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Biochemical Responses of *Bufo regularis* (Reuss, 1833) Tadpole Exposed to Butaforce® and Termex® Pesticides

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COE, HON, ICA, CLA and CDN conceived the study. COE, HON, ICA, CLA and JCM conducted the study. IOA, CDN, JCM and CLA did statistical analysis. COE, HON, IOA, JCM and CDN,wrote the manuscript. CDN supervised the study.

Key words
Pesticides toxicity, *Bufo regularis* tadpole, Biochemical parameters, Sublethal concentrations.

ABSTRACT

The study investigated the biochemical responses of *Bufo regularis* tadpoles exposed to two widely used agricultural pesticides, Butaforce® and Termex®. The Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total protein (TP) concentrations in homogenized muscle samples was determined by standard procedures. ALT, AST and ALP increased significantly in response to concentrations of Butaforce® (7.00, 9.00 and 11.00 μgL^{-1}) and Termex® (15.00, 20.00 and 25.00 μgL^{-1}) used. The TP decreased in response to the same concentrations of pesticides. The response of these biochemical parameters were dependent on concentration of the pesticides; it increased as concentration of pesticides was increased. This study showed that Butaforce® and Termex® are toxic to *B. regularis* tadpoles, and both induced concentration dependent biochemical changes indicative of muscle damage and depletion of protein synthesis. Both pesticides should be applied with extreme caution to avoid toxicity to non-target organisms.

INTRODUCTION

The quest to meet up with the increasing food demand of the growing human population has made man to introduce the use of different technologies and synthetic chemicals for improved yield by controlling pests and disease vectors. The substances used for control of pest are known as pesticides. The use of pesticides by man have adverse environmental effects whereby large percentage of agricultural pesticides never reach their target organisms instead they dispersed through air, soil and water (Murthy *et al*., 2013), where they may cause different kinds of damage (Ambreen and Javed, 2018). Studies have shown that 99% of applied pesticides go into the air, water and soil; and only 1% reach the target organisms (Somerville, 1987; Vogel *et al*., 2008; Dogan and Can, 2011). Aquatic biota are threatened by several environmental pollutants. According to Aspelin and Grube (1999), the discharge of synthetic pesticides into water bodies is of accelerating concern and is likely to continue and possibly increase in the near future.

Pesticides get into aquatic ecosystems in several ways which may be through direct application for the control of aquatic plants and animals; or they may reach the water body by accident as drift from nearby applications (Bohmont, 1997; Surekha *et al*., 2008). It has been reported that amphibian populations have been on the decline from the agricultural fields due to indiscriminate applications of pesticides (Mann *et al*., 2009; Natale *et al*., 2018). The most common of these are herbicides which account for approximately 80% of all the pesticides use (Ayansina and Oso, 2008). Butaforce® and Termex® are two widely used pesticides in Nigeria and other developing countries for the control of weeds and termite, respectively. Butaforce® is a trade name for commercial herbicide formulations of Butachlor (2-chloro-N-2, 6 diethylphenyl) acetamide (50% EC) used for the control of grasses in rice fields and many broad leaf weeds. Termex® is a trade name for termiticide emulsifiable concentrate formulation of imidacloprid for pre and post protection of building against termites and for the control of other pests such as cockroaches, ants, mosquito, spider, pineapple mealybug and black maize beetle. These chemicals sometimes find their way into

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aquatic environment where they could affect non-target organisms.

Biochemical parameters, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP) as well as the total protein have been used widely as indicators of pollution and stress in fish and other aquatic animals like *Bufo regularis* (Nwani *et al*., 2014). Studies have also shown that pesticides induced changes in biochemical system (Tongo *et al*., 2012; Nwani *et al*., 2014). The biochemical response of tissue to contaminants can be considered as a combination of the local event occurring within the area of injury and the systematic response induced by the local injury through hormonal neurons-endocrine pathways. Proteins are important as structural compounds, biocatalysts and hormones for growth and differentiations. Herbicides and insecticides have been shown to decrease protein level (Ravinder and Suryanarayana, 1988; Khan *et al*., 2003; Tongo *et al*., 2012). Omonona and Emikpe (2011) study on the clinico-pathological features associated with acute toxicity of lambda-cyhalothrin pesticide in adult toad (*Bufo perreti*) showed that there was a significant change in the biochemical parameters, ALT, ALP, AST and total protein of the exposed toad. ALT is an enzyme that helps to process proteins in living cell while ALP not only helps to break down proteins in the body but also liberates phosphate under alkaline conditions and is made in liver, bone, and other tissues (Singh *et al*., 2011). AST is an enzyme present in various parts of the body and its levels increase when there is damage to the tissues and cells where the enzyme is found. The present study aimed at determining the biochemical responses of *Bufo regularis* tadpole exposed to Butaforce® and Termex® pesticides.

