



**UNIVERSITI PUTRA MALAYSIA**

**THE EFFECT OF CARBOFURAN AND ENDOSULFAN ON THE  
AFRICAN CATFISH, *CLARIAS GARIEPINUS***

**ISAM ELDIN MOHAMED ELAMIN ABU ZEID**

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**By**

**ISAM ELDIN MOHAMED ELAMIN ABU ZEID**

**Thesis Submitted in Fulfilment of the Requirements for the  
Degree of Doctor of Philosophy in the Faculty of  
Science and Environmental Studies  
Universiti Putra Malaysia**

**February 2001**



## **DEDICATION**

To the memory of my late father, **MOHAMED ELAMİN** who left us alone while I am conducting this study, and to my beloved wife **GHADA** and sons **MOHAMED** and **RAZI** who were the source of inspiration and encouragement throughout the period of this study.



Abstract of the thesis presented to the Senate of Universiti Putra Malaysia  
in fulfilment of the requirement for the degree of Doctor of Philosophy

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**February 2001**

**Chairman: Assoc. Prof. Dr. Mohd. Arif Syed**

**Faculty: Science and Environmental Studies**

This study was undertaken to determine the toxic effects of sub-lethal concentrations of carbofuran and endosulfan on some behavioural, morphological, biaccumulation, biochemical, histopathological, and molecular aspects of the freshwater African catfish, *Clarias gariepinus*. The toxicity of carbofuran and endosulfan was ascertained by estimating the LC<sub>50</sub>.

The calculated 96-h LC<sub>50</sub> values of carbofuran and endosulfan for juveniles of *Clarias gariepinus* were found to be 10.4 p.p.m and 21.6 p.p.b respectively, under tropical condition. The test fish swam erratically, struggled to breath, often swam to the surface, followed by loss of equilibrium. The color of the skin became progressively pale during the period of the test, The liver glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and acetylcholinesterase (AChE) were determined photometrically. Within 16 days of treatment, the activity levels of GOT and GPT were significantly



( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ) increased by 203% and 121% for carbofuran and 167% and 195% for endosulfan respectively, whereas, AchE activity levels were inhibited following exposure to the test pesticides. With no exceptions, the *in vivo* and *in vitro* effects of carbofuran and endosulfan on GOT, GPT and AchE were qualitatively similar.

The pesticide concentration in the tissues was in the order of liver > intestine > gill > brain > muscle. The highest concentration of the test pesticides (353.47  $\mu\text{g/g}$  carbofuran and 1409.35  $\text{ng/g}$  endosulfan) were found in the liver 24 hours after treatment. Liver lesions were observed following exposure to both pesticides. The evidence of pesticide accumulation was elucidated by necrosis and damaged fish liver.

The depletion in the protein content was observed following exposure to carbofuran and endosulfan. The RNA concentrations were significantly decreased in *Clarias gariepinus* exposed to both pesticides, whereas, DNA concentrations tend to remain constant. The DNA molecular weight of the control fish was found to be about 16832 bp, whereas, the DNA molecular weights of carbofuran and endosulfan exposed fish were 14505 and 14505 bp respectively. The liver-somatic index decreased to 2.11 for carbofuran and 1.59 for endosulfan in comparison to control. The biochemical, molecular, bioaccumulation, histological, behavioural and morphological techniques employed in this study may be used to detect and assess any pesticidal pollution in the aquatic environment at an early stage of pollution.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

**KESAN KETOKSIKAN OLEH ENDOSULFAN DAN KARBOFURAN KE ATAS IKAN KELI AFRIKA, *CLARIAS GARIEPINUS***

Oleh

**ISAM ELDIN MOHAMED ELAMIN ABU ZEID**

**Februari 2001**

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Kesan ketoksikan endosulfan dan karbofuran ke atas perlakuan, morfologi, bioakumulasi, biokimia, histopathologi dan aspek molekul telah dikaji pada ikan keli Afrika dengan memberikan rawatan sublethal. Ketoksikan endosulfan dan karbofuran telah ditentukan nilainya dengan LC<sub>50</sub>.

