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The Effect of 2,4 Dimethylamine salt on the Blood, Liver and Muscle of *Oryclotagus* Cuniculus

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Abstract

Aminoforce containing 720g/l of 2,4-dimethylamine salt induced changes on some enzymes and electrolytes in the male *Oryclotagus cuniculus* (New Zealand rabbit) were assayed. The organisms were exposed to varying sub-lethal concentrations of the toxicant (720g/l). The concentrations were prepared by pipetting 0.4mls, 0.8mls and 0.12mls making it up to 1.5L clean water in a metal container to make 2.0 mgl^{-1}, 4.0 mgl^{-1} and 6.0 mgl^{-1}. Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Acid phosphatase (ACP) were assayed in the liver and blood. Results showed that aspartate amino transferase values in the liver and blood were significant ($p<0.05$) across the concentration of the toxicants. Aspartate amino transferase increased as the concentration of the toxicant increased in the liver, and decreased as the toxicant concentration increased in the blood. Alanine amino transferase in the blood and liver were akin to AST while ACP values increased in the blood and decreased in the liver as the concentration of the toxicant increased. Electrolytes (Sodium (Na^+), potassium (K^+) and magnesium (Mg^{2+}) ions) showed statistical deviation across the various concentration of the toxicants. Chloride ion values stabilized in the experimental group being not significantly different ($p>0.05$) across the various concentration of the toxicants. From the study, AST, ALT and ACP are suitable biomarkers for showing sub-lethal effect of aminoforce on *Oryclotagus cuniculus.* The effects recorded clearly unveiled the potential effect of this xenobiotics on *Oryclotagus cuniculus.* Therefore, exposure of *Oryclotagus cuniculus* to this toxicant will affect the organism's physiological responses and over prolong period of time it could lead to death. Additionally, via food chain man may be affected. The use of this toxicant close to rabbittory should be done with utmost caution.

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Introduction

Pesticides are chemicals used to destroy or kill, mitigate, control pests of different kind, including insects, rodents, weeds and other target organisms. A pesticide may be a chemical substance, or a biological agent (such as a virus or bacterium), antimicrobial, disinfectant or a device used against pests [1]. Pesticides in the environment could pose a risk to non-target organisms (plants and animals including humans) [2]. They do not only contaminate the ecosystem but also bioaccumulate in trace amounts in plant and animal tissues causing serious health hazards [3]. Feed and fodder offered to animals are often contaminated with pesticide residues [4, 5].

Aminoforces containing 720g/l with active ingredient 2,4-dimethyalamine salt (2,4-D) is one of the frequently used chemical agents in modern agriculture as a selective pre-emergent and post emergent systemic herbicide which reduces the population of broad leaved weeds and vegetation on farms or agricultural lands. Up until now, 2,4 dimethyamine salt remained one of the dominating and frequently used herbicides in the world for agricultural purposes, probably due to its low cost, selectivity, specificity and effectiveness in terms of its broad spectrum of weed control [6].

Pesticide residues such as 2,4-D can persist in the environment for long periods of time and are known to be persistent organic pollutants. They can sometimes bioaccumulate in domestic and farm animals like rabbit, fishes and plants, which are sources of nutrients and delicacies for human consumption. Several studies have equally addressed the toxic effect of pesticides in the function of several animal organs and tissues including the serum, liver, muscle and kidney with results indicating alteration in the levels of the biomarker parameters related to the various organs especially in fish [7-9].

Reproductive failures in fish and birds attributed to pesticides have been reported [8 -10]. The presence of the pesticides such as diazion, imidacloprid, paraquat dichloride, glyphosate among others in the aquatic ecosystem through the food chain can lead to bioaccumulation in fish and biomagnification in humans.

The toxicity of 2,4-D in the liver cells of rat determined by FTIR Spectroscopy revealed an alteration of the protein secondary structure by increasing random coil structures and turns and /or inducing a reduction in the β-sheet structure [11]. The authors also reported that the toxicant reduced protein, loosened lipid chain of membrane packings, caused lipid polarity and increased the formation of lipids with carbonyl compounds hydroperoxyl groups with a very pronounced liver enlargement. According to Amel et al. [12], 2,4-D exposed to rodents produces different toxicities such as reduction of acetylcholinesterase (AchE) activity, brain weight loss and testicular dysfunction. 2,4-D treatment impaired normal spermatogenesis in mice due to the disruption of cholesterol/testosterone homeostasis in leydig cells through peroxisome proliferator activated receptor A [13].