MATERIALS AND METHODS

Collection and maintenance of experimental animals

One hundred and eighty (n=180) tadpoles of freshwater *Bufo regularis* Reuss (family: Bufonidae; order: Anura) of mean body length, 2.01 ± 1.01cm and mean body weight, 0.10 ± 0.01g were collected using hand net. The tadpoles were collected from a stagnant pool in the Biological Garden of Department of Biology, Federal College of Education, Eha-Amufu, Enugu State, Nigeria. The tadpoles were transferred into 20 L plastic aquarium containing water collected from the pool where the tadpoles were caught and transported to Fishery Wet Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were acclimatized for fourteen days in three 500 L test plastic tanks prior to the experiment. Water was changed daily with well aerated tap water to remove faecal matter and other waste materials in order to reduce ammonia content of the water; the aquarium was also cleaned thoroughly. The tadpoles were fed with green algae three times weekly. Any dead tadpole was removed with forceps to maintain healthy water quality. The feeding of the experimental tadpoles were stopped 24 h before the commencement of acute toxicity test as recommended by Ward and Parrish (1982) and Reish and Oshida (1987). Ethical clearance was obtained from the Committee on Experimental Animal Care of Fishery Department, Ministry of Agriculture and Natural Resources, Enugu State, Nigeria. It was assigned the identification number, MANR/FD/2017/EC102. The guideline that was recommended by this Committee was carefully followed.

Procurement of chemicals

The herbicide, Butaforce®, and termiticide, Termex® were purchased from agrochemical shop in Ogbete Market, Enugu State, Nigeria. Stock solutions of a brand of butachlor trade-named Butaforce® liquid (China Agro Cropcare Co. Ltd, China) with NAFDAC No. A5-0268 and containing 50 % butachlor as active ingredient, and a brand of termex (Termex 350 SC®) containing 350 gw/v imidacloprid 30.5% SC (Nariman Point Mumbai Rallis, India Ltd.) were prepared.

Determination of sublethal concentrations

The 96 h LC_{50} values of Butaforce® and Termex® on *B. regularis* tadpoles were 0.42 mg/L and 1.13 mg/L, respectively following the probit analysis as described by Finney (1971). Based on the 96 h LC_{50} value obtained during the acute toxicity assay, three sublethal concentrations corresponding to $1/5^{th}$, $1/10^{th}$ and $1/20^{th}$ of 96 h LC_{50} of Butaforce® (7 µg/L, 9 µg/L, 11 µg/L) and Termex® (15 µg/L/, 20 µg/L/ and 25 µg/L/), respectively were used for the experiment.

Experimental design

A total of 120 acclimatized tadpoles were used. They were exposed to the test chemicals *in vivo*. Completely randomized design was used to divide the tadpoles into four groups, I–IV. The four groups were each replicated into three with each containing 10 tadpoles. Each of the replicates was contained in a 40 L glass aquarium of size 60×30×30 cm. This design was used for each of the test chemicals. For the experiment involving Butaforce® (50% butachlor), groups I, II and III, respectively were exposed to 7 µg/L, 9 µg/L and 11 µg/L of sublethal concentrations of the test chemical, respectively. Similarly, the experiment involving Termex® (350 gw/v imidacloprid 30.5% SC), groups I, II and III were exposed, respectively to 15 µg/L, 20 µg/L and 25 µg/L sublethal concentrations of the test chemical, respectively. The group IV for both test chemicals served as control; they were exposed to tap water that was free of the test chemicals. The test

solution was renewed daily (*i.e.* every 24 h). Samples were collected at intervals of 24 h starting from the first 24 h of exposure. The exposure lasted for a period of 96 h. Three tadpoles each from each of the four treatment groups were sacrificed on each sampling day. The sacrificed tadpoles were weighed and the muscle tissue obtained. The tissues were quickly rinsed in cold 0.9 % sodium chloride solution and immediately homogenized in pre-chilled potassium phosphate buffer (1: 10 w/v, 0.1M, pH 7.0). The homogenate was centrifuged for 20 min at 10,500 rpm under 4°C to obtain the supernatant which was stored at 4°C until when used for the estimation of biochemical parameters.

