REPRODUCTIVE PERFORMANCE OF MALE RABBITS (*Oryctolagus cuniculus*) CHRONICALLY EXPOSED TO NON-LETHAL DOSE OF METHOMYL

[1]Giselle B. Ramos* and [1]Roxan G. Eupeña

Abstract

Exposure to pesticides entails significant effects on the organisms' survival. This study was conducted to test whether chronic exposure of male rabbits (Oryctolagus cuniculus) to a non-lethal dose of the insecticide Methomyl (S-methyl N-((methylcarbamoyl)oxy) thioacetimidate) can have adverse effects on their reproductive performance. Male rabbits were treated for 30 days with an oral dose of 2 mg of Methomyl per kilogram body weight. These were then used to impregnate three untreated females. Reproductive performance of males was analyzed taking into account the effect on litter size, mean birth weight of offspring, percentage of embryo loss, percentage mortality of offspring one and two weeks after birth and total reproductive loss. Results showed that the treatment did not reduce the number and mean birth weight of the progeny nor did it cause gross external morphological abnormalities. Based on the counts of corpora lutea, the percentages of embryo loss in the two groups did not differ. However, within one week after birth, a significant increase in mortality of neonates was noted in the treated group and was increased further within the second week after birth. Therefore, the chronic exposure to methomyl did not reduce sperm production and viability; however, it induced dominant lethal mutations in spermatozoa. Those mutations did not affect embryonic development, but they exerted lethal effects on growth and survival of neonates. Hence, the study has generated evidence that Methomyl is mutagenic.

Keywords: *reproductive performance, male rabbits, Oryctolagus cuniculus, methomyl*

1.0 Introduction

Pesticides provide benefits and threats to organisms. Increase in food production, decrease in food cost, and consumer benefits on high-yield produce are some major benefits of pesticides. Nevertheless, they are toxic to organisms. Exposure to these chemicals implies significant effects on the organisms' survival. Thus, this study tests whether chronic exposure to non-lethal dose of methomyl on the reproductive performance of male rabbits (*Oryctolagus cuniculus*) may or may not cause adverse effects on their offspring.

Methomyl is a carbamate insecticide (ovicide/larvacide/adulticide) registered nationally for control of insects on a wide range of field, fruit, and vegetable crops, sod farms, and as a commercially fly bait (Erickson and Turner, 2003). Several studies have been conducted that show the adverse effects of pesticides/insecticides to animals' reproductive performance. Yousef et al., (1995) and Salem et al. (2008) reported a decline on bucks' (*Oryctolagus cuniculus*) body weight, libido, ejaculate volume, sperm concentration, semen initial

[1] *Surigao del Sur State University*

fructose and semen osmolality when they are exposed to sub-lethal dose of pesticides. Veeramachaneni (2002) also noted that there are adverse developmental effects of gestational exposure to anti-androgenic pesticides in male offspring, including impaired spermatogenesis, reduced testis and decreased mating ability. Moreover, the low dose exposure of female rabbits on pesticide Lindane during prenatal and lactation period has effects on spermatozoa ultrastructure of male offspring (Fausto, 2001). However, there is a dearth of information about the adverse effects of non -lethal doses of pesticides on the reproductive performance of male rabbits.

This study uses an experimental method to test the effects of methomyl on the reproductive performance of male rabbits chronically exposed to a low dose of the pesticide. Its primary concern is on the effects of chronic exposure of humans, particularly farmers and mammalian livestock. In addition, it focuses on chronic exposure to non-lethal doses which has not been studied as thoroughly as the acute toxicity effects of pesticides.

2.0 Materials and Methods

2.1 Test Animals and Experimental Conditions

All male and female animals used in this study were proven fertile in a preliminary test before the start of the experiment. Three mature males were treated daily with an oral dose of 2 mg of methomyl per kilogram body weight for one month, and then each was used to impregnate three untreated female rabbits. The control group consisted of three males and nine females. The male rabbits were used every other day to ensure that there is enough mount of sperm cells. Each of the test subjects were placed in separate cages to prevent overcrowding during the treatment period. On the onset of pregnancy of the tested females, the chronic treatment of three bucks began. After six weeks, all females of proven fertility were again mated to treated & control bucks.