Nilai LC<sub>50</sub> bagi rawatan 96 jam karbofuran dan endosulfan bagi *Clarias gariepinus* didapati masing-masing adalah 10.4 ppm dan 21.6 ppb. Ikan didapati menyelam tidak tentu hala, menghadapi masalah pernafasan, kerap muncul ke permukaan dan hilang dayaimbangan. Semasa ujikaji, warna kulit dilihat menjadi semakin pucat. Pada hati, enzim glutamat oxaloacetat transaminase (GOT), glutamat piruvat transaminase (GPT) dan asetilkolinesterase (AChE) ditentukan secara fotometrik. Semasa 16 hari rawatan, paras aktiviti GOT dan GPT adalah signifikan ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ). Paras masing-masing telah meningkat sebanyak 203% dan 121% untuk karbofuran, 167% dan 195% untuk endosulfan. Aktiviti AChE pula didapati terencat akibat pendedahan kepada racun

perosak tersebut. Dari pemerhatian *in vivo* dan *in vitro*, kesan karbofuran dan endosulfan ke atas GOT, GPT dan AchE adalah sama secara kualitatif.

Kandungan racun perosak pada tisu adalah tinggi menurut turutan hati>usus>insang>otak>otot. Kandungan tertinggi racun perosak (353.47µg/g karbofuran dan 1409.35ng/g endosulfan) di dalam hati, selepas 24 jam rawatan. Pemerhatian kerosakan hati telah dilakukan selepas dirawat racun perosak. Kesan pengumpulan racun perosak telah berjaya dibuktikan melalui kerosakan dan nekrosis pada hati tersebut.

Kandungan protein didapati berkurang pada ikan yang telah dirawat dengan carbofuran dan endosulfan. Jumlah RNA juga didapati menurun pada *Clarias gariepinus* yang terdedah kepada kedua-dua racun perosak ini manakala kepekatan DNA didapati tidak ada sebarang perubahan. Berat molekul DNA bagi kawalan adalah 16832 bp dan ikan terdedah kepada endosulfan dan carbofuran masing-masing berat molekulnya adalah 14505bp. Nisbah berat badan ikan kepada berat hati jika dibandingkan dengan kawalan didapati telah menurun kepada 2.11 bagi carbofuran dan 1.59 bagi endosulfan.

Kesimpulannya, teknik biokimia, molekul, bioakumulasi, histologikal, perlakuan dan morfologikal dapat digunakan bagi mengesan dan mengenalpasti pencemaran racun perosak di dalam persekitaran akuatik terutama di peringkat awal pencemaran berlaku.

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And above all to Almighty ALLAH, the Merciful and Benevolent.



I certify that an Examination Committee met on 2<sup>nd</sup> February 2001 to conduct the final examination of Isam El Din Mohamed El Amin Abu Zeid on his Doctor of Philosophy thesis entitled "The Effect of Carbofuran and Endosulfan on the African Catfish, *Clarias gariepinus*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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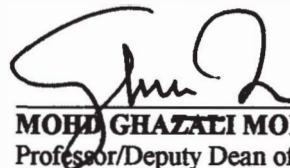
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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



