EFFICACY OF RODENTICIDES IN CONTROLLING MAJOR RAT SPECIES OF OIL PALM, *Rattus tiomanicus* (Miller) IN SUNGKAI, PERAK

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by

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LIST OF ABBREVIATIONS

µg	Microgram
g	Gram
mg	Milligram
kg	Kilogram
µm	Micrometer
mm	Millimeter
nm	Nanometer
cm	Centimeter
µl	Microliter
ml	Milliliter
ha	Hectare
ppm	Part per million
a.i	Active ingredient
psi	Pound per square inch (pressure)
rpm	Revolutions per minute
bw	Body weight
SE	Standard error
LD	Lethal dosage
FELDA	Federal land development authority
FASSB	Felda agricultural services Sdn. Bhd.
FGAR	First generation anticoagulant rodenticide
SGAR	Second generation anticoagulant rodenticide
NAR	Non-anticoagulant rodenticide
AR	Anticoagulant rodenticide
FFB	Fresh fruit bunch
US$	United States Dollar
RM	Ringgit Malaysia
HPLC	High performance liquid chromatography
UV	Ultra-violet

FLD	Fluorescence detector
SPSS	Statistical package for social sciences
ANOVA	Analysis of variance
CMR	Catch mark release
MDL	Minimum detection limit

KEBERKESANAN RACUN TIKUS UNTUK MENGAWAL SPESIES TIKUS UTAMA KELAPA SAWIT, *Rattus tiomanicus* (Miller) DI SUNGKAI, PERAK

ABSTRAK

Keberkesanan racun tikus komersial yang berbeza dalam mengawal spesis tikus sawit dominan, *Rattus tiomanicus* (Miller) telah dikaji dalam bentuk perbandingan antara enam jenis umpan tikus beracun yang terpilih daripada racun tikus antikoagulan generasi pertama, coumatetralyl, chlorophacinone dan warfarin, generasi kedua, brodifacoum dan flocoumafen, dan racun tikus bukan antikoagulan, cholecalciferol. Kajian keberkesanan umpan beracun tikus terpilih telah dijalankan melalui kajian makmal dan lapangan. Melalui kajian makmal, ujian tiada pilihan makanan menunjukkan umpan beracun flocoumafen adalah rawatan yang paling berkesan berbanding rawatan-rawatan lain di mana rawatan tersebut mencatat kadar kematian sepenuhnya terhadap keseluruhan sampel *Rattus tiomanicus*. Umpan beracun warfarin mencatatkan keputusan paling lemah dalam mengawal *R. tiomanicus* dalam kajian ini berbanding rawatan-rawatan lain dengan hanya mencatatkan 46.07% kadar kematian. Ujian pilihan makanan menunjukkan umpan beracun flocoumafen mencatatkan kadar kematian tertinggi berbanding rawatan-rawatan lain pada 55% manakala keputusan paling lemah dicatatkan oleh umpan beracun warfarin pada 10% kadar kematian. Umpan beracun flocoumafen mencatatkan ambilan umpan dan kadar selera tertinggi pada kadar 30.50% dan nisbah 1:2 berbanding rawatan-rawatan lain. Dalam pernilaian ini, umpan beracun warfarin mencatatkan paling kurang penerimaan (8.98%) dan kelazatan terendah (1:7) oleh sampel tikus yang dirawat berbanding rawatan-rawatan lain. Kesemua rawatan dalam kajian lapangan berjaya mengawal kerosakan kelapa sawit segar dibawah tahap kritikal (5%) kecuali umpan beracun warfarin.

Keputusan akhir menunjukkan kerosakan kelapa sawit segar oleh tikus dalam kawasan yang dirawat berjaya diturunkan ke paras bawah tahap kritikal berbanding kerosakan yang dicatat semasa pra-rawatan. Umpan beracun warfarin mencatatkan keputusan paling lemah melebihi tahap kritikal berbanding rawatan-rawatan lain. Peratus pengurangan kerosakan kelapa sawit segar menunjukkan hanya dua rawatan yang tidak mencapai 70% kawalan terhadap kerosakan kelapa sawit segar sepanjang kajian ini berlangsung, ia adalah umpan beracun brodifacum dan umpan beracun warfarin yang juga diformulasi menggunakan resepi yang sama. Keputusan kepelbagaian relatif populasi tikus menunjukkan semua kawasan kajian yang dirawat berjaya mencatatkan penurunan penangkapan tikus semasa pasca-rawatan berbanding yang dicatatkan semasa pra-rawatan. Kajian keracunan sekunder mencatatkan satu daripada empat burung pungguk daripada rawatan umpan beracun coumatetralyl telah terkesan akibat keracunan selepas memakan tiga ekor tikus beracun. Tanda dan simptom keracunan dicatatkan pada hari ke-enam selepas pemakanan tikus beracun. Sementara itu, burung-burung pungguk yang memakan tikus yang dirawat dengan umpan beracun cholecalciferol berjaya menempuh kajian ini dengan selamat dan sihat sehingga selepas enam bulan pemerhatian. Kandungan residu yang dikesan semasa analisis ke atas pellet yang dirawat dengan umpan beracun coumatetralyl adalah dalam julat 15.73-101.80 µg/hari manakala tiada pengesanan residu dalam pellet yang dirawat oleh umpan beracun cholecalciferol. Analisis ini menunjukkan bahawa coumatetralyl mampu berada didalam tisu burung pungguk lebih lama berbanding cholecalciferol. Oleh itu bahan aktif coumatetralyl mempunyai risiko lebih besar untuk menyebabkan keracunan sekunder ke atas burung pungguk berbanding bahan aktif cholecalciferol.

