



UNIVERSITI PUTRA MALAYSIA

***TOXICITY AND SUB-LETHAL EFFECTS OF BROMADIOLONE AND
CHLOROPHACINONE ON BARN OWL, *Tyto alba javanica* Gmelin
IN OIL PALM PLANTATIONS***

HASBER BIN SALIM

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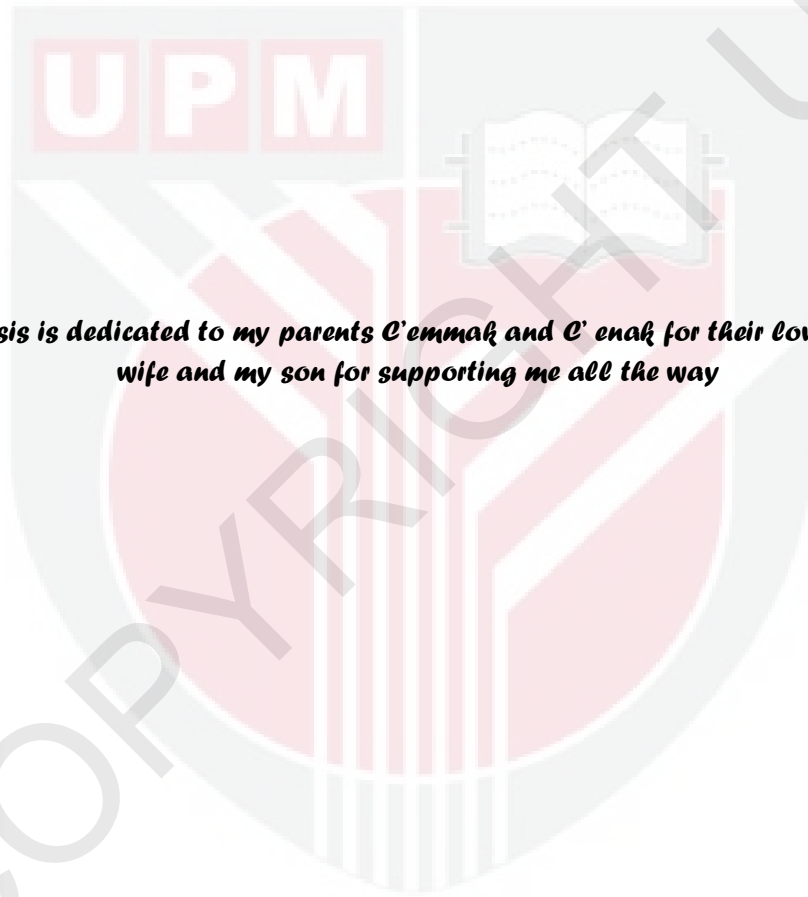
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By

HASBER BIN SALIM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

March 2014



This thesis is dedicated to my parents C'emmaq and C' enaq for their love, and my wife and my son for supporting me all the way



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

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**Chairman: Associate Professor Hafidzi Mohd. Noor, PhD
Faculty: Agriculture**

The toxicity and sub-lethal effects of two anticoagulant rodenticides; bromadiolone and chlorophacinone on barn owls, *Tyto javanica* were evaluated through aviary and field studies. In aviary study, *T. alba* showed behavioural aberrations less frequently took to flight and losing weight as early as on the fifth day after a meal of three rodenticide fed rats. Bromadiolone and chlorophacinone were found to have high degree of toxicity to *T. alba*. This finding is crucial since *T. alba* had been reported to consume up to three rats per night and this would certainly increase their potential exposure to secondary poisoning. HPLC analysis showed detection of residue in pellets regurgitated by *T. alba* can be used to indicate exposure of the latter to both compounds. However, blood residue method is limited to exposure duration of the compounds; the technique can only detect recent exposure to bromadiolone and chlorophacinone. The amounts of residue detected in the pellet samples for chlorophacinone were 69.9 to 81.6 µg/day or equivalent to 17.2 % to 27.4 % of the compound consumed and corresponding values for bromadiolone were 27.2 % to 34.5 % (72.24 to 85.77 µg/day). This suggests that the amounts of bromadiolone retained in the tissue of the rat were higher than chlorophacinone. Thus, a greater potential for secondary poisoning of bromadiolone than chlorophacinone can be deduced from this study.

