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SUBLETHAL IMPACT OF CHLOPYRIFOS EC 20% PESTICIDE ON METABOLITES AND ELECTROLYTES IN THE BRAIN AND INTESTINE OF NEW ZEALAND RABBITS (*ORYCLOTAGUS CUNICULUS*)

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ABSTRACT

The toxicity of pesticides to biodiversity have become a global phenomenon. Eighteen (18) adult male New Zealand Rabbits of 2.0±0.2kg were utilized in the bioassay. The pesticide (Immaculate Executioner containing Chlorpyriphos 20% EC) was exposed to three treatment groups (1ppm, 2ppm and 3ppm) and a control for 14 days. Compared to the control (at 0.00 ppm), results showed decline in metabolites like; Glyceride (TG) (0.89 to 0.55 µl), Total Creatinine (TC) (2.83 to 1.05 µl), Total Protein (6.93 to 4.81 µl), excepts for Total Cortisol level that appreciated from 62.83 to 77.97 µl. Also, significant decline in intestinal electrolytes was demonstrated for sodium (from 93.94 to 64.02 mmol/l), while Potassium (12.39 to 25.35 mmol/l) and Calcium (0.44 to 0.51 mmol/l) levels appreciated significantly. The aftereffect of this examination affirms the toxic effects of Chlorpyrifos on the brain and intestine of exposed animals and by extension, other contact animals in the food chain.

Key words: Rabbit, Pesticide, Chlorpyrifos, Brain, Intestine, Metabolites, Electrolytes.

1. INTRODUCTION

Pesticides are toxic chemical substance or a mixture of substances or biological agents that are intentionally released into the environment in order to avert, deter, control and/or kill and destroy populations of insects, weeds, rodents, fungi or other harmful pests [1]. The use of pesticide in modern agriculture has not only increased productivity, but caused adverse effects on the ecosystem when used excessively [2]. Pesticides work by attracting, seducing and then destroying or mitigating the pests. According to an estimate, about 5.2 billion pounds of pesticides are used worldwide per year [1].

Chlorpyriphos can be harmful if it is touched, inhaled, or ingested. Chlorpyriphos works by blocking an enzyme which controls messages that travel between nerve cells. When the enzyme is blocked, the nervous system can't send normal signals. This causes the nervous system to malfunction and this is how it eventually kills the pest [3]. The mechanism of action (toxicodynamics) of CPY involves activation by biotic transformation to CPYO, followed by covalent binding to the serine-hydroxyl in the active site of the acetyl cholinesterase molecule [4]. While this can occur in the environment [5], in animals this reaction is catalyzed by multifunction oxidase enzymes (MFO) and is important in the mode of action of CPY.

The key physical and chemical properties of Chlorpyrifos has short to moderate persistence in the environment as a result of several dissipation pathways that might occur concurrently. Primary mechanisms of dissipation include volatilization, photolysis, abiotic hydrolysis, and microbial degradation. Volatilization dominates dissipation from foliage in the initial 12 h after application, but decreases as the formulation adsorbs to foliage or soil [4]. Chlorpyrifos is very toxic to many bird species such as grackles and pigeons, and it is moderately toxic to others such as mallard ducks. Mallard ducks fed Chlorpyrifos laid fewer eggs and raised fewer ducklings [3].

The use of animals in eco-toxicological studies for the determination of lethal effects of industrial and anthropogenic chemicals to ascertain biomedical aberrations in living organisms has been recommended for perfecting and validating existing procedures [6, 7].

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New Zealand rabbits (*Oryctolagus cuniculus*) are frequently used in a variety of experiments, including orthopedics [6], and craniomaxillofacial surgery [2]. However, information on their handling and surgical procedures is sparse and limited. Chlorpyriphos is an organophosphate insecticide. Pure Chlorpyriphos is made up of white or colorless crystals [2]. Recent and past studies have revealed the toxicological profile and effects of the toxicant (Chlorpyriphos) and pesticides on both biotic and Abiotic components of the environment. But, based on the limited literature available on specific organ toxicological effect of in Rabbits, this research tends to focus on the toxicity of Chlorpyriphos on animal brain function through the analysis of biochemical aberrations caused by the toxicant in the brain of Rabbits.

2. MATERIALS AND METHOD

2.1 Sampling and Analysis

Healthy adult New Zealand Rabbits obtained from Kester Rabbit farm were used in the present study. They were transported individually in plastic baskets to a private rabbitoty for acclimatization. The rabbits were exposed to Chlorpyriphos 20% EC, for fourteen (14) days. A renewal bioassay was carried out throughout the experimental period of 14 days. The actual concentrations of toxicant (Chlorpyriphos 20% EC) to be used for the main experiment was determined through a range finding test. Also, the rabbits were also separated into compartments and cleaned at 24 hours intervals to maintain good hygiene during the experimental period.

