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*Full Length Research Paper*

# Teratogenic and embryotoxic effects of orally administered cypermethrin in pregnant albino rats

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**Lack of consensus in published works regarding cypermethrin's ability to produce teratogenic and embyotoxic effects has led to postulations. The aim of this study was to evaluate the teratogenic and embryotoxic effects of cypermethrin in 5% vegetable oil using albino rats. Acute toxicity study of cypermethrin was evaluated using Probit analysis method. Percentage mortality was calculated and $LD_{50}$ was determined. In addition, pregnant female rats in different groups received different oral doses from day 6 to 15 of gestation. Caesarean section was performed on day 20 to examine fetuses because their gestation period is between 21 to 22 days. Animals given higher doses experienced hyper-salivation, irritability, convulsion, respiratory distress and death, with on an $LD_{50}$ of 85.1 mg/kg body weight. Autopsy findings revealed vascular congestion and haemorrhage in different organs. Teratogenic and embryotoxic study revealed reduced fetal weights and with ecchymosis particularly in higher doses. The extent of fetal death and resorptions observed were not statistically significant compared to controls. Histological examination and skeletal staining technique showed no significant abnormalities despite the use of high doses of cypermethrin. The finding of ecchymosis is new, along with low birth weight, which appears to be a consistent effect of cypermethrin.**

**Key words:** Cypermethrin, teratogenicity, embryotoxicity, low birth weight, fetuses, malformation.

## INTRODUCTION

Infant mortality due to congenital malformation is now ranked second most important cause of death in developing countries (Kurinczuk et al., 2010; Cremonese et al., 2014). Factors implicated in the aetiology of infant mortality include maternal drug exposure and environmental exposures to chemical pollutants (Gorini et al., 2014). Exposure to pesticides is a thing of concern, as they have been associated with a number of fetal abnormalities with evidence linking them to central nervous system (CNS) malformations (Rull et al., 2006; Brender et al., 2010).

Cypermethrin is a synthetic pyrethroid insecticide widely used all over the world against a varied range of pests in agriculture, public health and animal husbandry (Al-Hamdani and Yajurvedi, 2010). Cypermethrin is a known neurotoxicant and primarily targets sodium channel in the nerve membrane of both CNS and peripheral nervous system (PNS) (Cox, 1996; Narahashi, 1996; Ahmad et al., 2011; Singh et al., 2012). Since its introduction, it has become one of the mostly used

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insecticides in the developing economies like Nigeria, Egypt, Saudi Arabia, India and the Caribbean (Assayed et al., 2010; Cremonese et al., 2014; Dewailly et al., 2014).

Cypermethrin is used to impregnate mosquito bed nets in West Africa to prevent malaria (Lim et al., 2011; N'Guessan et al., 2014) cypermethrin may range between 0.01 to 0.2mg/kg in food products of animal origin and up to 20 mg/kg in non-food products (US EPA, 1989; Cox, 1996). Thus, there is increased risk of food and environmental contamination, foreshadowing harmful effects to animals and humans particularly among those occupationally involved with the use of cypermethrin. Potential teratogenic effects of chemicals in humans are usually assessed using experimental animals (Woodruff et al., 2008). Assayed et al. (2010), reported toxic effects of cypermethrin when exposed to either target or non-target organisms. While the onset of signs of poisoning as was indicated by Singh et al. (2012), is characterized by salivation, tremors and increased startle response in experimental animals. Toxicity studies in rats have shown hematological abnormalities with depressed red blood cell count, white blood cell count and platelets.

Various researchers have reported sperm abnormality and genotoxicity (Elbetieha et al., 2001; Yousef et al., 2003; Manna et al., 2004; Al-Hamdani et al., 2010), and studies by Assayed et al. (2010) and Bhaskar et al. (2014), reported developmental malformations in animals and birds. Available epidemiological data indicates a causal relationship between pyrethroids exposure to pregnant women and subsequent congenital malformation of offspring (Ostrea et al., 2009; Cremonese et al., 2014).