EFFICACY OF RODENTICIDES IN CONTROLLING MAJOR RAT SPECIES OF OIL PALM, *Rattus tiomanicus* (Miller) IN SUNGKAI, PERAK

ABSTRACT

The efficacy of different types of rodenticides in controlling major rat species of oil palm, *Rattus tiomanicus* (Miller) was carried out in a comparative study among six types of rodenticide baits selected from first-generation anticoagulants: coumatetralyl, chlorophacinone and warfarin, second-generation anticoagulants: brodifacoum and flocoumafen, and non-anticoagulant rodenticide cholecalciferol. The study of the efficacy of selected rodenticide baits was conducted in both laboratory and field assessments. In the laboratory study, no-choice feeding study showed that flocoumafen bait was the most effective in this study compared to other treatments by recording complete mortality against *R. tiomanicus*. Warfarin bait recorded the lowest efficacy result in this study against *R. tiomanicus* by only recording 46.07% mortality. Choice feeding study results showed that flocoumafen bait recorded the highest mortality compared to other treatments at 55% while the least efficacy was recorded by warfarin at only 10% mortality. In addition, flocoumafen bait recorded highest bait acceptance at 30.50% and the most palatable bait at 1:2 compared to other treatment baits. In this evaluation, warfarin was the least effective (8.98%) and the least palatable bait (1:7) of the rats compared to other treatment baits. All treatment baits managed to control oil palm fresh rat damages below the threshold level (5%) except warfarin bait. The final result showed that fresh rat damages in treated areas dropped below the threshold level compared to the damages recorded earlier in pre-treatment. Warfarin bait recorded the least effective result, which was above the threshold level compared to other treatment baits. Percentage reduction of

fresh rat damages showed that only two treatment baits did not achieve more than 70% control over fresh rat damages throughout the study period, namely brodifacoum and warfarin baits. Relative abundance of rat populations showed all treated plots recorded reduction of rats captured in post-treatment sampling compared to pre-treatment sampling. Secondary poisoning feeding study showed that one of four barn owls from the coumatetralyl group were affected by secondary poisoning after consuming three poisoned-rats that consumed coumatetralyl bait. The sign and symptom of poisoning were recorded on the sixth day after feeding the poisoned rats. Meanwhile, all barn owls fed with rats treated with cholecalciferol survived and were healthy even after the six-month study period. The amount of residue detected during further pellet analysis of owls consuming rats treated with coumatetralyl were in the range of 15.73-101.80 µg/day meanwhile there was zero detection of residue in the pellets from owls consuming rats treated with cholecalciferol. This analysis showed that the coumateralyl compound was retained in the tissue of barn owls longer than the cholecalciferol compound, hence coumatetralyl pose a greater threat of secondary poisoning to barn owls compared to cholecalciferol.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Rat Problem in Oil Palm Plantations

Rodents, particularly rats, are a key pest of various major crop plantations in Malaysia such as cocoa, rice and oil palm. The rat problem in oil palm plantations in Malaysia started in the 1930s, approximately ten years after the first commercial plantations were established in the 1920s (Wood and Chung, 2003). Rat damages can cause substantial economic loss to the oil palm industry if this problem is not controlled. Singleton *et al*. (2003) reported that rat attacks caused 5-10% losses in rice and oil palm production in Asia. About 10% loss of palm oil production was estimated to be caused by rats (Chung, 2012). Rats attack oil palm regardless of growth stage. In immature palm, the damages are inflicted on the petiole (frond bases) and apical growing meristematic tissue (cabbage or palm heart). Damages on petiole suppress formation of frond and rats feeding on apical growing meristematic tissue eventually kill the palm. Whilst at the mature stage of palm, rat attacks on inflorescence and fruit bunch result in deformation of flowers and fruit damages leading to yield reduction (Wood, 1982a; Hafidzi and Saayon, 2001; Chung, 2012).

There are three major species of rats recorded in Malaysia responsible for fruit bunch damages in oil palm plantations. The three main species are *Rattus tiomanicus* (Miller), *Rattus argentiventer* (Robinson and Kloss) and *Rattus rattus diardii* (Jentik) (Wood, 1976). Each species is dominant in different stage of oil palm. *Rattus tiomanicus* is the common pest in oil palm and a dominant species especially in mature palms (Wood, 1968;

Buckle, 1997). *Rattus argentiventiver* originally is a native species in rice fields but this species can be found in nurseries and young oil palms (Wood, 1982b; Payne *et al*., 1985; Puan *et al*., 2011), whereas *R. rattus diardii* is usually associated with human activity and can be found in human habitation (Mohd, 1985; Buckle, 1997). This species was reported as a common pest from the 1980s, especially in plantations where *R. tiomanicus* was under control by baiting (Chung, 2012; Hafidzi and Saayon, 2001).

1.2 Management of Rats in Oil Palm

Management of rats came with various methods during early establishment of oil palms. Conventional rat control practice by plantation operators can be classified into two types: physical and chemical control. Physical control, such as trapping and hunting (human predation), were the cultural practice in oil palm plantations to control the rat populations (Wood and Chung, 2003). However physical control was identified as suitable in small scale areas but has been considered as non-practical in large areas because such methods did not cause much impact towards rat populations (Fitzwater, 1988; Chung, 2012). Application of chemical control during early establishment of oil palm was conducted by application of a mixture of non-anticoagulant rodenticide (NAR) bait from various chemical poisons such as sodium arsenite, thallium sulphate and zinc phosphide to control rat populations (Wood and Chung, 2003). The NARs offer rapid reduction in rat populations when applied due to its extreme level of toxicity resulting in instant death once consumed (Gupta, 2018).

Anticoagulant rodenticides (AR) were introduced in the early 1950s as an alternative toxicant to NAR. Acting as a chronic poison, AR is a solution to the weakness of NAR especially the way it is lethal to rats after being ingested. The rats which consumed ARs

do not express any symptom of toxicity until bait feeding reaches a lethal dose, thus preventing bait shyness (Bentley, 1972). ARs are also less harmful to humans and non-target animals as the antidote, vitamin K, is available in the market, and can prevent any symptoms from a poisoning incident from manifesting if administered in time (Lefebvre *et al*, 2017). Anticoagulant rodenticides are classified into two different classes, namely first-generation anticoagulant rodenticide (FGAR) and second-generation anticoagulant rodenticide (SGAR). Both class of rodenticides have different acute levels, application and impact to target species. The FGARs were labelled as multiple feed rat baits and SGARs as single feed rat baits. Rats need to consume more than a single bait of FGAR until their consumption reaches lethal dose, but rats generally cannot survive after they consume a single bait of SGAR. This is because SGARs have a higher acute level compared to FGARs (Chung, 2012).