Field studies were conducted to evaluate the sub-lethal effects of bromadiolone and chlorophacinone on breeding performance, growth performance of nestling, thinning of eggshell and home range of *T. alba* in oil palm plantations. The breeding performances (clutch size, brood size and fledging success) of *T. alba* in rodenticides treated areas were lower in comparison to rodenticide free area. The lower reproductive performance was the consequence of secondary poisoning on *T. alba*. This is substantiated by the detection of high residues in the pellets collected in both the rodenticide treated plots. Rodenticide free plot recorded the highest fledging success i.e. 71.42 % (n = 14) compared to chlorophacinone and bromadiolone treated plots at 42.85 % (n=14) and 35.71 % (n = 14) respectively. Liver autopsy of dead nestlings showed bromadiolone and chlorophacinone residue ranged from 0.18 µg/g to 0.41 µg/g wet weights indicating high exposure. Growth performance study indicated nestlings in the rodenticide free area recorded a higher body mass, longer tarsus, culmen and wings compared to the rodenticide treated plots. The study on the

effects of anticoagulant rodenticide on eggshell thickness showed 29.73 % (n= 37) and 5.35 % (n=56) of eggs collected from the treated plots contained bromadiolone and chlorophacinone residues respectively. The mean residue of bromadiolone and chlorophacinone ranged from 0.009 to 0.031 $\mu\text{g/g}$ wet weight. Bromadiolone and chlorophacinone residues can be transferred to the eggs of *T. alba*. However, the lower residue present in the albumen and the yolk did not seem to affect significantly egg shape, eggshell mass and eggshell thickness. The home range study that uses radio telemetry equipments showed home range sizes of *T. alba* in rodenticide free areas were larger compared to their counterparts in bromadiolone and chlorophacinone treated areas.

In conclusion both bromadiolone and chlorophacinone were found to have high degree of toxicity to *T. alba*. The field studies showed secondary poisoning risks produces detrimental sub-lethal effect on breeding performance and nestling growth of *T. alba*. The detection of residues in albumen and yolk showed residues from secondary poisoning can be transferred to egg of *T. alba*. The secondary poisoning risk of anticoagulant rodenticide also affects the home range size of *T. alba*.

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

**KETOKSIKAN DAN KESAN SUB-MAUT BROMADIOLONE DAN
CHLOROPHACINONE TERHADAP BURUNG PUNGGUK, *Tyto alba javanica*
Gmelin DI LADANG KELAPA SAWIT**

Oleh

HASBER BIN SALIM

Mac 2014

Pengerusi: Profesor Madya Hafidzi Mohd. Noor, PhD
Fakulti: Pertanian

Ketoksikan dan kesan sub-maut racun tikus anticoagulant, bromadiolone dan chlorophacinone terhadap burung pungguk, *Tyto alba javanica* telah dikaji melalui kajian dalam sangkar dan lapangan. Pada kajian sangkar, *T. alba* mengalami perubahan lakuan seperti kurang aktiviti penerbangan serta penurunan berat badan seawal lima hari selepas memakan tiga tikus-beracun yang diberikan. Bromadiolone dan chlorophacinone didapati memberi kesan ketoksikan yang tinggi terhadap *T. alba*. Penemuan ini penting kerana di lapangan *T. alba* telah dilaporkan mengambil sehingga tiga tikus semalaman dan ini akan meningkatkan potensi keracunan sekunder. Analisis HPLC menunjukkan pengesanan residu dalam pelet boleh digunakan untuk menentukan paras pendedahan racun anticoagulant terhadap *T. alba* di lapangan. Namun residu pada darah adalah terhad kepada tempoh pendedahan yang singkat. Jumlah residu dalam pelet bagi chlorophacinone adalah 69.9 to 81.6 µg/hari iaitu 17.2 % - 27.4 % daripada jumlah racun anticoagulan yang dimakan tikus. Sementara bagi bromadiolone sebanyak 27.2 % - 34.5 % iaitu bersamaan dengan nilai residu sebanyak 72.24 to 85.77 µg/hari. Keadaan ini menunjukkan bromadiolone disimpan lebih lama dalam badan tikus berbanding chlorophacinone dan ini akan memberi potensi keracunan sekunder yang lebih tinggi.