Cypermethrin, a synthetic pyrethroid can cross the placenta barrier, thereby affecting physiological functions associated with fetal neurological development (Dewailly et al., 2014). Earlier reports by Selevan et al. (2000) showed that fetuses are very sensitive to chemical toxicants, particularly during critical periods of development. There are different outcomes from the toxicity of cypermethrin by various study groups using same animal models, yet the results remained varied. In addition, exposure threshold for abnormalities observed are varied. Therefore, embryotoxicity will be dependent not only on the nature of compound or the amount but time and duration of exposure particularly during fetal development. The goal of this study was to evaluate the teratogenic and embryotoxic effects of cypermethrin, using multiple dose regimen obtained from $LD_{50}$ determination.

## MATERIALS AND METHODS

### Animals

Male and virgin female rats used for this study were obtained from the Animal House of the College of Medicine, University of Lagos, Nigeria. The study was approved by ethical committee of College of Medicine University of Lagos for the use of experimental animals. Their weights were between 120 to 220 g, and they acclimatized to the laboratory environment for 2-weeks in order to regularize their hormonal cycle. The National Research Council prescribed “Guide for the Care and Use of Laboratory Animals” was followed (National Research Council (NRC), 2011).

### Test chemical

Cypermethrin (viscous liquid) was obtained from Chemical and Allied Products Company (CAP) Plc, Ikeja, Lagos Nigeria. It was later dissolved in 5% vegetable oil purchased from the market. 100 mg of cypermethrin per ml of vegetable oil was prepared as stock. Using the body weight and desired dose, the volume was then determined from the concentration.

### Dose-range finding test

A range finding test was carried out to determine the dose-range for this study. Three rats per group were used and were administered with 40, 80 and 120 mg/kg body weight cypermethrin respectively. There were no mortalities in the 40 and 80 mg/kg groups, but group that received 120 mg/kg had 100% mortality.

### Acute toxicity studies

This study was performed with forty albino rats of both sexes which were divided into five groups consisting of eight animals per group. They were fasted overnight before the start of the experiment. Groups 1 to 4 were given cypermethrin orally at doses of 50, 60, 80, 100 mg/kg body weight respectively. The volume administered was based on the animal weight. The fifth group received 0.5 ml of vegetable oil and served as the control group. Animals were then allowed food and water ad-libitum after 30 min post administration. They were observed for 24 h for signs of toxicity and mortality. Dead animals were autopsied.

### Teratology evaluation

Twenty-five female virgin non-pregnant albino rats were used for the teratogenic and embryotoxic evaluation. They were divided into 5 groups, consisting of 5 rats each and exposed to 12 h of daylight and 12 h of night duration. Oestrous cycle was monitored for all animals before mating. For mating, two females with one male were placed in a cage overnight from 4.00 pm to 8.00 am the following day. In the morning of the next day, the male was removed and the females were examined for signs of mating by flushing the vagina with 0.9% saline using a pipette, and then examining the obtained solution microscopically. Mating was confirmed by the presence of sperm cell/plugs. The presence of spermatozoa is recorded as day zero of pregnancy and a daily increase in weight is further confirmation of pregnancy. The treatment schedule involved administration of the test chemical during organogenesis which is from day 6 to day 15 of gestation according to Organisation for Economic Co-operation and Development (OECD) guidelines (2001). Groups 1 to 4 received 85, 65.0, 42.5 and 21.2 mg/kg of cypermethrin in 5% vegetable oil respectively. The control group, (group 5), was given 0.5 ml of vegetable oil. The doses used were based on the results obtained from dose-range finding test and acute toxicity study. These doses were administered from day 6 to 15 for each animal and their weights were taken daily throughout the period of observation. Animals were also observed for signs of toxicity, if they were found to be severe, the experiment was terminated for that animal.

#### Post mortem procedure

The animals were sacrificed on day 20 of the gestation period by cervical dislocation, and then caesarean section was performed on the animals, in order to obtain the fetuses. Presence or absence of resorption sites was recorded and the numbers of dead or live fetuses were counted. Living fetuses were distinguished from the dead by reflex movement initiated by gently touching the fetus. Live fetuses were weighed and examined by a hand lens, to observe the cranium, face and muzzle, bilateral eye bulges, buccal cavity, limbs and tail to check for any malformations. The fetuses were euthanized and then placed in formalin in order to preserve them for skeletal examination and histological staining procedures. Tissues were taken from the fetuses and their mothers for histopathological studies as well.