During the main experiment, oral method was used to administer the toxicant into experimental animals, where three (1ppm, 2ppm and 3ppm) concentrations of toxicant was orally served to experimental Rabbits using a syringe on daily basis. The rabbits where handled with care and gloves were always worn when handling rabbits. They were always grasped by the skin in the dorso-cervical region, a fold of muscles at the dorsum of the upper part of the neck.

Picking rabbits up by the ears was deliberately avoided during the experiment as there is a high probability of causing cervical luxation and death. At the end of the 14 days exposure, the rabbits were killed and organs removed by surgical procedures to collect part of the target organ (Brain) and homogenized in a ceramic mortar with 0.5ml of perchloric acid. The mixture was centrifuged and supernatant transported to a chemical laboratory for analysis to determine the effect of Chlorpyrifos on metabolic parameters such as Total Triglyceride (TG), Total Creatinine (TC), Total Protein (TP) and Cortisol in the Brain of exposed Rabbits as well as electrolytes like Sodium ion (Na^+), Potassium ion (K^+) and Calcium ions (Ca^{2+}).

STATISTICAL ANALYSIS

The data gotten where analyze utilizing one-way analysis of variance (ANOVA) to check for significance level of different experimental parameters (biochemical, metabolic and electrolyte) assessed All analyses were conducted by means of the Statistical Package for Social Sciences (SPSS) version 23 software.

RESULTS AND DISCUSSIONS

Results on the toxicological effect of Chlorpyrifos on metabolites of the Brain of New Zealand Rabbit was reported for parameters such as; Total Glyceride, Total Creatinine, Total Protein and Cortisol (Table 1). Results on the effects of Chlorpyrifos on electrolytes like Calcium (Ca^{2+}), Potassium (K^+) and Sodium (Na^+) were presented (Table 2).

Tables 1: Effect of Chlorpyrifos on metabolites in the Brain of New Zealand Rabbit

Conc. of Chlorpyrifos(ppm)	T.G(µ/l)	TC(µ/l)	TP (µ/l)	Cortisol (µ/l)
0.00	0.89	2.83	6.93	62.83
0.01	0.79	1.58	5.24	72.40
0.02	0.80	1.76	13.31	69.06
0.03	0.55	1.05	4.81	77.97

Key: TG: Total Glyceride, TC: Total Creatinine, TP: Total protein: and C: Cortisol.

Table 2: Impact of Chlorpyrifos on Calcium (Ca^{2+}), Potassium (K^+) and Sodium (Na^+) in the Intestine of New Zealand Rabbits

Conc. of Chlorpyrifos (ppm)	Ca^{2+} (mmol/l)	K^+ (mmol/l)	Na^+ (mmol/l)
0.00	0.44	12.39	93.94
1.00	0.44	18.03	74.48
2.00	0.87	16.41	449.97
3.00	0.51	25.35	64.02

Key: Ca^{2+}: Calcium ion, K^+: Potassium ion, and Na^{2+}: Sodium ion

From the result obtained, the recorded values of total Glyceride, total Creatinine and total Protein significant declined from 0.79 µ/l to 0.55 µ/l as compared to control of 0.89 µ/l, 1.58 µ/l to 1.05 µ/l as compared to control of 2.83 µ/l, and 5.24 µ/l to 4.81 µ/l as compared to the control value of 6.93 µ/l, where as that of Cortisol appreciated through 72.40 µ/l to 77.97 µ/l as compared to 62.83 µ/l of the control respectively (Table 1). This result is a clear indication of the toxic effect of the toxicant (Chlorpyrifos) on metabolites in the brain of exposed animals. The declining values of metabolites recorded in this study is indicative of alterations in the processes of glycogen metabolism in the body which have very important implications for the proper functioning of the brain, especially the cooperation between astrocytes and neurons.

The recorded decrease in brain metabolites of exposed animals is also a pointer to the effect of glycogen deficiency which is a possible cause of hypoglycemia. Also, neuronal metabolic processes in the brain depends on activities of astrocytes, which produces lactate and activate glycolysis and glycogen metabolism on the account of metabolite deficiency to protect the brain from neuronal damage. Thus, the result of the current study reveals the toxic effect of the test chemical on brain function in exposed animals.

