COMMENSAL RAT PESTS AND THEIR CONTROL USING ANTICOAGULANT RODENTICIDES IN URBAN AREAS IN PENANG ISLAND

WAN NUR AMNI BINTI WAN AHMAD SUKRI

UNIVERSITI SAINS MALAYSIA

2018

COMMENSAL RAT PESTS AND THEIR CONTROL USING ANTICOAGULANT RODENTICIDES IN URBAN AREAS IN PENANG ISLAND

by

WAN NUR AMNI BINTI WAN AHMAD SUKRI

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

July 2018

ACKNOWLEDGEMENT

Alhamdulillah. All praise to the Most Gracious, Allah S.W.T for His bless and grace, I successfully completed this research. It would have not be possible to accomplish this study without support and guidance from several individuals during this journey.

I would like to express the heartiest gratitude to my respectable and considerate supervisor Dr. Hasber bin Salim for his guidance, encouragement and help throughout this study. I am thankful to my co-supervisors, Dr. Hamdan bin Ahmad and Prof. Dr. Shahrul Anuar bin Md. Sah for their brilliant ideas. My deepest gratitude to Prof. Abu Hassan bin Ahmad and Dr. Intan Haslina binti Ishak for their support and encouraging critics. Deepest appreciation to Barn Owl Research Group members, School of Biology staff; Mr. Muthu and School of Technology Industry Staff; Encik Maaruf for the help throughout this study.

Without love, support and encouragement from my beloved family I would have not be able to accomplish this study. Thank you to my mother, Hajah Che Aekah binti Omar and my siblings for the encouraging words and endless love. Thank you to my friends (Overthinkers and PHG) whom always with me throughout this lonely journey. Thank you so much.

My pillar of strength, my late father, Haji Wan Ahmad Sukri bin Wan Mohamad thank you so much for your endless love and support. Thank you and I love you.

Al-Fatihah.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

µg	Microgram
g	Gram
mg	Milligram
kg	Kilogram
nm	Nanometer
µm	Micrometer
cm	Centimeter
mm	Millimeter
µl	Microliter
ml	Milliliter
l	Liter
ppm	part per million
a.i.	active ingredient
psi	pound per square inch (pressure)
SPSS	Statistical Package for Social Science
SE	Standard Error
HPLC	High Performance Liquid Chromatography
APB	Apple and Peanut Butter
BPB	Bread and Peanut Butter
BFE	Bread and Fish Extract
DF	Dried Fish
DFPB	Dried Fish and Peanut Butter
FCL	Fried Chicken Leftover
S	Sausages
VPB	Vegetable and Peanut Butter

TIKUS KOMENSAL PEROSAK DAN KAWALAN MENGGUNAKAN RACUN ANTIKOAGULAN DI KAWASAN BANDAR DI PULAU PINANG

ABSTRAK

Taburan dan komposisi tikus komensal di Pulau Pinang telah dinilai melalui tangkapan langsung menggunakan kaedah perangkap hidup. Kajian pemilihan umpan juga dijalankan dan didapati tangkapan paling banyak (35.56%) telah direkodkan menggunakan perangkap yang diberi umpan ayam goreng berbanding umpan jenis lain setelah di kenal pasti melalui ANOVA satu arah ($p<0.005$). Dalam kajian ini, terdapat lima spesies tikus komensal yang berjaya ditangkap sepanjang kajian berlangsung. Keputusan mendapati, *Rattus norvegicus* merupakan spesies paling dominan di kawasan komersial manakala, *Bandicota bengalensis* merupakan spesies paling dominan di kawasan kediaman. Kajian keberkesanan di makmal, kesedapan dan penerimaan umpan racun antikoagulan iaitu bromadiolone dan chlorophacinone telah dinilai menggunakan *R. norvegicus* liar sebagai sampel. Bromadiolone dan chlorophacinone mencatatkan 100% kematian semasa ujian pemakanan tiada pilihan. Bagi ujian pemakanan pilihan, 77% kematian dicatatkan dengan penggunaan racun bromadiolone manakala 33% kematian dicatatkan dengan penggunaan racun chlorophacinone. Hasil kajian ini menunjukkan keberkesanan yang tinggi oleh kedua-dua racun terhadap tikus Norway. Racun bromadiolone merekodkan 24.12% penerimaan umpan oleh tikus manakala racun chlorophacinone merekodkan sebanyak 12.97%. Nisbah kesedapan umpan terhadap makanan normal menunjukkan bromadiolone

di nisbah (1:3) manakala chlorophacinone di nisbah (1:7). Hasil kajian mendapati penerimaan umpan beracun dan nisbah kesedapan menunjukkan formulasi racun bromadiolone adalah sangat menarik kepada tikus berbanding formulasi racun chlorophacinone. Seterusnya, kajian kerberkesanan di lapangan telah dilakukan selama 60 hari. Penerimaan umpan, impak rawatan terhadap populasi tikus dan penurunan aktiviti tikus telah dinilai sepanjang kajian ini dilakukan. Penerimaan umpan beracun bromadiolone adalah sebanyak 26% daripada keseluruhan umpan yang diletakkan selama tujuh hari pengumpanan. Bagi umpan beracun chlorophacinone, hanya 4% dimakan daripada keseluruhan umpan beracun diberikan tujuh hari umpan beracun diletakkan. Bilangan bangkai tikus dijumpai di kawasan kajian racun bromadiolone adalah sebanyak 15 ekor manakala di kawasan kajian racun chlorophacinone hanya seekor dijumpai. Penurunan populasi tikus adalah 83.57% selepas 60 hari racun di aplikasi bagi kawasan kajian racun bromadiolone manakala 55.94% penurunan di kawasan kajian racun chlorophacinone. Hal ini menunjukkan racun bromadiolone lebih berkesan berbanding racun chlorophacinone terhadap tikus komensal. Selain itu, analisis HPLC menunjukkan pengesanan residu dalam hati sampel tikus boleh digunakan untuk mengesan kedua-dua sebatian. Jumlah residu dikesan dalam hati sampel tikus untuk bromadiolone adalah sebanyak 0.00309±0.0010mg/ml atau bersamaan 12.88% daripada jumlah sebatian diambil semasa ujian pemakanan tidak pilihan. Manakala, residu kekal dalam hati sampel tikus bagi chlorophacinone adalah 0.0010±0.00004mg/ml atau bersamaan 5.56% daripada jumlah sebatian diambil semasa ujian pemakanan tidak pilihan. Hal ini menunjukkan jumlah sebatian bromadiolone kekal dalam hati tikus sangat tinggi berbanding chlorophacinone.

