

**TRANSFER OF PESTICIDE RESIDUES TO MICE  
OFFSPRING THROUGH PRENATAL AND  
LACTATION EXPOSURE**

By

**MARWA FAROUK AHMED MOHAMED GAD**

B.Sc. Agric. Sci. (Pesticide Chemistry), Alex., University (2003)

M.Sc. Agric. Sci. (Pesticides), Cairo University (2011)

**A Thesis Submitted in Partial Fulfillment  
Of  
The Requirements for the Degree of**

**DOCTOR OF PHILOSOPHY  
in  
Agricultural Sciences  
(Pesticides)**

**Department of Plant Protection  
Faculty of Agriculture  
Ain Shams University**

**2018**

**Approval Sheet**

**TRANSFER OF PESTICIDE RESIDUES TO MICE  
OFFSPRING THROUGH PRENATAL AND  
LACTATION EXPOSURE**

By

**MARWA FAROUK AHMED MOHAMED GAD**

B.Sc. Agric. Sci. (Pesticide Chemistry), Alexandria University, 2003  
M.Sc. Agric. Sci. (Pesticide Chemistry), Cairo University, 2011

**This Thesis for Ph.D. degree has been approved by:**

**Dr. Mounir Daoud Abd-Allah** .....

Prof. Emeritus of Pesticides, Faculty of Agriculture, Cairo University.

**Dr. Kadry Weshahy Mahmoud** .....

Prof. Emeritus of Pesticide Chemistry and Toxicology, Faculty of  
Agriculture, Ain Shams University

**Dr. Khaled Abdel Aziz Mohamed** .....

Prof. of Pesticide Chemistry and Toxicology, Faculty of Agriculture,  
Ain Shams University.

**Dr. Mohamed Ibrahim Abdel-Mageed** .....

Prof. Emeritus of Pesticide Chemistry and Toxicology, Faculty of  
Agriculture, Ain Shams University.

**Date of Examination:**    /    / 2018

# **TRANSFER OF PESTICIDE RESIDUES TO MICE OFFSPRING THROUGH PRENATAL AND LACTATION EXPOSURE**

By

**MARWA FAROUK AHMED MOHAMED GAD**

B.Sc. Agric. Sci. (Pesticide Chemistry), Alex., University (2003)

M.Sc. Agric. Sci. (Pesticides), Cairo University (2011)

**Under the supervision of:**

**Dr. Mohamed Ibrahim Abdel-Mageed**

Prof. Emeritus of Pesticide Chemistry and Toxicology, Dept. of Plant Protection, Faculty of Agriculture, Ain Shams University  
(Principal Supervisor)

**Dr. Khaled Abdel Aziz Mohamed**

Prof. of Pesticide Chemistry and Toxicology, Dept. of Plant Protection, Faculty of Agriculture, Ain Shams University

**Dr. Sameeh Abdel Kader Mansour**

Researcher Prof. Emeritus of Pesticide Chemistry and Environmental Toxicology, Pesticide Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

## ABSTRACT

**Marwa Farouk Ahmed Mohamed Gad. “Transfer of Pesticide Residues to Mice Offspring through Prenatal and Lactation Exposure”. Unpublished Ph.D. Thesis, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, 2018**

This study evaluates toxicity of three pesticides [atrazine (A), chlorpyrifos (C) and endosulfan (E)], and tertiary mixture (ACE) which were mixed in the rodent diet at their respective “Acceptable Daily Intake, ADI” (e.g., 31.0, 44.0 and 30.0 mg kg<sup>-1</sup>/bw/day, respectively), in addition to oral administration of vitamin E ( $\alpha$ -tocopherol; 100 ul/mouse). The experimental protocol included treatments during gestation and lactation periods separately. Either during gestation or lactation, the mouse dams were received one of the following treatments: (a) diet free of pesticides; (b) diet enriched with one of the tested pesticides (c) diet free of pesticides + oral vitamin E; and (d) diet enriched with one of the tested pesticides + oral vitamin E. Drinking water was allowed *ad libitum* up to 42 days. After the weaning, pups and dams were killed and selected organs and blood samples were collected for analyses. Compared with the control results either in dams or their pups, the tested pesticides induced high elevation in AST, ALT, ALP, urea and MDA and high decline in BuChE, SOD and CAT. Furthermore, the tested pesticides caused histopathological damage in the internal organs (e.g., liver, kidney, ovary and testis). The ameliorative effect of vitamin E, based on estimating the “Amelioration Index; AI” revealed the powerful effect of this vitamin in alleviating the oxidative stress exerted by the tested pesticides. The findings of this study may support the need to further investigating the adverse effects of exposure to low doses of commonly used pesticides, especially during pregnancy and breast-feeding as well as effects on newborn child.