**Isam El Din Mohamed El Amin**

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## TABLE OF CONTENTS

	Page
DEDICATION.....	ii
ABSTRACT.....	iii
ABSTRAK.....	v
ACKNOWLEDGEMENTS.....	vii
APPROVAL SHEETS.....	ix
DECLARATION FORM.....	xi
LIST OF TABLES.....	xvi
LIST OF FIGURES.....	xviii
LIST OF ABBREVIATIONS.....	xx
CHAPTER	
I INTRODUCTION.....	1
II LITERATURE REVIEW.....	4
Pesticides Problems in Malaysia.....	4
Pesticide Usage in Sudan.....	5
Toxicity of Pesticides to Fish.....	6
Behavioural Indicators of Pesticide Stress.....	7
Morphological Changes.....	8
Biochemical Changes.....	9
Effect of Pesticides on Fish Enzymes.....	9
Effect of Pesticides on other Biochemical Parameters in Fish.....	12
Histological Changes induced by Pesticides in Fish.....	14
Molecular Changes.....	15
Bioaccumulation of Pesticides in Fish.....	16
Techniques for Assessment of Stress on Aquatic Organisms.....	17
Life Cycle Tests.....	18
Toxicity Tests.....	18
Biological Markers and their Advantages in Environmental Monitoring.....	21
Effect of Pesticides on Catfish.....	23
Effect of Environmental Pollutants on African Catfish, <i>Claris gariepinus</i> .....	27
<i>Clarias gariepinus</i> as Test Organism.....	29
Carbofuran as Test Chemical and Fish Toxicity.....	30
The Toxic Effects of Endosulfan on Fish.....	34
Acetylcholinesterase (AChE) Inhibition.....	37
Mechanism of Inhibition of AChE by Carbamate and	



	Organophosphorus Insecticides.....	41
	Mechanism of Inhibition of AchE by Organochlorine Pesticides.....	41
	Alteration in Transaminase Enzymes as Indicators of Pesticides Stress.....	43
	Statement of the Problem.....	45
	Significance of the Study.....	47
	Objectives of the Study.....	50
<b>III</b>	<b>GENERAL MATERIALS AND METHODS.....</b>	<b>51</b>
	Experimental Design.....	51
	Acclimatisation of the Test Organism.....	52
	Water Quality Monitoring.....	52
	Experimental Set up.....	53
	Analysis of Different Parameters.....	54
	Data Collection.....	55
	Statistical Analysis.....	56
<b>IV</b>	<b>COMPARATIVE TOXICITY, BEHAVIOURAL AND MORPHOLOGICAL CHANGES INDUCED BY CARBOFURAN AND ENDOSULFAN IN THE AFRICAN CATFISH, <i>CLARIAS GARIEPINUS</i>.....</b>	<b>57</b>
	Introduction.....	57
	Materials and Methods.....	59
	Test Animal and Water.....	59
	Preliminary Toxicity Experiments.....	59
	Test Procedures.....	60
	Calculation of the LC <sub>50</sub> Values.....	61
	Results.....	62
	Discussion.....	68
	Conclusion.....	71
<b>V</b>	<b>BIOACCUMULATION OF RADIOLABELLED CARBOFURAN AND ENDOSULFAN IN DIFFERENT TISSUES OF AFRICAN CATFIS, <i>CLARIAS GARIEPINUS</i>.....</b>	<b>72</b>
	Introduction.....	72
	Materials and Methods.....	74
	Chemicals.....	74
	Test Organisms.....	74
	Test System and Sampling.....	74
	Determination of Pesticide Residues in Fish Tissues.....	75
	Statistical Analysis.....	76
	Results.....	76
	Discussion.....	81

Conclusion.....	86
<b>VI ENZYME MODULATION BY CARBOFURAN AND ENDOSULFAN IN THE LIVER OF AFRICAN CATFISH, <i>CLARIAS GARIEPINUS</i></b> .....	<b>87</b>
Introduction.....	87
Materials and Methods.....	88
Test Fish.....	88
Chemicals.....	89
Test Procedures.....	89
Preparation of Tissue Homogenate.....	90
Assay of the Test Enzymes.....	91
Statistical Analysis.....	91
Results.....	92
GOT, GPT and AchE Activity Levels of Control Fish.....	92
GOT Activity Levels in Fish Exposed to Pesticides.....	92
GPT Activity Levels in Fish Exposed to Pesticides.....	93
Effect of Pesticides on AchE Activity.....	102
Discussion.....	107
Conclusion.....	114
<b>VII HISTOLOGICAL CHANGES INDUCED BY CARBOFURAN AND ENDOSULFAN IN THE LIVER OF AFRICAN CATFISH, <i>CLARIAS GARIEPINUS</i></b> .....	<b>115</b>
Introduction.....	115
Materials and Methods.....	116
Test Fish.....	116
Light Microscopy.....	117
Transmission Electron Microscopy.....	117
Results.....	119
Light Microscopy.....	119
Transmission Electron Microscopy.....	119
Discussion.....	125
Conclusion.....	126
<b>VIII MOLECULAR CHANGES INDUCED BY CARBOFURAN AND ENDOSULFAN IN THE LIVER OF AFRICAN CATFISH, <i>CLARIAS GARIEPINUS</i></b> .....	<b>127</b>
Introduction.....	127
Materials and Methods.....	129
Analysis of Different Parameters.....	129
Statistical Analysis.....	130
Results.....	130