COMMENSAL RAT PESTS AND THEIR CONTROL USING ANTICOAGULANT RODENTICIDES IN URBAN AREAS IN PENANG ISLAND

ABSTRACT

The abundance and composition of commensal rats in Penang Island were evaluated using direct method of live-trap sampling method. Bait preferences of rat were also analyzed with the highest number of trap success (35.56%) recorded when live-traps were baited with fried chicken leftover compared with other type of baits as determined by one-way ANOVA ($p<0.05$). In this study, there were five different species of commensal rats caught throughout of the study. The dominant species in commercial areas was *Rattus norvegicus* and in residential areas was *Bandicota bengalensis*. The laboratory efficacy, palatability and bait acceptance of selected anticoagulant rodenticides, bromadiolone and chlorophacinone were evaluated using wild *R. norvegicus* as samples. Bromadiolone and chlorophacinone bait recorded 100% of mortality in no-choice feeding experiment. For choice-feeding test, 77% mortality rate was recorded for rats treated with bromadiolone bait meanwhile 33% of mortality was recorded on chloropacinone bait treatment. The results indicate high efficacy of both rodenticides against Norway rats. Bromadiolone bait recorded 24.12% bait acceptance while chlorophacinone recorded 12.97%. The palatability ratio of bait against normal diet showed bromadiolone at (1:3) while chlorophacinone at (1:7). The results of bait acceptance and palatability ratio indicated bait formulation of bromadiolone was highly attractive to rats as compared to chlorophacinone bait formulation. Next, the field efficacies of both rodenticides were evaluated for 60 days. The bait acceptance impact of the treatments on rat population and reduction of rat activity at the treatment sites were assessed throughout of the study. The bait acceptance for bromadiolone treatment was 26% from the total bait offered during seven days of baiting period. Whereas for chlorophacinone, only 4% from the total of bait offered was consumed during 7 days of baiting period. The number of carcasses found at bromadiolone treatment site was 15 meanwhile at chlorophacinone treatment site, only one carcass was found. The reduction of population was 83.57% at 60 days after treatment in bromadiolone treatment site whereas 55.94% in chlorophacinone treatment site. This indicates that bromadiolone was more effective compared to chlorophacinone against commensal rats. Furthermore, HPLC analysis showed the detection of residue in liver of rat samples can be used to indicate exposure of rats to both compounds. The amount of residue detected in liver of rat samples for bromadiolone was 0.00309±0.0010mg/ml or equivalent to 12.88% from the total compound consumed for 6 days in no-choice feeding tests. Meanwhile, residue retained in the liver of rat samples for chlorophacinone was 0.001±0.00004mg/ml or equivalent to 5.56% the total of compound consumed for 6 days in no-choice feeding. This suggests that the amount of bromadiolone compound retained in the liver of the rat was higher than chlorophacinone.

CHAPTER ONE

GENERAL INTRODUCTION

Rodent infestation is a severe problem in urban areas, affecting the quality of life, damaging the infrastructure and posing a health risk to the public by spreading various zoonotic diseases. They are also one of the important mammalian pests at the global level (Singelton and Petch, 1994). In Southeast Asia, rats have been identified as the major concern and threat to agriculture production (Grist and Lever, 1969; Hopf *et al.*, 1976; Hoque *et al.*, 1988; Geddes, 1992).

In urban areas, rodents usually found living in close association with human habitation are being categorized as commensal rodents (Sullivan, 1997). There are three dominant species of commensal rodents, the Norway rat (*Rattus norvegicus*), the roof rat (*Rattus rattus*) and the house mouse (*Mus musculus*) (Lund, 1994; Meyer, 2003). These species depend on essential elements near human settlement for sources of food, water, shelter and space (Sullivan, 1997). As they have a close association with humans, they cause a lot of damage and a reservoir of various zoonotic diseases transmissible to humans. Since many years ago, leptospirosis or rat-urine fever has become an endemic disease in Malaysia which is fatal to humans. Leptospirosis has become a major concern as the number of mortality cases have been increasing over time.

Many factors contribute to the rodent infestation in urban areas such as poor sanitation, poor waste management and abandoned properties. Rat control management should include all strategies in a suitable manner. There are several control strategies that has been practiced such as chemical control, biological control, physical control and IPM (Integrated

Pest Management). However, chemical control by using rodenticide is widely practiced to combat rat outbreaks. Rodenticides are convenient to use and usually provide fast reduction in rat numbers (Prakash, 1988).

Rodenticides are classified into two groups, anticoagulant and all other compounds (non-anticoagulants) (Whisson, 1996). Anticoagulant rodenticide can be divided into two groups depending on their chemical structure. The first group is indandione with members of chlorophacinone, diphacinone and pindone. The second group is coumarin and this group can be subdivided into first generation (warfarin, coumatetralyl and coumachlor) and second generation anticoagulants (bromadiolone, brodifacoum, difenacoum and flacomafen), which are more acutely toxic compared to the first generation (Whisson, 1996).

This study attempts to assess the species abundance of commensal rodent pests and efficacy of anticoagulant rodenticide use against these pests in Penang Island. The study of efficacy of anticoagulant rodenticides includes laboratory and field assessments. The findings from the trials contribute to the better management of rats in urban areas, in particular for pest management in Penang Island. Moreover, this study also assesses the persistence of residues of anticoagulant rodenticides treatments in rat liver samples through laboratory and field trials. The residues in rat liver samples were analyzed using HPLC (high performance liquid chromatography) to evaluate the effectiveness of the tested anticoagulants. This information will provide an evaluation of toxicity and poisoning risks to rat as a target animals and other non-target animals. Besides, the information is also useful for confirmation of bioaccumulation of residues in rat liver sample. Thus, this will provide information on the mechanism of anticoagulant rodenticides and the effectiveness against commensal rats in urban areas.