Estimation of biochemical parameters

ALT, AST and ALP concentrations in homogenized sample were determined according to the colorimetric method of Reitman and Frankel (1975) using Randox® kits. The absorbance was read at 540 nm. Total protein content in the muscle tissue was determined using a protein assay kit which adopted the Peterson's modifications of Micro-Lowry Method (Sigma Diagnostics, Missouri, USA).

Statistical analysis

The data was subjected to two-way analysis of variance (ANOVA) followed by *post-hoc* Tukey's test to determine the differences between concentrations and durations effects of the exposures. Statistical significance difference was considered at $p < 0.05$. Data was analyzed using SPSS® software version 20.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Effect of Butaforce®

The effect of sublethal concentrations of Butaforce® on biochemical parameters of *B. regularis* is shown in Table I. The four biochemical parameters evaluated, ALT, AST, ALP and TP showed variations in amounts in response to exposure of the tadpoles to Butaforce®. ALT concentration in all groups exposed to the three Butaforce® concentrations (7.00, 9.00 and 11.00 μgL^{-1}) were higher when compared to the unexposed control group. The difference was also significant at all cases at the end of 24, 48, 72 and 96 h ($p < 0.05$). At the end of 24 h exposure, ALT had its highest concentrations of 36.00 ± 0.15 U/L and 37.00 ± 0.58 U/L at 7.00 μgL^{-1} and 11 μgL^{-1} Butaforce® concentrations, respectively. At the end of the 48, 72 and 96 h exposure the difference in average ALT concentration between the groups exposed to Butaforce® and the unexposed control group was consistently in the order of magnitude 11.00 μgL^{-1} > 9.00 μgL^{-1} > 7.00 μgL^{-1}. The changes in ALT was distinctly dependent on concentration of Butaforce®.

Table I.- Effect of sublethal concentrations of Butaforce® on the biochemical parameters, alanine aminotransferase (ALT), aspartate aminotransfrase (AST), alkaline phosphate (ALP) and total protein (TP) in *Bufo regularis* tadpoles.

Parameters	Conc. (μgL^{-1})	Exposure duration (h)			
		24	48	72	96
ALT (U/L)	Control	22.33 ± 0.88^{a2}	19.00 ± 0.58^{a1}	22.00 ± 0.58^{a2}	17.67 ± 0.33^{a1}
	7.00	36.00 ± 1.15^{c12}	38.33 ± 0.88^{b2}	40.67 ± 0.33^{b2}	35.00 ± 0.58^{b1}
	9.00	30.33 ± 0.33^{b1}	42.67 ± 0.67^{c2}	44.00 ± 0.58^{c3}	41.33 ± 0.88^{c2}
	11.00	37.00 ± 0.58^{c1}	44.33 ± 0.88^{c2}	48.00 ± 0.58^{d3}	47.00 ± 0.58^{d3}
AST (U/L)	Control	19.00 ± 0.58^{a1}	17.33 ± 0.33^{a1}	19.67 ± 0.88^{a1}	17.33 ± 0.33^{a1}
	7.00	25.00 ± 0.58^{c2}	21.67 ± 0.33^{b1}	22.33 ± 1.20^{b1}	22.00 ± 0.58^{b1}
	9.00	22.00 ± 1.53^{b1}	26.33 ± 0.88^{c2}	28.67 ± 0.33^{c3}	30.33 ± 0.33^{c3}
	11.00	28.33 ± 0.33^{d1}	30.00 ± 0.58^{c1}	31.00 ± 0.58^{c2}	35.00 ± 0.58^{d3}
ALP (U/L)	Control	42.67 ± 1.20^{a1}	43.00 ± 1.53^{a1}	43.00 ± 0.58^{a1}	41.00 ± 0.58^{a1}
	7.00	61.67 ± 1.20^{b1}	64.00 ± 2.08^{b1}	68.00 ± 0.58^{b2}	69.00 ± 0.58^{b3}
	9.00	61.00 ± 2.51^{b1}	67.33 ± 0.88^{b2}	67.00 ± 1.53^{b2}	72.33 ± 0.88^{c2}
	11.00	66.33 ± 0.88^{b1}	67.33 ± 0.33^{b1}	71.67 ± 1.20^{c2}	74.67 ± 0.88^{c3}
TP (g/L)	Control	5.20 ± 0.23^{b1}	5.20 ± 0.21^{b1}	5.53 ± 0.44^{b1}	5.47 ± 0.22^{b1}
	7.00	2.70 ± 0.06^{a1}	2.43 ± 0.12^{a1}	1.83 ± 0.03^{a1}	1.40 ± 0.58^{a1}
	9.00	2.40 ± 0.25^{a1}	2.63 ± 0.09^{a1}	1.67 ± 0.08^{a1}	1.40 ± 0.58^{a1}
	11.00	2.13 ± 0.03^{a1}	2.67 ± 0.09^{a1}	1.63 ± 0.15^{a1}	1.30 ± 0.58^{a1}