#### Haematoxylin, eosin and skeletal staining technique

The tissue sections were prepared using a Shandon's automatic tissue processor and dewaxed using xylene and alcohol and then finally water. Tissues were stained with haematoxylin, to get a purplish colour, the cells differentiated from each other by treating with 1% acid alcohol and subsequently washed with water until blue coloured nuclei were obtained. A counter stain was done to pick up the other cell structures in the tissue using 1% aqueous eosin. The stained tissues were washed again, dehydrated in alcohol, cleared in xylene, and then mounted in DPX. Following maceration, the skeletal residues of the fetuses were transferred for staining, into a solution of 0.1% Alizarin Red S in 1% potassium hydroxide and left until the bones were fully stained to the desired colour, the skeletal fetal structures were transferred into pure glycerin and finally mounted in glycerin for examination according to the modified method of Menegola et al. (2001).

#### Data analysis

Results obtained were expressed as mean ± SD. Control values were compared with treatment groups by means of statistical package for the social sciences (SPSS) 19 using two-way one-way analysis of variance (ANOVA), followed by Tukey Post Ad-hoc test. $P< 0.05$ was considered statistically significant.

## RESULTS

### Acute toxicity

The results showed reduced locomotor activities of the animals, post administration of cypermethrin. Food and water consumption was normal for the animals treated with the 50 mg/kg body weight, 60 mg/kg body weight doses as well as for the controls. However, food intake was seen to have reduced for the animals on higher doses of 80 mg/kg body weight and 100 mg/kg body weight. Very obvious and worsening clinical manifestations like irritability increased startling response to mild stimuli and sound were observed. Among the highest dosage, group of 100 mg/kg body weight of cypermethrin salivation was prominent, as well as ataxia. Also, body tremour and convulsion were observed. At the terminal stage, the animals convulsed violently and had episodes of respiratory distress before they died with the presence of oily substance detected on their skin. $LD_{50}$ was calculated as 85.1 mg/kg using probit analysis method (Table 1). The histopathological study of the tissue sections from the dead rats revealed vascular congestion as a common feature in most of the tissues examined followed by haemorrhage noticed in the liver and kidney particularly in animals that was given 100 mg/kg body weight.

### Observations in pregnant rats

There were no apparent toxic responses in the rats when they were given cypermethrin days 6 to 15 of gestation, particularly for lower dosage groups. However, lethargy and diarrhea were observed in animals with the 85 mg/kg dose. Also observed, was middle ear disease among animals in the 85 and 65.0 mg/kg groups (middle ear disease is a condition characterized by abnormal tilting of the head to one side, and when the animal is picked up by the tail, there is 360 degrees rotation of its body). The results on the cumulative maternal weight increase observed during pregnancy are shown in Table 2. Low weight gain was observed in all treatment groups compared with the control and this observation was statistically significant ($p<0.05$). Mean percentage weight of pregnant mothers showed dose dependent decreases and appeared to be even lower in higher dosage groups, compared to the control group. One-way analysis of variance revealed that mean maternal change in weight of treated groups were significantly different ($p<0.05$) from that of control animals and within the groups (Figure 1).

### Teratogenic and development Findings

Abnormalities observed as presented in Table 3 were low birth weight and ecchymosis. These appeared as dark red spots on various parts of the body of the fetus and prominent with the group of animals treated with 85 mg/kg dose (group 1) and 65 mg/kg dose (group 2) of the test chemical. The mean fetal weight of the control (6.13g±1.3) was observed to be 2 to 3 times more than the mean fetal weight of treated groups showing statistical significant differences ($p> 0.05$) as presented in Table 4. An image showing the effect of cypermethrin on the development of the representative fetuses from the different treatment groups compared with that of the controls is shown in Figure 2. The figure shows that cypermethrin potentially reduced fetal body weight. A comparison between the control and experimental fetuses for morphological abnormalities using the hand lens showed that the experimental animals had no physical malformations but head size, bilateral eye bulges; tails, face and muzzles as well as other external appendages were not as prominent as that of fetuses in the control group. In addition, the crown-rump lengths for

