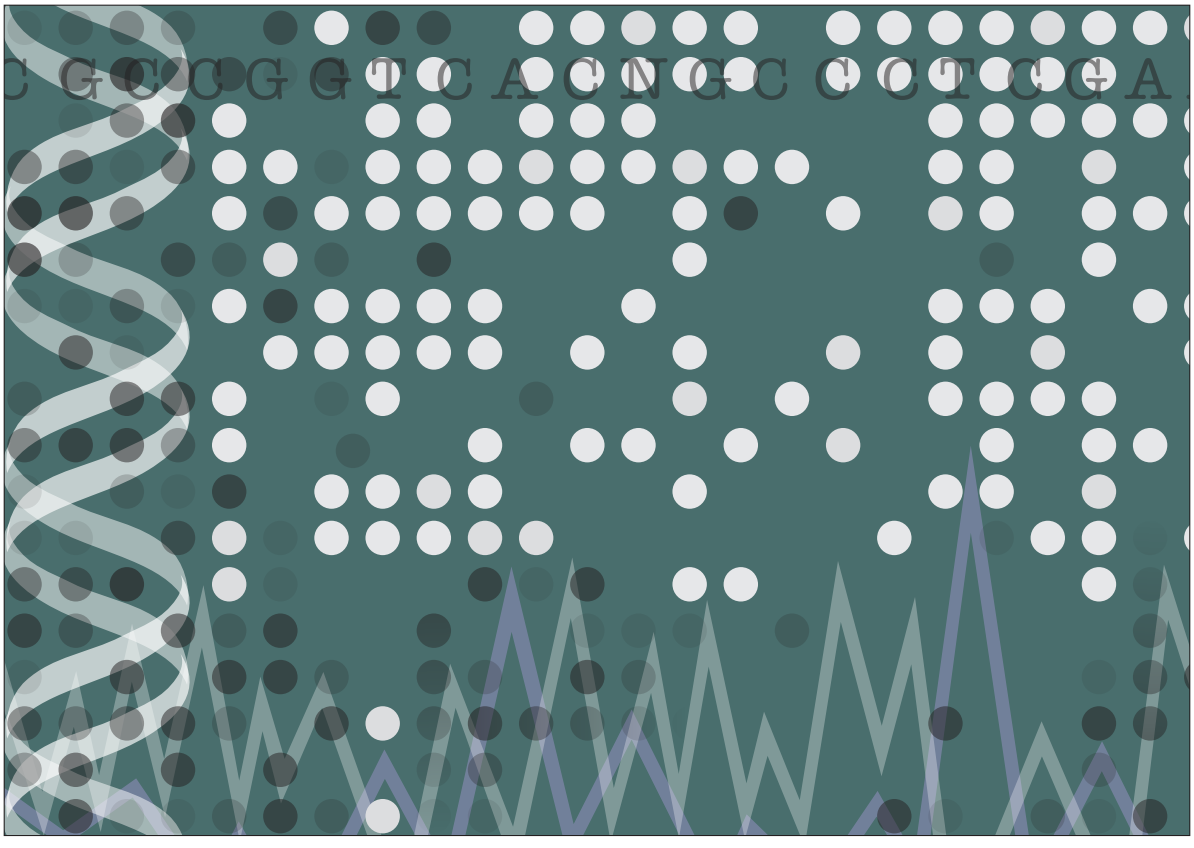

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Genotoxic Effect of Chlorpyrifos and Cypermethrin in Albino Rats

L.E. Okonko, E.V. Ikpeme and O.U. Udensi
Department of Genetics and Biotechnology, University of Calabar, PMB 1115, Calabar, Nigeria

Corresponding Author: L.E. Okonko, Department of Genetics and Biotechnology, University of Calabar, PMB 1115, Calabar, Nigeria