Different alphabetic superscripts show significant differences between Butaforce® concentrations along a column for each parameter while different numeric superscripts indicate significant differences across the rows and between durations of exposure as determined by Tukey's *post-hoc* test ($p < 0.05$). Values as mean ± SEM.

Table II.- Effect of sublethal concentrations of Termex® on the biochemical parameters, alanine aminotransferase (ALT), aspartate aminotransfrase (AST), alkaline phosphate (ALP) and total protein (TP) in *Bufo regularis* tadpoles.

Parameters	Conc. (µgL^{-1})	Exposure duration (h)			
		24	48	72	96
ALT (U/L)	Control	22.33 ± 0.88^{a2}	19.00 ± 0.58^{a1}	22.00 ± 0.58^{a1}	17.33 ± 0.33^{a1}
	15.00	32.67 ± 1.45^{b2}	31.00 ± 0.58^{b2}	33.00 ± 0.58^{b2}	24.33 ± 0.33^{b1}
	20.00	35.00 ± 0.58^{b2}	33.33 ± 1.76^{b12}	34.00 ± 0.58^{b2}	30.00 ± 0.58^{c1}
	25.00	37.00 ± 0.58^{c2}	37.00 ± 0.58^{c2}	39.67 ± 0.88^{c3}	31.00 ± 0.58^{c1}
AST (U/L)	Control	19.00 ± 0.58^{a1}	17.33 ± 0.33^{a1}	19.67 ± 0.88^{a2}	17.33 ± 0.58^{a1}
	15.00	26.33 ± 0.88^{b1}	25.00 ± 0.58^{b1}	24.00 ± 0.58^{b1}	24.33 ± 0.58^{ab1}
	20.00	29.00 ± 0.58^{b23}	25.00 ± 0.58^{b1}	27.00 ± 0.58^{c12}	30.00 ± 0.58^{c3}
	25.00	37.33 ± 0.33^{c2}	29.67 ± 0.33^{c1}	30.33 ± 0.33^{d1}	31.00 ± 0.58^{c1}
ALP (U/L)	Control	42.67 ± 1.20^{a1}	43.00 ± 1.53^{a1}	43.00 ± 0.58^{a1}	41.00 ± 0.58^{a1}
	15.00	62.33 ± 0.88^{b1}	60.00 ± 2.03^{b1}	60.00 ± 1.15^{b1}	58.67 ± 0.88^{b1}
	20.00	65.00 ± 0.58^{c1}	65.33 ± 0.33^{c1}	64.00 ± 0.58^{bc1}	65.00 ± 0.58^{c1}
	25.00	68.00 ± 0.58^{c1}	69.00 ± 0.58^{c1}	73.67 ± 6.70^{c1}	68.67 ± 0.88^{c1}
TP (g/L)	Control	5.20 ± 0.23^{b1}	5.20 ± 0.21^{c1}	5.53 ± 0.44^{c2}	5.47 ± 0.22^{d2}
	15.00	4.30 ± 0.15^{a3}	4.57 ± 0.12^{b3}	3.73 ± 0.12^{b2}	3.17 ± 0.03^{c1}
	20.00	4.83 ± 0.33^{ab3}	4.00 ± 0.06^{a2}	2.57 ± 0.88^{a1}	2.53 ± 0.03^{b1}
	25.00	4.60 ± 0.15^{a3}	3.67 ± 0.09^{a2}	1.83 ± 0.03^{a1}	1.73 ± 0.03^{a1}

Different alphabetic superscripts show significant differences between Termex® concentrations along a column for each parameter while different numeric superscripts indicate significant differences across the rows and between durations of exposure as determined by Tukey's *post-hoc* test ($p < 0.05$). Values as mean ± SEM.

