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Electro-Metabolic Aberrations in New Zealand Rabbit (*Oryclotagus cuniculus*) Induce by Chloropyrifos

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Abstract

The aim of this study was to unveil the effect of Chloropyrifos on *Oryclotagus cuniculus* electrolytes and metabolites. Twenty-four of the probe organism (mean weight, 1.8 – 2.00kg) were acclimatized to laboratory conditions for 14 days and then exposed to varying concentrations of the toxicant (1.00mgl^{-1}, 2.00 mgl^{-1} and 3.00 mgl^{-1}) in a renewal bioassay technique for 14 days. Samples were collected and analyzed following standard protocol. Results of blood electrolytes (K^+ and Na^+) values were significant ($p< 0.05$), overt fluctuation and diminution of values were recorded. A clear stabilization of values characterized Na^+ in the muscle, while Ca^{2+} values showed a significant deviation from the control. Cortisol in the brain and liver concentration were significant compared to the control group akin to brain and liver triglycerides. Essentially, triglyceride values decreased down in the experimental group (in a dose-dependent pattern). This report unveiled the hazards of pesticide use and the imperative of minimizing pesticide use or discovering pesticides that are safe with minimal impact on organisms.

Key words: Chloropyrifos, *Oryclotagus cuniculus*, electrolytes, metabolites

Introduction

Toxic substances released into the environment always lead to physiological aberrations if organisms come in contact with it. Physiological aberrations and adverse health problems have been documented over the years due to the careless handling of poisons/toxicants in the environment. Residues of pesticides, polycyclic aromatic hydrocarbons (PAH), petroleum substances, and heavy metals are major pollutants in the Niger Delta ecosystem. Pesticides by their nature are toxic compounds, and besides controlling pest they adversely affect the receiving environment and its associated biota (Ahmed and Gautam, 2014). Humans and animals can be directly exposed to pesticides by inhalation, injection, and dermal processes (Caroline, 1991). In addition, higher animals including humans take in the toxicants indirectly through consumption of vegetables and animals that have bioaccumulated the toxicant. According to Al-Amoudi (2012), there is convincing evidence that toxicants such as pesticides play a role in the development of some human cancers.

Organophosphate and carbamates constitute two classes of pesticides widely used in the last decades on a variety of crops and indoor pests (Zafiro-Pouluds et al., 2014). According to Britt (2000), organophosphate compounds have become widely used pesticides as replacement for the more persistent organochlorine insecticides. Basically, organophosphate-based pesticides are generally more toxic to vertebrates than other classes of insecticides and are chemically unstable or non-persistent in the environment. It is this later feature that brought them into agricultural use (Narayanan, 2004).

Chloropyrifos remains one of the most popular organophosphate insecticides fully embraced by farmers and non-farmers in the Niger Delta states of Nigeria (Inyang and Williams, 2019). According to the author, it is also available in small bottles in south-south states of Nigeria with a trading name *Otapiapia*, used domestically for killing insects.

Negative consequences have been reported when organisms are exposed to chloropyrifos. According to Uzun et al. (2010) and Ncibi et al. (2008), as a lipophilic molecule, chloropyrifos easily passes through the cells into the cytoplasm of eukaryotic cells. Once inside the cell, chloropyrifos induces damage to the cellular molecules. Chloropyrifos affects the nervous system of animals the same way it affects the target pest. Signs and symptoms can appear within minutes to hours after exposure. These effects can last for days or even weeks causing an alteration in the nervous system which could eventually lead to difficulty in breathing and even paralysis (Christensen et al., 2009).

Pesticides have been reported to have negative ecological consequences on biota and abiotic environment (Baticadoes and Tendeceie, 1991). Reproductive failures in fishes and birds attributed to pesticides have been reported in the literature. For example, Inyang (2008), have reported the effect of diazinon (a well-known organophosphate insecticide) on fish biochemical and hematological parameters. Inyang and Williams (2019) have reported chloropyrifos effect on biochemical and metabolic parameters in the New Zealand rabbit (*Oryclotagus cuniculus*). The presence of these pesticides in the aquatic ecosystem, through

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the process of food chain can lead to bioaccumulation in fish and biomagnifications in humans (Chindah, 2000).

Xenobiotics such as chloropyrifos that can alter physiological functions in rabbits and also pose a serious metabolic problem in humans, since the general physiology of rabbit is close to that of humans. A shift in functions of electrolytes, cortisol, and triglycerides will surely lead to serious metabolic paralysis, hence the essence of this present study was to unveil the effect of Chloropyrifos on *Oryclotagus cuniculus.*

Materials and Methods

Source of experimental animals and acclimatization

Adult *Oryclotagus cuniculus* for this study was obtained from a private rabbit farm at Mbiama, Rivers State, Nigeria. They were moved to the rabbitry unit in the Department of Biological Sciences, Niger Delta University, Bayelsa State, Nigeria. Twenty-four (24) rabbits weighing 1.8 – 2.0kg were used for the study. Acclimation of the rabbit (one per compartment) lasted for 14 days. The probe organisms were fed with 200g of synthetic growers' mash (pelletized) daily. 1.5L of water was also placed in each compartment of the rabbitry.