1.3 Secondary Poisoning of Barn Owls, *Tyto javanica javanica* Gmelin

Despite effectiveness against rat pests, there are growing concerns about the side effects produced by ARs, which indirectly affect non-target fauna through predation or scavenging on poisoned rats. Predators frequently reported in the case of secondary poisoning are mainly predators whose main diet are rodents. Preying on poisoned rats lead to risk of the predatory birds suffering secondary poisoning due to the residues of ingested AR by the rats that persist in tissue (Eason *et al.*, 2002; Walker *et al.*, 2008; Thomas *et al.*, 2011). Repeated exposure towards poisoned rodents cause an accumulation of poison residue in the body system of predatory birds which lead to secondary poisoning when the accumulated poison residue has reached lethal concentrations (Eason *et al*., 2002; Fisher *et al*. 2003; Hoare and Hare, 2006).

In Malaysia, secondary poisoning incidents are usually related to the barn owl, *Tyto javanica javanica* Gmelin. Barn owls were introduced as the main biological control of rats in oil palm plantations in Malaysia in the 1970s through natural propagation in order to reduce dependency on chemical control following the principle of integrated pest management (Rizuan *et al*., 2016). In Malaysia, Lee (1994) reported rodenticides from class SGAR (bromadiolone, brodifacoum and flocoumafen) and FGAR (warfarin) posed high degree of toxicity towards barn owls. While Hasber *et al*. (2014) added chlorophacinone to the list of highly toxic chemicals towards barn owls based on aviary and field studies.

Previous studies about secondary poisoning of barn owls in Malaysia by various researchers have showed a common repercussion of AR use towards the biological agent. There is a significant need for further research to identify less toxic rodenticides in order to be aligned with the practice of integrated pest management, whereby not only is the control of the pest population as the main concern, but natural biological control agents are spared and populations conserved.

1.4 Justification and Objectives

Chemical control through rodenticide application has been used as a main approach to combat rat infestations in oil palms in Malaysia since the 1960s (Lever, 1962; Wood, 1969). However, there is a lack of proper documentation and publication about the efficacy of rodenticides in this country, especially in laboratory and field studies since most of plantation research institutions only generate findings of their studies as internal

reports rather than publish them to public. Furthermore, existing publications are too outdated and very few publications after the 2000s can be acquired. A proper study is important on this matter to provide an update on new findings and to re-evaluate some of the information whether it is still relevant in recent times as rat pests is still one of the main problems in the pest management in oil palm. There is also a lack of knowledge on effect of rodenticides, particularly non-anticoagulant rodenticide namely cholecalciferol, on barn owls, though it is reported to be less toxic to non-target animals especially bird species (Marshall, 1984; Eason *et al.,* 2000; Erickson and Urban, 2004). Therefore, the objectives of this study were:

1. To evaluate the efficacy of commercial rodenticide baits against major rat pest in oil palm plantation, wood rat, *Rattus tiomanicus* through laboratory feeding study.
2. To study the field efficacy of selected rodenticide baits against rat populations in oil palm plantations.
3. To assess the secondary poisoning risk of anticoagulant and non-anticoagulant rodenticides on barn owl, *Tyto javanica javanica* (Gmelin) in captivity.

CHAPTER 2

LITERATURE REVIEW

2.1 Anticoagulant Rodenticides

The definition of rodenticides can be identified by the word itself. Rodents refer to small mammal pests and the suffix –cide means the substance which is used to kill. Rodenticides are pesticides which are used to kill or repel rodent pests such as rats, mice, squirrels, chipmunks and voles. The usage of rodenticides has become a normal practice in rat control in agriculture industries across the globe (Bentley, 1972; Buckle, 1994; Gupta, 2018). Planters choose rodenticides in dealing with rat problems because it provides a better solution compared to physical and cultural practices. In earlier days of rodent control, plantation operators-controlled rodents by applying acute poisons. Acute poison compound such as strychnine, white arsenic, yellow phosphorus, red squills and cantharides were used during earlier days of rodent control (Boelter, 1909; Lee and Kamarudin, 1987). Over time, acute poison compounds were more varied with zinc phosphide, thallium sulphate, alpha-napthylthiourea and flouroaceates available as other options for rodent control (Greaves, 1971; Lee and Kamarudin, 1987).

The advantage of using acute poisons is that the effect on rodents is fast and able to quickly knock down the rodents which come in contact with the poison. However, in terms of efficacy, the immediate effect of acute poisons with poisoning symptoms occurring before the animal can consume the lethal dosage lead to avoidance of the baits, known as bait-shyness syndrome. Furthermore, usage of acute poisons are highly hazardous to humans and animals which accidentally come in contact with the poisons as its efficacy is broad

spectrum (Gratz, 1973; Lam, 1982). In order to find safer rodenticides, anticoagulant rodenticides were introduced in the early 1950s as the main alternative to acute poisons. It was a major turning point in the rodent control industry as anticoagulant rodenticides dominated the market and were extensively used around the world (Bentley, 1972). Most planters switched to anticoagulant rodenticides not only because the rodenticides are safer but also more reliable in terms of efficacy.

Anticoagulant rodenticides are a chronic poison which lethal effects take place slowly and are cumulative over days as the animal ingests the poison. The effects are obviously different compared to acute poisons, as anticoagulant rodenticides work much more slowly and the post-effects only can be seen five to seven days after application. The anticoagulant poison is accepted without reluctance by the animal when it is included in the baits at low concentration (Rowe *et al*, 1970). Currently, anticoagulant rodenticides are divided into two classes which are distinguish mainly in terms of degree of toxicity, i.e., first-generation and second-generation anticoagulants. Anticoagulant rodenticides can also be divided into two types based on their chemical derivatives; hydroxycoumarin and indandione (**Table 2.1**). There are several first- and second-generation anticoagulants which belong to group hydroxycoumarin while only first-generation anticoagulants belong to the indandione group (Fishel, 2016).

