UNIVERSITI PUTRA MALAYSIA

EFFECT OF ENDOSULFAN AND CARBOFURAN ON ENZYME ACTIVITIES IN CLARIAS GARIEPINUS (AFRICAN CATFISH)

DARYANI

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EFFECT OF ENDOSULFAN AND CARBOFURAN ON ENZYME ACTIVITIES IN *CLARIAS GARIEPINUS* (AFRICAN CATFISH)

By

DARYANI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Partial Fulfillment of the Requirements for the Degree of Master of Science

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

EFFECT OF ENDOSULFAN AND CARBOFURAN ON ENZYME ACTIVITIES IN *CLARIAS GARIEPINUS* (AFRICAN CATFISH)

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March 2003

Chairperson : Assoc. Prof. Dr. Mohd Arif Syed

Faculty : Science and Environmental Studies

Determination of lethal concentration (LC_{50}) of carbofuran and the effect of a sub lethal concentration of endosulfan and carbofuran on glutathione S-transferase (GST), glutathione peroxidase (GPx) and ethoxyresorufin O-deethylase (EROD) activities were investigated in the liver and intestine (proximal, medial and distal) of *Clarias gariepinus* (African catfish).

The 96-h LC_{50} for carbofuran in present study was 1.63 mg/l. Endosulfan and carbofuran treatment resulted in a significant increase ($p<0.05$) of EROD, GST and GPx in liver and intestine (proximal, medial and distal) at the sub-lethal concentrations of 10.8 μg/l and 0.82 mg/l, respectively. Endosulfan enhanced GST GPx and EROD enzymes activity in the liver and intestine (proximal, medial and distal) of treated fish. The fish were examined every four days for twenty days. The results showed that all the enzyme activities were maximum after day four exposure and decreased until the termination of the experiment. The activity of GST in all of the organs of treated fish exposed to carbofuran decreased linearly after 2 hours

exposure until day 20. However GPx activity in all of the organs of treated fish exposed to carbofuran decreased after day 4 followed by an increase up to day 16 and decreased further until the termination of the experiment. The EROD activity in all of the organs exposed to carbofuran has a similar trend with that when exposed to endosulfan. Both activities, exposed to carbofuran and endosulfan increased until day 4 followed by a gradual decrease until day 20. In general, GST and GPx activity in all parts of the intestine of *Clarias gariepinus* (African catfish) is significantly higher than those in the liver. Compared to GST and GPx, EROD activity in the liver showed significantly higher activity than that found in the intestinal organs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk mendapatkan ijazah Master Sains

KESAN ENDOSULFAN DAN CARBOFURAN TERHADAP AKTIVITI ENZYME *CLARIAS GARIEPINUS* (IKAN KELI AFRIKA)

Oleh

DARYANI

Mac 2003

Pengerusi : Prof. Madya Dr. Mohd. Arif Syed

Fakulti : Sains dan Pengajian Alam Sekitar

Pengenalpastian ketoksikan oral akut (LC_{50}) carbofuran dan kesan kepekatan endosulfan dan carbofuran terhadap aktiviti enzim glutathione S-transferase (GST), glutathione peroxidase (GPx) dan ethoxyresorufin O-deethylase (EROD) telah dikaji didalam organ hati dan bahagian usus (proximal, medial dan distal) *Clarias gariepinus* (ikan keli Afrika).

Kesan pendedahan selepas 96 jam oleh carbofuran ialah 1.63 mg/l. Keputusan bagi rawatan endosulfan dan carbofuran jelas menunjukkan peningkatan aktiviti ($p<0.05$) bagi GST, GPx dan EROD dalam hati dan bahagian usus (proximal, medial dan distal) pada kepekatan sublethal 10.8 µg/l and 0.82 mg/l secara berturutan. Endosulfan telah merangsangkan peningkatan aktiviti enzim GST, GPx dan EROD di dalam hati dan bahagian usus (proximal, medial dan distal) pada ikan yang dirawat. Aktiviti enzim pada ikan diuji setiap 4 hari selama 20 hari. Keputusan menunjukkan aktiviti enzim mencapai tahap maksimum selepas 4 hari pendedahan