**Keywords:** Atrazine; Chlorpyrifos; Endosulfan; Pesticide Mixture; Vitamine E; Pregnant Mice ; Lactating Mice; Offspring; Oxidative Stress

## ACKNOWLEDGEMENT

All praises and thanks are due to **ALLAH**, who blessed me with kind professors and colleagues, and gave me the support to complete this thesis.

First of all, i have to record my deep appreciation to late Professor **Dr. Zidan H. Zidan**, Professor of Pesticide Chemistry and Toxicology, Faculty of Agriculture, Ain-Shams University, who shared preparation of the protocol of this thesis. I dedicate the results of this investigation to his spirit.

I am highly grateful and indebted to **Dr. Mohamed I. Abdel-Mageed**, Professor of Pesticide Chemistry and Toxicology, Faculty of Agriculture, Ain-Shams University, and **Dr. Sameeh A. Mansour**, Professor of Pesticides and Environmental Toxicology, National Research Centre, for their kind supervision, extreme cooperation, valuable guidance and continuous encouragement throughout the work of this thesis.

I would like to express my sincere appreciation to **Dr. Khaled A. Mohamed**, Professor of Pesticide Chemistry and Toxicology,, Faculty of Agriculture, Ain-Shams University, for his valuable guidance, encouragement and patience with me throughout editing, reviewing of this thesis.

I would like to express my gratitude to **Dr. Laurence Gamet-Pyrastre**, Professor of Food Science and Technology, TOXALIM, INRA/INP/UPS Research Centre in Food Toxicology, Toulouse, France, for sharing the protocol of this study, and introduced facilities and requirements for conducting the experimental work of animals in her institute.

Thanks are also due to **Dr. Doha A. Mohamed**, Professor of Food Science & Nutrition, National Research Centre, for supervising the biochemical analyses and reviewing the results.

Thanks are dedicated to the National Research Centre, who supported the work of this thesis and to the staff member of the Pesticide Chemistry Department for their continuous encouragement.

I cannot disclose this acknowledgment without thanking of my family for their patience, moral support and continuous encouragement.

# CONTENTS

	Page
<b>LIST OF TABLES.....</b>	<b>III</b>
<b>LIST OF FIGURES.....</b>	<b>IX</b>
<b>1. INTRODUCTION .....</b>	<b>1</b>
<b>2. REVIEW OF LITERATURE .....</b>	<b>5</b>
2.1. Adverse effects of exposure to atrazine.....	6
2.2. Adverse effects of exposure to chlorpyrifos.....	17
2.3. Adverse effects of exposure to endosulfan.....	27
2.4. Adverse effects of exposure to mixture.....	32
2.5. Protective effect of vitamin E.....	39
<b>3. MATERIALS AND METHODS.....</b>	<b>48</b>
3.1. Chemicals .....	48
3.2. Preparation of pesticide enriched feed .....	50
3.3. Animals.....	51
3.4. Experimental groups.....	52
3.5. Blood and body organs .....	55
3.6. Biochemical analyses .....	55
3.7. Histopathological studies .....	56
3.8. Detection of pesticide residues in mice liver .....	56
3.9. Statistics analysis .....	58
<b>4. RESULTS AND DISCUSSION.....</b>	<b>59</b>
4.1. Atrazin .....	59
4.1.1. Body and organs weights .....	59
4.2. Chlorpyrifos.....	64
4.2.1. Body and organs .....	64
4.3. Endosulfan .....	69
4.3.1. Body and organs weights from hibiscus and black mulberry	69
4.4. Mixture .....	74
4.4.1 Body and organs .....	75