The specific objectives of the study were:

1. To identify commensal rodents in selected urban areas in Penang island, their abundance, and bait preferences.

2. To evaluate the efficacies of chlorophacinone and bromadiolone against Norway rats, *Rattus norvegicus*,in laboratory condition.

3. To evaluate the field efficacies of chlorophacinone and bromadiolone against commensal rats in Penang Island.

4. To analyze the residues of anticoagulant rodenticides in liver samples or rats using high performance liquid chromatography (HPLC) analysis.

CHAPTER TWO

LITERATURE REVIEW

2.1 Rodentia

Almost 40% of mammal species are from Rodentia, the largest order of mammals that comprised 1063 species from 28 families (Aplin *et al.*, 2003; Singelton *et al.*, 2003). The name of order is derived from Latin word, *rodere* meaning to gnaw. The rats gnaw on hard surface to keep their incisors in remarkable length as their teeth growth rate is about 5 inches per year (Barnett, 1963). The incisors teeth are important for the rodent to eat, collect material to build nest, carry food and for defense purposes (Hanson, 2006).

True rodents are believed to have originated in Asia (Hanson, 2006) and about 54 million years ago the first rodent appeared in fossil record in Asia and North America during end of Paleocene era (Meng *et al.*, 1994). Rodents such as Norway rats, roof rats and house mice are from family of Muridae (Hanson, 2006). The genus *Rattus* first emerged within the Muridae family about 3.5 to 56 million years ago (Furano and Usdin 1995, Verneau *et al.*, 1998). The genus was native to the Mediterranean countries, the Middle East, India, China, Japan, and Southeast Asia including the Philippines, New Guinea and Australia (Krinke, 2000). There are 56 species within the genus *Rattus* (Feldhamer *et al.*, 2004). The Norway rat (*Rattus norvegicus*) and the roof rat (*Rattus rattus*) are originated from Asia. However, for Norway rats are probably originated from northern China and Mongolia, where wild rats still live in burrows (Hanson, 2006). *Rattus rattus* originated further south in the Indo – Malayan region (Krinke, 2000). Norway rats travelled to Europe with human back then and become a common species in Europe and America today (Hanson, 2006).

2.2 Rodent as a Pest

Rodents from family Scuiridae (squirrels), Rhizomyidae (bamboo rat), Muridae (rats and mice) and Hysteridae (porcupines) are reported as the pests in agricultural sectors (Prakash, 1988). Genus *Rattus* from family Muridae was known as the major pest in agriculture (Faizul, 2008) and urban area (Prakash, 1988) and caused economic losses by their infestation. They damage a lot of things such as plastic materials and woods by their gnawing acts. Rats also can contaminate food by their urine or feces. This can cause medical importance to human as they can transmit various diseases (Prakash, 1988). Rat is a pest in urban settlement because of the ability to destroy property, contaminate food and source for infectious diseases such as leptospirosis, murine typhus, salmonellas and many more (Yong, 1982; Prakash, 1988). They also have high adaptability to changing environment and high reproduction rate to allow them to coexist with human (Gerozisis and Hadlington, 2005).

Rats are not only pest in urban area but also in agriculture sectors. In Malaysia, oil palm and paddy are the crops commonly attacked by rats and they caused significant damage and yield reduction of the crops (Singleton and Petch, 1994; Turner and Gillbanks, 2003). Nowadays, rodents can cause major losses to agricultural production even though many controls and treatment have been done. Herwigs (2003) stated that there are almost 2000 species of rodents but only limited numbers of them caused problem in agriculture. For example in Africa there are 77 species that caused damage to agriculture and most of them from family of Muridae (Fiedler, 1986). There are three invasive species for agriculture sectors in Malaysia, *Rattus tiomanicus* (wood rat), *Rattus argentiventer* (rice field rat) and *Rattus rattus diardii* (roof rat) (Hafidzi and Saayon, 2001).

Rice is one of the major crops for certain countries in Asia (Prakash, 1988). Rat damages rice field by cutting and pulling up the new planted plants and also they feed on developing rice grains (Stuart and Singelton, 2016). In Korea, the damages caused by field rodent reported to be 14.2% but in coastal area, *R. norvegicus* caused 60 to 80% losses (Bai *et al.*, 1971; Shin, 1974). *Bandicota bangelensis* and *B. indica* caused 5 to 10% damages in deep water rice field in India and also Burma (Poche et al, 1980; Catlin and Yassin, 1981; Walton and Brooks, 1981). It was reported that annual losses is between 10-12% in Indonesia, 2-5% in Malaysia, 6-7% in Thailand, and less than 10% in Vietnam (Singleton and Petch, 1994, Singleton *et al.*, 1999, Boonsong *et al.*, 1999).

Meanwhile, rats also caused damages to oil palm plantation on immature and matured palms. Rats damaged the oil palm tree by tearing the leaves for nesting materials, eating the inflorescences and fruits of the palm (Wood, 1982). Damages to inflorescence can effects the pollination process while damages to fruits can cause loss in yield (Wood, 1982). Apart from attacking matured plants, rat also causing damages to young plant by feeding on apical tissue and disturb the development of the young shoots (Hafidzi and Saayon, 2001).

The economic losses due to rats in the oil palm were estimated at 108kg of oil per hectares in a year or about 5% of a good plantation yield. This estimate is obtained by multiplying the average amount of fruits eaten by a healthy rat in captivity divided by the rat population per ha (Wood, 1976). In Malaysia, oil palm is one of the major crops to suffer a significant loss due to rats (Khoo, 1984). According to Hoque *et al.* (1988) *Rattus tiomanicus* is the main species that attack matured oil palm plantation. Wood and Liau (1977) added that rats can cause major loss in oil palm production during high infestation with total loss of 240kg of oil per hectare in a year. Chung (2012) reported that severe rat damage on young palms could cause between 20% to 30% damage for the new plantings and the total fresh fruit bunch yield during the first twelve months after maturity. It is 20% lower than those from palms with no damage (Chung, 2012).