Table 2.1: Chemical derivatives and type of anticoagulant rodenticides

Chemical Derivatives	Active Ingredient	Class of anticoagulant	
		First Generation	Second Generation
Hydroxycoumarin	Warfarin	✓	
	Coumatetralyl	✓	
	Brodifacoum		✓
	Flocoumafen		✓
Indandione	Bromadiolone		✓
	Chlorophacinone	✓	

There have been several studies in the literature reporting on mode of action of every rodenticide when it is consumed by the animal (Bentley, 1972; Thijssen, 1995; Merola, 2002; Frankova *et al.*, 2019). Researchers have studied the effect of different rodenticides on the consumer especially rats. The studies suggested that every rodenticide produce different modes of action in order to kill the consumer. Acute poisons such as zinc phosphide, red squill and cyanogas-calcium cyanide have different modes of action which attack vital parts of the body system and eventually kill the consumer (Lee and Kamarudin, 1987). However, anticoagulant rodenticides such as warfarin, coumatetralyl and brodifacoum have a much more similar mode of action where the effects are chronic but lethal to the consumer.

Anticoagulant rodenticides work as anti-vitamin K in the body system of the consumer, preventing blood clotting from happening resulting in death due to excessive blood loss (Berny, 2011). Lee and Kamarudin (1987) described acute poison rodenticides gave immediate effect after consumption by the animal. Contrary to acute poisons, chronic poisons (anticoagulant rodenticides) effects are delayed for a few days (five to seven days)

after being consumed (Deykin, 1970). Baert *et al*. (2012) reported that the delayed action in anticoagulants prevent bait shyness syndrome and helped to control rodent pest. Delayed action occurs due to cumulative effects of anticoagulants which lethal effects take place after the poison dosage consumed by rodent pests reach lethal levels.

Introduction of anticoagulant rodenticides in the early 1950s were deemed successful by Berny (2011) to replace acute poisons. According to Bradbury (2008), the application of anticoagulant rodenticides is widely practiced around the world to manage commensal rodents in order to protect human health, property and agriculture crop. Even though anticoagulants were introduced in the 1950s, it only started to be used in Malaysia in the 1960s, with warfarin as the first anticoagulant (Lever, 1962). Since then, anticoagulant rodenticides have gained popularity among the plantation operators and/or plantation owners owing to the positive result of baiting in oil palm, rice field and cocoa plantations (Wood, 1969; Wood 1971; Han and Bose, 1980). Over time, anticoagulant rodenticides have developed and there are plenty of options with various first- and second-generation anticoagulants available in the market (**Table 2.2**).

Table 2.2: Commercial anticoagulant rodenticides registered and available in Malaysia.

Class of rodenticides	Type of anticoagulant	Trade name
First generation	Warfarin	• Ebor baits® • King Kong®
	Coumatetralyl	• Racumin®
	Chlorophacinone	• Butik S®
Second generation	Bromadiolone	• Butik G2®
	Brodifacoum	• Ebor 2030® • Matikus®
	Flocoumafen	• STORM®

2.1.1 Selected Rodenticide Baits in the Study

In general, there were various rodenticide, regardless anticoagulant or non-anticoagulant, that commonly used to combat commensal rodents in Malaysia. Anticoagulant rodenticides were widely used in Malaysia (Wood, 1969; Wood, 1971; Han and Bose, 1980, Hasber *et al.,* 2014) where the rodenticides which commonly found in the market were warfarin, coumatetralyl, cholorophacinone, bromadiolone, brodifacoum and flocoumafen. Nevertheless, non-anticoagulant as fast acting rodenticide also were chose to provide short term solution to rodent problem. However, the choice was limited to only zinc phosphide since other fast acting rodenticides such as fluoroacetamide and calcium cyanide were banned by Malaysian authority (Jabatan Pertanian Malaysia, 2018).

As for the selected rodenticide bait for this study, the selection were including only six rodenticides which are commonly found in Malaysian market. Selected rodenticide baits in this study belonged to the coumarin group compound except chlorophacinone (indandione) and cholecalciferol. Most of the rodenticide baits are available in the Malaysian market and permitted by the government to be used in agriculture sectors except for cholecalciferol bait (widely used in urban sector).

2.1.1.1Anticoagulant Rodenticides

Coumatetralyl

Coumatetralyl is a hydroxyl coumarin derivative which is classified as a first-generation anticoagulant (Fisher *et al*., 2004). This compound was developed originally by Farbenfabriken Bayer A. G. in Germany (Schultze, 1965, Fisher, 2005). The chemical compound was used to counter warfarin-resistant rats which were reported in Scotland and Wales (Great Britain, Ministry of Agriculture, Fisheries and Food, 1968). It is widely

used in many countries against rat pests in urban and agriculture settings (Lund, 1972; Jin and Chen, 2006; Andru *et al*., 2013). Even though it was developed purposely to combat rat pests, coumatetralyl is also used by some plantation operators to reduce the population of big vertebrate pests such as wild boar, *Sus scrofa cristatus* in Pakistan (Khan *et al*., 2017).

There have been several laboratory trials conducted to measure the efficacy of coumatetralyl against several species of rats. A series of laboratory trials of coumatetralyl against female *Rattus norvegicus* Winstar led by Penny Fisher in 2004 found that the chemical compound was lethal against the species with 83% of the samples dead after 4 days of feeding (Fisher *et al*., 2004). The reason 17% of the samples managed to survive was because they didn't consume more than 32 mg/kg, which is the LD_{99} for *Rattus norvegicus* Winstar proposed by Hone and Mulligan (1982) which was used by the author as a reference for the laboratory trial. In general, house mice, *Mus musculus* are less susceptible to coumatetralyl compared to rats. A study conducted by the same researcher, Fisher (2005) showed that more than 1000 mg/kg was needed to kill half of total sample of house mice at two different doses of coumatetralyl bait, 375 and 500 ppm, compared to previous research by the author where 32 mg/kg of coumatetralyl was sufficient to kill 99% of rats sample. The author also estimated the maximum amount of baits needed to be consumed by house mice to achieve LD_{50} were 66.40 g (375 ppm) and 49.80 g (500 ppm). There is scant report of coumatetralyl against wood rats, *R. tiomanicus* both in the laboratory and field. The latest report about coumatetralyl against wood rats is by Andru *et al*. (2013) in a study of resistance among *Rattus tanezumi*, also known as the oriental house rat, where wood rats co-existed in the site of the study in oil palm plantations. In

the published report, the authors revealed that wood rats were susceptible against coumatetralyl compared to oriental house rats which developed a resistance gene against the chemical compound.