**Table 1.** Acute toxicity studies results used to determine the $LD_{50}$ of cypermethrin in albino rats.

| Dose (mg/kg body weight) in 5% veg oil | Log dose | Number of death | % Response | Probit |
|---|---|---|---|---|
| 50 (Group 1) | 1.69 | 0/8 | 0 | 0 |
| 60 (Group 2) | 1.77 | 0/8 | 0 | 0 |
| 80 (Group 3) | 1.90 | 2/8 | 25 | 4.3 |
| 100 (Group 4) | 2.0 | 8/8 | 100 | - |
| 0.5 ml veg. oil (Group 5) | - | 0/8 | 0 | 0 |

**Table 2.** Comparism between control group maternal weight and experimental groups treated with cypermethrin throughout the gestation period.

| Groups | Day zero (Mean±SD) | Day 6 (Mean±SD) | Day 15 (Mean±SD) | Day 20 (Mean±SD) |
|---|---|---|---|---|
| 85 mg/kg body weight | 158.3±16 | 165±13.2 | 166±16 | 178±10.4* |
| 65 mg/kg body weight | 158.7±29.5 | 166.2±24.9 | 173.7±23.9 | 183.7±28.4* |
| 42.5 mg/kg body weight | 143.3±11.5 | 146.6±15.3 | 163.3±38.8 | 176.6±44.8* |
| 21.25 mg/kg body weight | 130±1.4 | 135±7 | 154±14.1 | 167.5±31.8* |
| Control (0.5 veg oil) | 118.3±7.6 | 128.3±2.8 | 148.3±20.2 | 158.3±30.5 |

(*p<0.05), What about p value = 0.05.

**Table 3.** Showing teratogenic and embyotoxic effects observed in fetuses whose mothers were administered with different doses of cypermethrin compared with control.

| S/N | Dose (mg/kg body weight) in 5% veg. oil | Number of implantation | Number of resorption | Number of dead fetus | Number of viable fetus | Anomalies observed |
|---|---|---|---|---|---|---|
| 1 | 85 | 17 | 1 | - | 16 | Ecchymosis |
| 2 | 65 | 19 | - | - | 19 | Ecchymosis |
| 3 | 42.5 | 18 | 2 | 1 | 15 | - |
| 4 | 21.2 | 14 | 1 | - | 13 | - |
| 5 | Control (0.5 ml of oil) | 19 | - | - | 19 | - |

**Table 4.** Showing total number of fetuses and mean fetal weights in the control group and treatment groups.

| Group | Dose (mg/kg body weight) in 5% veg. oil | Number of pregnant rats | Total number of fetuses | Mean weight of fetuses (g) |
|---|---|---|---|---|
| 1 | 85 | 5 | 16 | 2.9± 0.8* |
| 2 | 65 | 5 | 19 | 3.1±0.7* |
| 3 | 42.5 | 5 | 15 | 3.43±0.6* |
| 4 | 21.2 | 5 | 13 | 2.06±0.3* |
| 5 | Control (0.5 ml Veg Oil | 5 | 19 | 6.13±1.3 |

(*p<0.05).

the fetuses whose mother were treated with various doses of cypermethrin were reduced, perhaps due to reduced body weight/size. Some of the observed differences were in one or two fetuses which were not significant (Table 5).

## Histological and skeletal changes

Histological examination of tissue sections of rat fetuses with haematoxylin and eosin showed that there were no defects in the organs of the rat fetuses whose mothers were treated with the various doses of cypermethrin (Table 5). Most of the organs like the liver, kidney, heart, lungs and small intestine did not show any significant abnormal development. Haemorrhage was observed in the liver and heart of three foetuses from different groups and was not consistent with dose or was it a common feature in most of the tissues examined. These observations were not statistically significant. The bones were in the cartilaginous phase of development and the neural tissues were normal.

