a]	(0.62-0.94)[b]	(0.48-0.58)[b]
LC80	2.38	1.98	0.85	0.59
	(1.26-389.66)[a]	(1.11-118.63)[a]	(0.71-1.27)[b]	(0.54-0.68)[b]
LC90	3.32	2.96	1.08	0.7
	(1.52-1773.66)[a]	(1.41-641.24)[a]	(0.85-1.95)[a]	(0.62-0.87)[b]
Note: Lethal concentration values in rows with different letters significantly differ at p< 0.05.				

Table 4: Lethal concentrations (LC_{10-90}) of Termex depending on exposure time (24-96h) for *Bufo regularis* tadpoles.

Point	Concentrations (mg/L) At Various Exposure Times (95% Confidence Intervals)			
	24h	48h	72h	96h
LC_{10}	1.4	1.01	0.82	0.73
	(1.09-2.00)[a]	(0.69-1.17)[a]	(0.61-0.95)[a]	(0.16-0.94)[a]
LC_{20}	1.79	1.29	0.01	0.85
	(1.48-5.66)[a]	(1.09-1.52)[a]	(0.83-1.12)[a]	(0.29-1.05)[a]
LC_{30}	2.13	1.54	1.16	0.94
	(1.66-13.22)[a]	(1.34-2.08)[a]	(1.02-1.29)[a]	(0.45-1.16)[b]
LC_{40}	2.48	1.8	1.32	1.04
	(1.84-27.6661)[a]	(1.52-2.84)[a]	(1.19-1.50)[a]	(0.62-1.29)[a]
LC_{50}	2.85	2.07	1.48	1.13
	(2.00-55.18)[a]	(1.69-3.86)[a]	(1.33-1.776)[a]	(0.80-1.50)[a]
LC_{60}	3.28	2.4	1.66	1.23
	(2.00-55.18)[a]	(1.87-5.28)[a]	(1.47-2.09)[a]	(0,97-1.88)[a]
LC_{70}	3.53	2.79	1.89	1.35
	(2.27-156.66)[a]	(2.07-7.39)[a]	(1.62-2.52)[a]	(1.10-2.56)[a]
LC_{80}	4.54	3.34	2.18	1.5
	(2.62-555.60)[a]	(2.34-11.00)[a]	(1.82-3.21)[a]	(1.22-3.84)[b]
LC_{90}	5.79	4.29	2.67	1.74
	(3.01-1862.71)[a]	(2.76-19.11)[a]	(2.11-4.50)[a]	(1.37-6.92)[b]
Note: Lethal concentration values in rows with different letters significantly differ at p<0.05				

Table 5: Acute toxicity studies of some pesticides in tadpoles.

Pesticide	Assay	Result	References
Glyphosate	96 h LC_{50}	5.38mg/L in *P. albonotatus* tadpole	Simioni et al. [21]
Glifosan	96 h LC_{50}	20.81mg/L in *O. septentrionalis* tadpole	Reyes et al. [24]
Permethrin	96 h LC_{50}	2.50µg/L in *Rana temporaria* tadpole	Johansson et al. [29]
Esfenvalerate	96 h LC_{50}	7.30µg/L in *Rana temporaria* tadpole	Johansson et al. [29]
Herbicide vision®	96 h LC_{50}	11.50mg/L in *Rana pipiens* tadpole	Wojtaszet and Staznik [30]
Endosulfan	96 h LC_{50}	0.074mg/L *Rana dalmtina* tadpole	Manuela et al. [6]
Spent engine oil	96 h LC_{50}	2915mg/L in *Amietophoryous regularis* tadpole	Amaeze et al. (2014)
Unused engine oil	96 h LC_{50}	7353mg/L in *Amietophoryous regularis* tadpole.	Amaeze et al. (2014)
Endosulfan	96 h LC_{50}	0.730mg/L adult *B. regularis*	Ezemonye and Tango [20]
Diazinon	96 h LC_{50}	0.438mg/L adult *B. regularis*	Ezemonye and Tango [20]
Butaforce	96 h LC_{50}	0.42mg/L in *Bufo regularis* tadpole	This study
Termex	96 h LC_{50}	1.13mg/L in *Bufo regularis* tadpole.	This study
Dimethoate	96 h LC_{50}	37.37ppm in *Hyla arborea* larvae	Ferah and Ugur (2005)

