EFFECT OF SOME INSECTICIDES (METHOMY AND IMIDACLOPRID) ON EXPERIMENTAL RATS

By

NAGLA KAMAL YOUNES HASSAN

B.Sc Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., Egypt, ١٩٩٨

M.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., Egypt, ٢٠٠٤

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences

(Biochemistry)

Department of Biochemistry
Faculty of Agriculture
Cairo University
EGYPT

٢٠٠٨

APPROVAL SHEET

EFFECT OF SOME INSECTICIDES (METHOMY AND IMIDACLOPRID) ON EXPERIMENTAL RATS

Ph.D. Thesis
By

NAGLAA KAMAL YOUNES HASSAN

B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., Egypt, ١٩٩٨

M.Sc. Agric. Sci (Biochemistry), Fac. Agric., Cairo Univ., Egypt, ٢٠٠٤

Approved by:

Dr. HASSAN MOHAMED SALEM .....
Professor of Biochemistry ,Fac. Agric., Cairo University.

Dr. ABDEL-KADER MORSEY ABDEL-SAMAD .....
Professor of Biochemistry, Fac. Agric., Cairo University.

Dr. NAGEY ABDEL-SALAM IBRAHIM .....
Professor of Pesticides, Cent. Agric., Pesticides Lab.

Date: / /٢٠٠٨

SUPERVISION SHEET

EFFECT OF SOME INSECTICIDES (METHOMY AND IMIDACLOPRID) ON EXPERIMENTAL RATS

Ph.D. Thesis

By

NAGLAA KAMAL YOUNES HASSAN

B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., Egypt, ١٩٩٨

M.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., Egypt, ٢٠٠٤

SUPERVISION COMMITTEE

Dr. HASSAN MOHAMED SALEM

Professor of Biochemistry, Fac. Agric.,Cairo University

Dr. MOHAMAD SAAD ABDEL-LATIF

Lecture of Biochemistry, Fac. Agric., Cairo University

Dr. OLFAT ABDEL-LATIF SAYED

Senior Researcher of Pesticides, Cent. Agric., Pesticides Lab.

Name of Candidate: Naglaa Kamal Younes Hassan **Degree:** Ph.D.
Title of Thesis: Effect of some insecticides (methomyl and imidacloprid) on experimental rats.
Supervisors: Prof. Dr.Hassan Mohamed Salem, Dr. Mohamed Saad Abdel-Latif and Dr. Olfat Abdel-Latif Sayed
Department: Biochemistry
Branch: Biochemistry
Approval: ١٥ / ٣ /٢٠٠٨

ABSTRACT

The effect of daily oral administration of Carbamate (Methomyl) ,at ٠,٢ , ٢ and ٥ mg/kg b.w and neonicotinoid (Imidacloprid), at ٠,٥ ,١ and ٥ mg/kg b.w insecticides on male albino rats for ٢٨ successive days, followed by ١٥ days recovery were studied . The most important histopathology and biochemical parameters of the serum ; alanine aminotranseferase (ALT) , aspartate aminotraseferase (AST) ,alkaline phosphatase activities (ALP) , total protein , protein profile (albumin and globulin) , urea , creatinine , cholesterol , triglycerides , cholinesterase activity were investigated.

The activities of AST, ALT were significantly increased at all tested times in cumulatively dose related manner .No significant changes were observed in alkaline phosphatase enzyme activity compared with the control. On the other hand serum total protein , Protein Profile (albumin and globulin) recorded highly significant decrease in all treatments with methomyl and imidacloprid . A highly significant increase in the blood urea and creatinine with two tested insecticides in the following order, methomyl and imidacloprid after treatments ١٤ days were observed.

The levels were decreased during the ٢١, ٢٨ and ٤٥days. methomyl significantly increased the rat serum triglycerides and cholesterol at different used concentrations for ١٤ ,٢١ ,٢٨ and ٤٥ days but imidacloprid significantly decreased the rat serum triglycerides and cholesterol at different used concentrations for ١٤ ,٢١ ,٢٨ and ٤٥ days .compared with the control .

Methomyl and imidacloprid treatments significantly reduced the rat cholinesterase activity at different used concentrations. The highest residues of methomyl and imidacloprid in organs after treatments for ٣٠ days were detected in the liver, kidney, brain and testes. At the end of experiment period recovery (٤٥ days) residues were reduced in different organs.