**Table 5.** Showing effects on foetal external development, histological and skeletal changes from day 6 to 15 of gestation during administration of cypermethrin to pregnant rats.

| Parameters | Dose of cypermethrin (mg/kg body weight) | | | | |
|---|---|---|---|---|---|
| | Control | 21.25 | 42.5 | 65 | 85 |
| Total no. of live foetuses | 19 | 13 | 15 | 19 | 16 |
| **Foetal external observations** | | | | | |
| Head size | 0 | 0 | 0 | 1 | 0 |
| Face and muzzle | 0 | 0 | 0 | 0 | 0 |
| Bilateral eye bulges | 0 | 0 | 0 | 1 | 0 |
| Limbs | 0 | 0 | 0 | 0 | 0 |
| Tails Length | 0 | 0 | 1 | 0 | 1 |
| Crown-rump length | 0 | 0 | 1 | 0 | 1 |
| **Histological changes** | 19 | 13 | 15 | 19 | 16 |
| Liver | - | + | - | + | + |
| Lungs | - | - | - | - | - |
| Kidney | - | - | - | - | - |
| Heart | - | + | - | + | - |
| **Skeletal abnormalities** | | | | | |
| Skull malformation | 0 | 0 | 0 | 1 | 0 |
| Vertebrae column | 0 | 0 | 1 | 0 | 0 |
| Ribs | 0 | 0 | 0 | 0 | 0 |
| Phalanges | 0 | 0 | 0 | 0 | 0 |

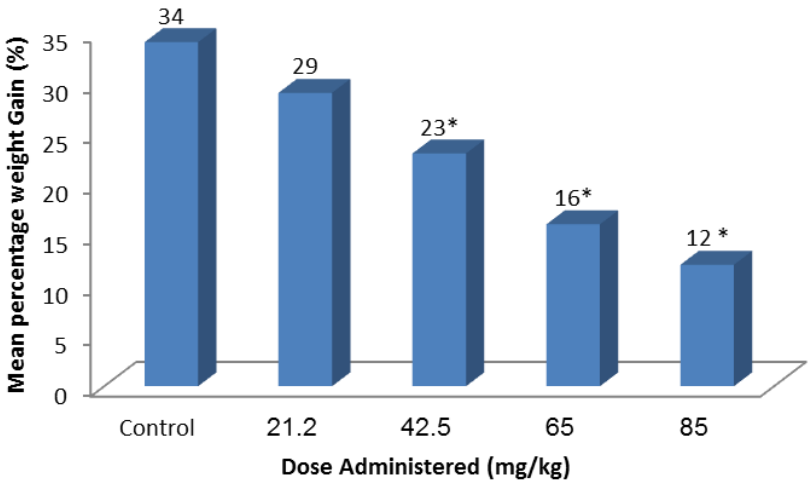


**Figure 1.** Showing mean percentage weight gain from day 6 of gestation to day 20 in different treatment groups compared to the control, (*p<0.05).

## DISCUSSION

The reports available on long and short term toxicity studies of cypermethrin indicate that it is toxic to both target and non-target organisms including humans (Al-Hamdani et al., 2010; Uggini et al., 2012; Cremonese et al., 2014). The acute toxicity test results obtained from the present study is therefore not unexpected. Tests results from this study showed mortality at high dose levels of 80 and 100 mg/kg, with 25 and 100% mortality

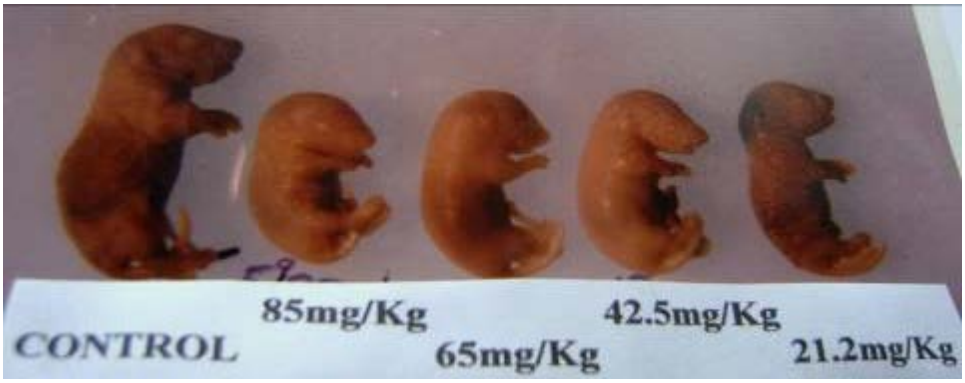


**Figure 2.** Photograph showing comparism between harvested fetuses from control group and those from the treatment groups**.**