There is also limited information on toxicity of coumatetralyl towards non-target animals. Worthing and Hance (1991) reported coumatetralyl was toxic only to chickens, *Gallus gallus* with a chronic LD_{50} reported at 50 mg/kg. For mammals, Dobson (1973) suggested coumatetralyl was highly toxic towards domestic pig, *Sus scrofa* with LD_{50} as low as 1.0 mg/kg. The only report about secondary poisoning of coumatetralyl in reptile species was reported by Weir *et al*. (2016) against western fence lizards, *Sceloporus occidentalis* which was administered orally and resulted in high LD_{50} that exceeded 1750 mg/kg. In another report by Fisher *et al*. (2004), the researcher implied that coumatetralyl was safe to biological control agents barn owls, *Tyto javanica javanica* based on an aviary study where the raptors were fed coumatetralyl-fed rats for six consecutive days. They reported that the barn owls survived after the post-feeding observation of 30 days.

Chlorophacinone

Chlorophacinone is an indandione derivative which is classified as a first-generation anticoagulant rodenticide. The chemical compound was originally developed into rodenticides in the early 1960s in France (Lam, 1982; Fisher, 2005). Chlorophacinone is widely used in Europe and the United States (Bentley, 1972). Chlorophacinone was stated by Lund (1971) as "rather more toxic" than warfarin at same concentrations. The chemical compound was found highly toxic towards *M. musculus* and *R. norvegicus (*Vanag *et al*., 1965; Rowe and Redfern, 1968). Fishel (2016) stated that generally chlorophacinone was toxic to rats at oral doses with LD_{50} of 3.15 mg/kg. Laboratory rats were highly affected

by toxicity of chlorophacinone with LD_{50} as low as 0.95 mg/kg (Jackson and Ashton, 1992) and can increase up to 6.2 to 11.0 mg/kg (Erickson and Urban, 2004). There were two different level of LD_{50} for Norway rats, *R. norvegicus* which was as low as 0.80 mg/kg as stated by Jackson and Ashton (1992) to 5.0 mg/kg as recorded by Clark (1994). House mice, *M. musculus* also had a low LD_{50} for chlorophacinone ranging from 1.0 to 6.0 mg/kg which proved the statement from Vanag *et al*. (1965) and Rowe and Redfern (1968).

Chlorophacinone can cause secondary poisoning towards non-target animals especially birds. Chlorophacinone was toxic towards northern bobwhite, with a reported LD_{50} at 258 mg/kg (EPA, 1998a) while Clark (1994) recorded LD_{50} values of more than 100 mg/kg and 430 mg/kg for ring-necked pheasants and red-winged blackbirds respectively. There were also several laboratory studies of secondary hazards of the compound to birds by Sterner (1981), Radvanyi *et al*. (1988) and Riedel *et al*. (1991) which reported that surviving birds showed severe signs of poisoning such as increased blood coagulation, external bleeding and internal hematoma detected on the tested birds.

Warfarin

Warfarin (4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one) is a coumarin derivative rodenticide, belonging to the class of first-generation anticoagulant (Erickson and Urban, 2004). The chemical compound was developed around the 1940s and 1950s by Wisconsin Alumni Research Foundation (WARF) in the United States (Merola, 2002). Warfarin was the first developed anticoagulant rodenticide and is widely used around the world to control commensal rodent pests (Fisher, 2005).

There have been a lot reports regarding warfarin-resistant issues around the world. The first report of warfarin-resistance happened in Scotland in 1958 where resistant *R.*

norvegicus populations were discovered and the issue emerged in some parts of Europe and the United States (Boyle, 1960; Drummond, 1966; Jackson and Kaukeinen, 1972; Lund, 1988). In Malaysia, warfarin-resistant rats were first reported by Wood and Chung (1990) where they discovered resistance in *R. tiomanicus* in oil palm plantations in 1982 near Klang, Selangor. Other than rats, house mice, (*M. musculus/ domesticus*) also developed resistance towards warfarin in several countries (Wallace and MacSwinney, 1976; Ashton and Jackson, 1984). Toxicity of warfarin against rats was measured using *R. norvegicus* (lab strain) which LD_{50} was different depending on the sex of the rats, with LD_{50} of male *R. norvegicus* higher than female. Hagan and Radomski (1953) reported the LD_{50} for male *R. norvegicus* was 323 mg/kg and 58 mg/kg for females, while Erickson and Urban (2004) reported LD_{50} values for male *R. norvegicus* was 100 mg/kg and females was 8.7 mg/kg. Basically, female *R. norvegicus* are more highly susceptible towards warfarin than males. Fisher (2005) stated in his report that LD_{50} of warfarin against *M. musculus* was 374 mg/kg and there was no difference in level of susceptibility among different sex of mice. This showed that *M. musculus* was less susceptible to warfarin compared to *R. norvegicus.*

Toxicity risk of warfarin towards non-target animals is also high, especially for bird species such as mallard duck, northern bobwhite and chicken, with mallard ducks (LD_{50}: 620 mg/kg) and chickens (LD_{50}: 942 mg/kg) being more susceptible to warfarin compared to northern bobwhites (LD_{50}: > 2150 mg/kg) (Erickson and Urban, 2004).