General bioassay technique

A range-finding test was carried out to ascertain the exact concentration to be used for the definitive test, hence 1.00 mgl^{-1}, 2.00 mgl^{-1}, and 3.00 mgl^{-1} were used. Four rabbits were exposed to each concentration of the toxicant. A renewed bioassay technique was employed.

The definitive test was carried out based on the results of the trial test. The concentration was prepared by pipetting 7.5ml, 15ml, and 22.5ml from the original concentration of the toxicant and making it up to 1.5L borehole water in a metal container to make respectively 1.00 mgl^{-1}, 2.00 mgl^{-1}, and 3.00 mgl^{-1}. There were four treatment levels with four replicates. The experiment lasted for 14 days.

The probe organism was sacrificed after blood collection and target organs (liver, muscle and brain) were obtained for analysis of electrolytes and metabolites. To each sample of metabolic analysis, 5ml of perchloric acid was added while physiological saline and deionized water were used for the preservation and stabilization of electrolyte samples. All samples were centrifuged at the rate of 3000rpm for 15 minutes. The supernatant was stored at -2^{o}C using plain bottles.

Electrolyte samples were assayed via Logarwarny et al. (2006) and APHA (1998) methods, while metabolites (cortisol and triglycerides) were assayed via Hyams and Carey (1998) and Bucolo and David (1973) respectively.

Statistical Analysis

The data obtained were expressed as mean ± standard error. Analysis of variance (ANOVA) was used to show the significant difference, and where variations exist, Duncan Multiple range test (DMRT) was used to discern the source of the observed variation.

Results and Discussion

Blood electrolytes

Table 1 presents the activities of blood and muscle electrolytes of *Oryclotagus cuniculus* exposed to sublethal concentration of chloropyrifos for 14 days. Electrolytes value in the blood fluctuateی down in the experimental group while some stabilized i.e the values of the control and experimental groups were not significant ($p>0.05$). Sodium ions (Na^{+}) were not significant except at 3.00 mgl^{-1}. The last concentration of the toxicant recorded 160.3mmol/l compared to the control (114.89mmol/l). A slight retrogression in values was recorded in blood K^{+}. The first two concentrations (Table 1) recorded similar values while the last concentration almost stabilized compared to the control value. Ca^{2+} values were not significant ($p>0.05$).

Basically, electrolytes partake in various physiological processes in cells and tissues in terrestrial and aquatic organisms. Electrolytes facilitate the passage of fluid between and within cells through a process known as osmosis and play a role in regulating the functioning of the neuromuscular, endocrine, and excretory systems in fishes (Inyang et al., 2016). Sodium (Na^{+}) and Calcium (Ca^{2+}) values stabilized except at the last concentration (3.00mgl^{-1}). Similar results were also reported by Inyang (2008) and Luscova et al. (2001) when they exposed *Clarias gariepinus* and common carp to diazinon. A slight decrease in values of potassium (K^{+}) at the first two concentrations was observed while blood Ca^{2+} concentration was stable compared to the control. A shift from the value obtained in the control group indicates an overt sign of the effect of chlorpyrifos on the probe organism electrolytes. A decrease in values of K^{+}, Mg^{+} and Na^{+} was reported by Abdel-Ghany et al. (2016) when they exposed fenitrothion (a well-known organophosphate insecticide) to vital organs of rats. A shift in electrolytes may lead in some instances to muscle shivering, tremors, and paralysis, especially if accompanied by the miscellaneous polymer effects indicated by the decreases in electrolytes like sodium, potassium, calcium, phosphorus, and magnesium.

Muscle electrolytes

Apparent aberration of values was recorded in the muscle electrolytes (Na^{+} & Ca^{2+}). Sodium values were significant ($p<0.05$). Experimental group values showed a clear deviation from the control. The experimental group values increased as the concentration of the toxicant increases except at 3.00 mgl^{-1} (the last concentration), hence the values were not dose-dependent. Ca^{2+} values decrease down in the experimental group except at 1.00 mgl^{-1}.

Vertebrate muscle contraction relies much on the ability of Ca^{2+} to bind with a regulatory protein called troponin, thus action enables a cascade of processes to emanate which always leads to muscle contraction. Therefore, a shift in the concentration of Ca^{2+} (increase or decrease) will surely affect muscle function. According to Hall and Cameron (1981), chronic K^+ depletion in rabbits results in a loss of skeletal muscle K^+, with an associated rise in tissue Na^+. The present study witnessed a rise in muscle Na^+ while overt diminutive values were recorded in Ca^{2+} electrolytes. Miller and Harley (2004) opined that the nervous system controls Ca^{2+} levels in the skeletal tissue, thereby exerting control over contraction. Chloropyrifos may have affected the nervous system or a case of inhibition of Ca^{2+} in the skeletal muscle.