dan semakin menurun sehingga tamat tempoh pendedahan. Aktiviti GST di dalam kesemua organ pada ikan yang dirawat dengan carbofuran menunjukkan penurunan garis lurus selepas pendedahan 2 jam sehingga hari ke-20. Aktiviti GPx di dalam semua organ pada ikan yang telah dirawat dengan carbofuran menunjukkan penurunan selepas 4 hari diikuti peningkatan sehingga hari ke-16 dan penurunan semula sehingga tamat tempoh pendedahan. Aktiviti EROD di dalam organ yang telah dirawat dengan carbofuran mempunyai keluk yang sama dengan endosulfan. Kedua-dua aktiviti terhadap rawatan dengan carbofuran dan endosulfan meningkat sehingga hari ke-4 dan diikuti penurunan secara perlahan sehingga hari ke-20. Secara umumnya aktiviti GST dan GPx di dalam kesemua bahagian usus ikan *Clarias gariepinus* (keli Africa), adalah lebih tinggi dari hati. Manakala aktiviti EROD dalam hati menunjukkan lebih tinggi berbanding di dalam bahagian organ usus.

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I certify that an Examination Committee met on 5th March 2003 to conduct the final examination of Daryani on her Master of Science thesis entitled " Effect of Endosulfan and Carbofuran on Enzyme Activities in *Clarias gariepinus* (African Catfish)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination committee are as follows:

Radzali Muse. Ph.D.
Associate Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

Mohd. Arif Syed. Ph.D.
Associate Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Nor Aripin Shamaan. Ph.D.
Associate Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Juzu Hayati Arshad. Ph.D.
Associate Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Johari Ramli. Ph.D.
Associate Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

GULAM RUSUL RAHMAT ALI, Ph.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 29 MAR 2003

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Mohd. Arif Syed. Ph.D.
Associate Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Chairman)

Nor Aripin Shamaan. Ph.D.
Associate Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

Juzu Hayati Arshad. Ph.D.
Associate Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

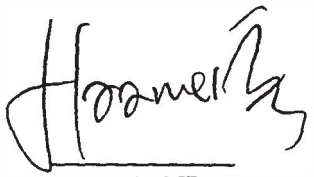
Johari Ramli. Ph.D.
Associate Professor,
Faculty of Science and Environmental Studies
Universiti Putra Malaysia
(Member)

AINI IDERIS, Ph.D.
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 8 MAY 2003

DECLARATION

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



DARYANI

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
AHH	Aryl hydrocarbon hydroxylase
AhR	Aryl hydrocarbon receptor
ARNT	Aryl hydrocarbon nuclear transferase
BSA	Bovine Serum Albumin
BaP	Benzo(a)pyrene
BPB	Bromophenol blue
CDNB	1-chloro-2,4-dinitrobenzene
CO	Carbon monoxide
CYP	Cytochrome P450
DCNB	1,2-Dichloro-4-nitrobenzene
DEN	Diethylnitrosamine
DNA	Deoxyribonucleic acid
e.u.	Enzyme per unit
EA	Ethacrynic acid
EDTA	Ethylenediaminetetra acetic acid
ER	Endoplasmic reticulum
EROD	7-ethoxyresorufin O-deethylase
g	gram
GSH	Glutathione (reduced form)
GSSG	Glutathione disulphate (oxidised)
GPx	Glutathione peroxidase
GST	Glutathione S-transferase
HCl	Hydrochloric acid
HSP90	Heat-shock protein 90
H_3PO_4	Orthophosphoric acid
H_2O_2	Hydrogen peroxide
K_2HPO_4	dipotassium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
KCl	Potassium chloride
L	liter
MACA	Malaysian Agriculture Chemical Association
MARDI	Malaysian Agricultural Research and Development Institute
mg	Milligram
mL	Millilitre
mM	Millimolar
MO	Monooxygenase
mRNA	messenger ribonucleic acid

