INTERNATIONAL JOURNAL OF NATURAL AND APPLIED SCIENCES (IJNAS), VOL. 5, NOS.1& 2 (2010); P. 6 – 11, 1 TABLE, 1 FIG.

# Effect of Ridomil on sperm parameters of male albino rats

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**ABSTRACT**

This study was undertaken to determine the effect of Ridomil, a fungicide widely used by Cocoa farmers in Cross River State, Nigeria on sperm parameters of male albino rats. Twelve male albino rats of 10 weeks old were used as the mammalian model for this sub-chronic toxicity study. They were assigned to three groups of four rats each and treated with Ridomil at 0, 50 and 100 mg/Kg BW/day respectively, four times a week for nine weeks, via direct mixing in the animal ration. After nine weeks of treatment, the rats were sacrificed and the epididymes were dissected out, weighed and processed for epididymal sperm parameters. Sperm count, sperm motility, sperm viability, sperm morphology and pH of semen did not show any significant differences (P>0.05) between the control and treatment groups. In conclusion, the pesticide RIDOMIL GOLD®, did not cause much changes in rat sperm parameters at the doses used in this sub-chronic toxicity study.

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## INTRODUCTION

Since the introduction of DDT, pesticides and other agrochemicals have played a major role in crop protection, food preservation, and the control of vector borne diseases (Mathews, 1975). Despite intensive research into alternative methods, chemical control via pesticides use is still the most effective method of controlling pests, and continues to enjoy increasing patronage year after year, in Nigeria and other countries of the world (Asogwa and Dongo, 2009).

Many farmers in Nigeria, especially cocoa farmers now use pesticides of one form or another (Tijani, 2006). Unfortunately though, many farmers fail to adopt "best practices" in pesticide use, and are not aware about the possible consequences of its mismanagement. Inadequate capacity for effective post marketing surveillance, insufficiency of agro support services, smuggling, and poor farmer/public enlightenment are some of the problems associated with pesticides use in the country (Keri, 2009).

Ridomil is a widely available, relatively cheap commercial fungicide used in controlling black pod disease of cocoa (Asogwa and Dongo, 2009), caused by the fungus *Phytopthora spp*. It is a registered product of Syngenta Agro AG, Switzerland, and is very popular among cocoa farmers. The specific Ridomil formulation used in this study is Ridomil Gold Plus 66WP and has the following composition as per label claim: metalaxyl (6%) and Copper I oxide (60%). Oral $LD_{50}$ (rat) for Ridomil Gold® Plus 66 WP as given by the manufacturer is 1183 mg/kg (male) and 1001mg/kg (female) (Syngenta Material Safety Data Sheet, 20-3-02).

Cocoa pods and leaves are eaten by many locals in Nigeria. With the high pesticide residues on Nigerian cocoa products and the use of banned pesticides by cocoa farmers as reported by the National Agency for Food and Drug Administration and Control (NAFDAC) (Tijani, 2006), there is need for sustained research interest on the toxic effects of pesticide formulations utilized on crops, as a major component in the overall quest for food safety and security in the country. This is important, as the deleterious effects of pesticides on animal systems have been documented by several researchers (Farag *et al*., 2000; Handy *et al*., 2002; Gokalp *et al*., 2005; Kalender *et al*., 2005). Fisch and Goluboff (1996) in their studies on geographic variations in sperm counts associated low sperm counts in agro-workers to exposure to pesticides, and mentioned Nigeria as one of the countries where this was notable. There is paucity of published work on the effects of Ridomil on sperm quality and other parameters associated with male reproduction. This research was therefore conducted to evaluate the effect of Ridomil on sperm parameters of male albino rats.

## MATERIALS AND METHODS

### Animals

Male albino rats of 10 weeks old weighing 170 – 210 g (ScoutPro SPU601, Ohaus) were obtained from the Animal house of Department of Zoology and Environmental Biology, University of Calabar, Calabar. In a Completely Randomized Design (CRD), the animals were divided into three equal groups; A, B, and C, comprising four animals per group. To minimize weight variation, the experimental groups were constituted such that the initial group weight ranged from 185.26g – 190.00g.

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The animals were housed in conventional wire mesh cages under standard laboratory conditions (temperature 25-30$^0$C, 12 hours light and 12 hours darkness cycle) in Experimental Animal Laboratory of Department of Genetics and Biotechnology, University of Calabar. They were allowed free access to water and commercial feed throughout the period of the experiment. One week of acclimatization was allowed before commencement of treatment. Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of animals (Carbone, 2004).

**Chemicals**

RIDOMIL GOLD® 66 WP (Syngenta Agro AG) (with 6% metalaxyl and 60% copper I oxide according to label claim) was purchased from Egoob Chemicals, Watt market Calabar. All stains and reagents were of analytical grade, and purchased at Globules Science Company, Mayne Avenue, Calabar.