Brodifacoum

Brodifacoum (3-[3-(4'-bromo [1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2H-1-benzopyran-2-one) is a coumarin derivative which was developed as a

rodenticide in 1976 and classified as a second-generation anticoagulant (Fisher, 2005). Brodifacoum was introduced in response to the warfarin-resistance issue which emerged in the late 1950s (Boyle, 1960; Drummond 1966; Jackson and Kaukeinen, 1972; Lund, 1988; Wood, 2001). Brodifacoum is widely used around the world to combat commensal rodents and also overcome resistance issues (Fisher, 2005).

The chemical compound was highly toxic to rodent pests. Wild *R. norvegicus* are highly susceptible to brodifacoum with low LD_{50} of 0.27 mg/kg (Godfrey, 1985). Wild *M.musculus* are also reported to be susceptible towards this compound with LD_{50} slightly higher than *R. norvegicus*, at 0.52 mg/kg (O'Connor and Booth, 2001). However, susceptibility levels for lab strain of both species were slightly different. *Rattus norvegicus* lab strain had a LD_{50} that was slightly higher than the wild strain, with the values differing according to sex, i.e., 0.41 mg/kg for males and 0.56 mg/kg for females (US EPA, 1998). LD_{50} of lab strain of *M. musculus* was slightly lower than the wild strain, at 0.4 mg/kg (Godfrey, 1985). Toxicity risk of brodifacoum is high especially towards birds. LD_{50} of birds ranged between 0.26 -10 mg/kg, making birds considered susceptible towards the chemical compound (EPA, 1998a; Godfrey, 1986).

Flocoumafen

Flocoumafen (4-hydroxy-3-[3-[4-[[4-(trifluoromethyl)phenyl]methoxy]phenyl]-1,2,3,4-tetrahydronaphthalen-1-yl]chromen-2-one) is a coumarin derivative developed into a rodenticide and reported to be used as a rodenticide in 1984 against commensal and agriculture rodents (Bowler, 1984; Fisher, 2005). Flocoumafen was developed intentionally to combat warfarin resistant rats as another second-generation anticoagulant (Wood, 2001).

Flocoumafen was highly toxic towards commensal rodents. During the first application in 1984, *R. norvegicus* was among the species reported susceptible towards flocoumafen regardless of normal strain or resistant strain (Bowler, 1984). House mice, *M. musculus* are also reported to be susceptible towards flocoumafen, with the toxicity values similar with difenacoum (LD_{50} 0.8 mg/kg) (Tomlin, 2000; Fisher, 2005). LD_{50} of *R. norvegicus* (lab strain) was low (0.25 mg/kg) according to Huckle *et al*. (1989) but WHO (1995) reported a slightly higher LD_{50} value for rats (0.46 mg/kg). Rice field rats, *R. argentiventer* are reported to be a susceptible species with a low LD_{50} which value differs according to sex, i.e., 0.25 mg/kg for males and 0.37 mg/kg for females (Lam, 1990). Another agriculture rodent pest, wood rats, *R. tiomanicus* are also susceptible with low LD_{50} values of 0.28 mg/kg (male) and 0.42 mg/kg (female).

There is no report on toxicity of flocoumafen towards non-target fauna except by Newton *et al*. (1994) where the authors studied the toxicity of the rodenticides by feeding five barn owls with flocoumafen-dosed mice. All tested barn owls survived the trial except for one owl which died from haemorrhaging five days after the final poisoned rat was given. This indicates that flocoumafen can still harm non-target fauna especially birds, though there are no other reports about toxicity of flocoumafen towards non-target fauna.

2.1.1.2 Non-anticoagulant rodenticides

Cholecalciferol

Cholecalciferol is a sterol (vitamin D_3) which is classified as a non-anticoagulant rodenticide (Erickson and Urban, 2004). This compound was developed as a rodenticide in 1984 by Bell Laboratories, Inc., Wisconsin, led by Edward F. Marshall. The rodenticides were intentionally developed due to the raised issue in difficulty to control

commensal rodents, e.g. house mice (Bull, 1983), with existing anticoagulant rodenticides. New Zealand (Eason *et al*., 1993; 2010b), Europe (synonym with application in mixture with coumatetralyl) (Tobin *et al*., 1993; Pospischil and Schnorbach, 1994) and the United States (Eason *et al*., 2000; Baldwin *et al*., 2014) are countries which have established the use of cholecalciferol as a rodenticide.

Cholecalciferol was reported to be effective against Norway rats, *R. norvegicus* and house mice, *M. musculus* when orally administered with acute oral LD_{50} of 43.6 mg/kg and 42.5 mg/kg respectively based on old data before it was commercialized as a rodenticide (Marshall, 1984). However, the value of acute oral LD_{50} of cholecalciferol for Norway rats and house mice were proven reliable with the data still being used as reference by several researchers for the past 26 years when it comes to the study of the chemical compound against both species (Eason *et al*., 1993; Eason *et al*., 1994; Jolly *et al*., 1995; Erickson and Urban, 2004; Eason *et al*., 2000; 2010a). Feeding studies of cholecalciferol against both species were proven effective in a series of laboratory studies by Marshall (1984) and 16 years later by Eason *et al*. (2010a) when all the samples died during the study after consuming the offered bait. Other than rats and mice, efficacy of cholecalciferol against brushtail possums, *Trichosurus vulpecula* are well documented in a series of study conducted in New Zealand. Acute oral toxicity LD_{50} of possum was stated by Jolly *et al*. (in press) to be below 20 mg/kg. The exact value of acute oral toxicity of possums was mentioned by Godfrey (1985), cited by Eason and Spurr (1995) at 0.17 mg/kg.

Toxicity towards non-target animals have been reported in a few published reports. Cholecalciferol toxicity risk towards dogs, *Canis familiaris* was low with LD_{50} ranging between 80-88 mg/kg (Marshall, 1984; Eason and Ogilvie, 2009). Mallard ducks are

reported to have LD_{50} value greater than 2000 mg/kg, hence mallard ducks are unlikely to suffer secondary poisoning (Marshall, 1984; Eason *et al*., 1993).