Key word : Methomyl , imidacloprid , Histopathology , Biochemical , rats , animals

ACKNOWLEDGEMENT

Many thanks and praise be to great Allah most gracious who shined my way and supported me with patience and perseverance to fulfill this humble work.

I wish to express my sincere thanks, deepest gratitude and appreciation to ***Dr. Hassan Mohamed Salem and Abdel Kader Morsy Abdel-Samad*** *Professors of Biochemistry Faculty of Agriculture, Cairo University for suggesting the problem, supervision, continued assistance and their guidance through the course of study and revision the manuscript of this thesis. Sincere thanks to* ***Dr. Mohamed Saad Abdel- Latif*** *Professors of Biochemistry Faculty of Agriculture, Cairo University for sharing in supervision.*

Many thanks and gratitude are also extended ***Dr. Olfat Abdel – Latif Sayed*** *Senior Researcher of pesticides central Agric .Pesticides Lab, for sharing in supervision.*

Many thanks and gratitude are also extended to ***Dr. Kamal El-Din Abdel-Hamid*** *Researcher ,* ***Dr. Ibrahim El-Dosoky and .Dr. Basem El-Sayed*** *Senior Researcher in pesticides central Agric .Pesticides Lab, for sharing in supervision.*

Many thanks and gratitude are also extended to ***Dr. Eman Abdl – El-Mattaleb*** *Senior Research in pathology Department animal Health Research institute .*

INTRODUCTION

Pesticides have been useful in agriculture pest control, there is considerable risk for human health and damage to ecosystems (Moreno *et al.*, ٢٠٠٧). Carbamates inhibit the enzyme acetyl cholinesterase (ACHE) which is present in erythrocyte and plasma in man (Rama and Jaga, ١٩٩١and Padilla *et al.*,٢٠٠٧). Carbamates affect on the human central nervous system (Hoogduijn *et al.*, ٢٠٠٦), also cause significant changes in total serum lipids glucose, protein levels AST, ALT, acid phosphatase and alkaline phosphatase activities in mammals (Sadek *et al.*,١٩٨٩;Fayez and Kilgore,١٩٩٢ and Chevalier *et al.*,١٩٩٣). They affect liver glucose ٦-phosphatase and liver succinic acid dehydrogenase (Fayez and Kilgore,١٩٩٢) Kidney and liver AST and ALT activities(Kiran *et al.*,١٩٨٨ and Saleh, ١٩٩٠a) . Carbamates have toxic symptoms and physiological changes in different animals. Toxic effects of carbamates were noticed in frogs and birds (Mullie *et al.*,١٩٩١)and suspected cause of death in ducks (Yuningshi and Dan,١٩٨٥).Methomyl cause high significant increase in the blood urea ,uric acid and creatinine in rats (Zidan *et al.*, ١٩٩٨).

Imidacloprid is a neonicotinoid insecticide which produces neurotoxicity through binding or partial binding to specific areas of the nicotinic acetylcholine receptor(Anatra-Cordone and Durkin ,٢٠٠٥). Imidacloprid is an agonist at the nicotinic acetylcholine receptor ,and as

such it is highly effective against many sucking insects (Worthing,١٩٩٤;Elbert *et al*.,١٩٩٨).This investigation was undertaken to study the effect of methomyl (carbamate) and imidacloprid(neonicotinoid) insecticides on liver function (ALT ,AST and alkaline phosphatase (ALP) activities , total protein , albumin and globulin, , kidney function (urea and creatinine), lipid profile (cholesterol and triglycerides) , cholinesterase activity and histopathological in male albino rats .

REVIEW OF LITERATURE

١. Imidacloprid

a. Effect of imidacloprid on body weight

Block (١٩٨٧) examined the subchronic toxicity of imidacloprid (٩٢,٨ %) in Beagle dogs by administrating it through the diet for a period of ٤ weeks which included four group of dogs, each containing two males and two females. The doses were ٠, ٢٠٠, ١٠٠٠ and ٥٠٠٠ ppm, which corresponded to ٠, ٧,٣, ٣١ and ٤٩ mg/kg /day . All animals in the ٥٠٠٠ ppm dose group died or were sacrificed prior to the completion of the study. The first dog died after only ٢ days of the treatment; the other three dogs died on day ١٨ or day ٢٤. The clinical signs for the dying animals included marked reduction in food intake, weight loss (up to ٤٢ %) .The lower tested dose of imidacloprid ١٠٠٠ ppm in the body weights of these dogs was not affect by the treatment.