Discussion.....	134
Conclusion.....	136
<b>IX GENERAL DISCUSSION AND CONCLUSION.....</b>	<b>137</b>
<b>BIBLIOGRAPHY.....</b>	<b>147</b>
<b>APPENDICES.....</b>	<b>186</b>
<b>Appendix A: Transformation of percentage to probits.....</b>	<b>187</b>
<b>Appendix B: Tissue Processing by an Automatic                   Tissue Processor.....</b>	<b>188</b>
<b>BIODATA OF THE AUTHOR.....</b>	<b>189</b>





## LIST OF TABLES

Table		Page
1	LC <sub>50</sub> of some pesticides for different species of catfish.....	24
2	Carbofuran acute toxicity (LC <sub>50</sub> ) for some fish species.....	32
3	Endosulfan acute toxicity (LC <sub>50</sub> ) for some fish species.....	36
4	Percentage of dead fish, empirical probits, and approximate expected probits for the estimation of LC <sub>50</sub> of carbofuran for <i>C. gariepinus</i> .....	64
5	Percentage of dead fish, empirical probits, and approximate expected probits for the estimation of LC <sub>50</sub> of endosulfan for <i>C. gariepinus</i> .....	64
6	Bioaccumulation (µg/g dry wt.) of radiolabelled carbofuran in different tissues of <i>C. gariepinus</i> .....	78
7	Bioaccumulation (ng/g dry wt.) of radiolabelled endosulfan in different tissues of <i>C. gariepinus</i> .....	78
8	<i>In vivo</i> effect of sub-lethal concentrations of carbofuran and endosulfan on the activity levels (U/l) of GOT in the liver of <i>C. gariepinus</i> .....	94
9	<i>In vivo</i> effect of sub-lethal concentrations of carbofuran and endosulfan on the activity levels (U/l) of GPT in the liver of <i>C. gariepinus</i> .....	96
10	<i>In vitro</i> effect of sub-lethal concentrations of carbofuran and endosulfan on the activity levels (U/l) of GOT in the liver of <i>C. gariepinus</i> at different incubation time.....	98
11	<i>In vitro</i> effect of sub-lethal concentrations of carbofuran and endosulfan on the activity levels (U/l) of GPT in the liver of <i>C. gariepinus</i> at different incubation time.....	100
12	<i>In vivo</i> effect of sub-lethal concentrations of carbofuran	



	and endosulfan on the activity levels ( $\mu\text{mole}/\text{min}/\text{mg}$ protein) of AchE in the liver of <i>C. gariepinus</i> .....	103
13	<i>In vitro</i> effect of sub-lethal concentrations of carbofuran and endosulfan on the activity levels ( $\mu\text{mole}/\text{min}/\text{mg}$ protein) of AchE in the liver of <i>C. gariepinus</i> at different incubation time.....	105
14	Effect of carbofuran and endosulfan on total protein (mg/g wet wt.), total RNA and DNA ( $\mu\text{g}/\text{g}$ wet wt.), RNA/DNA ratio and liver somatic index in the liver of <i>C. gariepinus</i> at 16 days exposure.....	132
15	Transformation of percentage to probits.....	186
16	Tissue processing by an automatic tissue processor.....	187