2.2 Economic importance of rats in agriculture in Malaysia

Economic impact inflicted by rats was a major concern for researchers and planters as a significant amount of loss was incurred due to rat attacks. The rats in mature oil palm plantation attacks ripe and unripe Fresh Fruit Bunch (FFB) as their main diet consist of oil palm fruit (Puan *et al.*, 2011). Not only FFB, they also cause damage to post-anthesis male inflorescences by chewing the inflorescences spikelets to consume developing grubs / pupae of pollination weevils, *Elaeidobius kamerunicus* as source of protein (Chung, 2012). The damage done is not only inflicted on male inflorescence but also female inflorescence by feeding on the spikelet, which affect the pollinating activity and in serious cases, affecting the development of fruit bunch if necessary, control measure are not taken (Hafidzi and Saayon, 2001). At immature stage of oil palm, petiole that forms the fronds is the most favourite parts of rats which stunted the formation of the fronds, while at nursery stage, the rats feeding on apical tissue kill or affecting the development of young shoots (Hafidzi and Saayon, 2001).

Wood (1976) stated in his paper that rat damages on oil palm fruit cost 5% loss of oil palm production. This statement was repeated in the researchers' subsequent reports in 1978 and 1984. Another researcher, Liau (1990) updated the oil palm production losses, with an increase of estimation from 5% to 10% after taking into account the detached fruitlets which are accessible for rats to carry away. Chung (2012) stated that damage to crops may reach 25 - 100% in certain conditions where serious outbreaks take place and for oil

palm production, an estimated 7-24% damage can happen due to rat attacks. In further discussion made in the same report, the researcher concluded that palm oil production was highly affected by yield of oil palm FFB. The yield of FFB affected by rat attacks cost 10% loss of palm oil production annually, and the researcher took an example from 2009 where Malaysia suffered oil loss per hectare valued at about US$ 311.67.

Hafidzi and Saayon (2001) studied 12 different oil palm estates to assess the rate of losses caused by rat attacks and the researchers reported that annual loss incurred by estate owners were as low as RM 4/ha per annum when the oil palm estate faced least serious damage from rat attacks. However, the loss incurred by estates when the rat infestations were serious reached as high as RM1200/ha per annum. Based on several reports reviewed, rat infestations can be more serious in recent times due to extensive commercial plantations growing bigger in a larger scale compared to previous years as this lucrative industry gets more attention from many parties.

2.3 Dominant rats in oil palm plantation

Rats is a key pest in oil palm plantations in Malaysia. Wood rats, *Rattus tiomanicus* Miller (formerly known as *Rattus* jalorensis) is the dominant species of rats in oil palm plantations in this country especially in matured palms (Wood, 1968; Hafidzi and Saayon, 2001). According to Wood and Chung (2003) and Chung (2012), *R. tiomanicus* is not only the main rodent pest in oil palm plantations in Malaysia, but it also was the first rodent pest discovered in oil palm plantations in Malaysia when the first report of rat damage on oil palm fruit bunches was documented in the 1930s. *R.tiomanicus* can be found in secondary forest, coastal forest, scrub, woodland, garden, orchards and grassland (Chung, 2012; Lim *et al.*, 2015). Chung (2012) described *R.tiomanicus* as a good climber which

one of the reason where it can spend much time on the palm tree. *R.tiomanicus* also inhabit under frond piles where it live in shallow burrow (Chung, 2012; Lim, 2015).

Apart from that, there are two other species of rats which co-exist as pests in the oil palm plantations, also commonly found in Malaysia. There are rice field rats, *R. argentiventer* and house rats, *R. r. diardii* (Hafidzi and Saayon, 2001, Chung, 2012, Wood and Singleton, 2015). *R.argentiventer* described as living in open areas, grassland, rice fields area, nurseries and young oil palms (Wood, 1982c; Hafidzi and Saayon, 2001; Chung, 2012; Lim *et al.,* 2015). Unlike *R.tiomanicus, R.argentiventer* is a poor climber and it's ability to climb only limited to some tree (Chung, 2012). Despite of poor climbing ability, *R.argentiventer* was described by Chung (2012) as a good burrower which usually builds an extensive underground burrow in well-drained soil for nesting (Wood and Singleton, 2015; Lim, 2015). *R. r. diardii* often found near human settlements or area where human activities exist such as town, villages, garden, farms and living quarters near plantation areas (Medway, 1978; Chung, 2012; Wood and Singleton, 2015). The species was described gradually common in oil palm plantations primarily in areas where *R. tiomanicus* is being controlled by rat baits (Soh *et al.*,1982; Mohd, 1985).

2.3.1 Morphology of dominant rats in oil palm plantation

Previous studies by Harrison (1974), Payne *et al.* (1985), Francis (2008) and Lim (2015) have listed the details of the morphology (features and measurement) of *R. tiomanicus*, *R.argentiventer* and *R.r.diardii* for identification purposes. Features and measurements references are very useful to downsize the species identification since there are more than one species that may exist in oil palm plantation.

Rattus tiomanicus

Different details were reported especially in features such as belly colour, with different researchers stating different colour of *R. tiomanicus* belly, varying from clean white (Harrison, 1974) to slightly off-white (Francis, 2008). In spite of different information provided, it is not really significant as a recent study by Lim (2015) proved that the details about the colour of *R. tiomanicus* belly from different researchers can be accepted as the finding in their study revealed that the belly colour of *R. tiomanicus* is normally pure white with occasionally dull white or yellowish white. The dorsal part of *R. tiomanicus* can be greyish-brown (Chung, 2012) or olive-brown (Lim, 2015) smooth fur with short spines. The colour of the tail varies from dark brownish (Chung, 2012) to uniformly dark (Lim, 2015). **Table 2.3** shows the features and measurement of body parts of *R. tiomanicus* for identification and screening purposes.