The mean AST concentrations in tadpoles exposed to Butaforce® were all higher compared to the unexposed control group. The difference was significantly between the control and each of the exposed groups (7.00, 9.00 and 11.00 µgL^{-1}) at the end of 24, 48, 72 and 96 h of exposure ($p < 0.05$). The pattern of variation in AST concentration was similar to that of ALT. The mean concentration of AST at the end of 24 h was higher in groups exposed to 7 µgL^{-1} and 11.00 µgL^{-1} of Butaforce® compared to the control and 9.00 µgL^{-1} group. The mean concentrations of AST at the end of the other time intervals (48, 72 and 96 h) were in the order of magnitude 11.00 µgL^{-1} > 9.00 µgL^{-1} > 7.00 µgL^{-1}. The change in AST concentration was dependent on concentration of Butaforce®.

The changes in concentration of ALP in tadpoles exposed to Butaforce® followed a similar pattern as ALT and AST (Table I). The mean ALP concentrations in the three groups exposed to Butaforce® were each higher compared to the unexposed control group at all the time intervals considered. The difference was significant between the control and each of the groups exposed to 7.00, 9.00 and 11.00 µgL^{-1} ($p<0.05$). The change in concentration of ALP was indicative of concentration dependent response where the increase in concentration of Butaforce® to which the tadpoles were exposed resulted in greater effect on ALP concentration.

The mean concentration of total protein (TP) in all the groups exposed to Butaforce® was low compared to the unexposed control at the end of 24, 48, 72 and 96 h. The difference between each of the exposed groups and the control was significant at all cases of comparison ($p<0.05$). The changes in TP were not dependent on concentration of Butaforce or duration of exposure.

Effect of Termex®

The effect of sublethal concentrations of Termex® on the biochemical parameter of *B. regularis* tadpoles is presented in Table II. The concentration of the four biochemical parameters evaluated, ALT, AST, ALP and TP varied in response to exposure of the tadpoles to Termex®. The changes were dependent on concentration of Termex®, but independent on duration of exposure. ALT, AST and ALP at the end of 24, 48, 72 and 96 h were higher in the three groups exposed to concentrations of Termex (15.00, 20.00 and 25.00 µgL^{-1}) than the unexposed control group. The differences were all significant ($p<0.05$). Total protein was similarly lower in all three groups exposed to Termex® than the unexposed control group at the end of 24, 48, 72 and 96 h. The difference between each group and control at these time intervals was significant ($p<0.05$).

DISCUSSION

The present study evaluated the biochemical

responses of *B. regularis* tadpoles exposed to Butaforce® and Termex®. The results of *B. regularis* tadpoles exposure to different sublethal concentrations of Butaforce® (7.00 µg/L^{-1}, 9.00 µg/L^{-1} and 11.00 µg/L^{-1}) and Termex® (15.00 µg/L^{-1}, 20.00 µg/L^{-1} and 25.00 (µg/L^{-1}) in the present study indicate that tadpole can be used as bio-indicator of contaminations. The intensive application of pesticides for increased agricultural production and for other purposes pose adverse effects on non-target organisms. Non-target organisms, such as amphibian breed during rainy season when pesticides are applied to the land for control of weed, insects and other pests (Paskora *et al*., 2011).

The results obtained on biochemical parameters in this study showed significant increase ($p < 0.05$) in ALT, AST, and ALP in *B. regularis* tadpoles exposed to Butaforce® and Termex® pesticides when compared with the control group. ALT, AST and ALP increased as the concentration of Butaforce® was increased from 7.00 µg/L^{-1} to 9.00 µg/L^{-1} and 11.00 µg/L^{-1}, and as that of Termex® was increased from 15.00µg/L^{-1} to 20.00 µg/L^{-1} and 25.00µg/L^{-1}. The elevated levels of ALT as observed in exposed tadpoles is indicative of toxicity-induced muscle damage, and the resultant leakage of the enzyme from this tissues (Singh *et al*., 2011). The high level of ALT suggests tissue injuries. A similar finding was reported by Gungordu *et al*. (2013) where it was observed that ALT increased significantly in tadpoles exposed to Benzothiazole organophosphorus pesticides compared to control group. The increase in the AST level in this study possibly resulted from muscle damage in the exposed tadpole compared with the control. Similar result was observed by other researchers on different pesticides (Omonona and Emikpe, 2011; Gungordu *et al*., 2013).