Rabbits are used widely in varieties of research paradigms due to their smaller structure, docile demeanor, and second only to mice and rats, they are the next most commonly used species in research [14]. The general physiology of rabbits is similar to humans; therefore, the rabbits are used as model organisms for human diseases [15]. Xenobiotics such as 2,4-D that affect rabbits, rats, and mice could have serious effect on humans.

Agricultural fields, residential and commercial lawns, roadsides and parks are suspected sites that bring about the direct human exposure to 2,4 –D. The mode of exposure may be through non-dietary ingestion, inhalation, (dust/vapour), and skin contact. 2, 4–D contaminated soil, drinking water, and foodstuff are also other main routes of the chemical exposure [16]. The contaminated air can bring about itching, irritation, burning sensation of the skin, shortness of breath and upper lung burning sensation. Additionally ingestion of contaminated water can result in diarrhea, vomiting, skeletal muscle injury, headache, irritation and hypertension [16]. About 2 to 8-fold increment in the incidence of non-Hodgkin's lymphoma has been reported among farmers in Canada and USA, who frequently used 2,4-D [17].

Therefore, the present study was designed to evaluate the consequences of aminoforce containing 720 g/l of 2,4 Dimethylamine salt on Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Acid phosphatase (ACP) in the blood and liver, and electrolytes such as Sodium (Na^+), Potassium (K^+), Chloride (Cl^-) and Magnesium (Mg^{2+}) in the muscle of

Oryclotagus cuniculus (New Zealand rabbit).

Materials and Methods

The New Zealand rabbit (*Oryclotagus cuniculus*) for this study were obtained from a private rabbit farm at Mbiama, Rivers State, Nigeria. They were transported individually in plastic baskets to the Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, where the assays were carried out from October to December, 2019. A total 25 healthy adult *Oryclotagus cuniculus* weighing 1800g to 2000g per weight were used in the present study. Each rabbit occupied each compartment of the rabbitory for 10 days acclimation. They were fed with 200 g of synthetic growers' march (pelleted) daily. A metal container containing clean water (1.5 L) was kept in each compartment.

Range Finding Test

A range finding test (trial test) was carried out to determine the actual concentration of the toxicant (aminoforce containing 720 g/l of 2,4 - Dimethylamine salt) to be used for the main experimental run. The trial test rabbits were grouped into three groups. Each group which contain triplicate rabbit was exposed to 1.00 mgl^{-1}, 2.0 mgl^{-1} and 3.00 mgl^{-1} of the toxicant. The trial test lasted for 14 days. The test solution in each compartment were renewed daily.

Definitive Test (Main Test)

A sub-lethal dose of the toxicant needed for the main experiment was determined based on the result of the range finding test. Three different concentrations viz: 0.04 mls, 0.08 mls and 0.12 mls were exposed to the probe organism from the original concentration of 720 g/l to make 2.0 mgl^{-1}, 4.0 mgl^{-1} and 6.0 mgl^{-1} i.e. concentration of the toxicant used. In addition, a control was set up viz: 0.0 mgl^{-1}. Each of the experimental group contain triplicate New Zealand rabbit. The main experiment lasted for 21 days. The test solution in each compartment were renewed daily akin to trial test period.

Blood samples were collected from the ear veins before the probe organisms (New Zealand rabbit) were killed and dissected and target organs were obtained (liver and muscle). About 0.5 g of each of the organs were macerated with a ceramic mortar. 5 ml of physiological saline was used stabilize each sample for enzyme analysis, while deionized water was used stabilize samples meant for electrolyte analyses. All samples were centrifuged at the rate of 3000 rpm for 15 minutes. The supernatant was then removed and stored in plain bottles at -20 ^{0}C for analysis. Sodium and potassium ions were assayed via APHA [18] method. Chloride ion assay was carried out using Malmstadt and Winefordner [19] method, while Mg^{2+} assay was carried out via Macintyre and Davidsson [20] method. In the liver and blood samples, aspartate amino transferase and alanine amino transferase were analysed using the colorimetric technique previously described by Zimmerman [21], while ACP analysis was carried out by the method described by Andersch and Szcypinski [22].