2.2 Parameters Examined

The evaluated parameters on the reproductive performance of male rabbits include: litter size, mean birth weight of offspring, percentage of embryo loss, percentage mortality of offspring one and two weeks after birth, and total reproductive loss. Offspring were weighed and examined morphologically at birth for any external defects. All neonatal progeny were observed for two weeks. Proper care was administered to ensure their survival during the conduct of the study. Percentage mortality at embryonic stage was based on the number of corpora lutea that were scored after postpartum laparotomy of the doe. Embryo loss was calculated by subtracting the number of corpora lutea from the number of neonates.

2.3 Statistical Analysis

The experiment was laid out using a completely randomized design with three replicates. The effects of treatment on offspring in terms of: litter size, birth weight, neonatal mortalities, embryo loss and total reproductive loss were assayed using one-tailed t-test with the assumption that chronic exposure to non-lethal dose of methomyl on the reproductive performance of male rabbits (*Oryctolagus cuniculus*) may or may not cause adverse effects on their offspring.

3.0 Results and Discussions

As presented in figures 1a and 1b, results show that the one-month daily exposure of male rabbits to a non-lethal oral dose of methomyl did not reduce the number and mean weight of the progenies at birth nor cause any abnormalities in their gross external morphology. One doe in the treated group had 100% mortality of neonatal offspring for unknown reasons. However, as shown in table 1, the chronic treatment of the bucks with a non-lethal dose of Methomyl significantly increased the percentage mortality of neonatal offspring.

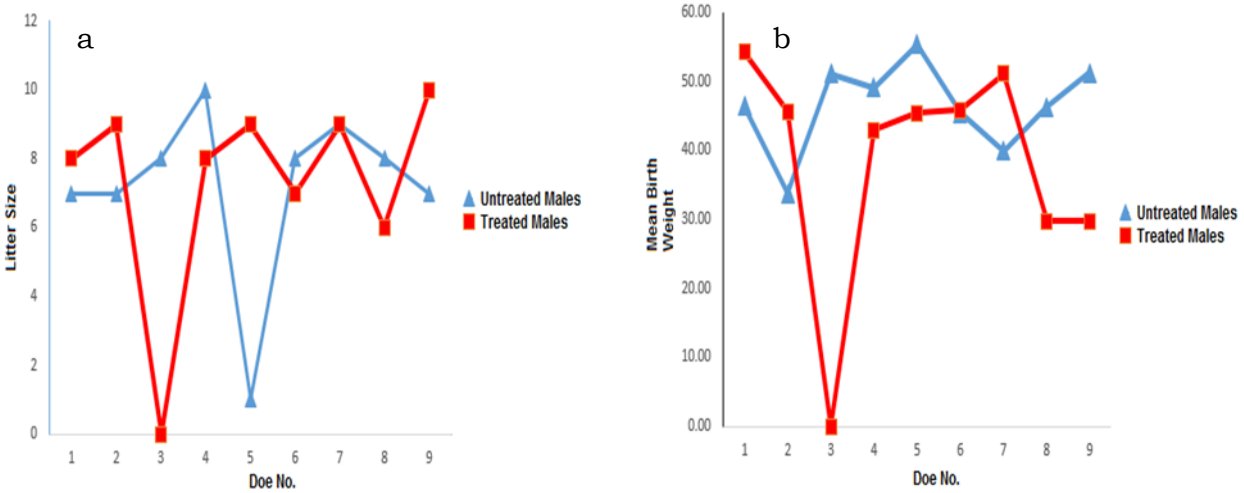

Figure 1. Mean (a) litter size and (b) birth weight of offspring of untreated females impregnated by untreated males and those treated with non-lethal dose of Methomyl