## II

	Page
4.5. Biochemical alterations due to the tested insecticides.....	80
4.5.1. Aspartate amino transferase (AST) .....	80
4.5.2. Alanine amino transferase (ALT) .....	84
4.5.3. Alkaline phosphatase (ALP) .....	89
4.5.4. Buteryle Choline Esterase (BuChE) .....	92
4.5.5. Urea (mg/dl) .....	97
4.5.6 Malondialdehyde (MDA nmol/g tissue) .....	101
4.5.7. Super Oxide Dismutase (SOD u/g tissue) .....	105
4.5.8. Catalase (CAT in serum $\mu\text{mol}/\text{min}/\text{ml}$ ) .....	109
4.6. Assesment of antioxidative stress of the tested pestecticides and the ameliorative effect of vitamine E .....	115
4.6.1. Atrazine .....	115
4.6.2. Chlorpyrifos.....	118
4.6.3. Endosulfan.....	121
4.6.4. Mixture .....	124
4.7. Histopathological studies on the tested pesticides.....	127
4.7.1. Histological effect of atrazine .....	129
4.7.2. Histological effect of chlorpyrifos.....	134
4.7.3. Histological effect of endosulfan .....	139
4.7.4. Histological effect of mixture.....	144
4.8. Determination of pesticide residues in liver tissues.....	148
4.9. The thesis in a glance.....	150
4.9.1. Atrazine .....	152
4.9.2. Chlorpyrifos.....	152
4.9.3. Endosulfan.....	153
4.9.4. Mixture .....	153
<b>5. SUMMARY AND CONCLUSION .....</b>	<b>156</b>
<b>6. REFERENCES .....</b>	<b>160</b>
<b>ANNEX .....</b>	<b>187</b>
<b>7. ARABIC SUMMARY .....</b>	

### III

#### LIST OF TABLE

<b>Table No.</b>		<b>Page</b>
<b>Table 1.</b>	Pesticides used in the present study .....	<b>49</b>
<b>Table 2.</b>	Experimental groups, abbreviations (symbols) and system of dosing used throughout the study.....	<b>53</b>
<b>Table 3.</b>	Experimental groups, abbreviations (symbols) and system of dosing used throughout the study.....	<b>54</b>
<b>Table 4.</b>	Final body and organ weights of mouse dams and their pups treated with the atrazine, with and without supplementation of vit E, during gestation periods. ....	<b>60</b>
<b>Table 5.</b>	Relative organ weights of mouse dams and their pups treated with atrazine with and without Supplementation of vit E, during gestation periods.....	<b>61</b>
<b>Table 6</b>	. Body and organ weights of mouse dams and their pups treated with atrazine, with and without supplementation of vit E, during lactation periods. ....	<b>62</b>
<b>Table 7.</b>	.Relative body and organ weights of mouse dams and their pups treated with atrazine, with and without Supplementation of vit E, during lactation periods. ....	<b>63</b>
<b>Table 8.</b>	.Body and organ weights of mouse dams and their pups treated with chlorpyrifos, with and without supplementation of vit E, during gestation periods. ....	<b>65</b>
<b>Table 9.</b>	Relative Body and organ weights of mouse dams and their pups treated with chlorprifos with and without supplementation of vit E, during gestation periods. ....	<b>66</b>
<b>Table 10.</b>	Body and organ weights of mouse dams and their pups treated with chlopyrifos, with and without supplementation of vit E, during lactation periods. ....	<b>67</b>
<b>Table 11.</b>	Relative Body and organ weights of mouse dams and their pups treated with chlorpyrifos with and without supplementation of vit E during lactation periods. ....	<b>68</b>
<b>Table 12.</b>	Body and organ weights of mouse dams and their pups treated with the endosulfan, with and without supplementation of vit E, during gestation periods .....	<b>70</b>
<b>Table 13.</b>	Relative Body and organ weights of mouse dams and	<b>71</b>