Statistical Analysis

SPSS software version 16 was employed to unveil the analysis of the data. The data were expressed as mean ± standard error and one-way analysis of variance was carried out at α=0.05 and Duncan multiple range test (DMRT) was used to separate the means.

Results and Discussion

Enzymes

Table 1 shows the effect of *Oryclotagus cuniculus* blood and liver enzymes (ACP, ALT and AST) when exposed to 2,4-D (aminoforce) for 21 days. The concentration of AST in the liver were statistically different ($P<0.05$) across the various concentration of the toxicant. Values progress down the experimental group, albeit not in a dose dependent pattern, while blood AST values receded down the experimental group. The lowest blood AST value was recorded at 2.0 mgl^{-1} (54.65±2.01 µ/l) compared to the control that had 96.59±1.10 µ/l. Liver ALT values showed a remarkable deviation from the control, which progressed in a dose dependent pattern down the experimental group. The highest value (697.99 µ/l) of liver ALT was recorded at the highest concentration (6.00 mgl^{-1}) (Table 1). Blood ALT values showed significant. However, multiple comparison showed no significant variation between 0.00 mg/l and 2.00 mg/l, and between 4.00 mg/l and 6.00 mg/l concentration of the toxicant. The blood ALT recorded were diminutive compared to the control, albeit values appreciated down the experimental group in a dose dependent pattern. Retrogressive values were recorded in liver ACP (albeit not dose dependent) quite

Table 1. Blood and liver enzymes (ACP, ALT and AST) of *Oryclotagus cuniculus* exposed to 2,4-D (aminoforce) for 21 days (mean±SD)

Conc. Of 2,4 D (mgl^{-1})	AST (µ/l)		ALT (µ/l)		ACP (µ/l)	
	Liver	Blood	Liver	Blood	Liver	blood
0.00	24.18±1.09^{c}	96.89±1.10^{a}	20.61±4.09^{c}	136.02±5.21^{a}	65.93±1.79^{a}	0.36±0.00b^{c}
2.00	22.67±0.91^{c}	54.65±2.01^{d}	344.29±10.21^{b}	105.86±10.10^{c}	36.21±0.11^{b}	0.17±0.24b^{c}
4.00	344.29±4.31^{a}	78.40±1.01^{b}	616.10±9.37^{a}	120.62±6.01^{b}	17.72±1.03^{c}	4.17±0.24^{a}
6.00	125.27±3.20 b	67.93±0.09^{c}	697.99±12.20^{a}	129.83±8.10^{b}	19.42±3.80^{c}	1.47±0.00^{b}

In the column, means with different superscripts are significantly different ($p<0.05$)

unlike blood ACP that recorded elevated values at the last two concentrations (4.00 and 6.00 mgl^{-1}).

Many researchers have reported effects of xenobiotics on organism's enzymes [23 - 26]. According to Edsall [27], these biochemical changes or characteristics of blood or organs are among the most important indices used in assessing the status of the internal environment of a fish. Luskova et al. [28] added that changes in metabolites and biochemical processes of the organism resulting from the effect of various pollutants, makes it possible to study the mechanisms of the effect of these substances.

The elevated values of liver AST in this study have some trend with the reports of Inyang et al. [9], Aly and El-Gendy [15], Edori [29], Inyang and Williams [30]. This elevation of enzymes in the experimental group compared to control is a clear indication of the effect of 2,4 –D on the *Oryclotagus cuniculus.* According to Ambali et al. [31], the disruption of transaminases from the normal values denotes biochemical impairment and lesions of tissues and cellular function because they are involved in the detoxification process, metabolism and biosynthesis of energetic macromolecules for different essential functions. Desmet and Blust [32] exposed *Cryprinus carpio* to cadmium for 21 days and observed elevation of the activities of AST and ALT in the liver, kidney and gill and opined that the elevation is a result of proteolysis which is intended to increase the role of proteins in the energy production during cadmium stress. Kaur and Dhauju [33] reported a significant increase in the activities of AST, ALT and ALP in the liver of albino rats exposed to monoclotophos, methyl parathion and dimethoate given orally for 90 days, and inferred that such increase is an indication of cellular toxicity of these organophosphate causing a release of the enzymes into the blood. Additionally, Kumar et al. [34] suggested that such increase might have resulted from stepped-up transamination, where amino acids are used to generate intermediate for tricarboxylic acid cycle in an attempt to cope with the energy crises during stress cause by the toxicant.