2.2.1 Economic Importance of Rats in Urban Areas

Town, cities and suburbs areas have high density of people and building structures. Rodents or rats in particular have become adapted to diverse habitats, from the agriculture fields to buildings and human dwellings. The development of urban environments suits their ecological requirements and they are very good at exploiting the urban opportunity (Meyer, 2003). In Penang islands, rat is considered as fourth major pest after cockroach, mosquitoes and ant. Penang Municipal Council received a lot of reports from public regarding the nuisance cause by the rats (Nurul Liyana, 2008).

Urban rats have the ability to destroy structures and consumer goods and become threats to human and commerce (Tobin and Fall, 2004). Pimentel *et al*. (2000) reported the economic cost of rat damage was greater than any other invasive animal species. Moreover, urban rats can cause some degree of economic loss to country, company and individual by causing to fire hazards. The most common structural damages were gnawing of walls, floors, doors and chewing electrical appliances and components (Almeida *et al*., 2013). On a large scale, the rats caused fire by nibbling on plastic insulator of live cable causing electrical short circuits, burning down the house as reported in the news (Daily Mail UK, 2014).

2.2.2 Public Health Issues

Rodent pests in Malaysia have been long considered as a threat to public health and agriculture production (Zain *et al.*, 2012; Wood and Chung, 2003). Rats eat and contaminate large amounts of foods, damages structures and building and also spread diseases to the livestock and human being. Rat also can cause traumatic and emotional problem to certain people (Zahner *et al.*, 1985). Rodent pest in particular rats play important role as a source for infectious diseases that can pose a significant health risk to people such as plague, leptospirosis, murine typhus, salmonellosis and rat-bite fever (Prakash, 1988). Black Death (1330 – 1352) was the most devastated epidemic that spread by rat and its flea, which spread plague to human throughout Asia and Europe (Hanson, 2006). *Yersinia pestis* is a plague bacteria that carried by flea on the many different rodent species which include the *R. rattus* and *R. norvegicus* (Hanson, 2006). These two species have a close association with human and caused plague outbreaks among people.

Presently, leptospirosis is a serious disease that is becoming a major concern is leptospirosis. It can be a fatal threat to human that are exposed to water source contaminated with rat urine containing leptospirosis virus (Benacer *et al.,* 2016). Leptospirosis which is commonly known as rat-urine fever is an endemic disease in Malaysia and recently has received increasing attention mainly due to several recent incidents that have resulted in human mortality (Thayaparan *et al.*, 2013). The Health Ministry of Malaysia reported 3,665 leptospirosis cases in 2013 with 69 deaths recorded, a whopping 85.5% increase from the 1,976 cases recorded in 2012, with 58 deaths (the Star, 1st June 2013). The leptospirosis cases had skyrocketed to 8291 cases in 2015 (the Star, 12th July 2017). Benacer *et al.* (2016) reported *R. rattus* and *R. norvegicus* were reported as the reservoir for leptospirosis virus in urban area in Malaysia.

2.3 Commensal Rats

Commensal rats have been a growing concern among urban residents. The ideal conditions for the increasing of commensal rodent population were because of the availability of food and water sources as well as the harborages (Yap *et al.*, 1999; Meyer, 2003). It is not clear when this commensalism between human and rats began: it could have started thousands years back (Krinke, 2000). The commensal rat species are the Norway rats (*R. norvegicus*), roof rats (*R. rattus*) and the house mouse (*M. musculus*) which are distributed worldwide (Lund, 1994; Meyer, 2003). *Rattus norvegicus* and *R. rattus* tend to settle along routes of human migration (Yoshida, 1980). Lesser bandicoot rat (*B. bengalesis*) is the commensal rat in central Asia and *R. exulans* is a common commensal rats around Pacific (Meyer, 2003).

The status of commensal rats were documented by Harrison (1957) whereby from 10,000 small mammals collected from Kuala Lumpur, 72% were *R. rattus*, and 15% were *M. musculus*. However, *R. norvegicus* were largely found at coastal towns such as Penang and Langkawi as reported by Medway (1969). The most commonly found rodents in urban areas in Malaysia are the roof rat (*R. rattus*), the Norway rat (*R. norvegicus*) (Zain *et al.*, 2012) and *Bandicota* spp which is the latter restricted to the northern peninsular Malaysia e.g. Kedah, Perlis and Penang (Nurul Liyana *et al.*, 2011). Nurul Liyana *et al*. (2011) also reported that rat pests in Penang are Norway rat (*R. norvegicus*), roof rat (*R. rattus*), house mouse (*M. musculus*) and bandicoot rat (*B. bengalensis*).

Figure 2.1 shows the general morphological characteristics of commensal rats in urban areas; source taken from CDC (2010). The species of commensal rats were identified based on physical features such as the body size, tail length, size of ears and eyes and the shape of the snout. Besides the general characteristics of commensal rats, the identification of the species can be done by using the shape of their dropping (CDC, 2010) as shown in **Figure 2.2**. The shape and size of dropping were varies between species.

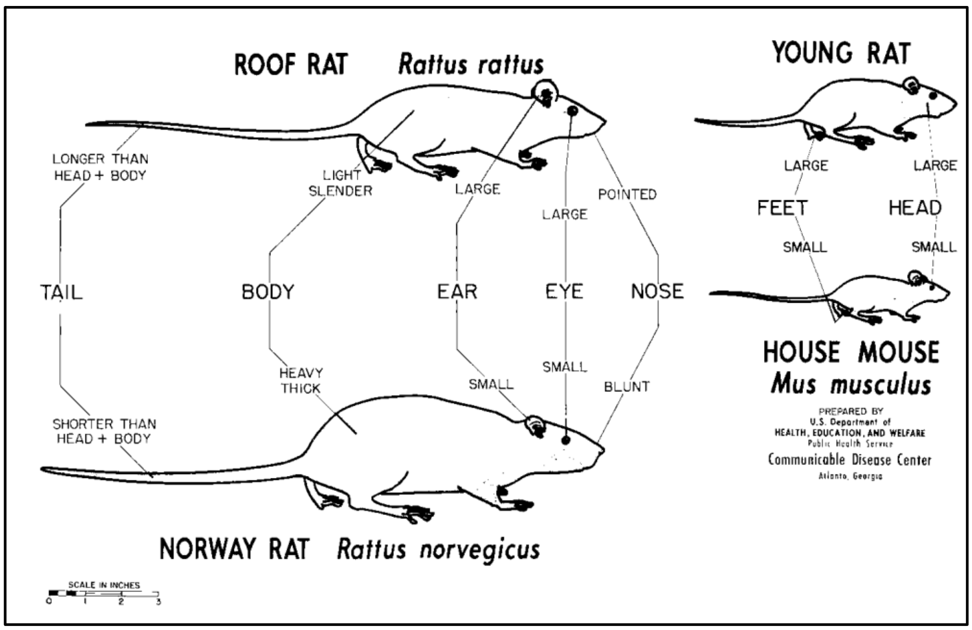


Figure 2.1. The general characteristic to identify commensal rats. Source adopted from CDC (2010).