## LIST OF FIGURES

Figure		Page
1	Schematic diagram of the possible directions of movement and fates of a pollutant after it has been absorbed into the bloodstream of fish.....	17
2	Chemical formula of carbofuran.....	31
3	Chemical formula of endosulfan.....	35
4	Diagrammatic representation of the reaction of acetylcholinesterase with its normal substrate and organophosphate or carbamate inhibitors .....	42
5	Determination of the LC <sub>50</sub> of carbofuran for <i>C. gariepinus</i> .....	65
6	Determination of the LC <sub>50</sub> of endosulfan for <i>C. gariepinus</i> .....	66
7	Morphology of the African catfish, <i>C. gariepinus</i> (control).....	67
8	Morphological changes in the African catfish, <i>C. gariepinus</i> exposed to carbofuran.....	67
9	Morphological changes in the African catfish, <i>C. gariepinus</i> exposed to endosulfan.....	67
10	Bioaccumulation of radiolabelled carbofuran in different tissues of <i>C. gariepinus</i> .....	79
11	Bioaccumulation of radiolabelled endosulfan in different tissues of <i>C. gariepinus</i> .....	80
12	<i>In vivo</i> effects of carbofuran and endosulfan on the GOT activity levels in the liver of <i>C. gariepinus</i> .....	95
13	<i>In vivo</i> effects of carbofuran and endosulfan on the GPT activity levels in the liver of <i>C. gariepinus</i> .....	97
14	<i>In vitro</i> effects of carbofuran and endosulfan on the GOT	



	activity levels in the liver of <i>C. gariepinus</i> .....	99
15	<i>In vitro</i> effects of carbofuran and endosulfan on the GPT activity levels in the liver of <i>C. gariepinus</i> .....	101
16	<i>In vivo</i> effects of carbofuran and endosulfan on the AchE activity levels in the liver of <i>C. gariepinus</i> .....	104
17	<i>In vitro</i> effects of carbofuran and endosulfan on the AchE activity levels in the liver of <i>C. gariepinus</i> .....	106
18	Light micrograph of control <i>C. gariepinus</i> liver.....	121
19	Light micrograph of carbofuran exposed <i>C. gariepinus</i> liver.....	121
20	Light micrograph of endosulfan exposed <i>C. gariepinus</i> liver.....	122
21	Electron micrograph of control <i>C. gariepinus</i> liver.....	123
22	Ultrastructural changes in the liver of <i>C. gariepinus</i> exposed to carbofuran.....	123
23	Ultrastructural changes in the liver of <i>C. gariepinus</i> exposed to endosulfan.....	124
24	Effect of pesticides on DNA pattern in the liver of <i>C. gariepinus</i> .....	133



## LIST OF ABBREVIATIONS

AchE	Acetylcholinesterase
ANOVA	Analysis of variance
Bp	Base-pair
DDVP	Dichlorvos
DO	Dissolved oxygen
EDB	Ethylene dibromide
EROD	Ethoxyresorufin O-deethylase
GOT	Glutamate oxaloacetate transaminase
GPT	Glutamate pyruvate transaminase
H & E	Hematoxylin and Eosin
H	Hepatic cell
HCH	Hexachlorocyclohexane
IAEA	International Atomic Energy Agency
LC <sub>50</sub>	Lethal concentration that results in 50% death.
LSC	Liquid scintillation counter
LSI	Liver-somatic index
MATC	Maximum acceptable toxicant concentration
MFO	Mixed function oxygenase
N	Nucleus
NC	Necrotic cell