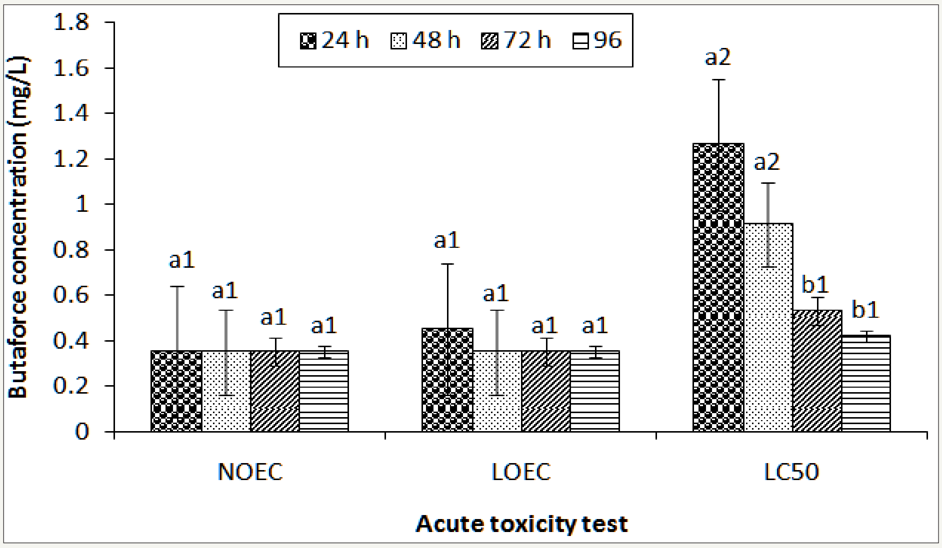

Figure 3: Acute toxicity testing statistical endpoints in *Bufo regularis* tadpole exposed to butaforce at different durations (24h, 48h, 72h and 96h).

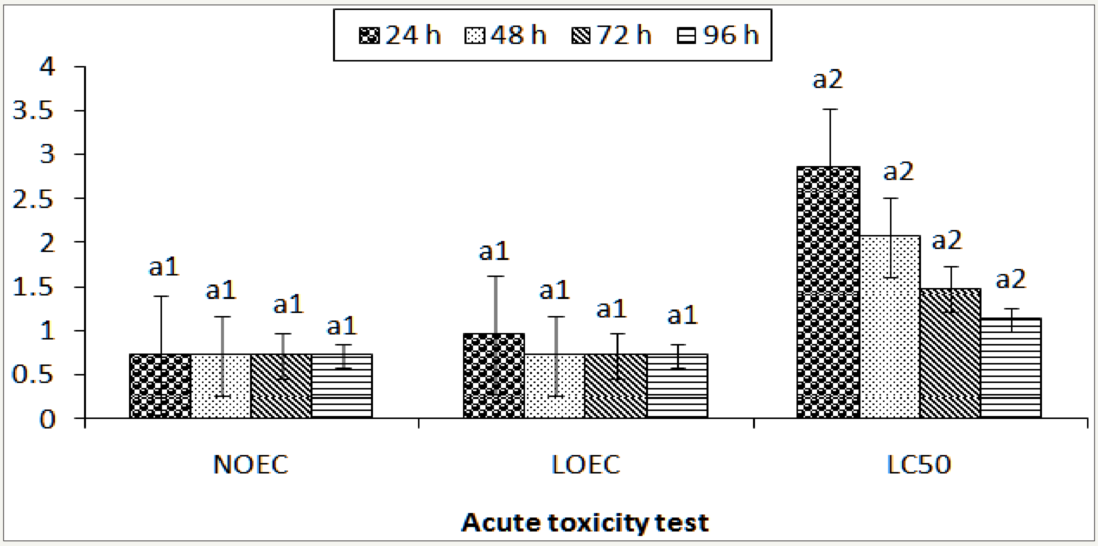

Figure 4: Acute toxicity testing statistical endpoints in *Bufo regularis* tadpole exposed to termex at different durations (24h, 48h, 72h and 96h).

Conclusion

In conclusion, both butaforce and termex pesticides are lethal to *B. regularis* tadpole and mortality is dependent on concentration and exposure duration. The two pesticides (butaforce and termex) should be applied with caution to avoid eco-toxicological effect on non target organisms such as tadpoles in the aquatic ecosystems.

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