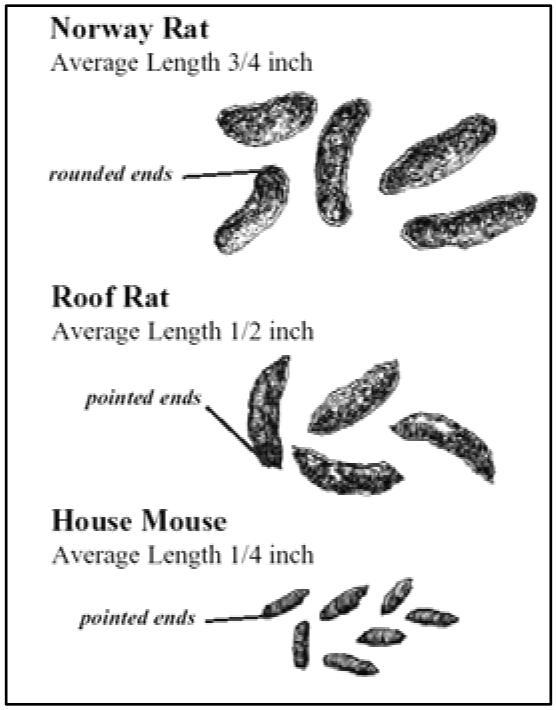


Figure 2.2. The identification of commensal rats species based on the shape and size of dropping. Source adopted from CDC (2010).

2.3.1 *Rattus rattus*

The roof rat (*R. rattus*) is large in size about 110-200mm of head to body length with average of body weight of 180g (Medway, 1969). The hind foot length is about 30-38mm. The roof rat has soft spines fur with brown to black in color (Medway, 1969; Harrison, 1974). This species has long tail about 85-119% longer than their body length (Medway, 1969; Harrison, 1974). The ears are prominent and large and the eyes are big and protruded (Medway, 1969; Harrison, 1974). The roof rat has a pointed snout with average skull length of 41mm (Medway, 1969; Harrison, 1974; Yong, 1982). The number of mammae for this species are 10, 2 pairs of mammae at pectoral and 3 pairs at inguinal (Yong, 1982).

2.3.2 *Mus musculus*

Mus musculus or the house mouse is small in size about 50-80mm of head to body length with average weight of 10g (Medway, 1969). The hind foot length is about 14-17mm (Medway, 1969; Harrison, 1974). The house mouse has a soft fur which greyish to brown in color and on the lower abdomen, the color is plain grey to white (Medway, 1969; Harrison, 1974). The tail length of the house mouse is about 90-120% of their body length (Medway, 1969; Harrison, 1974). The ears are large and prominent and the eyes are small in size (Medway, 1969; Harrison, 1974). The shape of snout for *M. musculus* is pointed with average skull length of 19mm (Medway, 1969; Harrison, 1974; Yong, 1982). The number of mammae for this species are 10, 2 pairs of mammae at pectoral and 3 pairs at inguinal part of their lower abdomen (Yong, 1982).

2.3.3 *Bandicota bengalensis*

Bandicota bengalensis or known as lesser bandicoot rat is huge in size with head and body length about 150mm to 250mm with average of weight about 400g (Medway, 1969; Harrison, 1974). The hind foot length is about 32-42mm which first inner digit of hind foot bears a normal claw as in other digits (Yong, 1982). The lesser bandicoot rat fur is in dark greyish to brown color with grey color on the lower abdomen and the fur for this species is long and stiff spines (Medway, 1969; Harrison, 1974; Yong, 1982). The tail is slightly shorter in length about 70-95% of the body length (Medway, 1969; Harrison, 1974). This species has small eyes and ears. The shape of snout is blunt with average skull length of 46mm (Medway, 1969; Harrison, 1974; Yong, 1982). The number of mammae for this species are 12 which 3 pairs of mammae at pectoral and 3 pairs at inguinal part of their lower abdomen (Yong, 1982).

2.3.4 *Rattus norvegicus*

Rattus norvegicus or known as Norway rat is large in size with head and body length about 150mm to 200mm with average of weight about 250g (Medway, 1969; Harrison, 1974). The hind foot length is about 34-42mm (Medway, 1969; Harrison, 1974). Norway rat fur is in greyish to brown color with grey color on the lower abdomen and has a soft spine fur. The tail is slightly shorter in length about 80-100% of the body length with slightly darker in color at the above part of the tail and paler in color at below (Medway, 1969; Harrison, 1974). This species has small eyes and ears. The shape of snout is blunt with average skull length of 50mm (Medway, 1969; Harrison, 1974;Yong, 1982). The number of mammae for this species are 12 which 3 pairs of mammae at pectoral and 3 pairs at inguinal part of their lower abdomen (Yong, 1982).

The Norway rat lives in colonies and divided into two social systems for females and males. Female social system: Wild females may raise young alone (Telle, 1966; Schultz and Lore 1993), or up to six reproductive females may share a ground burrow, each with a separate nest chamber. The females are often related through the mother's side and they may collectively nurture their young (Steineger, 1950; Calhoun, 1962; Barnett, 1975; Schultz and Lore, 1993; Moore, 1999). Male social system is territorial depending on the population density per square meter especially at low population densities (Barnett, 1958). In a system of territorial males, each male has a separate home range and defending the burrow which consist of one or more females (Barnett, 1958; Hanson, 2006). Rat on its own territory would protect the burrow and attack any intruder (Lott, 1984). At high population densities, (e.g. 1 rat per 5 square meters) territories become too costly to maintain due to intruder pressure (Hanson, 2006).