Liver ACP values decreased significantly down the experimental group compared to the control. Inyang [23], Inyang et al. [9] reported decreased concentration of ACP when diazinon and aluminum phosphide were exposed to *Clarias gariepinus* and *Paraphiocephalus obscures* respectively. The overt diminutive values recorded are caused by the toxicant and is significantly a clear sign of inhibition. Inhibition of the effect of the toxicant on the ACP infers a breakdown in the kreb's cycle (TCA) intermediates, since transamination which provides the keto acid (α-ketogulatarate) is inactive and α-ketogulatarate is one of the intermediates in the TCA cycle [29]. According to Jawale [26], Sherekar and Kulkarni [35], ACP is hydrolytic lysosome of foreign materials and the enzyme can hydrolyze the phosphorus ester in acidic medium. The authors added that increase or decrease in the lysosome enzyme activity depends upon the concentration of the pesticide the probe organisms are exposed to.

The values observed in this study indicates that some complications arising from the effect of the

toxicant in the liver cells. Liver AST unveiled elevated values, while blood AST recorded decreased values denoting tissue damage and inhibition occurring concurrently in the tissues of the probe organism. Kalender et al. [36] reasoned that the susceptibility of liver tissues to the stress resulting from exposure to pesticides is a function of overall cellular balance between the degree of oxidative stress and the antioxidant capacity.

Electrolytes

Table 2 shows the effect of *Oryclotagus cuniculus* muscle electrolytes when exposed to 2,4-D (aminoforce) for 21 days. Sodium (Na^+) values in the muscles were significant ($p<0.05$), and progressed down the experimental group compared with the control and it was dose dependent. Potassium (K^+) values unlike sodium retrogresses down the experimental group in a dose dependent pattern. The least was recorded at the highest concentration (6.0 mgl^{-1}) i.e. 8.62 ± 0.06 mmol/l compared to the control that recorded 13.30±0.01 mmol/l. Chloride values were not significant ($p>0.05$) while Mg^{2+} showed a slight decrease down the experimental group (not in a dose dependent pattern).

Minerals are mainly responsible for the maintenance of osmotic pressure in the blood and proper functioning of all types of tissues [37]. Christensen and Tucker [38] opined that the basic function of electrolytes in the body lies in controlling fluid distribution, inter and extra cellular acid-basic equilibrium, maintaining osmotic pressure of body fluids and normal neuromuscular irritability. The elevated value of Na^+ in this study is caused by the toxicant. A slight shift in the values of electrolytes from normal may lead to serious physiological defect. Toxic metals and pesticides can alter the electrolytes in the blood, tissue and organs of organisms [39]. Ca^{2+} K^+ and Na^+ ions functionally participate in maintaining normal irritability of the heart, muscles and nerves as well as selective permeability of the cell membrane [40]. Therefore, significant elevation of Na^+ can lead to hyperfunction of the muscle tissues (also known as hypernatraemia). This progressive value recorded for sodium in the muscle fibres may lead to loss of weight occasioned by excessive dehydration overtly attributed to increased muscular activity and stress.

A significant decline in the values of K^+ unveiled 2,4-D effect on muscle tissue. Low levels of K^+ could lead to a condition known as hypokalemia. According to Weiner et al. [41], hypokalemia can exacerbate hepatic encephalopathy by increasing renal ammoniagensis and hence increasing systemic ammonia level. Additionally, Na^+ and K^+ are constantly diffusing through the plasma membrane, moving from regions of high concentration to regions of lower concentration. However, the concentration of Na^+ and K^+ on the two sides of the membrane remains constant due to the action of the sodium-potassium pump which is provided by ATP [42]. Retrogressive values as recorded in this study will surely affect membrane potential (electrolytes balance) and alter Na^+ and K^+ ion channel and also neuron communication in cells.