## IV

Table No.		Page
	their pups treated with endosulfan, with and without supplementation of vit E, during gestation periods. ....	
<b>Table 14</b>	Body and organ weights of mouse dams and their pups treated with Endosulfan, with and without supplementation of vit E, during lactation periods. ....	<b>72</b>
<b>Table 15.</b>	Relative Body and organ weights of mouse dams and their pups treated with endosulfan with and without supplementation of vit E during lactation periods. ....	<b>73</b>
<b>Table 16.</b>	Final body and organ weights of mouse dams and their pups treated with the mixture, with and without supplementation of vit E, during gestation periods. ....	<b>76</b>
<b>Table 17.</b>	Relative organ weights of mouse dams and their pups treated with mixture with and without supplementation of vit E, during gestation periods. ....	<b>77</b>
<b>Table 18.</b>	Body and organ weights of mouse dams and their pups treated with mixture, with and without supplementation of vit E, during lactation periods. ....	<b>78</b>
<b>Table 19.</b>	Relative body and organ weights of mouse dams and their pups treated with mixture ,with and without Supplementation of vit E ,during lactation periods. ....	<b>79</b>
<b>Table 20.</b>	Activity of aspartate amino trasferase (AST) in serum of mouse dams and their pups following exposure during gestation to atrazine (ATZ), chlorpyrifos (CPF), endosulfan(END) and their mixture (MIX), with and without administration of vitamin E (V.E.). ....	<b>81</b>
<b>Table 21.</b>	Activity of aspartate amino trasferase (AST) in serum of mouse dams and their pups following exposure during lactation to atrazine (ATZ), chlorpyrifos (CPF), endosulfan(END) and their mixture (MIX), with and without administration of vitamin E (V.E.). ....	<b>83</b>

<b>Table No.</b>		<b>Page</b>
<b>Table 22.</b>	Activity of alanine amino transferase (ALT) in serum of mouse dams and their pups following exposure during gestation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E.....	<b>85</b>
<b>Table 23.</b>	Activity of alanine amino transferase (ALT) in serum of mouse dams and their pups following exposure during lactation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E.....	<b>87</b>
<b>Table 24.</b>	Activity of alkaline phosphatase (ALP) in serum of mouse dams and their pups following exposure during gestation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E....	<b>89</b>
<b>Table 25.</b>	Activity of alkaline phosphatase (ALP) in serum of mouse dams and their pups following exposure during lactation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E....	<b>91</b>
<b>Table 26.</b>	Activity of buteryle Choline Esterase (BuChE) in serum of mouse dams and their pups following exposure during gestation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E. ....	<b>93</b>
<b>Table 27.</b>	Activity of buteryle Choline Esterase (BuChE) in serum of mouse dams and their pups following exposure during lactation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E. ....	<b>95</b>
<b>Table 28.</b>	Concentration of urea (mg/dl) in serum of mouse dams and their pups following exposure during	

## VI

Table No.		Page
	gestation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E...	98
<b>Table 29.</b>	Concentration of urea (mg/dl) in serum of mouse dams and their pups following exposure during lactation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E...	100
<b>Table 30.</b>	Activity of malondialdehyde (MDA nmol/g tissue) in serum of mouse dams and their pups following exposure during gestation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E. ....	102
<b>Table 31.</b>	Activity of malondialdehyde (MDA nmol/g tissue) in serum of mouse dams and their pups following exposure during lactation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E. ....	104
<b>Table 32.</b>	Activity of super Oxide dismutase ( SOD u/g tissue) in tissue of mouse dams and their pups following exposure during gestation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E. ....	106
<b>Table 33.</b>	Activity of super Oxide dismutase ( SOD u/g tissue) in tissue of mouse dams and their pups following exposure during lactation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E. ....	108
<b>Table 34.</b>	Activity of catalase (CAT $\mu$ mol/min/ml) in serum of mouse dams and their pups following exposure during gestation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E...	110
<b>Table 35.</b>	Activity of catalase (CAT $\mu$ mol/min/ml) in serum of	113