Eiben (١٩٨٨a) evaluated the toxicity of imidacloprid (٩٢,٨ %) in mice (١٠ / sex / dose) for period of ١٠٧ days . The dietary levels were ١٢٠, ٦٠٠ and ٣٠٠٠ ppm , which reportedly corresponded to ٧٧ , ٣٩٧ and

٢٣٢٣ mg/kg /day (males) and ٩١, ٤٥٣ and ٣٠٧٥ mg/kg /day (females). The animals in the ٣٠٠٠ ppm dose group were in poor general condition, had rough coats and markedly lower body weights. The average body weight of the males and females in this group was ١٥ % and ٢٧ % (P≤٠,٠١) respectively, lower than the control. The food consumption at this dose was distinctly higher (١١ % males and ٥١ females) compared to the control, thus indicating that the reduction in body weight was caused by the treatment. Lower body weight (٥ %, P≤٠,٠١) was also reported for the males at the ٦٠٠ ppm dose group.

In two chronic toxicity /oncogenicity studies, imidacloprid (٩٥,٣ % a.i) was administered to mice (٥٠/sex /dose) for a period of ٢٤ months. The dietary levels were ٠,١٠٠, ٣٣٠ ٠r ١٠٠٠ ppm (Watta –Gebert ,١٩٩١a) and ٠ and ٢٠٠٠ppm (Watta –Gebert ,١٩٩١b). Ten more mice /sex /dose were used for interim examinations after ١٢ months of treatment. The two studies had similar protocols and therefore, were evaluated together. The reported average daily doses were ٠,٢٠ ,٦٦ ,٢٠٨ or ٤١٤ mg/kg/day for males and ٠, ٣٠ , ١٠٤ ,٢٧٤ and ٤٢٤ mg/kg /day for females . The doses were based on the mean daily food consumption ranging from ٦,٢ to ٦,٥ g/male /day and from ٧,٤ to ٨,٥ g/female day. This food intake represented about ٢٢ – ٢٨ % of the body weight of an adult mouse.

Pauluhn (١٩٨٩) assessed imidacloprid (٩٥,٢ %) for subchronic inhalation toxicity in Wister rats. Ten rats /sex /dose were exposed by head /nose only to imidacloprid in the from of dust. The exposure time was ٦ hours /day, ٥ days /week over a period of ٤ weeks. The concentrations of imidacloprid were ٠, ٥,٥, ٣٠,٥ and ١٩١,٢ mg/m^{٣} /day. The control groups received air alone. The principal toxicological findings were the reduction in body weight gains (٦ –٩ P≤٠,٠١)of the male rats from the ١٩١,٢ mg /m^{٣} /day group and the concentration-dependent increase of ٧- ١٤ in the absolute and relative (to body weight) liver weights of females treated with ٣٠,٥ and ١٩١,٢ mg /m^{٣} /day imidacloprid .

Ruf (١٩٩٠) administered imidacloprid to Beagle dogs (Bor: Beag strain; ٤ dogs /sex /dietary level) as food mash at doses of ٠, ٢٠٠, ٦٠٠ or ١٨٠٠ ppm for ١٣ weeks. The ١٨٠٠ ppm produced a drastic reduction in body weight (٨- ٢٠ % less than control) within the first ٤ weeks .This effect was, at least in part, due to the ٣٠ – ٥٤ % decrease in the food intake .Because of the low food consumption, the concentration of imidacloprid was thereafter reduced from ١٨٠٠ to ١٢٠٠ppm until the completion of the study. Nevertheless, the average body weight in the high-dose animals remained lower than the control by ٦ % (females) and ٩ % (males)at the completion of the study at week ١٣.

Eiben (١٩٩١) found that chronic exposure of Wister rats to ١٨٠٠ ppm imidacloprid resulted in substantial reduction in body weights in both sexes at all times .The weight decline reached maximum of ١١ - ١٢ %, (P≤٠,٠١) at week ١٠. About ٥ to ٨ % (P≤٠,٠١) decrease in body weight was observed in males and females at the ٩٠٠ ppm dietary level. The reduction in the body weight was clearly treatment –related .