Stabilization of values were recorded in Cl^- while Mg^{2+} values showed a marked concentration (4.00 mgl^{-1} and 6.00 mgl^{-1}). These results were also obtained by

Table 2. Muscle electrolytes of *Oryclotagus cuniculus* exposed to 2,4-D (aminoforce) for 21 days (mean±SD)

Conc. Of 2,4 D (mgl^{-1})	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	Mg^{2+} (mmol/l)
0.00	1.95±0.00^b	13.30±0.21^a	26.62±0.82^a	0.85±0.00^a
2.00	2.70±0.01^b	11.95±0.12^b	26.20±0.03^a	0.82±0.00^a
4.00	4.25±0.10a^b	9.84±1.01^c	26.60±0.00^a	0.62±0.01^b
6.00	5.05±0.08^a	8.62±0.06^{cd}	26.20±0.07^a	0.6±0.09^b

In the column, means with different superscripts are significantly different ($p<0.05$)

Inyang et al. [43] when the authors exposed *Clarias gariepinus* (juveniles) to dichlorvos. Luskova et al. [28] also recorded similar results when the author exposed common carp to diazinon. The toxicant used in this study may be less toxic to Cl^- and Mg^{2+} ions due to the concentration of the toxicant used. The stabilization of some electrolyte according to Ogamba et al. [44] could be a stress induced response occasioned by the effect of animals exposed to toxicant which could activate certain physiological and metabolic mechanisms that may lead to a rapid uptake of electrolytes from water and food material or a reduction of ion influx.

Conclusion

The data obtained from this study allows us to conclude that aminoforce containing 2,4-dimethylamine salt elicits profound alteration in AST, ALT and ACP (both in liver and blood). Again the electrolytes precisely Na^+ and K^+ in the experimental group showed overt changes from the control, therefore exposure of *Oryclotagus cuniculus* to this toxicant will surely affect the organism's physiological state. Additionally, via the food chain man may be affected. The use of 2,4-dimethylamine salt close to rabbittory should be done with utmost caution.

References

1. USEPA (United States Environmental Protection Agency). (2008). Pesticide home page. http// www.epa.gov/0pp0001/
2. Hamilton D., Ambras, A., Dieterle, R., Felsot, A., Harris, C. Petersen, B., Rack, K., Wong, S.S., Gonzalez, R., Tanaka, K., Earl, M., Roberts, G., and Bhula, R. 2004. Pesticide residue in food-acute dietary exposure. *Pest Manag Sci.,* 60, 311–339
3. John, P.J., Bakore, N and Bhatnagar, P. (2001). Assessment of organochlorine pesticides residue levels in dairy milk and buffalo milk from Jaipur city, Rajasthan, India. *Environment International, 26* (4), 231-236.
4. Sandhu, T. S. (1980). Pesticide residues in foods, *Indian Dairyman*, 32**,** 61–63.
5. Raikwar, M.K. and Nag, S.K. (2003). Organochlorine pesticide residues in animal feeds, In: Proceedings of 40th Annual Convention of Chemists. *Indian Chemical Society,* (4) 127.
6. Material Safety Data Sheet (MSDS). (2018). Fact sheet of aminoforce containing 2,4-Dimethylamine salt SL.
7. Inyang, I. R., Izah, S. C., and Ntaka, C. M. (2018). Effect of imidacloprid on total protein, albumin and electrolytes in *Heterobranchus bidorsalis*. *Environmental analysis and Ecology Studies.,* 4(3), 1-4.
8. Inyang, I. R., Izah, S. C., and Okpogholor, K. D. (2019a). Impact of aluminum phosphide on the transferases in liver and muscle of *Parophiocephalus obscures*. *Journal of Plant and Animal Ecology, 1* (4),1-6.
9. Inyang, I. R., Izah, S. C., and Suobo, K. (2019b). Effect of phenol on the kidney and liver biochemical and metabolites of *Clarias gariepinus*. *Noble International Journal of Scientific Research, 3*(3), 33-40.
10. Bosveld, A. T. C., Gradener, J., Van Kampen, M., Murk, A. J, Evers, E. H.G. and Van den Berg, M. (1993). Occurrence and effects of PEBs, PCDDs, PCDFs in hatchlings of the common tern (*Sterna hirundo*). *Chemosphere 27,* 419-427.
11. Dakhakhni, T.H., Raouf, G.A., and Qusti, S.Y. (2016). Evaluation of the toxic effect of the herbicide 2,4-D on rat hepatocytes: an FT-IR Spectroscopic study. *Eur. Biophy. J. 45*(4), 311-320.
12. Amel, N., Wafa, T., Samia, D., Yousra, B., Issam, C., Cheraif, I., Attia, N., and Mohamed, H. (2016). Extra virgin olive oil modulates brain dacosahexaenoic acid level and oxidative damage cause by 2,4-dichlorophenoxyacetic acid in rat. *J. Food Sci Technol. 53,* 1454-1464.
13. Harada, Y., Tanaka, N., Ichikawa, M., Kamijo, Y., Sugiyama, E., Gonazalez, F.J., and Aoyama, T 2016. PPAR α-dependent cholesterol/testosterone disruption in Leydig cells mediates 2, 4, dichlorophenolacetic acid-induce testicular toxicity in mice. *Arch. Toxicol. 90,* 3061-3071.
14. Karen, M., and Froberg-Fejka, L. (2014). A review of the physiology and behaviour of the laboratory rabbit." Available: https:// www.laboratoryequipment.com/article/2014/05/

