Clinico-pathological effects of single oral dose of cypermethrin in guinea pigs

AO Omonona¹*, TA Jarikre² & AT Adetuga¹

¹. Department of Wildlife & Ecotourism Management, University of Ibadan
². Department of Veterinary Pathology, University of Ibadan

*Correspondence: Tel.: +2348037258481, E-mail: ao.omonona@gmail.com

Abstract
The effect of environmental intoxication and single oral ingestion of cypermethrin was studied in the guinea pig through exposure to sublethal concentrations of the pesticide. The clinical, haematologic, and biochemical changes were monitored within 72 hours of exposure. Haemocytometric method was used to evaluate the erythrogram and leucogram changes, and microscopy for histopathology. There were significant increases in the eosinophil count, ALP, creatinine, cholesterol, globulin and albumin and decrease in urea levels and thrombocytopenia (P>0.05). There were diffuse moderate vascular congestion and mononuclear cellular infiltrates in the interstitium of the lungs, and degeneration in the liver and kidney. Single oral ingestion of cypermethrin altered biochemical parameters commensurate with tissue changes. Cypermethrin exposure and intoxication of animals in the environment may contribute to morbidity and ecotoxicity.

Keywords: Cavia, Cypermethrin, Environment, Toxicity, wildlife

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Introduction
The demand for pesticide products and their contributions towards agricultural yield and efficiency are clearly remarkable. Besides being beneficial, their use has also resulted in the manifestation of health related problems in especially contact personnel, farm animals and wildlife (Bhunya & Pati, 1990; Raj et al., 2013). About 90% of pesticides applied to farmlands never reach the intended targets and, as a result, many other organisms especially wildlife sharing the same environment or habitat as pests may be accidentally poisoned (Sparling et al., 2001). This could result in ill health of wildlife which could be aggravated by stress or any other environmental toxicities and opportunistic infections (Akegbejo, 1996; Omonona & Kayode, 2011).

Pesticide abuse leads to detection of residues in fish and other wildlife due to diffusion from site of application and with risk to wildlife species (Casida et al., 1983). Repeated use of herbicides also caused changes in plant communities and survival of undesirable species. The contamination of water bodies results from leaching, runoff, drift from spray application, or contamination of wells at different tolerable levels to the ecology (Khan & Law, 2005). These observations warrant a study to investigate effect of single oral toxicity of cypermethrin at agricultural formulations with traceable environmental contamination to wildlife. Acute pesticide poisoning to wildlife may take place over a relatively short time. The toxic oral dose of cypermethrin in mammals is greater than 100-1000mg/kg and the potential acute oral dose is between 1-10g (Oslen, 1994).

Ethical use of animals, like guinea pig (Cavia porcellus), had thrown light into sufferings of man and animals in general (D’Erchia et al., 1996; Nowak, 1999). Human or animal exposure to pesticides could result from ingestion, occupational hazard or environmental contact, which on some occasions
lead to endocrine disruption in the host (White et al., 1994; Khan & Law 2005). Casida et al. (1983), white et al. (1994), Khan et al. (2003), Shah et al. (2007), Ezemoneye & Tango (2010) and Omonona & Emikpe (2011) studied aspects of pyrethrin to demonstrate their metabolism, pharmacological characteristics, and toxicity. Haratym-Maj (2002), Manna et al. (2004) and Shah et al. (2007) reported toxicity of cypermethrin through assay of oxidative enzymes in mice, rats and rabbits respectively. There is need to know the effect of single oral ingestion with any ensuing haematological and histological changes of agricultural formulation of cypermethrin to wildlife. This investigation is set to demonstrate the clinico-pathological effect of cypermethrin in non-target species using guinea pig.

Materials and Methods
Study design and animals
Twelve guinea pigs (Cavia porcellus) were bought from a local market in southwestern Nigeria. The animals were screened for blood and faecal parasites, stabilized with variety of forages and formulated commercial feed for a period of seven days at room temperature (25±1°C) and relative humidity of 50±5%. Clean water was supplied ad libitum and their weights (average 0.4 kg) taken with a sensitive weighing scale (Pelouze, model SP5). Experimental protocols met the requirements of ethical guidelines on the care and use of laboratory animals.