Wild rat have an average lifespan of about 1 year (Jackson, 1982). Whitaker (1980) reported that some wild rats can reach age 3 years old, but such individuals are very rare. High adaptation or lack of specificity are the main reasons for this species survived in urban area (Lund, 1994). The recovery of urban Norway rat populations depended on how severely the initial population were reduced (Emlen *et al.*, 1948). After eradication process occurred (100% reduction), the population will recover within 2 years. However, in moderate reduction, the rat population started recovering early and increased about 4% from original number of population every month (Hanson, 2006).

Norway rats eat any type of food either from plant or meat (Barnett, 1963; Timm, 1994). They select only nutritious food and balance diet for their daily consumption (Timm, 1994). Timm (1994) also added Norway rats require 15 to 30 ml of water daily and food items in household garbage offer balanced diet and moisture needs. Both wild and laboratory *R. norvegicus* have exploratory and sampling behavior as they sample the unfamiliar food first before consuming (Barnett, 1958). They also follow trails left by other to find the food and attracted to food that have “rat breath” on it (Barnett, 1963). Rat breath or odors cues left at feeding sites facilitated the food preference in Norway rats (Galef and Heiber, 1976; Laland and Plotkin, 1991).

Rat mating system is determined by the changes of population density. At low densities the mating system is mostly polygynous one male mate with multiple females meanwhile, at high densities the mating system becomes polygynandrous; multiple males mate with multiple females, through group mating and rushing (Hanson, 2006). In this case large groups of males compete among themselves to copulate sequentially with an oestrus female (Moore, 1999). Usually male with larger testicles deposited more sperm and has a higher chance to produce offspring (Moller, 1989). Social and mating systems may also change with food supply distribution, spatial qualities of the landscape, predation levels, and other factors (Lott, 1984). One matured female can produce six to twelve litters per time and born 21 to 23 days after conception (Timm, 1994). Female can go into heat again at least 10 hours after giving birth (Gilbert *et al.*, 1985).

According to Timm (1994), Norway rats were first introduced in United States around 1775 and spread all over the places wherever humans reside. *Rattus norvegicus* has been distributed throughout the world by transportation or by stowing away (Lund, 1994). They live in close association with human either in sub-urban or urban areas by making nests and burrow under the buildings (Timm, 1994). They preferred wet and moist places for example sewer, ponds and riverbank (Traweger and Slotta-Bachmayr, 2005).

2.4 Control Measure

Several control measures have been practiced to control rats both in urban and agricultural areas such as cultural, mechanical, biological and chemical control, with varying success (Prakash, 1988). However, chemical controls by using anticoagulant and non-anticoagulant rodenticides are most commonly used for rodent control both in urban and agricultural areas.

A chemical control by using rodenticide is the main control method against rat populations both in urban and agriculture areas in Malaysia (Lam, 1982). The attributes of rodenticide such as effective result with quick reduction in pest numbers and convenience to use has make them a popular method to deal with rat problem (Lam, 1982). Commercial chemical rat baits are available in several form and formulation such as in loose meals form, wax block, pellet and also in liquid form. Chemical control also can be in powder or dust form such as the tracking powder. The toxicant powder can be placed at the rat trails and the powder will be picked up by their feet. They get poisoned by ingesting the powder during grooming process. The uncommon chemical control is by fumigation process. This treatment is very toxic and lethal to human.

Other than chemical control, physical methods in controlling rats have been long recognized and effective in reducing rat population. The method of controlling rats is by mechanical proofing, physical barrier and trapping (Singelton *et al.*, 1999). One of the oldest ways of controlling rats are by using traps (Lam, 1982). Traps are widely used in developing countries in both agriculture and urban area (Singelton *et al.*, 1999). Trapping is effective in small area but too costly and laborious over large scales (Lam, 1982). Glue boards or sticky traps are the alternative to the snap traps or live traps. Glue boards are available in various sizes and should be place in rat runaway to trap them.

Another method that can be employed in dealing with rats is by biological control method. An early biological control for controlling rats in agricultural areas was to try to establish ‘‘cat farms’’ (Bunting, 1939). However it was not successful and the method was not developed further (Wood and Chung, 2003). The most well-tested and environmentally friendly method of rodent population control is the rearing of barn owls (*Tyto alba*) on plantations (Duckett, 1989). The barn owl has adapted itself very well to oil palm plantations, where it survives on a diet comprising of 99.4% rats (Smal, 1990). The use of wild barn owls in artificial nesting boxes for the control of rodent pests has been suggested a few decades ago and is implemented in many regions of the world (Taylor, 1994). Studies in Malaysia have reported that barn owl is highly efficient in controlling rats in agricultural areas (Duckett and Karuppiah, 1990; Hafidzi and Saayon, 2001). Integrated pest management with biological control using *Tyto alba* (barn owl) has been adopted since the late 80's against rat populations in paddy and oil palm plantations (Chung, 2012) but not in urban area. The potential of barn owls as biological control of rat populations in urban areas have been studied in South Africa and they are able to adapt close to human habitation and establish breeding populations (Meyer, 2003).

2.5 Rodenticides

Rodenticides provide a very effective tool for increasing mortality, which is one of the key demographic processes in population dynamics. The rodenticides must be efficacious while relatively safe for other non-target animals and human. The poisons can be classified into two groups: all other compounds (non-anticoagulants) and anticoagulant (Murphy, 2007). There are many different active ingredients registered as rodenticides in Malaysia. There are two types of rodenticides; acute poison and chronic poison.

Acute poison damaged the function of vital organs and system of the animals such as respiratory, nervous, renal system, liver and metabolic pathways (Lee and Kamarudin, 1987). The usage of acute poisons started early nineteenth century using arsenous oxide, strychnine and thallium sulphate (Jack, 1921) as these poisonous effects were highly hazardous, they were banned subsequently (Lee and Kamarudin, 1987). Nowadays, zinc phosphate is still available and being used in rice field. However, the usage of acute poison has disadvantage. Acute poison can cause bait shyness during baiting program as the poisoning symptoms appeared before the animal consumed a lethal dose (Lee and Kamarudin, 1987).