**Administration of Test Substance**

1/10 of the known acute oral $LD_{50}$ in rat can usually be used for sub chronic toxicity tests in this experimental model (Hogdson *et al*, 1998). In this study, 100mg/Kg was chosen as the high dose, as it does not exceed 1/10 of the stated $LD_{50}$ of the pesticide. The weights of the animals were determined once every week and this was used to determine the amount of Ridomil administered. Group A was the experimental control and received 0mg of the pesticide. while groups A and B received Ridomil at 50 and 100 mg/Kg BW/day respectively, four times a week for nine weeks, via direct mixing in 30% of the daily feed consumption and given in the morning (8:00am), to ensure the consumption of the entire treatment dose; before the remaining feed was given later in the afternoon (1:00pm) (Ekaluo *et al*., 2009). After nine weeks of treatment the rats were sacrificed under mild chloroform anaesthesia and the epididymes were dissected out, weighed and processed for epididymal sperm count, sperm motility, sperm viability, sperm head abnormality and pH of semen

**Sperm count**

The epididymal sperm samples were obtained by macerating known weights of epididymes in physiological saline in the ratio of 1:10 weight by volume. After vigorous pipeting to release the sperm cells, the suspension was filtered using an 80µm stainless mesh. Epididymal sperm count was obtained by cytometry using the improved Neubauer cytometer ($2.5\times10^{-4}$ mm$^3$; Hawksley, England). After placing a cover slip, a fine bore capillary tube was used to fill up the cytometer with sperm suspension. Counting was done under ×40 lens in a light microscope (HM-LUX, Leitz Wetzler, Germany). Cells touching the left and top border of each square were not counted to avoid double counting. The count was expressed as million/ml of suspension (Ekaluo *et al*., 2008).

Sperm count ($\times10^6$/ml) = number of cells counted ×10(dilution factor) ×cytometer volume

**Sperm motility**

Two drops of epididymal sperm suspension were put on a microscope slide and cover slip was placed. The number of motile cells divided by the total number of spermatozoa counted under ×40 lens in a light microscope was expressed as a percentage (Adeeko and Dada, 1998).

**Sperm viability**

The sperm viability was determined using the eosin-nigrosin staining technique (Björndahl *et al*., 2003). 2ml of the sperm suspension was mixed with equal volume of the stain and smeared on glass slides. Five air-dried smears were prepared for each sample and examined under ×40 lens in a light microscope. Live sperm excluded the stain and appeared white while dead sperm took up the stain and appeared pink. The number of live cells divided by the total number of cells was expressed as a percentage.

**Sperm head abnormality test**

2mls of the sperm suspension was mixed with equal volume of 1% eosin Y solution (10:1) for 30 minutes and air-dried smears were prepared on glass slides for the sperm head abnormality test. Five air-dried smears were prepared for each sample and examined under ×40 lens in a light microscope. The slides were examined for sperm head abnormalities (Nahas *et al*, 1989; Mori *et al*, 1991; Ekaluo, 2003) and the number of abnormal sperm heads in every 200 spermatozoa observed on each slide was expressed as a percentage (Ekaluo, 2003).

**pH of semen**

Immediately after dissection, a puncture was made in the Epididymis with a sterile pin. The sperm smear on the pin was rubbed on a pH paper of range 4.0 – 10.0. The colour change corresponds to the pH and was read from the paper.

**Statistical analysis**

All data generated were subjected to Analysis of Variance (ANOVA) to check for significant differences between the treatment groups.

## RESULTS AND DISCUSSION

Up until the 5$^{th}$ week of treatment with Ridomil, none of the animals exhibited any noticeable signs or symptoms of pesticide toxicity. By the 6$^{th}$ week however, rats in group C (100mg/Kg BW) displayed sign of dullness, oversleep and faster breathing rate sometimes. These are some signs of pesticide poisoning according to USEPA (1999). Feed and water intake was however not adversely affected by the pesticide.

Pesticides have been shown to impact negatively on sperm parameters (Farag *et al*., 2000; Okamura *et al*., 2005; Issam *et al*., 2009) and are

regarded as important contributors to the perceived global decline in sperm densities (Carlson *et al*., 1992; Fisch and Goluboff, 1996).