Again the observed reduction in total protein level in *B. regularis* tadpoles exposed to Butaforce® or Termex® may be related to the inhibition of protein synthesis in muscle tissues as a result of toxicity caused by both pesticides. In addition, utilization of available protein in the body physiology to compensate for the increased energy demand imposed by the toxicant may result in depletion of total protein level (Dogan and Can, 2011). Protein is very important building blocks of all cells and tissues and is required for body growth and development. Thus when there is decrease in protein levels as a result of Butaforce® and Termex® pesticides, inducement of the body growth and development of tadpole is inhibited. The alteration of protein contents in tadpoles could be used as biomarker for monitoring the physiological status of the tadpoles. Similar to the present observations decrease in total protein in the plasma have been reported in amphibian and other vertebrate exposed to other pesticides, such as diazinon on adult toad (Tongo *et al*., 2012), and malathion on common Indian toad *Bufo melanosticus* (Mahananada and Mohanty, 2012). Amaeze *et al*. (2014) observed decrease in total protein content of the *Amietophrynus regularis* tadpoles exposed to refined petroleum and unused spent engine oil.

CONCLUSION

Butaforce® and Termex® are toxic to *B. regularis* tadpoles and induced biochemical changes in the exposed tadpoles. The biochemical changes were indicative of toxicity-induced muscle damage and the leakage of the enzymes ALT, AST and ALP from muscle tissues. It was also indicative of toxicity-induced depletion of protein synthesis. The toxic effect of Butaforce® and Termex® each depends on the concentration to which the tadpoles are exposed. Both pesticides should be applied with extreme caution to avoid toxicity to non-target organisms.

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Statement of conflict of interest

The authors declare no conflict of interest.

REFERENCES

Amaeze, N.H., Onadeka, A. and Nwosu, C.C., 2014. Comparative acute toxicity and oxidative stress responses in tadpoles of *Amietophrynus regularis* exposed to refined petroleum products, unused and spent engine oils. *Afr. J. Biotechnol.*, **13**: 4251-4258. https://doi.org/10.5897/AJB2014.14093

Ambreen, M. and Javed, M., 2018. Pesticide mixture induced DNA damage in peripheral blood erythrocytes of freshwater fish, *Oreochromis niloticus. Pakistan J. Zool.,* **50**: 339-346.

Aspelin, A.L. and Grube, A.H., 1999. *Pesticides industry sales and usage 1996 and 1997 market estimates*. EPA733-R-99-001, Office Pesticide Programs, Washington DC.

Ayansina, A.D.V. and Oso, B.A., 2008. Effect of organic amendments on microbial biomass of a tropical soil treated with some herbicides. *Int. J. Biol. Chem. Sci*., **2**: 417-424.

Bohmont, B.L., 1997. *The standard pesticide user's guide,* 4th edition. Prentice Hall, Upper Seddle River, N.J.

Dogan, D. and Can, C., 2011. Hematological,

biochemical and behavioural responses of *Oncorhychus mykiss* to dimethoate. *Fish Physiol. Biochem.*, **37**: 951-958. https://doi.org/10.1007/s10695-011-9492-1

Finney, D.J., 1971. *Probit analysis*. Cambridge University Press, Cambridge.

Gungordu, A., Sireci, N., Kucukbay, H., Birhanli, A. and Ozmen, M., 2013. Evaluation of in-vitro and in-vivo toxic effects of newly synthesized benzimidazole-based organo-phosphorus compounds. *Ecotoxicol. environ. Safe.,* **87**: 23-32. https://doi.org/10.1016/j.ecoenv.2012.10.007

Khan, M.Z., Nazia, M., Fatima, F., Rhilla, T. and Gabol, K., 2003. Comparison in effect of lambda-cyhalothrin with permethrin on cholinesterase activity in wildlife species, *Rana cyanophlyctis* and *Rana tigrina* (Ranidae: Amphibian) *Bull. Pure appl. Sci.*, **22A**: 43-49.