Table 1. Percentage mortality of neonatal offspring of untreated males and those treated with non-lethal dose of Methomyl

No. of weeks	Treatments	Mean of Neonatal Mortality	Computed t-value	Interpretation
One week	Untreated	18.34 ± 34.10	-1.755	significant
	Treated	47.53 ± 34.39		
Two weeks	Untreated	18.34 ± 34.10	-4.1669	highly significant
	Treated	75.0325 ± 18.7365		

The results imply that treated male parents did not directly exhibit symptoms of toxicity but such symptoms were observed in their offspring. The significant mortality rates in neonatal offspring of treated males as early as one week and up to two weeks after birth indicate that the treatment caused adverse effects on the development and survival of the offspring soon after birth. The neonates that died exhibited retarded growth and progressive weakness with time. The study conducted by El-bendary (2014) showed that the genotoxic effect of methomyl using polymorphism of glutathione S-transferase (GST) could affect the specific tissues; and these compounds

are more reactive than in normal tissues. These phenomena are especially evident in the kidneys according to Ćorić et al., (2010). These more reactive intermediates damage the kidney tissues directly, and active mammalian glutathione S-transferase (GST) enzymes are required for the formation of such intermediates that could be contributed to the causal effect of neonatal deaths observed in this study. Moreover, Guanggang et al. (2012) cited that methomyl is a strongly genotoxic agent that induces cell DNA damage and apoptosis in vitro at sublethal concentrations. And according to Wang, et al. (1998), the mutagenic potential of N-nitroso-N-methylcarbamates was much higher than those of many other known mutagenic nitroso compounds, as well as some non-nitroso mutagenic alkylating agents as observed in a study using Chinese hamsters. Mugford and Kedderis (1997) as cited by Wang, et al. (1998) pointed out that chemicals that produce DNA damage indirectly through cytotoxic mechanisms have been reported, however, since N-nitroso methylcarbamates (NOCs) also induced a very high frequency of gene mutation, they probably do not damage cells through this mechanism. Although the offspring that died were not autopsied in this study, it is likely that the causes of death were attributed to serious defects in the respiratory, circulatory, digestive or excretory organs due to mutations induced by methomyl in sperm cells of the bucks. Furthermore, the treatment did not cause embryo loss as reflected in fig. 2. The mean percentage of embryo loss observed in both treated and control groups which was about 38% which falls within the normal rate of embryo loss in rabbits which is 30-40% (Lebas et al., 1986). Embryo losses are usually very extensive that in general only 60 to 70 percent of the eggs will become live rabbits (Lebas et al., 1997).