Source of cypermethrin
The cypermethrin (CYPERFORCE®) used was collected from the Practical Year Training Unit PYTP, of the Faculty of Agriculture and Forestry, University of Ibadan. The pesticide (CYPERFORCE®) commonly applied on the farm lands for the training of students, is a synthetic pyrethroid and contact insecticide containing Cypermethrin 10% EC manufactured by Gharda Chemicals Limited, B-27 Midd Dombivli (E), 421 203 Dist thane Maharashtra, India having batch number CMN100E1230B and NAFDAC number A5-0108. The toxicity class of this pesticide is II.

Exposure to cypermethrin
After the seventh day of stabilization, the animals were divided into three experimental groups E1, E2, E3 and administered 0.07 to 0.24ml of the pesticide based on estimated sublethal dosage of 50mg/kg, 60mg/kg and 70mg/kg body weight respectively (WHO/FAO, 1992; Oslen, 1994). Another group E0 served as control. The cypermethrin was dissolved in a vehicle (corn meal oil) and administered orally to the guinea pigs using an improvised gastric tube of 22mm gauge.

Collection, analysis of blood and tissue samples
The animals were bled via ocul sinus within 72 hours post exposure to cypermethrin. The bleeding was done using capillary tube into plain sample bottles and bottles containing 1mg of anticoagulant EDTA (ethylene diamine tetra acetate) from each of the animals across the groups. The animals in the control group (unexposed) were bled prior to ingestion of corn meal to serve as negative control and likewise 72 hours post oral administration of corn meal. The blood samples were then immediately taken to the clinical pathology laboratory for haematological and serum biochemical analysis.

Haematological parameters such as packed cell volume (PCV), haemoglobin concentration (Hb) and red cell counts (RBC) were determined using standard techniques as described by Coles (1986) and Omonona & Emikpe (2011). Other haematologic indices were subsequently estimated. The biochemical parameters (total protein, albumin, alanine aminotransferase, cholesterol and glucose) were determined using haemocytometric while alkaline phosphatase (ALP) by colourimetry, Urea and Creatinine by diacetyl reaction methods (Bartley 2001).

Representative animal from each group was euthanized in chloroform chamber before necropsy. Tissues from the lung, liver and kidney were collected, immersed in 10% buffered formalin and processed routinely for histopathology (Cooper, 2002; Akpavie, 2004). Microscopic evaluation of tissue changes was done using light microscope (CX21).

Statistical analysis
Values obtained were presented as Mean ± Standard Deviation, and then subjected to one way analysis of variance and Bonferoni post-test. P values <0.05 were considered significant.
Results
Clinically, the cypermethrin exposed groups showed signs of incoordination, anorexia, mild hair loss 48 hours and insignificant weight loss 72 hours post exposure irrespective of the dosage used.
Haematologically, there was slight decrease in the Packed Cell Volume (PCV) at the highest dosage (E3) but was not significant. The changes observed in Haemoglobin concentration (Hb) and red blood cell counts (RBC) across the exposed groups were also insignificant (p>0.05). There was significant moderate leucocytosis characterized by neutrophilia and lymphocytosis in groups E2 and E3 respectively (p<0.05). The decreases in platelet count of the cypermethrin-exposed groups were also significant (Table 1 and Fig 1).

Biochemically, there were mild increases in total protein, alkaline phosphatase (ALP) and alanine transaminase (ALT) activities, albumin-globulin ratio, creatinine and cholesterol values within the cypermethrin exposed groups. There was also decrease in urea levels. The changes in ALP, creatinine, urea, cholesterol, globulin, and albumin were statistically significant (P<0.05) (Table 2 and Fig 2).

Histopathological findings showed that there was diffuse pulmonary vascular congestion and mononuclear cellular infiltrates in the interstitium of the lungs (Plate I), mild sinusoidal congestion, and vacuolar degeneration of hepatocytes in the liver (Plate II). Glomerular atrophy, interstitial congestion, and tubular necrosis with casts in the kidney (Plate III) of cypermethrin exposed guinea pigs were observed.