Table 1: Activities of blood and muscle electrolytes of *Oryclotagus cuniculus* exposed to sublethal concentration of chloropyrifos for 14 days

Blood			Muscle		
Conc. of chloropyrifos (mgl^{-1})	Na^+ (mmol/l)	K^+ (mmol/l)	Ca^{2+} (mmol/l)	Na+ (mmol/l)	Ca^{2+} (mmol/l)
0.00	114.89 ± 0.09^{ab}	11.03 ± 0.0^{a}	3.01 ± 0.00^{a}	38.97 ± 0.05^{b}	0.74 ± 0.00^{b}
1.00	110.06 ± 0.08^{ab}	$7.03\ \pm0.02^{b}$	3.01 ± 0.01^{a}	93.50 ± 0.10^{a}	0.91 ± 0.01^{a}
2.00	114.68 ± 0.05^{ab}	7.54 ± 0.02^{b}	3.03 ± 0.03^{a}	97.30 ± 0.30^{a}	0.54 ± 0.02^{c}
3.00	160.31 ± 0.13^{a}	10.0 ± 0.02^{a}	3.24 ± 0.01^{a}	89.56 ± 0.05^{a}	0.57 ± 0.03^{c}

Means with the same superscript within a column indicate no significant difference ($p<0.05$)

Metabolic parameters (Cortisol and Triglyceride)

Brain cortisol values were in a progressive pattern as the concentration of the toxicant increased except at 2.00 mgl^{-1} (Table 2). The highest value was recorded at 3.00 mgl^{-1} (77.97 ± 0.02 mg/dL) compared to the control that had 62.83 ± 0.10 mg/dL. Liver cortisol also recorded a progressive increase except at 1.00 mgl^{-1}. Brain and liver triglycerides values were significant ($p<0.05$). Diminutive values were obtained as the concentration of the toxicant increased. The experimental group values decreased in a dose-dependent pattern (Table 2).

Cortisol

The glucocorticoids such as cortisol help in regulating overall metabolism and concentration of blood sugar. They also function in defence responses to infection or tissue injury (Miller and Harley, 1994). Apparent deviation of cortisol concentration observed in this study could be a result of a disturbance in the physiological functions of various organs and tissues of the probe organism. According to Akio (2016), an increase in cortisol level in *Clarias lazera* exposed to dimethoate is attributed to low carbohydrate in the probe organism system, as a result, increased cortisol level to promote gluconeogenesis. Barton and Iwama (1991) reported that a low carbohydrate diet plus malathion in the fish system leads to the acceleration of cortisol levels. Taylor et al. (2005) also opined that cortisol promotes gluconeogenesis and liver glycogen formation. The authors also added that cortisol also raises blood glucose levels and partakes in protein metabolism. Apparent deviation of values from the cortisol in this present study is an indication of distortion of metabolic stability in the probe organism caused by the xenobiotics.

Triglyceride

Brain and liver triglycerides recorded clear retrogressive values in the experimental group. A similar result was also obtained by Akio (2016). Triglycerides are one of the commonest lipids and are further classified as fats or oils, according to whether they are solid (fats) or liquids (oils) at 20ºC (Taylor et al., 2005). In this study, a statistical decrease in triglycerides values in the brain and liver tissues may be a result of stress-induced by chloropyrifos which in order to compensate for the loss of carbohydrates, more lipid is released for physiological functions. Triglycerides are used to evaluate nutritional status and lipid metabolism in animals.

Table 2: Activities of cortisol and triglycerides in the brain and liver of *Oryclotagus cuniculus* exposed to sublethal concentration of chloropyrifos for 14 days

Cortisol (mg/dL)			Triglyceride (mg/dL)	
Conc. of chloropyrifos (mgl^{-1})	Brain	Liver	Brain	Liver
0.00	62.83 ± 0.10^{ab}	59.77 ± 0.04^{c}	0.89 ± 0.00^{a}	1.72 ± 0.00^{a}
1.00	72.40 ± 0.08^{a}	53.72 ± 0.01^{c}	0.79 ± 0.01^{ab}	1.64 ± 0.01^{ab}
2.00	69.06 ± 0.02^{ab}	61.97 ± 0.05^{b}	0.68 ± 0.01^{b}	1.51 ± 0.00^{ab}
3.00	77.97 ± 0.02^{a}	76.65 ± 1.00^{a}	0.35 ± 0.00^{c}	0.96 ± 0.02^{b}

Means with the same superscript within a column indicate no significant difference $p<0.05$)

Conclusion:

Our research unveiled serious metabolic aberrations in the probe organism. The probe organism physiology is close to that of humans, hence care should be taken on indiscriminate use of chloropyrifos close to *Oryclotagus cuniculus*. These metabolic parameters could serve as a biomarker of chloropyrifos effect in the New Zealand rabbit. Ultimately, these findings overtly indicated the hazards of pesticide use and the importance of minimizing pesticide use close to domesticated organisms.

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