ABSTRACT

This study was designed to evaluate genotoxic effect of chlorpyrifos and cypermethrin, singly and in combination, in albino rats. Thirty-six albino rats were randomly divided into three groups (A, B and C) of four rats per treatment (chlorpyrifos, cypermethrin and chlorpyrifos+ cypermethrin). Group A was the control and received distilled water and feed only. Groups B and C received 5 and 10 mg kg^{-1} b.wt. of each treatment, respectively. Treatment was administered via oral gavage and lasted for a period of 65 days. The 24 h after administering the last dosage, rats were sacrificed and bone marrow samples were collected for evaluation. The result revealed that pesticide treatment reduced polychromatic erythrocytes and the ratio of polychromatic erythrocytes to normochromatic erythrocytes significantly, while normochromatic erythrocytes and micro-nucleated polychromatic erythrocytes increased significantly ($p<0.05$) compared with the control. However, the treatment had no significant ($p>0.05$) effect on micro-nucleated normochromatic erythrocytes. These findings therefore indicate that chlorpyrifos and cypermethrin, singly and especially in combination, could be mutagenic.

Key words: Chlorpyrifos, cypermethrin, genotoxicity, bone marrow, albino rats

INTRODUCTION

The use of pesticides has substantially improved the social and economic well being of inhabitants of developing countries through significant increase in food production and the effective mitigation of vector borne diseases. At the same time, however, public concerns over the indiscriminate application of pesticides and their possible adverse effect on animal and human health, as well as the environment has risen remarkably. Worldwide application of these chemicals is increasing continually as they have become a major part of the ecosystem. About 5.6 billion pounds of active pesticide ingredients are used yearly throughout the world (USEPA., 2001). Post market surveillance has shown that about 25 million agricultural workers globally experience unintentional pesticide poisoning annually (Alavanja *et al*., 2004).

It has been reported that pesticides available in the market may cause cancer in humans (Bonner *et al*., 2005). Zahm and Ward (1998), showed that cancers such as leukemia, neuroblastoma, sarcoma, lymphoma and cancer of the brain and testes are linked to pesticide exposure. These chemicals may also alter the genetic materials of organisms which may in turn affect them and their off spring (Manjula *et al*., 2006). Pesticides are reactive compounds that tend to form covalent bonds with various nucleophilic centres of cellular biomolecules, including DNA (Simoniello *et al*., 2008; Poletta *et al*., 2009). Because of their biological activity, the indiscriminate

se of these chemicals may cause undesired effects in humans. For instance, the induction of DNA damage can potentially lead to adverse reproductive conditions, cancer and many other chronic diseases (Meinert *et al*., 2000).

Since, mutation occurs spontaneously or under the influence of certain external factors like broad spectrum chemicals including pesticides, genotoxicity testing becomes necessary to assess the mutagenicity of chemicals in order to protect the human gene pool and identify potential carcinogens. To assess the genetic damage induced by physical and chemical agents including pesticides, various test systems have been described in bacteria, in mammalian cells *in vitro* and in plants (Celik *et al*., 2005). Arguably, the most reliable genotoxicity evaluation for human health risk is conducted in mammals by inducing chromosomal aberrations and micronucleus. In view of this premise, this present study was designed to assess the genotoxic effect of chlorpyrifos and cypermethrin, singly and in combination in bone marrow cells of male albino rats.

MATERIALS AND METHODS

Experimental site/materials: This experiment was carried-out in the year 2014 in the Department of Animal house of Genetics and Biotechnology University of Calabar, Calabar, Nigeria.

Chlorpyrifos and cypermethrin were purchased from Department of Agrochemicals Ministry of Agriculture, Calabar, Nigeria.

Experimental animals/procedure: Thirty six sexually mature male albino rats of body weight ranging from 170-200 g were purchased from the Department of Physiology, University of Calabar, Nigeria. They were kept in aluminum cages covered with wire mesh. They were fed with growers mash and allowed unrestricted access to clean water. The rats were allowed to acclimatize for one week. They were handled in accordance with the guidelines for care and use of laboratory animals as modified by the Department of Animal Genetics research committee.

The treatment was laid out in a 3×3 factorial experimental format using Completely Randomized Design (CRD). Group A serve as the control and received only distilled water. Groups B and C were administered 5 and 10 mg kg^{-1} b.wt. of each treatment, respectively. Treatment was administered through oral gavage and lasted for a period of 65 days. Animals were sacrificed under chloroform anesthesia 24 h after the last dose was administered.

Bone marrow micronucleus assay: This method evaluates the genotoxicity of any mutagen. It has been accepted as a standard and a reliable *in vivo* assay to screen chemicals for their genotoxicity (Hodge and Sterner, 1956). The method described by Schmid (1976) was modified slightly and used to analyze micronuclei (MN) in polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) of rat bone marrow.