Kajian lapangan juga telah dijalankan untuk menilai kesan sub-maut bromadiolone dan chlorophacinone terhadap prestasi pembiakan, tumbesaran anak burung, penipisan kulit telur dan tingkah laku penerbangan *T. alba* di ladang kelapa sawit. Prestasi pembiakan (purata telur, purata anak dan anak burung berjaya terbang) *T. alba* di kawasan yang dirawat dengan racun antikoagulan adalah lebih rendah berbanding dengan kawasan bebas racun antikoagulan. Prestasi pembiakan yang lebih rendah adalah disebabkan oleh kesan keracunan sekunder terhadap *T. alba*. Ini dibuktikan oleh pengesanan residu yang tinggi dalam pelet yang diambil dari kedua-dua kawasan kajian yang diumpan dengan racun antikoagulan. Kawasan tanpa rawatan menunjukkan jumlah anak burung yang kekal hidup adalah tinggi iaitu 71.42 % (n = 14) berbanding kawasan umpanan chlorophacinone dan bromadiolone iaitu hanya 42.85 % (n = 14) dan 35.71 % (n = 14). Autopsi ke atas hati anak burung menunjukkan residu bromadiolone dan chlorophacinone menunjukkan julat dari 0.18 g/g hingga 0.41 g/g berat basah menunjukkan tahap keracunan sekunder yang tinggi. Selain itu, kajian tumbesaran menunjukkan anak burung di kawasan tanpa

rawatan mempunyai kadar pertumbuhan dan penambahan yang lebih tinggi bagi berat badan, tarsus, culmen dan panjang sayap berbanding anak burung dikawasan rawatan racun antikoagulan. Keputusan kajian kesan racun antikoagulan terhadap penipisan cengkerang telur menunjukkan 29.73 % (n = 37) dan 5.35 % (n=56) telur terbiar dari kawasan pengumpanan dengan bromadiolone dan chlorophacinone mengandungi residu diantara 0.009 ó 0.031 g/g berat basah. Ini menunjukkan residu bromadiolone dan chlorophacinone boleh dipindahkan kepada telur *T. alba*. Namun, kuantiti residu yang rendah di dalam telur tidak menjejaskan secara ketara bentuk telur, berat kulit telur mahupun menyebabkan penipisan kulit telur *T. alba*. Kajian banjaran kediaman yang menggunakan peralatan radio telemetri menunjukkan *T. alba* di kawasan tanpa pengumpanan mempunyai banjaran kediaman lebih besar berbanding kawasan rawatan yang dirawat dengan bromadiolone dan chlorophacinone.

Secara kesimpulannya, kedua-dua racun antikoagulan iaitu bromadiolone dan chlorophacinone mempunyai kesan ketoksikan yang tinggi terhadap *T. alba*. Kajian lapangan juga menunjukkan keracunan sekunder memberi kesan sub-maut yang negatif terhadap prestasi pembiakan dan tumbesaran anak burung. Pengesanan residu pada putih dan kuning telur juga menunjukkan residu boleh dipindahkan ke dalam telur *T. alba*. Akhir sekali, Risiko keracunan sekunder juga mmberi kesan terhadap saiz banjaran kediaman *T. alba*

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I certify that a Thesis Examination Committee has met on 05 Mac 2014 to conduct the final examination of Hasber bin Salim on his thesis entitled "Toxicity and sub-lethal effects of bromadiolone and chlorophacinone on barn owl, *Tyto alba javanica* Gmelin in oil palm plantations" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Lau Wei Hong, PhD

Senior Lecturer

Faculty of Agriculture

Universiti Putra Malaysia

(Chairman)

Rita Muhamad Awang @ Rita Suryadi, PhD

Professor

Faculty of Agriculture

Universiti Putra Malaysia

(Internal Examiner)