review-physiology-and-behavior-laboratory-rabbit.

15. Aly, N. and El-Gendy, R. 2014. Impact of parathion exposure on some biochemical parameters in rabbit as a non-targeted organism. *Alexandria Journal of medicine 51*, 11-17.
16. Ganguli, A., Choudhury, D., and Chakrabarti, G. (2014). 2,4-Dichlorophenoxyacetic acid induced toxicity in lung cells by disruption of the tubulin-microtubule network. *Toxicol. Res. 3,* 118-130.
17. Zahm, S.H., and Blair, A. (1992). Pesticides and non-Hodgkin's lymphoma. *Cancer Res. 52,* 5485 – 5488.
18. American Public Health Association (APHA) (1998). Standards methods for examination of water and waste water. Washington DC.
19. Malmstadt, H.V., Winefordner, J.D (1959). Determination of chloride in blood serum, plasma or other biologic fluids by a new rapid precision method. *Clinical Chemistry*, 5(4), 284–296
20. Macintyre, L., and Davidsson, D. 1958. The production of secondary potassium depletion, sodium retention, Nephrocalcinosis and Hypercalcaemia by Magnesium deficiency. *Biochem. J., 70*(3), 456-462.
21. Zimmerman, A. C. (1978). Biochemisty of phosphatase and transaminase in *Cyprinus carpio* exposed to carbontetrachloride. *Journal of Aquatic Science,* 23, 178-183.
22. Andersch, M. A., and Szcypinski, A. J. (1947). A colorimetric method for determination of acid phosphatase from serum. *Amer. J. Pathd. 17,* 571-574.
23. Inyang I.R 2008. Haematological and biochemical responses of *Clarias garipinus* to diazinon. Ph.D thesis, Rivers State University of Science & tech. Port Harcourt, Rivers State.
24. Ayalogu, O. E., Igboh, N. M., and Dede, E. (2001). Biochemical changes in th serum and liver of albino rats exposed to petroleum samples (Gasoline, Kerosene, and Crude petroleum). *Journal of Applied Science and Environmental Management, 5*(1), 97 -100
25. Modesto, K. A., and Martinez, C. B. R. (2010). Effect of roundup Transorb on fish: Haematology, antioxidant defenses and acetylcholinesterase activity. *Chemosphere, 81,* 781 -787.
26. Jawale, C. A. (2016). Consequence of organophosphate dimethoate pesticide on acid phosphatase activity in Gills of freshwater fish, *Catla catla. Flora and Fauna*. *22*(1), 89-92.
27. Edsall, C. C. (1999). A blood chemistry profile for lake trout. Journal of Aquatic Animal Health, 11, 81-86.
28. Luskova, V., Svobodova, M. and Kolarora, J. (2002). The effects of diazinon on blood biochemistry of carp (*Cyprinus carpio*) ACTA VET. BRON. 71: 117-123.
29. Edori, O.S. 2006. Effect of Nonoclotophos (an organophosphorus insecticide) on organosomatic indices and enzymes activity of hybrid catfish. Msc thesis, Rivers State university of Sci & Tech. Port Harcourt, River State.
30. Inyang I.R and Williams E.B. 2019. Biochemical and metabolic changes in new Zealand rabbit (*Oryclotagus cuniculus*) induced by chloropyrifos. *Sumerianz Journal of Biotechnology, 2*(8),70 -74.
31. Ambali, S., Akanbi, D., Igbokwe, N., Sittu, M, Kawu, M., and Ayo, J. (2007). Evaluation of sub-chronic chloropyrifos poisoning on haematological and serum biochemical changes in mice and protective effect of vitamin C. *Journal of Tropical Science, 32* (20), 11-20.
32. De Smet, H., and Blust, R. (2001). Stress responses and changes in protein metabolism in carp (*Cyprinus carpio*) during cadmium exposure. Ecotoxicol. Environ. Saf.48(3):255-262.
33. Kaur, S., and Dhanju, C. R. (2004). Enzymatic changes induced by some organophosphorus pesticides in female rats. Indian Journal of Experimental Biology. 42(10):1017-1019.
34. Kumar, S. V., Fareedullah, M. Sudhakar, Y., Venkateswarlu, B., and Kumar, E. A. (2010). Current review on organophosphorus poisoning. *Achieves of Applied Science Research, 2*(4), 199-215.
35. Sherekar, P. Y., and Kulkarni, K. M. (1987). Studies on the acid and alkaline phosphatase activity of