The animals were sacrificed, lower abdomen and limbs were incised and the femora were cleaned and separated from the hip joint. The ends of the femur were trimmed and a blunt needle was pushed to pierce the marrow cavity. Bone marrow was flushed into a tube containing 0.9% saline. Then, the suspension was made up to 5 mL and centrifuged at 1500 rpm for 10 min. The clear supernatant was discarded, while 2-3 drops of fetal calf serum were added to the pellet and mixed thoroughly. Smears were made on sterile coded slides using a drop of the suspension. The slides were air dried, fixed in absolute methanol and stained using May-Grunwald Giemsa method (D'Souza *et al*., 2005). Micronuclei were identified as dark blue staining bodies in the cytoplasm of

polychromatic and normochromatic erythrocytes. Two thousand PCEs and NCEs each were scored per animal for the presence of MN using light microscope. The frequency of micro-nucleated cells was expressed in percentage (Marzouk *et al*., 2012).

Statistical analysis: All data obtained were subjected to analysis of variance (ANOVA) using PASW Version 18.0. Least Significant Difference (LSD) was used to separate the means that were significant at $p<0.05$.

RESULT

Pesticide treatment had significant effect ($p<0.05$) on polychromatic erythrocytes (PCE), Normochromatic Erythrocytes (NCE), micro-nucleated polychromatic erythrocytes (MPCE) and the ratio of polychromatic erythrocytes to normochromatic erythrocytes (P/N ratio). But no significant effect ($p>0.05$) was observed for micro-nucleated normochromatic erythrocyte (MNCE).

The different pesticide treatment reduced PCE significantly. The least numerical value for PCE (38.75%) was observed in rats administered 10 mg kg^{-1} b.wt. of chlorpyrifos+cypermethrin, compared with rats in the control group (50.25%) which was significantly higher. Meanwhile, pesticide treatment increased NCE significantly, though the mean value observed in rats of the control group was not significantly different from those recorded in treated rats. A similar trend was noticed wherein pesticide treatment increased MPCE in rats treated with pesticide. Rats in the control group had 1.5% as mean value of MPCE, whereas, the highest mean value (5.0%) was recorded in rats administered 10 mg kg^{-1} b.wt. of chlorpyrifos+cypermethrin (Table 1).

However, pesticide treatment reduced P/N ratio significantly. Rats in the control group had the highest P/N ratio (1.12) compared with those treated with pesticide. It was also observed that rats administered 10 mg kg^{-1} b.wt. of chlorpyrifos+cypermethrin had the lowest P/N ratio (0.66), although this was not significantly different from values in other groups except those in the control group (Table 1).

DISCUSSION

Application of pesticides is often regarded as an easy, cheap and quick means of eradicating pests. The use of these chemicals has scaled up production of crops as well as the control of vectors and diseases. Consequently, indiscriminate application of these chemicals has given rise to serious health and environmental concerns.

Pesticides have been implicated to alter the genetic materials of organisms they come in contact with, which could in turn affect them and their off spring (Manjula *et al*., 2006). Mutation occurs spontaneously or under the influence of certain physical or chemical agents such as pesticides, therefore, genotoxicity testing becomes necessary to assess their mutagenicity, identify potential carcinogens and protect the human gene pool. Micronuclei assay is an important tool used to

Table 1: Micronuclei formation and P/N ratio of control and pesticide treated rats