Chronic poisons are categorized as slow acting poison as their effects are seen 5 – 7 days after the lethal dose (Deykin, 1970). In Malaysia, chronic poisons are anticoagulants such as warfarin, coumachlor, coumatetralyl, chlorophacinone, brodifacoum and bromadiolone (Lee and Kamarudin, 1987). The registered rodenticides use in Malaysia falls into three categories, first generation anticoagulant, second generation anticoagulant and non – anticoagulant rodenticides.

2.5.1 Non-anticoagulant Rodenticides

Non-anticoagulant can be a hazardous compound and can kill rodent instantly (eg, arsenic and zinc phosphate) (Lam, 1982). Zinc phosphide was first registered in 1947 and very toxic to mammals as it can damage the heart, brain, kidney and liver (Albretsen, 2004). There are some non-anticoagulant which are moderately hazardous and toxic to mammals such as cholecalciferol that can be used against anticoagulant resistant rats (Lund, 1974; Lam, 1982). Cholecalciferol is a vitamin D3 and was first registered as rodenticide in 1984 (EPA, 1998; Rumbeiha, 2006). Rumbeiha (2006) also added that toxic doses of cholecalciferol will lead to the excessive amounts of calcium in blood system that will lead to organ failure and death. However, these acute rodenticides has some advantages or disadvantages either in relation to toxicity, acceptability, safe usage or secondary poisoning hazards (Brooks, 1973; Lam, 1982).

2.5.2 Anticoagulant Rodenticides

Anticoagulant rodenticides were first discovered in the 1940's and introduced in Malaysian market in middle of 1980's and have since become the most widely used ingredients for commensal and agricultural rodent pest control (Noor Hisham *et al.*, 2007). The mechanism of anticoagulant rodenticides are by inhibiting the production of vitamin K in the liver that cause internal bleeding and death after a few days of consumption (Lam, 1982).The inhibition of vitamin K1 epoxide reductase will inhibits the prothrombin and other clotting factors (Lee and Kamarudin, 1987).

A variety of compounds and delivery mechanism exists, with second generation anticoagulants giving the best results (Buckle, 1994). There are two groups of anticoagulant rodenticides which are coumarins and indandione that have different chemical structures. Each of the groups were sundivided into two classes; first generation and second generation of anticoagulant rodenticides (Joermann, 1998; Valchev *et al.*, 2008). Currently, the registered rodenticide that available in the market are warfarin and coumatetralyl (first generation anticoagulant from coumarin group), chlorophacinone and diphacinone (first generation anticoagulant from indandione group), bromadiolone, brodifacoum and flocoumafen (second generation anticoagulant from coumarin group) (Lam, 1982).

The earliest first generation rodenticide is warfarin. Apparently its wide and frequent usage has developed resistance in rat population (Wood and Chung, 2003). Anticoagulant resistant in *R. norvegicus* was first discovered at Scoltland in 1958 because of continues treatments with warfarin and diphacinone (Boyle, 1960). Other warfarin resistant cases reported were in England and Wales back in 1960 (Drummond, 1966; Drummond and Bentley, 1967). However in Malaysia, only *R. rattus diardii* was reported to be resistance towards anticoagulant rodenticides (Lam *et al.*, 1982). Hence, second – generation anticoagulant rodenticide has been applied to overcome the resistance of first generation rodenticides (Chung, 2012). Second generation anticoagulant rodenticide were derived from coumarin same as group as warfarin but are approximately 100-1000 times more acutely toxic (Erickson and Urban, 2004).

Table 2.1 shows the list of registered anticoagulant rodenticides in Malaysia for controlling rodent pest in agricultural and commensal sites. Currently, there are six anticoagulant rodenticides of Coumarin group and two from Indandione group are registered and available in the market. According to the Pesticides Board of Malaysia (2017), bromadiolone and chlorophacinone are the most popular rodenticides manufactured as rat bait employed in oil palm plantations against rat populations. Two types of formulations which come as granule in sachet and block bait are manufactured with recommended concentration by the authority.

Apart from hastening the development of resistance, effort is made to switch back to first generation of anticoagulant rodenticides of different active ingredients for instance chlorophacinone and coumatetratyl. This is especially important and critical in agricultural habitats whereas application of highly toxic rodenticide can lead to secondary poisoning of predators, raptors and scavengers that may have access to the rodenticides baits (Dowding *et al.*, 2010). Despite some public concern about the use of chemical compounds, rodenticides certainly will continue to play a role in rodent management in agriculture (Buckle, 1994). In order to be effective, rodenticides must be applied at the correct point, both in space and in time (Tobin *et al.*, 1997; Stenseth *et al.*, 2001).

Table 2.1. List of anticoagulant rodenticides registered in Malaysia.

Group	Class	Chemical Name	Concentration mg a.i/kg bait (trade name)
Coumarins	1st Generation	Warfarin	500 (KG22®, TIKUMIN®)
		Coumatetralyl	375 (RACUMIN®)
	2nd Generation	Bromadiolone	25 (DSP®, Q-RAT®) 50 (EBOR®, BUTIK G2®, LANIRAT®, RATOX®)
		Brodifacoum	50 (ARATTA®, JASPER®) 30 (ECHO®, MATIKUS®)
		Difenacoum	50 (RATPUS®)
		Flocoumafen	50 (STORM®)
Indandiones	1st Generation	Chlorophacinone	25 (DSP®, TRIXON®) 29 (CHECK®) 50 (BUTIK S®) 100 (CHECK BAIT®)
		Diphacinone	50 (YASODION®)

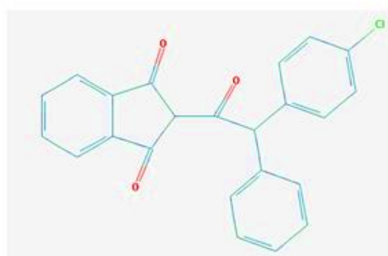
Source: Pesticide Board Malaysia (2017)

2.6 Rodenticides Selected for the Study

There are two rodenticides selected for this study which is chlorophacinone and bromadiolone. Both are anticoagulant rodenticides but from two different generation. **Figure 2.3** shows the chemical structure and properties of chlorophacinone. Chlorophacinone is from the first generation of anticoagulant rodenticides meanwhile bromadiolone is from the second generation. Chlorophacinone, 2-[1-(*p*-chlorophenyl)-1-phenyl] acetyl-1, 3-indanedione, anticoagulant was developed in France (Lam, 1982). It is widely used in Europe and United States and found to be more toxic to Norway rats (Rowe and Redfern, 1965; Lund, 1977; Lam, 1982).