Mohammed Zakaria Hussein, PhD

Associate Professor

Faculty of Forestry

Universiti Putra Malaysia

(Internal Examiner)

Graham Richard Martin, PhD

Professor

Centre for Ornithology Research

University of Birmingham

United Kingdom

(External Examiner)

NORITAH OMAR, PhD

Associate Professor and Deputy Dean

School of Graduate Studies

Universiti Putra Malaysia

Date: 21 April 2014

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Hafidzi Mohd Noor, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Dzolkhifli Omar, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Azhar Kasim, PhD
Associate Professor
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LIST OF ABBREVIATIONS

FELDA	Federal Land Development Authorities
FASSB	Felda Agricultural Services Sdn Bhd
PPPTR	Pusat Penyelidikan Pertanian Tun Razak (Tun Razak Research Center)
HPLC	High Performance Liquid Chromatography
a.i.	Active Ingredient
ANOVA	Analysis of Variance
SAS	Statistical Analysis System
LD ₅₀	Lethal Dose
LOD	Limit of Detection
LOQ	Limit of Quantitation
QC	Quality Control
UV	Ultra Violet
IUPAC	International Union of Pure and Applied Chemistry
g	Gram
µg	Microgram
kg	Kilogram
mg	Milligram
µL	Microliter
mL	Mililliter
L	Liter
mm	Millimeter
cm	Centimeter
km	Kilometer
PPM	Part Per Million
Ha.	Hectare
v/v	Volume/volume
w/w	Weight/weight
S.P.E	Solid Phase Extraction
EDTA	Ethylenediaminetetraacetic acid
e.g.	<i>exempli gratia</i> , for example
et al.	et alii, and other
S.E	Standard Error

CHAPTER 1

INTRODUCTION

1.1 Anticoagulant rodenticides and barn owls, *Tyto alba*

In Malaysia, anticoagulant rodenticides have become a key solution to the threat of rodent pest particularly in the agricultural sector such as paddy fields and oil palm plantations. Anticoagulant rodenticides, in particular bromadiolone and chlorophacinone has been reported as the most effective and widely used in rat control practice in oil palm plantations (Noor Hisham *et al.*, 2007). However, in line with the Malaysian oil palm industry and its commitment to produce sustainable palm oil through the implementation of the Integrated Pests Management (IPM), effective anticoagulant rodenticides has been employed in combination with biological control using barn owls, *Tyto alba javanica* (Lam, 1982). Duckett (1984) proposes the idea of using IPM for controlling rat pest in which anticoagulant rodenticides were recommended to ensure that the pest was controlled while sustaining sparing the natural predator, *T. alba* for an environmental friendly rodent pest approach.

1.2 The Problem

Chemical control using anticoagulant rodenticides integrated with biological control using *T. alba* has been adopted since the late 80s against rat populations in Malaysian oil palm plantations (Wood and Chung, 2003). However application of anticoagulant rodenticides (e.g. bromadiolone and chlorophacinone) in combination with the natural propagation barn owls has raised concerns of potential secondary poisoning to the latter. Monitoring programmes on exposure of anticoagulant rodenticides in United States, Canada and Europe have shown evidence of extensive contamination of anticoagulant rodenticides in raptors (e.g. Stone *et al.*, 2003; Thomas *et al.*, 2011; Sanchez-Barbudo *et al.*, 2012). Contamination with anticoagulant rodenticides is considered to be one of the most detrimental threats to wildlife.

Raptor and predator populations have been reported declining in numbers when anticoagulant rodenticides were introduced in farmlands against rodent pests (e.g. Elmeros *et al.*, 2011; Gabriel *et al.*, 2012). In Malaysia, researchers have proposed combining *T. alba* with application of first generation anticoagulant rodenticide warfarin to control rats; speculating that the poison was not hazardous to barn owls (Duckett, 1984). However, prolonged exposure triggers resistant of rats to warfarin (Lam, 1982). New and more toxic anticoagulant e.g. chlorophacinone, and bromadiolone have been introduced in the early 1980s to deal with rodenticide resistance (Khoo *et al.*, 1991).