<b>NRC</b>	<b>National Research Council</b>
<b>NRCC</b>	<b>National Research Council of Canada</b>
<b>NU</b>	<b>Nucleolus</b>
<b>PCB</b>	<b>Polychlorinated biphenyl</b>
<b>PCP</b>	<b>Pentachlorophenol</b>
<b>SAC</b>	<b>Safe application concentration</b>
<b>SD</b>	<b>Standard deviation</b>
<b>SDS</b>	<b>Sodium dodecylsulfate</b>
<b>SEM</b>	<b>Scanning electron microscope</b>
<b>TCDD</b>	<b>Tetrachlorodibenzo-p-dioxin</b>
<b>TEM</b>	<b>Transmission electron microscope</b>
<b>UPM</b>	<b>Universiti Putra Malaysia</b>



## CHAPTER I

### INTRODUCTION

The growing demand for increased food productivity to meet the needs of the global population has led farmers to use sophisticated agricultural technology in which pesticides play a crucial role. Pesticide use has a positive and dramatic impact on agricultural production through protection of crops against insects, pests and diseases. The extensive use of pesticides in agriculture has given rise to criticisms in recent years, due to their persistent nature in the environment (Hernandez *et al.*, 1993), and accumulation in different tissues of plants (Kaplan, 1999), animals (Miao *et al.*, 2000) and human beings (Saleh *et al.*, 1998). Therefore, the use of pesticides is a mixed blessing, while their benefits for preventing crop losses (Kacew *et al.*, 1996) and saving human lives (Emerson *et al.*, 1999) are well recognised, they very often result in unwanted side effects. Therefore, the identification of pesticides which are effective against pests and at the same time relatively safe to human and non-target organism is of considerable importance.

Among the numerous environmental impact of the application of pesticides in agriculture are their undesirable effect on the aquatic fauna of freshwater courses (Barlas, 1999). Pesticides have the potential to enter the aquatic environment by direct spraying or broadcast of granular formulations,

drift deposition of sprayable formulations, and in runoff water from treated field (Sharma, 1990). Redeposition from the atmosphere is another route of entry, as some studies have shown the presence of pesticides in rain water (Richards *et al.*, 1987). The pollution of the aquatic environment by pesticides is known to pose a constant threat to fish by altering their habitat, behavioural pattern, growth, and reproduction (Jarvian *et al.*, 1977). Fish is extremely sensitive to pollutants and exhibit a very high bioaccumulation rate of dissolved chemicals relative to their concentration (Al-Yousuf *et al.*, 2000). Fish may accumulate pollutants and pass them to human beings through food causing chronic or acute diseases (Adeyeye *et al.*, 1996). The importance of fish as one of the major source of cheap and available protein-rich food for human being is recognised (Begum and Vijayaraghavan, 1996). The nutritional value of different fishes depends on their biochemical composition; protein, amino acids, vitamins, and mineral contents (Ganeson *et al.*, 1989). Pesticides affect these biochemical composition and may cause biochemical and physiological changes in different fish tissues (Ramaswamy *et al.*, 1999).

Due to increasing pesticide applications, it has become necessary to evaluate their hazards and develop biological indicators of aquatic contamination. Various approaches have been used to evaluate or predict the effects of environmental stress on fish. The most common of these are laboratory tests of acute and chronic toxicity. Although these approaches are valuable for achieving such objectives as formal water quality criteria (Adams, 1990), however, they



lack ecological realism (Cairns, 1981). Recently, researchers used the biological markers approach to detect the effect of pollutants in the environment (Hoque, 1997). Biological markers permit the detection of stress-related factors that are biologically and ecologically variable. They provide early signals of adverse ecological effects as they use the lower levels of biological organisation, evaluate the specific response for each type of environmental stress as well as the overall integrated response, and can predict and evaluate the ecological significance and chronic stress (Adams, 1990). Changes in fish such as behavioural (Rice *et al.*, 1997), morphological (Richmond and Dutta, 1992), biochemical (Juzu *et al.*, 1998; Abu Zeid *et al.*, 1997), histological (Dhanapakiam and Premlatha, 1994), and molecular (Thomas, 1990) have been attributed to pesticides. These changes have the potential to be used as possible biological markers for the assessment of pollution in the aquatic environment.