respectively. An extrapolated $LD_{50}$ of 85.1 mg/kg was calculated suggesting that cypermethrin is moderately toxic to rats, a non-target organism. The present findings are similar to those reported by Coombs et al. (1976), Pronk et al. (1996), EMEA (2002) and Raj et al. (2013), who showed that oral acute toxicity study for this compound varied from 82 to 4000 mg/kg in rats. Suggesting a wide variation in toxicity that could be attributed to the different mixtures of isomers contained and their ratios in the test materials as well as other factors which includes the strain of the animal, age, sex, type of solvent vehicle used in the study and environmental conditions (WHO, 2004).

The observed clinical signs and symptoms seen in the present study which included muscular weakness, irritability, refusal of feed and respiratory distress as well as the convulsion before death particularly for the animals administered with 80 and 100 mg/kg doses are similar to those of Manna et al. (2004) and Iyaniwura and Okonkwo (2004). However, extrapolating these results to human risk management in cypermethrin exposure is daunting task.

The present findings have therefore, highlighted the fact that developmental toxicity is an important issue because of rising congenital malformations among infants as was also noted by other researchers such as Winchester et al. (2009) and Ahmad et al. (2012). Developmental abnormalities that mainly reduced fetal weight and ecchymosis after prenatal exposure to cypermethrin as seen in the present investigation is similar to the findings of Murkunde et al. (2012) but contrary to findings by other studies such as Assayed et al. (2010), Elbetieha et al. (2001), Rustamov and Abbasov (1994) and Anwar (2003). Their findings recorded drastic fetal abnormalities. However, abnormalities such as the difference in the mean fetal weight between the controls and those of cypermethrin treated pregnant rats agrees with earlier findings by EMEA (2002) who reported reduced litter size and weights in a three generations study of rats after administration of doses that produced reduced body weight gains in their parents. However, it is contrary to an IPCS-WHO (1989) report, which indicated that oral administration of 70 mg/kg per day of cypermethrin in 5% corn oil to rats did not produce any teratogenic effects particularly at doses that caused maternal toxicity. Thus, suggesting a variation in research findings, and this could lead to postulations and estimations. There is however, evidence from researches showing significant association between cypermethrin exposure and low birth weight (Hanke et al., 2003).

In addition, teratogenic effects have been reported in some other non-target species like birds and aquatic organisms (IPCS, 1989; Cox, 1996; Uggini et al., 2012; Yu et al., 2013) with such reports being consistent on the issue of low birth weight irrespective of the dose employed. Earlier findings by Shaw et al. (1999) and Bell et al. (2001) documented that exposures to pregnant women enhanced risks of giving birth to malformed babies, while Cremomese et al. (2014) linked increased infant mortality rates to pesticide consumption which they attributed to CNS and cardiovascular system (CVS) malformations. This suggests that exposure to cypermethrin can be critical in areas of heavy usage. Although, cypermethrin is rapidly metabolized into phenoxybenzoic and carboxylic acids, none of these has been implicated in any adverse events (Dewailly et al., 2014). The likelihood that cypermethrin does have the potential, to bring about teratogenic and embryotoxic effects even at low levels of exposures is conceivable

## Conclusion

The results obtained from this study appear to be at variance with other studies but still revealing potential teratogenicity and embryotoxicity effects of cypermethrin. Yet again, exposure to cypermethrin has displayed a wide variability of responses as documented in literature. This findings show that there are still gaps about desciptive

cypermethrin toxicity in both humans and animals exposures. The lack of consensus in various studies has given room to different interpretations and postulations. These studies have employed different vehicles, doses, and a variety of test animals treated in different experimental conductions yet with different conclusions. However, due to extensive consumption of cypermethrin worldwide in different human activities, its use should be with measured caution, particularly in the face of increasing infantile mortality due to congenital abnormalities.

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## Conflict of interest

The author declares no conflict of interest and received no funding from any agency or organisation.

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