The actions of more recent rodenticides are more toxic and exhibit relatively longer biological half-lives in tissues (e.g. liver) (Erickson & Urban, 2004). Both characteristics enhance the potential of compounds to cause secondary poisoning to *T. alba* that takes rats in 98 % of their diet (Duckett, 1984).

1.3 Toxicity and sub-lethal risks

There is growing concern about the risk of secondary poisoning of anticoagulant rodenticides to non target animals (e.g. Gabriel *et al.*, 2012). Reports of non- target wildlife contamination and toxicosis following the use of anticoagulant rodenticides has increased worldwide (Eason *et al.*, 2002). In North America and Europe, secondary poisoning of first and second generation anticoagulant rodenticides (e.g. chlorophacinone and bromadiolone) to non-target species that ingest poisoned-rodent have been documented in a variety of wild birds (e.g. Walker *et al.*, 2008b; Thomas *et al.*, 2011) and mammals (e.g. Fournier-Chambrillon *et al.*, 2004; Elmeros *et al.*, 2011). However, all anticoagulants residue data are derived from opportunistic sampling of tissue, particularly liver, from those animals found dead in field situations. Therefore, the impact of poisoning on predator populations could be underestimated. Thus, captive studies approach have been used to determine the real risks of secondary poisoning by chlorophacinone and bromadiolone to predatory and scavenger birds (e.g. Wylie, 1995) and mammals (e.g. Sage *et al.*, 2010).

Destructive method through sampling of tissue (e.g. liver) from respective non targets that required the animal to die has been employed in order to investigate secondary poisoning hazards of anticoagulant rodenticides. However, this method is less favoured due to ethical reasons (Fisher *et al.*, 2004; Ward, 2008). Hence, several non-invasive techniques have been developed to determine the risks of anticoagulant rodenticides exposure in animals, such as evaluation of residue presents in eggs (Fisher, 2009; Mario and Grazia, 2010) and in pellets regurgitated by barn owls (e.g. Eadsforth *et al.*, 1996). However, the study of sub-lethal effects of anticoagulant rodenticides (bromadiolone and chlorophacinone) to barn owls by using non invasive technique (egg, blood and regurgitated pellet) is largely unexplored.

An ecological assessment to study secondary poisoning has been evaluated and proposed These include changes in population (Brown *et al.*, 2002; Ward, 2008), study on reproductive performances of animals (Mendenhall *et al.*, 1983), foraging and ranging behaviour (Hegdal *et al.*, 1986; Naim *et al.*, 2012b), and growth performances of nestling (Naim *et al.*, 2011). Yet again, the information on ecological assessment on the effects of anticoagulant rodenticides, chlorophacinone and bromadiolone through secondary poisoning to *T. alba* in oil palm plantations is still limited.

1.4 Justification and Objectives

Anticoagulant rodenticides, chlorophacinone and bromadiolone have been used and integrated with biological control using barn owls, *T. alba* since late 80s and 90s for rat control in Malaysia (Noor Hisham *et al.*, 2007). However, comprehensive information on the lethal and sub-lethal effects of anticoagulant rodenticides from chronic secondary exposure to *T. alba* in oil palm environment are largely unexplored. There is a lacking of information on toxicity, sub-lethal effects, health status, reproductive and breeding performance and growth developments on *T. alba*. Based on this information, it is pertinent to evaluate probable secondary poisoning of *T. alba* as a result of rodenticides (chlorophacinone and bromadiolone) baiting in oil palm. The specific objectives of this study are

1. To study the toxicity and sub-lethal risks of bromadiolone and chlorophacinone exposure in captive barn owls, *T. alba*;
2. To determine the sub-lethal effects of bromadiolone and chlorophacinone on breeding performances of *T. alba* in oil palm plantations;
3. To investigate the sub-lethal exposure of bromadiolone and chlorophacinone on the survival and the growth rate of nestlings of *T. alba* in oil palm plantations;
4. To trace evidence of secondary poisoning from the rodenticides residues deposited in *T. alba* eggs and their symptoms as measured in the thinning of those eggshells;
5. To evaluate the effects of the bromadiolone and chlorophacinone on the home range size of *T. alba* in oil palm plantation environments.



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