	Chlorpyrifos			Cypermethrin			Chlorpyrifos+cypermethrin		
Parameters	A	B	C	A	B	C	A	B	C
PCE (%)	50.25±0.75^{b}	45.00±2.08^{a}	39.00±1.73^{a}	50.25±0.75^{b}	42.25±1.79^{a}	39.25±0.48^{a}	50.25±0.75^{b}	41.75±1.10^{a}	38.75±1.75^{a}
NCE (%)	45.00±2.04^{a}	47.25±1.25^{a}	52.75±2.49^{a}	45.00±2.04^{a}	51.25±2.56^{a}	58.75±2.72^{a}	45.00±2.04^{a}	48.50±1.44^{a}	59.50±1.66^{a}
MPCE (%)	1.500±0.29^{a}	2.500±0.65^{a}	4.250±0.63^{a}	1.500±0.29^{a}	2.000±0.41^{a}	4.250±0.85^{a}	1.500±0.29^{a}	2.750±0.48^{a}	5.000±1.08^{a}
MNCE (%)	0.250±0.025^{a}	0.500±0.50^{a}	0.750±0.48^{a}	0.250±0.25^{a}	0.500±0.29^{a}	0.750±0.25^{a}	0.250±0.25^{a}	0.750±0.48^{a}	0.750±0.47^{a}
P/N ratio	1.120±0.66^{b}	0.950±0.04^{a}	0.740±0.06^{a}	1.120±0.66^{a}	0.830±0.07^{a}	0.660±0.03^{a}	1.120±0.66^{b}	0.850±0.04^{a}	0.640±0.02^{a}

Values are presented as Mean±SEM. Means followed by the same case letter along the horizontal array indicate no significant difference ($p>0.05$). A: control (0 mg kg^{-1} b.wt.), B: 5 mg kg^{-1} b.wt. of pesticide, C: 10 mg kg^{-1} b.wt. of pesticide

represent the consequence of chromosomal aberrations induced during the preceding mitotic divisions in erythroblasts (Matter and Grauwiler, 1975). Exposure to genotoxic substances damages chromosomes or components of the mitotic spindle leading to the formation of micronuclei. Thus, micronuclei may be formed from chromosomal materials fragmented during mitosis and not incorporated in the daughter nuclei at completion of telophase (Asare *et al*., 2012). In the present study, the result obtained revealed genotoxic effect of chlorpyrifos and cypermethrin in albino rats. Result for micronucleus assay showed that pesticide treatment had significant ($p<0.05$) effect on PCE, NCE, MPCE and P/N ratio but had no significant ($p>0.05$) effect on MNCE. It was further observed that pesticide treatment reduced PCE and P/N ratio significantly and increased NCE and MPCE compared with the control. The frequency of PCE in bone marrow cells provides an index of mitotic activity (Jayashree *et al*., 1994). Decline in PCE could be due to the suppression of proliferative activity of the bone marrow or suppression of erythrocyte production (Manjula *et al*., 2006). This reduction also suggests cell death. The formation of micronuclei demonstrates that a considerable amount of genetic information is no longer available to the cell and thus results in cell death (Jayashree *et al*., 1994). Increase in frequency of micro-nucleated cells reflects the damage caused by agents that induce chromosome breakage and non-disjunction. Since, these effects have been correlated with tumor initiation, the micronucleus test is appropriate for screening potentially genotoxic environmental agents (Jayashree *et al*., 1994). Meanwhile, reduction in P/N ratio due to pesticide treatment indicates that they have cytotoxic effect (Manjula *et al*., 2006). The result of this study is in agreement with the findings of Manjula *et al*. (2006) who reported that endosulfan increased MPCE and reduced PCE and P/N ratio significantly at 9 and 12 mg kg^{-1} b.wt. Prasad *et al*. (2009) also reported that intraperitoneal administration of glyphosate at 25 and 50 mg kg^{-1} b.wt., increased micronuclei induction significantly in rats. Furthermore, Marzouk *et al*. (2012) demonstrated that repeated administration of tribenuron-methyl increased the frequency of micronuclei formation in bone marrow of rats. Celik *et al*. (2005) also demonstrated that lambda-cyhalothrin induced a significant dose-related increase in micronuclei formation in bone marrow of Wistar rats at concentrations of 3.06 and 6.12 mg kg^{-1} b.wt. Similarly, Kocaman and Topaktas (2009) reported that alpha-cypermethrin at 5 and 10 μg mL^{-1} induced micronuclei formation in human peripheral blood lymphocytes, thus suggesting that the insecticide has genotoxic potential. On the contrary, Saleh *et al*. (2010) reported that thiocyclam did not cause any significant increase in MPCE of rat bone marrow cells. In the same vein, Alshehri (2014) reported that there was no significant increase in the frequency of micronuclei formation in bone marrow cells of rats exposed to methidathion.

The findings of this study therefore indicate that chlorpyrifos and cypermethrin, singly and in combination, have genotoxic potential in albino rats and thus could be mutagenic.

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