methyl parathion expose fish. *Channa orientalis. Uttar Pradesh Journal of Zoology 7*(2), 154-159.

36. Kalender, S., Uzun, F. G., Durak, D., Drmir, F., and Kalender, S. (2010). Malathion-induced hepatoxicity in rats: the effects of vitamin C and E. *Food and Chemical Toxicity, 48*(2), 633-638.
37. Johnson, D. W. (1968). Pesticides and Fishes—A Review of Selected Literature. Journal Transactions of the American Fisheries Society, 97 (4), 63 – 105.
38. Christensen, G. M., and Tucker, J. H. (1976). Effects of selected water toxicants on the in vitro activity of fish carbonic anhydrase. *Chem Biol Interact., 13 (*2),181-192.
39. Inyang, I. R., and Jenakumo, C. (2017). Changes in muscle and gastrointestinal tract electrolytes of *Parophiocephalus obscures* exposed to aluminum phosphide. *Bulletin of Trend in Biological Science, 1,* 23-26.
40. Inyang, I. R., and Patani, D. E. (2015). Haematological aberrations and electrolyte Stabilization in *Heterobranchus bidorsalis* induced by rhonasate 360SL containing glyphosate. *Nigerian Journal of Agriculture, Food and Environment*, *11*(3), 28-31.
41. Weiner, I. D., Mitch, W. E., and Sands, J. M. (2015). Urea and ammonia metabolism and the control of renal nitrogen excretion. *Clinical Journal of the American Society of Nephrology, 10*(8), 1444-1458.
42. Miller. S.A and Harley, J.P. 1994. Zoology (2nd) Edition. Wn.C. Brown publ. Oxford, England. 528-529.
43. Inyang, I. R., Ogamba, E. N., and Frank, V. E. (2013). Biochemical changes and electrolyt stabilization in *Clarias garipinus* (Juveniles) induced by dichlorvos. *International Journal of Biochemistry, 108,* 244-248.
44. Ogamba, E. N., Inyang, I. R., and AlforGod, S. S. 2011. Alteration in the levels of ions in muscle and liver of African catfish *Clarias gariepinus* exposed to paraquat dichloride. *Current Research Journal of Biological Sciences, 3*(6), 547-549.