Table 2.3: Features and measurement of body parts of *R. tiomanicus*

References	**Harrison (1974)**	**Payne et al. (1985)**	**Francis (2008)**	**Lim (2015)**
Head + Body length (mm)	100 - 180	140 - 188	140 – 190	80 - 160
Tail length (mm/ % of Head + Body)	mm not provided / 80 – 110%	120 – 181 mm / 75 – 120%	150 – 200 mm / 95 – 120 %	85 – 170/ % not provided
Hind foot length (mm)	26 - 34	28 – 35	28 – 35	27 – 34
Skull length (mm)	41	34.3 – 36.9	34 - 45	Not provided
Weight (g)	110	78 - 125	55 - 150	Not provided
Belly colour	Clean white	White	Pure white / slightly off - white	Pure white/ occasionally dull white or yellowish white
No of mammae – pectoral	2 pairs	Total of 10 mammae	2 pairs	Total of 10 mammae
No of mammae – inguinal	3 pairs		3 pairs	

(Source: Chung Gait Fee (2012))

Rattus argentiventer

Belly colour of *R.argentiventer* was described generally as grey in colour according Harrison (1974), Payne *et al.* (1985), Francis (2008) and Lim (2015), however the details grey colour description was varies from uniform pale grey (Harrisom, 1974), wholly silver grey (Payne *et al.,* 1985; Francis, 2008) and silvery grey with or without darker streak in the middle (Lim, 2015). The dorsal part of *R.argentiventer* was described as either pale brown to orange brown with spiny fur (Chung, 2012) or olive brown with black hairs intermixed among the brown fur (Lim, 2015). The colour of tail varies from entirely dark brownish (Chung, 2012) to uniformly dark (Lim, 2015). **Table 2.4** shows table description the features and measurement of body parts of *R. argentiventer*.

Table 2.4: Features and measurement of body parts of *R. argentiventer*

References	**Harrison (1974)**	**Payne et al. (1985)**	**Francis (2008)**	**Lim (2015)**
Head + Body length (mm)	110 - 200	140 - 210	140 – 210	140 - 220
Tail length (mm/ % of Head + Body)	mm not provided / 85 – 119%	130 – 192 mm / 80 – 125%	130 – 205 mm / 80 – 125 %	130 – 220/ % not provided
Hind foot length (mm)	28 - 38	32 – 36	30 – 40	35 – 38
Skull length (mm)	41	35.1 – 39.7	35 - 45	Not provided
Weight (g)	190	85 – 180	85 - 240	Not provided
Belly colour	Uniform pale grey	Wholly silver grey	Wholly silver grey	Silvery grey with or without a darker streak in the middle
No of mammae – pectoral	3 pairs	Total of 12 mammae	3 pairs	Total of 12 mammae
No of mammae – inguinal	3 pairs		3 pairs	

(Source: Chung Gait Fee (2012))

Rattus rattus diardii

Belly colour description of *R. r. diardii* was varies from light grey to reddish brown (Harrison, 1974), pale brown to dark grey brown (Payne *et al.*, 1985) or buffy brown (Francis, 2008). The upper or dorsal part of *R.r.diardii* was normally brown in colour with spiny fur while the tail is entirely brownish (Chung, 2012). **Table 2.5** shows table description identification for the features and measurement of *R. r. diardii* body parts.

Table 2.5: Features and measurement of body parts of *R. r. diardii*

References	Harrison (1974)	Payne et al. (1985)	Francis (2008)	Lim (2015)
Head + Body length (mm)	110 - 200	122 - 219	105 – 215	Not provided
Tail length (mm/ % of Head + Body)	mm not provided / 85 – 119%	121 – 220 mm / 95 – 120%	120 – 230 mm / 90 – 120 %	Not provided
Hind foot length (mm)	30 - 38	32 – 39	26 – 40	Not provided
Skull length (mm)	41	33.8 – 42.9	33 - 43	Not provided
Weight (g)	180	100 - 200	100 - 200	Not provided
Belly colour	Light grey to reddish brown	Pale brown to dark grey brown	Buffy brown	Not provided
No of mammae – pectoral	2 pairs	Total of 10 mammae	2 pairs	Not provided
No of mammae – inguinal	3 pairs		3 pairs	

(Source: Chung Gait Fee (2012))

2.3.2 Diet composition of dominant rats in oil palm plantation

In oil palm plantations, the main diet of *R. tiomanicus* mostly consist of oil palm mesocarp. An earlier study by Wood and Liau (1984a) led to the first conclusion about the main or favourite diet of *R. tiomanicus*, where they discovered that average 96% of the content found in stomachs of 5372 individual *R. tiomanicus* consisted of oil palm mesocarp.

Further study was carried out by Liau (1990) and reported consumption of oil palm mesocarp of *R. tiomanicus* was 4.3g/rat, while the other two species, *R. argentiventer* and *R. r. diardii*, consumed 8.6g/rat and 9.9g/rat respectively. These species have different average consumption due to their body mass, i.e., the higher the body mass, the greater the amount of consumption (Wood and Liau, 1984a). As their preferred diet was proven to consist of oil palm mesocarp, this provide a strong reason related to their increase in population in oil palm plantations where the accessibility towards oil palm fruits is high, which make rats numerically response to it (Puan *et al*., 2011).

2.4 Secondary poisoning of non-target animals

The issue of secondary poisoning was raised when people were looking for better anticoagulant rodenticides to combat commensal and agriculture rodent pests. The emergence of warfarin-resistant rats in the late 1950s led to the introduction of SGAR (brodifacoum, bromdiolone, flocoumafen, difethialone, difenacoum) also known as "superwarfarin" (Rattner *et al*., 2014). People turned to second-generation anticoagulants which are more potent in order to overcome warfarin-resistant rats which became a growing problem across the globe. The rodenticides were successful in combating the resistance problem (Merola, 2002). However, a major issue involving anticoagulant rodenticides that has raised concern among researchers and plantation operators is when chemical compounds in the baits cause secondary poisoning towards non-target fauna after its implementation (Elmeros *et al*., 2011; Sanchez-barbudo *et al*., 2012).

Secondary poisoning is defined as non-target fauna consumption of the residue of the poison from tissues of the target pests. More than 300 reports about secondary poisoning harm on non-target fauna have been documented around the world (Erickson and Urban,