Table 1: Haematological parameters (Mean ±SD) from the cypermethrin exposed and unexposed guinea pigs

<table>
<thead>
<tr>
<th>PCV</th>
<th>Hb</th>
<th>RBC</th>
<th>WBC</th>
<th>Plt</th>
<th>Lym</th>
<th>Neut</th>
<th>Mon</th>
<th>Eosin</th>
<th>MCV</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>33.9±4.3</td>
<td>10.9±1.2</td>
<td>5.5±0.1</td>
<td>5.7±1.1</td>
<td>1.0±0.2</td>
<td>4.3±0.9</td>
<td>1.2±0.4</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td>62.5±6.0</td>
</tr>
<tr>
<td>E2</td>
<td>33.7±3.6</td>
<td>11.0±1.3</td>
<td>5.6±1.0</td>
<td>6.1±1.4</td>
<td>1.1±0.2</td>
<td>4.1±0.6</td>
<td>1.7±0.9*</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td>60.8±4.6</td>
</tr>
<tr>
<td>E3</td>
<td>32.9±4.1*</td>
<td>10.7±1.1</td>
<td>5.5±0.9</td>
<td>5.9±2.6</td>
<td>0.9±0.5</td>
<td>4.3±2.0</td>
<td>1.3±0.7</td>
<td>0.2±0.1*</td>
<td>0.9±0.1</td>
<td>60.5±3.4</td>
</tr>
<tr>
<td>E0</td>
<td>33.5±0.5</td>
<td>10.85±0.3</td>
<td>5.5±0.5</td>
<td>5.0±0.1</td>
<td>1.2±0.1</td>
<td>3.9±0.2</td>
<td>1.2±0.1</td>
<td>0.1±0.1</td>
<td>0.1±0.0</td>
<td>61.2±0.3</td>
</tr>
</tbody>
</table>

* Values with stars are significant at p < 0.05

Packed Cell Volume- PCV (%), Haemoglobin Concentration-Hb (g/dl), Red Blood Cell-RBC (*10^12/ µL), White Blood Cell-WBC (*10^3/ µL), Platelet Count-Plt (*10^10/ µL), Lymphocytes-Lym, Neutrophils-Neut, Monocytes-Mon, Eosinophils-Eosin, Mean Cell Volume-MCV (fl), Mean Cell Haemoglobin Concentration-MCHC (pg)

Table 2: Serum biochemical values (Mean ±SD) from the cypermethrin exposed and unexposed guinea pigs

<table>
<thead>
<tr>
<th>TP</th>
<th>Alb</th>
<th>Glob</th>
<th>A:G</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>Urea</th>
<th>Creat</th>
<th>Gluc</th>
<th>Choles</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>7.0±0.9</td>
<td>4.4±0.7</td>
<td>2.7±0.5</td>
<td>1.6±0.3</td>
<td>41.1±2.6</td>
<td>30.1±2.5</td>
<td>96.0±18*</td>
<td>14.4±0.5*</td>
<td>0.8±0.3*</td>
<td>121±4.7</td>
</tr>
<tr>
<td>E2</td>
<td>6.8±0.6</td>
<td>4.4±0.2</td>
<td>2.4±0.5a</td>
<td>1.8±0.3</td>
<td>41.4±2.0</td>
<td>30.2±2.2</td>
<td>96.4±18*</td>
<td>14.8±1.0</td>
<td>0.6±0.3</td>
<td>119±7.0</td>
</tr>
<tr>
<td>E3</td>
<td>6.8±0.8</td>
<td>4.4±0.4</td>
<td>2.2±0.6b</td>
<td>1.9±0.6</td>
<td>41.6±1.6</td>
<td>30.4±0.9</td>
<td>97.8±17b</td>
<td>15.1±1.2</td>
<td>0.8±0.3b</td>
<td>122±5.0</td>
</tr>
<tr>
<td>E0</td>
<td>6.8±0.2</td>
<td>4.1±0.1</td>
<td>2.7±0.1</td>
<td>1.5±0.0</td>
<td>40.0±0.0</td>
<td>28.5±0.5</td>
<td>87.5±3.8</td>
<td>15.0±0.0</td>
<td>0.5±0.1</td>
<td>119±2.8</td>
</tr>
</tbody>
</table>

Values with superscript (*) are significant while different super script (a,b) are significantly different (p<0.05).