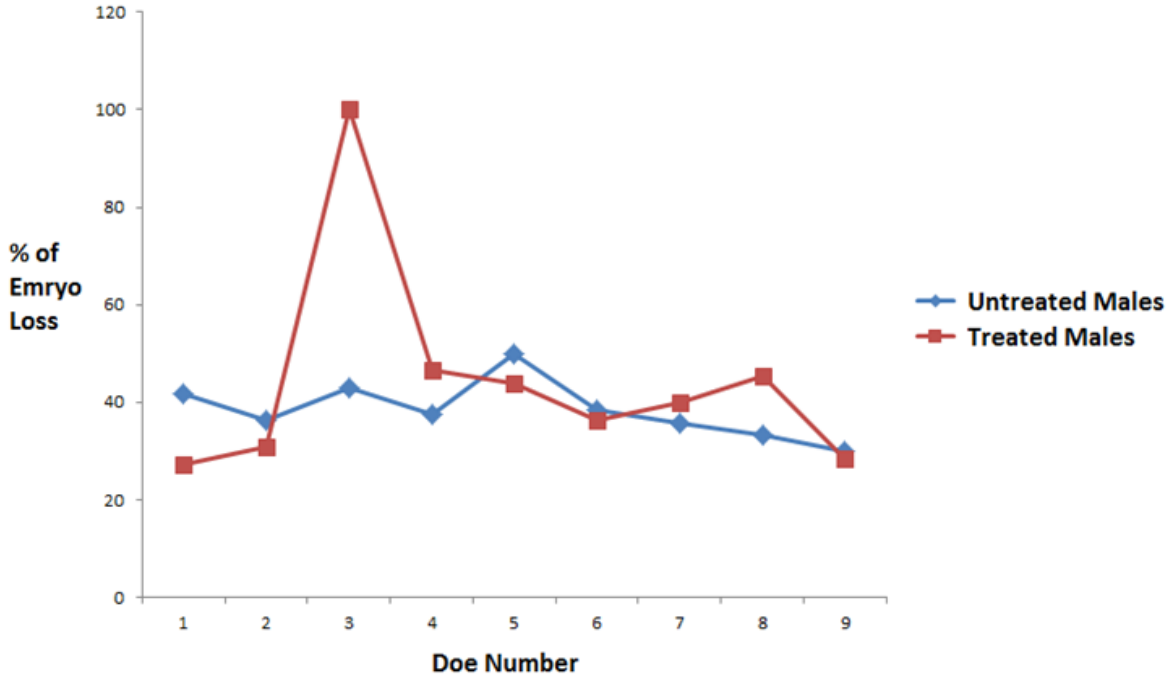

Figure 2. Percentage of embryo loss in progeny of untreated and treated males with non-lethal dose of Methomyl

Table 2. Embryonic loss and neonatal death after birth of progeny of untreated males and treated males with non-lethal dose of Methomyl

No. of weeks	Treatments	Mean of Neonatal Mortality	Computed t-value	Interpretation
One week	Untreated	37.24 ± 17.74	-3.212	highly significant
	Treated	69.87 ± 24.78		
Two weeks	Untreated	23.619 ± 33.20	-5.29	highly significant
	Treated	86.03 ± 12.19		

There was no reduction in the number of progeny of treated males at birth, hence it appears that the chronic exposure to a low dose of methomyl had no effect on sperm production or viability of spermatozoa as depicted in table 2. Rather, the embryonic loss and neonatal death after birth could be attributed only to dominant lethal mutations induced by the treatment in the spermatozoa of treated males. This is in consonance with study of Fausto et al. (2001) on the exposure to a low dose of Lindane which induces certain toxicity on spermatozoa ultrastructure of male rabbits, even though general toxic symptoms were not observed in the animals.

The result of this study is interesting because the dose used has been declared as the Maternal No Observed Effect Level (NOEL) in rabbits (E.I. DuPont de Nemours, 1989). Moreover, the highest dose of methomyl tested in rabbits did not cause teratogenic or embryo toxic effects. Besides, a dietary dose of 2.5 or 5mg/kg of methomyl fed to rats for three generations caused no adverse effect on reproduction, nor was there any evidence of congenital abnormalities; the NOEL in this study was 5 mg/kg (Hayes, 1982). The observed dominant lethal effect of methomyl shows that it can interact with DNA and cause mutations.

Bonatti, et al. (2006) established that methomyl and the methomyl-containing technical formulation "Lannate 25" products induced dose-dependent increases in chromosome aberrations and micronuclei. In addition, in a study conducted by Wang et al.,(1998),N-nitroso methylcarbamates (NOCs) are highly mutagenic to mammalian cells. Also, methomyl showed cytogenetic potential in human lymphocytes *in vitro* as indicated by an increase in micronuclei and chromosomal aberrations. However, it did not show mutagenicity or caused primary DNA damage in bacterial or mammalian cells *in vitro* and in an *in vivo* rat bone marrow chromosomal study (The International Programme on Chemical Safety-IPCS, 1996). Interestingly, the present findings are in contrast to previous reports that methomyl is not mutagenic in several assays including Ames test, a reverse mutation assay, a recessive lethal assay, DNA damage studies, an unscheduled DNA synthesis assay, and *in vivo* and *in vitro* cytogenetic assays (Hayes, (1982); DuPont Agricultural Products, (1991) thus, further researches must be undertaken to resolve conflicting results in evaluating potential risks of insecticides to human health.