Mw	Molecular weight
M	Molar
NADH	Reduced nicotinamide adenine dinucleotide
NaN_3	Sodium azide
NaOH	Sodium hydroxide
PAH	Poly aromatic hydrocarbon
PB	Phenobarbital
PCbs	Polychlorinated biphenyls
ppb	Part per billion
ppm	part per million
SDS	Sodium dodecyl sulphate
TCDD	2,3,7,8-tetra chlorodibenzo-p-dioxin
Tris	Tris (hydroxymethyl) aminomethane
U	Unit
UV	Ultra violet
µg	Microgram
µl	Microliter
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
XRE	Xenobiotic regulatory element

CHAPTER I

INTRODUCTION

Agricultural production of both arable crops and livestock is an activity of primary importance to developing countries. The growing world demand for food due to the increased population had led to greatly increased crop and animal production in these countries. It has led farmers to use sophisticated agricultural technology in which pesticides play a critical role. Residual levels of pesticides can constitute, in some cases, an important source of pollution, mainly if they have been used for a long time. Their mobility through air and water, their accumulation and transformation in the environment and finally, their biomagnification, constitute a real risk to human health, wildlife and the environment (Ruzafa et al., 2000). Pesticides can enter the aquatic environment by a number of routes including spillages, inappropriate disposal of diluted pesticides and runoff into drains and rivers. In the aquatic ecosystem, there is a continuous interchange of pesticides between land, sediment, sediment-water interface, interstitial waters and air-water interstitial and aquatic organisms. Data from various countries, through directed research on water-quality monitoring systems and accident reports, indicate that incidents of pollution in the aquatic environment by pesticides are frequent (OECD, 1986).

Utilization of biochemical factors to evaluate responses to pollutants, especially to carcinogenic compounds, such as PAHs and PCBs, has increased

considerably over the past 15 years. Alteration in biochemical systems are often more sensitive indicators than those at higher levels of biological organization. Depending upon the function of the systems affected and the nature of the responses, biochemical perturbations can indicate whether additional effects (at the organ or individual level) are likely to occur (Stegeman, et al., 1992). Relationships between exposure to chemical contamination and alterations in several biochemical processes in fish may allow the use of the latter as biomarkers of exposure and early responses to chemical contaminants (Stien et al., 1998).

The fish can provide excellent models to study the *in vivo* and *in vitro* effects of mutagens and carcinogens in the natural environment. It has been reported that feral population of fishes which exhibit extraordinary high prevalence of hepatic tumours, preneoplastic lesions and nonneoplastic hepatic lesions, have been identified at highly urban and industrialized sites in North America (Harshbarger and Clark, 1990). Freshwater catfish from industrialized tributaries of the Great Lakes exhibited hepatic tumours frequencies that ranged from 28-44% and frequent occurrences of epidermal papillomas were reported in the catfish as well as white suckers *Catostomus commesoni* from same geographic area (Wirgin and Waldman, 1998). A marine flatfish species at highly contaminated sites in Puget Sound, WA, showed hepatic tumour levels that ranged from 15 to 30% (Malins et al, 1988).

A number of such biomarkers was used in the present study. Cytochrome P450-dependent mono-oxygenase enzymes (CYPIA), conveniently measured as 7-ethoxyresorufin O-deethylase (EROD) and glutathione S-transferase (GST), are two

biomarkers that have been used extensively as they are induced by poly aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and certain dioxins (Stegemen, 1981; Stegemen and Lech, 1991; Stegemen et al., 1992). Enhanced levels of antioxidant biomarkers, e.g. glutathione peroxidase (GPx) and the associated reduced glutathione (GSH), function to remove oxyradicals derived from exposure to organic xenobiotics, including aromatic diols and quinines, nitroaromatics, bipyridyl herbicides and metal (Stegemen et al., 1992). Exposure to PAHs, PCBs, chlorinated hydrocarbons and metals have been shown to correlate with the increased activity of antioxidants in fish (Livingstone, 2001).