TP-Total protein (g/dl), Alb-Albumin (g/dl), Glob-Globulin (g/dl), A:G-Albumin globulin ratio, AST- Aspartate Amino transferase (U/l), ALT-Alanine Aminotransferase (U/l), ALP-Alkaline Phosphatase (U/l), Urea- Blood Urea Nitrogen (mg/dl), Creat-Creatinine (mg/dl), Gluc- Glucose (mg/l) and Choles- Cholesterol (mg/l)
Figure 1: A plot of the haematological parameters across groups

Plate I: Photomicrograph of the lungs from *Cavia porcellus*. 1- 72 hours post-exposure to cypermethrin showing diffuse interstitial congestion and mononuclear cellular infiltrates (arrow). 2- Control showing normal alveoli and septa (arrow). HE x100

Figure 2: A plot of the serum biochemical parameters across groups
Plate II: Photomicrograph of the liver. 3- 72 hours post exposure to cypermethrin showing diffuse hepatocellular vacuolar degeneration (arrow). 4- Control. HE x150, 300.

Plate III: Photomicrograph of the kidney from Cavia porcellus. 5- 72 hours post exposure to cypermethrin showing extensive coagulation necrosis of renal tubules and ectasia (arrow). 6- Control. HE x100

Discussion
The toxicity of pyrethrin insecticides to animals has received much attention in recent years because animals exposed to these insecticides exhibited changes in their physiological activities (Oslen 1994; Sakr et al., 2002). In this work, the haematology, serum biochemistry and histological changes induced by sub-lethal doses of cypermethrin were assessed in guinea pigs to mimic the acute effects of this pyrethroid in non-target wildlife species. The haematological and serum biochemical findings especially as observed in the unexposed animals are in consonance with the observations in the Nigerian local guinea pigs (Oluwaniyi et al., 2001).
Cypermethrin intoxication in rats at doses between 160-300mg/kg inhibited ATP in the rat liver tissue following single and repeated oral dosing (El-Toukhly & Girgis, 1993). The toxic oral dose in mammals according to Oslen (1994) is more than 100mg/kg, comparable to trace amounts in agricultural formulation and those used in this study, may cause devastating effects. Mild hair loss, though nonspecific, was observed in most of the exposed animals. The observed incoordination in the animals was similar to the muscular tremor and ataxia reported by Sakr et al. (2002). Animals were observed to have lost minimal weight in most of the exposed groups 72 hours post exposure. These symptoms of cypermethrin toxicity are quite typical of its neurotoxic properties which were observed by Ray (1982), Khan et al. (2003), and Ezemonye & Tongo (2010) but differ from the findings of Raj et al. (2013). The haematological changes and moderate leukocytosis suggest probably its involvement in circulatory disturbance and cause of tissue damage demonstrated histologically. These are similar to the findings of Handerson & Parkinson (1981) and Prakash et al. (2009). The increased enzyme activity (ALP and ALT) suggests induction of hepatocellular injury as this organ is involved in metabolism of the pesticide. The increase in creatinine level suggests renal insufficiency. The low albumin observed may be due to loss from renal insufficiency or low synthesis sequel to liver injury. The decrease in globulin level suggests an impaired immune system by this pyrethroid, while thrombocytopenia may predispose to bleeding tendencies. Cypermethrin administered at 1/14th lethal dose had significant adverse effects on a number of immunological functions in rats (Tulinska et al., 1995). It is known to suppress the immune system, inhibiting the formation of antibodies to disease-producing microbes (Khan & Law 2005). This finding underscores the depressing effect of cypermethrin and cause of susceptibility to opportunistic diseases in contact animals and wildlife. The ALP and globulin changes are comparable to those reported by Schwetz et al. (1973) and Manna et al. (2004). The biochemical alterations induced by cypermethrin toxicity could be largely attributed to induction of oxidative stress during biotransformation in the liver (Prakash et al., 2009). Similar alterations were also reported by Atamanalp et al. (2002) in rainbow trout, Haratym-Maj (2002) in mice, Jayakumar et al. (2008) in Rana spp using different lethal concentrations.

In conclusion, single sublethal oral exposure of cypermethrin altered biochemical parameters in the guinea pig with commensurate tissue changes. Thus, haematological and biochemical values are good indices in assessing the health status of animals, especially wildlife in our environment. Further studies of repeated pyrethroid ingestion in the tropical environment may be important. The exposure or intoxication of animals to cypermethrin may contribute to morbidity in contact animals and ecotoxicity problems.

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References
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