Chemical name: 2-[1-(*p*-chlorophenyl)-1-phenyl] acetyl-1, 3-indanedione

Chemical structure:



Class: Indandione anticoagulant

Molecular formula: $C_{23}H_{14}O_3Cl$

Molecular weight: 373.8

Physical state: Solid

Melting point: 140℃

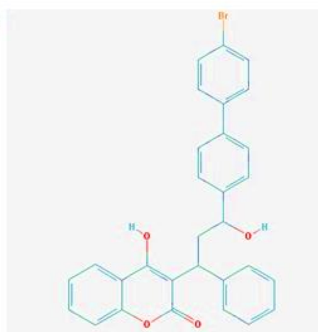
Solubility: 20-34ppm

Figure 2.3 Chemical structures and selected physical and chemical properties of the Chlorophacinone (Erickson and Urban, 2004; PubChem Compound Database, 2017)

Figure 2.4 shows the chemical structures and properties of bromadiolone. Bromadiolone is the new potent hydroxyl coumarin derivative, 3-[3-4'-bromo (1,1'-biphenyl)-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one (Lam, 1982; Erickson and Urban, 2004) is highly toxic to rats and mice (Lund, 1977). Both anticoagulant is widely used in agriculture such as in oil palm plantations and rice fields but rarely used in urban area. According to the Pesticides Board of Malaysia (2017), the most popular rodenticides manufactured as rat bait in oil palm plantations were bromadiolone and chlorophacinone. The common formulations used were granules in sachet and block bait formulation with recommended concentration by the authority.

Chemical name: 3-[3-4'-bromo (1,1'-biphenyl)-4-yl)-3-hydroxy-1-phenylpropyl]-4-hydroxy-2H-1-benzopyran-2-one

Chemical structure:



Class: Coumarin

Molecular formula: $C_{30}H_{23}BrO_4$

Molecular weight: 527.4

Physical state: Solid

Melting point: 200-210°C

Solubility: 12ppm in water at 20°C

Figure 2.4 Chemical structures and selected physical and chemical properties of the bromadiolone (Erickson and Urban, 2004; PubChem Compound Database, 2017).

2.6.1 Toxicity of Bromadiolone towards Rodents

Bromadiolone was effective against *R. norvegicus* by acute oral at 0.005% in bait with LD_{50} less than 2mg/kg (Poche, 1988). Erickson and Urban (2004) reported LD_{50} for laboratory rat, *R. norvegicus* were 0.56 to 0.84 mg/kg meanwhile for laboratory mouse, *M. musculus* LD_{50} was 1.75 mg/kg. The acute oral toxicity of bromadiolone in laboratory rat was 0.56mg a.i/kg and for laboratory mice was, 1.75mg a.i/kg (EPA, 1998). Bromadiolone residue in rodents caused secondary poisoning of wildlife such as foxes (Sage *et al.,* 2010), mink (Fournier-Chambrillon *et al*., 2004) and barn owls (Albert and Wilson, 2009; Hasber *et al*., 2014). These predators were reported to be high risk from secondary poisoning by bromadiolone because of their heavy predation on rodents. Many research were done to study the secondary poisoning hazard of bromadiolone to non-targets raptors and scavengers either in laboratory studies, field evaluations and monitoring survey (Elmeros *et al*., 2011; Gabriel *et al*., 2012; Sanchez-Barbudo *et al*., 2012).

2.6.2 Toxicity of Chlorophacinone towards Rodents

Chlorophacinone was effective against laboratory rat *R. norvegicus* with LD50 of 6.2mg/kg whereas for wild Norway rat LD50 was 5.0mg/kg as reported by Erickson and Urban (2004). For *M. musculus*, lethal dosage of chlorophacinone were 1.0mg/kg to 6.0mg/kg (Erickson and Urban, 2004). Toxicity hazards of chlorophacinone studies in both laboratory (Sterner 1978; Marsh and Howard, 1986; Fisher and Timm, 1987; Bachhuber and Beck, 1988; Askham 1988; Riedel *et al.* 1991; Askham and Poche, 1992) and monitoring programs (Fournier-Chambrillon *et al.,* 2004; Berny *et al.,* 1997; Proulx, 2011; Sanchez-Barbudo *et al*., 2012) were demonstrated that non-target animals from predators and scavengers which that include rodents in their diet have a high risk of exposure to secondary poisoning.

2.7 Analytical Determination of Anticoagulant Rodenticides Residue in Rat Samples

It is required for the analytical test of anticoagulant rodenticides residue in animal tissue for diagnosis. The most common technique is by analyzing the anticoagulant residue in the internal organs such as the liver, lymph, brain and pancreas (Erickson and Urban, 2004). Residue are essentially absent from the poisoned animal's stomach content as reported by Armentano *et al*. (2012). However, anticoagulant are known to have high affinity for liver tissue and contains the highest level of residues compared to other organs of poisoned animal (Parmer *et al*., 1987; Fauconnet *et al.*, 1997; Fisher *et al*, 2004; Armentano *et al*., 2012). The second anticoagulant are much more persistent in animal tissue than the first generation anticoagulant after several day exposure (Erickson and Urban, 2004). The persistence of anticoagulant residue in liver for first generation anticoagulant such as warfarin and coumatetralyl can last up to 6 months (Eason*et al.*, 1999). Whereas for second generation anticoagulant rodenticides such brodifacoum and bromadiolone, the residue persist up to 12 months (Eason*et al*., 1999).

The repetition of exposure for anticoagulant rodenticide will cause bioaccumulation inside the primary consumer and it will circulate for a longer time (Belleville, 1981). Erickson and Urban (2004) added, the high concentration circulate in the body after absorption and rapidly established in the liver and other tissue as bioaccumulation. Proportion of any ingested dose of anticoagulant rodenticide bound in the liver and it is slowly excreted