Table 1 shows the effects of Ridomil on sperm parameters of rats, after 9 weeks of treatment. Sperm count of rats showed no significant differences (P>0.05) between the control and the pesticide treated groups. But there appeared to be a slight dose dependent decrease in sperm count from group A (0mg/Kg BW) down to group C (100mg/Kg BW). This may indicate a slight ability to affect sperm count, given sufficient exposure time, or higher dose. Sperm motility and pH of semen did not show any significant differences (P>0.05) between the treatment groups, and no trend appeared obvious. Group C (100mg/Kg BW) had the lowest sperm viability value (88.89%) and the highest value for sperm head abnormality (4.5%), even though these were not significantly different from control (0mg/Kg BW). It appears then, that Ridomil may not be considered a disrupter of spermatogenesis in rats at these doses. Ridomil did not cause any significant changes (P>0.05) in Testes and Epididymes weights of rats (Table 1). Although control rats (0mg/Kg BW) had the highest body weight at the end of the experiment, this was not statistically different (P>0.05) from that of animals in treatment groups B (50mg/Kg BW) and C (100mg/Kg BW). It is fair to think that in a chronic study, perhaps significant differences in body weight might have been obtained, as chronic administration of pesticides have been known to cause significant changes in body weight of animals.

In conclusion, RIDOMIL GOLD®, did not cause much changes in rat sperm parameters at the doses used in this sub-chronic toxicity study. Further research is needed on the effects of pesticides like Ridomil that are commonly used in our environment in order to establish the animal systems upon which they exert the most effects. This will help to enable proper precautions to be taken by agro-workers, especially in the light of the possibility of synergism between pesticides.

**Table 1. Effect of Ridomil on Sperm Parameters, Body, Testes and Epididymes Weights of Rats after Nine Weeks of Treatment.**

| Parameter | A (0mg/Kg BW) | B (50mg/Kg BW) | C (100mg/Kg BW) |
|---|---|---|---|
| **Sperm count (×10$^6$/ml)** | 6.82$^a$±0.16 | 6.79$^a$ ±0.10 | 6.63$^a$ ±0.37 |
| **Sperm motility (%)** | 75.91$^a$ ±3.50 | 67.46$^a$ ±4.01 | 79.03$^a$ ±3.24 |
| **Sperm viability (%)** | 91.13$^a$ ±2.45 | 91.27$^a$ ±1.60 | 88.89$^a$ ±2.46 |
| **Sperm head abnormality (%)** | 3.67$^a$ ±0.71 | 3.60$^a$ ±0.91 | 4.50$^a$ ±0.43 |
| **pH of semen** | 7.10$^a$ ±0.05 | 6.97$^a$ ±0.10 | 7.00$^a$ ±0.00 |
| **Initial body weight (g)** | 190.00$^a$ ±19.58 | 186.25$^a$ ±22.95 | 189.00$^a$ ±7.40 |
| **Final body weight (g)** | 290.25$^a$ ±15.30 | 236.50$^a$ ±29.00 | 244.33$^a$ ±12.17 |
| **Testes weight (g)** | 1.19$^a$ ±0.03 | 1.13$^a$ ±0.02 | 1.17$^a$ ±0.03 |
| **Epididymes weight (g)** | 0.34$^a$ ±0.01 | 0.34$^a$ ±0.01 | 0.36$^a$ ±0.01 |

Values are presented as Mean ± SEM. Values across the table with similar superscript are not significantly different at 5% based on ANOVA.

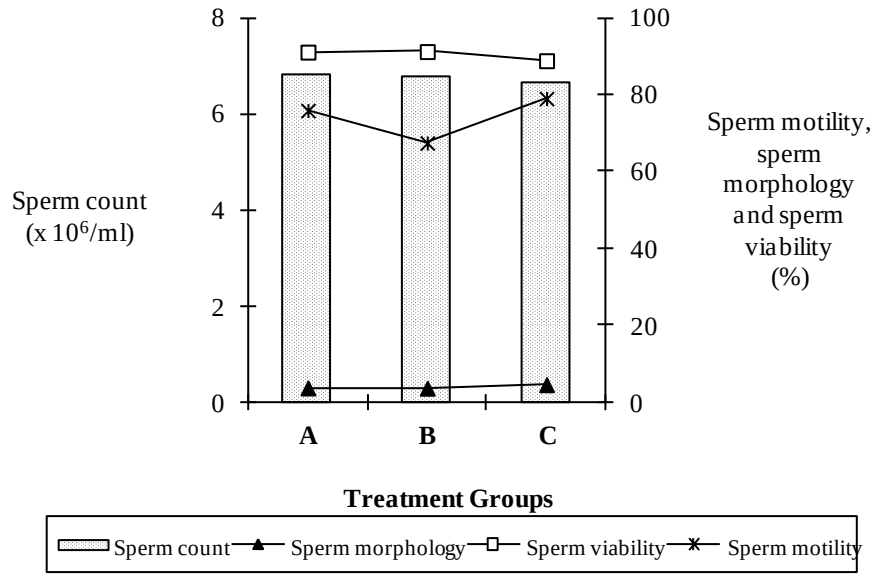


Fig. 1. Relationship between sperm count, sperm motility, sperm head abnormality and sperm viability in rats treated with Ridomil at 0mg/Kb BW (A), 50mg/Kg BW, and 100mg/Kg BW for nine weeks. No significant differences (P>0.05) was noticed between the groups.

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