Becker and Biedermann (١٩٩٢) examined the developmental toxicity of imidacloprid in the rabbit. Mated Chinchilla rabbits (١٦ /dose level) were treated by gavage from Gestation day (GD) ٦ through ١٨ with daily dosage of ٨, ٢٤ and ٧٢ (mg/kg /day). The mean body weight was decreased by ٤ % on day ٢ of treatment (GD ٨). The weight loss became significantly lower than the controls within ٥ days of treatment (٨ -١١ %, P≤٠,٠١).The fetuses from these dams had a reduced body weight (١٠ % , P≤٠,٠١)and delayed ossification . The next lower dose (٢٤ mg/kg /day) caused a decrease in food consumption (١٦ % P≤٠,٠١) and a reduction in body weight gain of the dams (٣٣ % , not statistically significant) , compared to control animals .

Sheets (١٩٩٤)administrated imidacloprid (٩٨,٨ %) to Fischer -٣٤٤ rats (١٢/sex /dose) at dietary levels of ٠, ١٥٠ ,١٠٠٠ and ٣٠٠٠ for a period of ١٣ week , During most of the exposure period , imidacloprid caused a reduction in body weights in both sexes at ١٠٠٠ (up to ٥ %

,females ; ٨ % , males ; P≤٠,٠٥) and ٣٠٠٠ ppm (up to ٩ % , females ; ١٧ % males ; P≤٠,٠٥) . This effect was due, at least in part , to a decreased in food consumption (up to ١٣ % and ٢٩ %)for the animals at ١٠٠٠ ppm and ٣٠٠٠ ppm , respectively .

b. Effect of imidacloprid on liver functions

Eiben (١٩٨٨b) found higher serum AP activity (up to, ٤٧ % P≤٠,٠١) in mice males and females treated with ٣٠٠٠ ppm imidacloprid.

Allen *et al* .(١٩٨٩) administered imidacloprid through the diet for period of ٥٢ weeks to Beagle dogs (٤/ sex/dietary level) at ٢٠٠, ٥٠٠, ١٢٥٠ ppm. The ١٢٥٠ ppm dose was increased to ٢٥٠٠ ppm from week ١٧ to the end of the treatment. Effects at ١٢٥٠ /٢٥٠٠ ppm included an increase in the metabolic activity in the liver and liver cytochrome P-٤٥٠ enzymes for both sexes (٥١ -٩٣ % P≤٠,٠١).

Pauluhn (١٩٨٩) studied the effects of imidacloprid on Wistar rats after ٤- weeks of inhalation .Head /nose-only were exposed to imidacloprid clearly induced hepatocellular damage and impairment of the liver function in females. The activities of the serum ALT, AP and bilirubin were measured by ٢٥ %, ٧٠ and ٢٥ % P≤٠,٠١), respectively. Finally, marked induction of the hepatic mixed function oxidases (MFO) of ٣٤- ٨٣ % (P≤٠,٠١) was observed in the animals treated with ٣٠,٥ and ١٩١,٢ mg/m^{٣} /day imidacloprid.

Suter *et al*. (١٩٩٠) examined the effects of imidacloprid (٩٥ .٣ %) on reproduction and development in two generation, two-litter study in Wistar rats. The dietary doses were ١٠٠, ٢٥٠ and ٧٠٠ ppm. Liver enzymes participating in the biotransformation were of xenobiotics (cytochrome P-٤٥٠, O-demethylase and N-demethylase were also induced in the maternal animals (up to ٣٧ % , P≤٠,٠١)at ٢٤ months .

Eiben (١٩٩١) observed no changes in liver morphology in Wister rats exposed to imidacloprid. While there were some indications of liver toxicity for both sexes at ١٨٠٠ ppm based on alteration in serum chemistry. These included elevated activities of serum AP (up to ٣٧ %, P≤٠,٠١) at ٦, ١٢ and ١٨ months and aspartate aminotransferase (AST, ٤٣٪ P≤٠,٠١).

Haschek and Rousseaux (١٩٩٨) concluded that the following changes in the serum chemistry of the rats exposed to ٢٤٠٠ ppm imidacloprid were indicative of hepatotoxicity . Elevated activities in the serum of alkaline phosphatase (AP, ١٥ % P≤٠,٠١) and alanine aminotransferase (ALT , ٢٥ % P≤٠,٠١). Decreased levels of protein (٨ % P≤٠,٠٥), albumin (٦ % P≤٠,٠٥).

El-Kashoury (١٩٩٩) found that imidacloprid caused a significant decrease in ALT and ALP activates in rats .