4.0 Conclusions

Chronic exposure of non-lethal oral dose of methomyl does not exhibit direct effects to male rabbits but it induced dominant lethal

mutations that resulted to mortality of the neonatal offspring. Based on the results, the claim that methomyl is a non-genotoxic and non-mutagenic substance should be reevaluated.

Acknowledgement

The author* is grateful to Dr. Cesar A. dela Seña, Ph.D. of MSU-Marawi, her thesis adviser, panel members of the Biology Department and Prof. Diogenes Apao for the critical comments made and brilliant ideas shared in the study.

References

Bonatti, S., Bolognesi, C., Degan, P. & Abbondandolo, A. (2006). Genotoxic effects of the carbamate insecticide methomyl. I. *In vitro* studies with pure compound and the technical formulation "Lannate 25". *Environmental and Molecular Mutagenesis, 23(4), 306-311.* doi: 10.1002/em.2850230408.

Ćorić, V., Plješa-Ercegovac, M., Matić, M., Krivić, B., & Šuvakov, S. (2010). The role of GSTM1 and GSTT1 polymorphism in patients with renal cell carcinoma. *J. Medi Biochem 29: 204–210.* doi: 10.2478/v10011-010-0025-8.

DuPont Agricultural Products. (1991). *MSDS for methomyl technical.* DuPont, Wilmington, DE.

DuPont Agricultural Products. (1991). *MSDS for lannate insecticide.* DuPont, Wilmington, DE.

El-bendary, H. M. (2014). Biochemical effects of methomyl on experimental animals. *Applied Science Reports, 1(2), 2014: 74-78.* doi:10.15192/ PSCP.ASR.2014.1.2.7478

Erickson, W. and Turner, L. (2003). *Methomyl analysis of risks to endangered and threatened salmon and steelhead.* Retrieved from www.epa.gov/espp/litstatus/effects/ methomyl-analysis.pdf on May 5, 2013 .

Fausto, A.M., Morera, P., Margarit, R., & Taddei, A. R. (2001). Sperm quality and reproductive traits in male offspring of female rabbits exposed to Lindane (g-HCH) during pregnancy and lactation. *Reprod Nutr Dev., 41 (3), 217-225. PMID: 11592719.*

Hayes, W. J. (1982). *Pesticides studied in man.* Baltimore, MD: Williams and Wilkins.

The International Programme on Chemical Safety (IPCS) (1996). *Methomyl* (Environmental health criteria ; 178).

Lebas, F., Coudert, P., de Rochambeau, H., Thebault, R.G. (1986). *The rabbit.* Food & Agriculture Organization of the United Nations, Rome.

Lebas, F., Coudert, P., de Rochambeau, H., Thebault, R.G. (1997). *The rabbit husbandry, health and production.* Food & Agriculture Organization of the United Nations, Rome.

Salem, M. H., Zahraa Abo-Elezz, Abd-Allah, G. A., Hassan, G. A. & Shaker, N. (2008). Effect of organophosphorus (dimethoate) and pyrethroid (deltamethrin) pesticides on semen characteristics in rabbits.

Journal of Environmental Science and Health Part B., 23 (3): 279-90. doi: 10.1080/03601238809372604

Veeramachaneni, D. (2002, September 30). *Anti-androgenic pesticides: Impact on male reproduction.* 2002 Progress Report.

U.S. Environmental Protection Agency. (1987). *Health advisory: Oxamyl*. Office of Drinking Water, Washington, DC.

Yousef M.I., Salem, M.H., Ibrahim, H.Z., Helmi, S., Seehy, M.A., Bertheussen, K. (2008). Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits. *Journal of Environmental Science and Health, 30(4), 513-534. doi: 10.1080/03601 239509372951.*