Cytochrome-P450 dependent monooxygenases (MO) system participates in the phase I detoxification of exogenous compounds; 7-ethoxyresorufin is a model substrate for the cytochrome-P450IA dependent MO action, called 7-ethoxyresorufin O-deethylase (EROD), which can be specifically induced by compounds with relatively flat molecular structures, such as planar polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). EROD activity in several fish species has been shown to be a good indicator of exposure to organic micro pollutants, because of the strong correlation observed with doses of toxicants both in laboratory and field studies (Eggens et al., 1995). CYPIA1 expression is transcriptionally regulated in fish and has been quantified in biomarker. Studies at the mRNA, CYPIA protein and associated catalytic activities of EROD and AHH levels. The mechanisms of CYPIA transcription in fish are probably very similar to those in mammals (Wirgin and Waldman, 1998).

This study is undertaken to study the toxicity of endosulfan and carbofuran on *Clarias gariepinus* and the effect of sub lethal concentrations (LC_{50}) of the carbofuran and endosulfan on the activities of glutathione S-transferase (GST), glutathione peroxidase (GPx) and 7-ethoxyresorufin O-deethylase (EROD). In this study, *Clarias gariepinus* (African catfish) a tropical freshwater fish was chosen. The choice of the fish in this study was based on its wide distribution in tropical and sub-tropical areas and availability of the cultures species which is never being exposed to any pesticide contamination. This study was expected to determine aquatic environment pollution level of pesticides using suitable biomarker. It was also expected that the pollution level could be reduced and the human health risk exposed by this pollution could also be mitigated.

CHAPTER II

LITERATURE REVIEW

Growing demand for food as a result of increased human population has led to a substantial increase in the production and use of agro-chemicals, such as pesticides and fertilizers. The increased use of various types of pesticides has led to concerns regarding the potential for contamination of environmental media (i.e., water, sediment, and biota) and the associated effects on wildlife and human health.

Approximately 1000 pesticide formulations are used throughout the world today for virtually all forms of agricultural commodity. The annual worldwide agricultural use of pesticides has been estimated to be in the order of 5 million tons, of which about 70 per cent is used for agriculture and the remainder by public health agencies and government agencies for vector control and for domestic purposes (Shaw and Chadwick, 1998). Despite the use of pesticides, about 35 per cent of the crops are lost. Nearly 50 per cent of food in the world may be lost annually despite all pest control procedures. It has been estimated that 0.1 per cent of the pesticides applied to crops reach the target pests; thus, more than 99 per cent of the applied pesticides have the potential to affect non- target organisms and to become widely dispersed in the environment. Although not yet well documented, pesticide-induced chronic toxicity is emerging as a public health concern including cancer, reproductive impairment and irreversible neurotoxicity (Shaw and Chadwick, 1998).

Pesticides Contamination in the Environment in Malaysia

In Malaysia, the usage of pesticides is extensive and the number of pesticides which are available on the market is large (Balasubramaniam and Yunus, 1974). Currently there are over 300 retail outlets involved in the manufacture, formulation and packaging of pesticides (MACA, 1989). In 1980, sale of pesticides amounted to RM 160 million. This increased to RM 237 million in 1984 and to nearly RM 300 million in 1988 (MACA, 1989). In Malaysia, pesticides are predominantly used in the plantation industries (rubber and oil palm), vegetable growing and public health control and in rice cultivation. The major insecticides used are γ-BHC, endosulfan, chlorpyrifos, carbofuran and carbaryl in rice field (Ooi, 1974), while malathion, resmethrin and abate are used for public health control (Yap, 1974).

Some herbicides have been shown to be toxic to paddy field fish in Malaysia (Ooi and Lo, 1990). Paddy field fish forms an important protein source for the rural people in west Malaysia (Gill, 1980). The estimated per capita consumption of fish in Malaysia was at 30 kg per annum in 1986. This consumption rate is higher than in 1974 where the value was at 26 kg per annum. Given this estimate, fish production has to increase substantially in order to meet the national requirement (Omar et al., 1990). Widespread use of pesticides in paddy fields has resulted in the decline of paddy field fish production. Paddy field fish production in Krian, Perak has been reported to decline over the past few years because of a pesticide (Ooi and Lo, 1990). It was also reported that fish killed in the Muda river was caused by the contamination of the river water with pesticide residues from effluents of pesticides