On the other hand, the increase in the level of Cortisol from 62.83 to 77.97 as shown in table 1 above could be a

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possible indication of stress induced by the introduction of the toxicant which is one of the major factors of release of Cortisol in the body. It also reveals an increased metabolic or muscular activity and consequent damage to brain neurotransmitters and other neural effects caused by the introduction of the toxicant under prolonged exposure in animals.

More so in animals, metabolic imbalance and disturbances could lead to neuropsychiatric disorders and consequent brain death. Thus, the significant decline in metabolic parameters in the brain of exposure rabbits is an indication of the toxic effect of Chlorpyrifos to brain metabolites thereby altering the normal brain neurotransmission. This could lead to effects such as insulin resistance, diabetes in the case of humans, neuro-degeneration and other known neuropsychiatric disorders. It is therefore confirmed that Chlorpyrifos is toxic to the brain of exposure Rabbits (New Zealand Rabbits).

From Table 2 above, the values of sodium (Na^+) in small intestine declined from 93.94 at 0.00ppm to 64.02 at 3.00ppm. This decrease was dependent on increasing concentration of toxicant (Chlorpyrifos), thus dose dependent. That of Potassium (K^+) increased from 12.39 to 25.35 as compared to the control while Calcium (Ca^{2+}) slightly increased from 0.44 to 0.51. These observed alterations of electrolytes in the organs of exposed animals as compared to the control are similar to that reported by Ogamba *et al.,* [8], when 2, 4–Dichlorophenoxyacetic acid was exposed to C*larias gariepinus* and recorded electrolytes aberrations in the blood, liver and muscles of exposed fishes.

The values of sodium Na^+, calcium Ca^{2+} and potassium ions (k^+) in the sampled organ (Intestine) of the exposed animal (New Zealand Rabbit) as observed in table 1 above varies considerably with the control values at various concentrations. This probably indicates Critical imbalance of body electrolyte which is capable of causing severe osmotic instabilities that can lead to loss of water, which in turn leads to haemo-concentration and circulatory collapse, all due to the toxicant effect (Chlorpyrifos) to exposed rabbits.

Sodium and potassium are essential for the activity of many enzymes and have been implicated in the transport of ATP which participates in several metabolic processes. Na+ and K+ ATpase, are located in the cell membrane and have been found to be involved in the active transport of Na+ and K+ across the cell membrane. Thus, the variations observed in the values of sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) in the small intestine as compared to the control in table 1 of this study at various concentrations of toxicant (Chlorpyrifos) could indicate possible damage to the functions of osmotic and ionic balance in exposed Rabbits. Inyang *et. al* [9], also reported similar case of electrolyte aberration in African catfish exposed to sublethal concentrations of Diazinone. The irregularity in the values of electrolytes was again reported by Ogamba *et al*., [9] in 2011 when fishes were exposed to similar concentrations of the toxicant.

Normal colonic mucus contained high concentrations of sodium as opposed to the findings of the current study and this simply imply that low sodium absorption could influence reduced water and electrolyte conservation in the body of exposed animal colon. Also, the observed increase in the values of other electrolytes such as Calcium and Potassium from 0.44 to 0.51 and 12.39 to 25.35 measured in this experiment above the control values is an indication of electrolyte transport impairment caused by toxicant effect of toxicant (Chlorpyrifos), excessive mucus excretion, and changes in potential difference of exposed animals which is capable of causing fecal electrolyte losses in diarrhoea.

The ultration of nerve impulse by Chlopyrifos pesticide observed in our study is consistent with the study of Cox [10]. According to Cox [10], this effect can also increase gill permeability to ions or lateral line imbalance in fishes and hormonal disorder by affected endocrine organs through toxicant attack. These distortions in the level of electrolytes such as sodium, potassium and calcium observed in the Small Intestine of the exposed animals (New Zealand Rabbits) could emanate to some clinical conditions such as hypernatraemia, hypokalaemia, hyponatraemia and hyperkaelamia etc.

CONCLUSION

This study demonstrates that the inclusion of sublethal concentrations of Chlorpyrifos up to 1ppm, 2ppm and 3ppm in drinking water may have induced metabolic effect in the brain of exposed animals which have the potential to impact or alter biochemical and nervous system stability of animals. It is therefore imperative that further research be carried out to ascertain the effect of Chlorpyrifos on animals in order to improve the information available on Chlorpyrifos toxicological profile. By doing so, adequate measures should also be put in place to control and regulate the incessant use of organophosphate pesticides such as Chlopyrifos on the environment in order to protect existing biodiversity and endangered species on our environment.

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