## VII

<b>Table No.</b>		<b>Page</b>
	mouse dams and their pups following exposure during lactation to atrazine, chlorpyrifos, endosulfan and their mixture, with and without administration of vitamin E...	
<b>Table 36.</b>	Assessment of oxidative stress of the pesticide atrazine and the ameliorative effect of vitamin E based on measured biochemical parameters from gestation experimental results. ....	116
<b>Table 37.</b>	Assessment of oxidative stress of the pesticide atrazine and the ameliorative effect of vitamin E based on measured biochemical parameters from lactation experimental results. ....	117
<b>Table 38.</b>	Assessment of oxidative stress of the pesticide chlorpyrifos and the ameliorative effect of vitamin E based on measured biochemical parameters from gestation experimental results. ....	119
<b>Table 39.</b>	Assessment of oxidative stress of the pesticide chlorpyrifos and the ameliorative effect of vitamin E based on measured biochemical parameters from lactation experimental results. ....	120
<b>Table 40.</b>	Assessment of oxidative stress of the pesticide endosulfan and the ameliorative effect of vitamin E based on measured biochemical parameters from gestation experimental results. ....	122
<b>Table 41.</b>	Assessment of oxidative stress of the pesticide endosulfan and the ameliorative effect of vitamin E based on measured biochemical parameters from lactation experimental results. ....	123
<b>Table 42.</b>	Assessment of oxidative stress of the pesticide mixture and the ameliorative effect of vitamin E based on measured biochemical parameters from gestation and lactation experimental results. ....	125

## VIII

Table No.	Page
<b>Table 43.</b> Assessment of oxidative stress of the pesticide mixture and the ameliorative effect of vitamin E based on measured biochemical parameters from gestation and lactation experimental results. ....	126
<b>Table 44.</b> Histopathologic changes based on scoring severity of injury in different organs from mouse dams and their pups following exposure to pesticide atrazine (ATZ) with and without vitamin E during gestation. ....	130
<b>Table 45.</b> Histopathologic changes based on scoring severity of injury in different organs from mouse dams and their pups following exposure to pesticide atrazine (ATZ) with and without vitamin E during lactation. ....	133
<b>Table 46.</b> Histopathologic changes based on scoring severity of injury in different organs from mouse dams and their pups following exposure to pesticide chlorpyrifos (CPF) with and without vitamin E during gestation. ....	137
<b>Table 47.</b> Histopathologic changes based on scoring severity of injury in different organs from mouse dams and their pups following exposure to pesticide chlorpyrifos (CPF) with and without vitamin E during lactation. ....	138
<b>Table 48.</b> Histopathologic changes based on scoring severity of injury in different organs from mouse dams and their pups following exposure to pesticide endosulfan (END) with and without vitamin E during gestation. ....	142
<b>Table 49.</b> Histopathologic changes based on scoring severity of injury in different organs from mouse dams and their pups following exposure to pesticide endosulfan END with and without vitamin E during lactation. ....	143

## IX

<b>Table No.</b>		<b>Page</b>
<b>Table 50.</b>	Histopathologic changes based on scoring severity of injury in different organs from mouse dams and their pups following exposure to pesticide mixture with and without vitamin E during gestation.....	<b>145</b>
<b>Table 51.</b>	Histopathologic changes based on scoring severity of injury in different organs from mouse dams and their pups following exposure to pesticide mixture with and without vitamin E during lactation. ....	<b>147</b>
<b>Table 52.</b>	Percent of change in some biochemical parameters in the tested mice treated with the tested pesticides: comparisons. ....	<b>151</b>

## LIST OF FIGURES

Fig. No.		Page
<b>Fig. 1</b>	Effect of different insecticide treatments on AST activities in serum of mouse dams and their pups in gestation. ....	82
<b>Fig. 2</b>	Effect of different insecticide treatments on AST activities in serum of mouse dams and their pups in lactation. ....	84
<b>Fig. 3.</b>	Effect of different insecticide treatments on ALT activities in serum of mouse dams and their pups in gestation. ....	86
<b>Fig. 4</b>	Effect of different insecticide treatments on ALT activities in serum of mouse dams and their pups in lactation. ....	88
<b>Fig. 5</b>	Effect of different insecticide treatments on ALP activities in serum of mouse dams and their pups in gestation. ....	90
<b>Fig. 6</b>	Effect of different insecticide treatments on ALP activities in serum of mouse dams and their pups in lactation. ....	92
<b>Fig. 7</b>	Effect of different insecticide treatments on BuChE activities in serum of mouse dams and their pups in gestation. ....	94
<b>Fig. 8</b>	Effect of different insecticide treatments on BuChE activities in serum of mouse dams and their pups in lactation. ....	96
<b>Fig. 9</b>	Effect of different insecticide treatments on urea concentration in serum of mouse dams and their pups in gestation. ....	99
<b>Fig. 10</b>	Effect of different insecticide treatments on urea concentration in serum of mouse dams and their pups in lactation. ....	101