Mahananada, M.R. and Mohanty, B.P., 2012. Toxicity on biochemical and ematolgical Parameters in *Bufo melanostictus* (Schneider) (common India toad) exposed to malathion. In: *Pesticides-Advances in chemical and botanical pesticides* (ed. R.P. Soundararajan). InTech Open. https://doi.org/10.5772/46231

Mann, R.M., Hyne, R.V., Choung, C.B. and Wilson, S.P., 2009. Amphibians and agricultural chemicals: Review of the risks in a complex environment. *Environ. Pollut*. **157**: 2903-2927. https://doi.org/10.1016/j.envpol.2009.05.015

Murthy, K.S., Kiran, B.R. and Venkate-Shwarlu, M., 2013. A review on toxicity of pesticides in fish. *Int. J. Open Sci. Res.,* **1**: 15-36.

Natale, G.S., Vera-Candioti, J., Ruiz de Arcaute, C., Soloneski, S., Larramendy, M.L. and Ronco, A.E., 2018. Lethal and sublethal effects of the Primicarb-based formulation Aficida® on *Boana pulchella* (Dumeril and Bibron, 1841) tadpoles (Anura, Hylidae). *Ecotoxicol. environ. Safe*., **147**: 471-479. https://doi.org/10.1016/j.ecoenv.2017.09.007

Nwani, C.D., Ifo, C.T., Nwamba, H.O., Ejere, V.C., Onyishi, G.C., Oluah, S.N., Ikwuagwu, O.E. and Odo, G.E., 2014. Oxidative stress and biochemical responses in the tissues of African catfish *Clarias gariepinus* juvenile following exposure to primextra herbicide. *Drug Chem. Toxicol.*, **158**: 1528-6014.

Omonona, A.O. and Emikpe, B.O., 2011. Clinicopathological features associated with acute toxicity of lambda-cyclothrin pesticide in adult toads (*Bufo perreti*). *J. Adv. environ. Biol.,* **5**: 808-813.

Paskova, V., Hilscherova, K. and Blaha, L., 2011. Teratogenicity and embryotoxicity in aquatic organisms after exposure to pesticides role of oxidative stress. *Rev. environ. Contam. Toxicol*., **211**: 25-61. https://doi.org/10.1007/978-1-4419-8011-3_2

Ravinder, V. and Suryaanarayanan N., 1988. Pesticides induced biochemical alterations in a freshwater catfish.(*Clarias batrachus*). *Indian J. Comp. Anim. Physiol*., **6**: 5-12.

Reish, D.L. and Oshida, P.S., 1987. *Manual of methods in aquatic environment research*, Part 10: *Short-term static bioassays*. FAO Fisheries Technical Paper No. 247.

Reitman, S. and Frankel, S., 1957. Determination of serum transaminase. *Am. J. clin. Pathol.,* **28**: 56-59. https://doi.org/10.1093/ajcp/28.1.56

Singh, A., Bhat, T.K. and Sharma, O.P., 2011. Clinical biochemistry of hepatotoxicity. *J. clin. Toxicol.*, **S4**: 001. https://doi.org/10.4172/2161-0495.S4-001

Somerville, L., 1987. Monitoring degradation products. In: *Pesticides effects on soil microflora* (eds. L. Somerville and M.P. Greaves). Taylor and Francis Publisher, London.

Surekha, R., Lakshmi, D., Suvarnalatha, M. Jaya, M., Aruna, S., Jyothi, K., Narasimba, G. and Venkateswarlu, K., 2008. Isolation and characterization of a chlorpyrifos degrading bacterium from agricultural soil and its growth response. *Afr. J. microbiol. Res.*, **2**: 26-31.

Tongo, I., Ezemonye, L. and Ochei, U., 2012. Diazinon mediated biochemical changes in the African toad (*Bufo regularis). J. Xenobiot.,* **2**: e4. https://doi.org/10.4081/xeno.2012.e4

Vogel, J.R., Majewski, M.S. and Capel, P.D., 2008. Pesticides in rain in four agricultural watersheds in the United States. *J. environ. Qual.,* **37**: 1101-1115. https://doi.org/10.2134/jeq2007.0079

Ward, G.S. and Parrish, P.R., 1982. Toxicity tests. In: *Manual of methods in aquatic environment research,* Part 6. FAO Technical Paper No. 185, pp. 1-23.