SECONDARY POISONING OF CAPTIVE BARN OWLS, *Tyto alba javanica* THROUGH FEEDING WITH RATS POISONED WITH CHLOROPHACINONE AND BROMADIOLONE

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ABSTRACT

The potential secondary hazards of two anti-coagulant rodenticides commonly used in Malaysian oil palm plantations were evaluated through feeding trials with captive barn owls. A total of 12 adult barn owls (six pairs) were assigned to two rodenticide treatments and a control. The two treatments were rats fed with bromadiolone (0.005% a.i) and chlorophacinone (0.005% a.i) respectively. All rodenticide treated owls received four poisoned rats at Day 1, 3, 5 and 7 and a non-poisoned rat on each intervening day for 30 days. Each barn owl of the control group received a non-poisoned rat throughout the study. The reliability of a non-invasive technique such as an estimation of anti-coagulant rodenticide residue in regurgitated pellets and blood samples, were also evaluated. Barn owls showed behavioural aberrations such as coarse breathing, frequent closing of the eyes and reduced flying activity as early as Day 5 after consuming three poisoned rats. The weight recorded at Day 7 after treatment showed that all treated owls registered a reduction in weight. The owls in the control group on the contrary gained weight. Bromadiolone and chlorophacinone were found to have high degree of toxicity on captive barn owls. After feeding the birds with as few as four poisoned rats in a week the signs of toxicity in birds such as haemorrhages (beak) and haematoma (wing) were found. This finding is very crucial since barn owls have been reported to consume up to three rats per night and this would certainly increase their potential exposure to lethal secondary poisoning. The detection of residue in the pellets regurgitated by barn owls can be used to indicate exposure of the latter to both compounds. However, as the blood residue method is limited to the exposure duration of the compounds, this technique can only detect recent exposure to bromadiolone and chlorophacinone. The amount of residue detected in the pellet samples for chlorophacinone was 69.9 to 81.6 µg per day or equivalent to 17.2% to 27.4% of the compound consumed and corresponding value for bromadiolone was 27.2% to 34.5% (72.24 – 85.77 µg per day). This suggests that the amount of bromadiolone retained in the tissue of the rats was higher than that of chlorophacinone. Thus, barn owls face a greater potential for secondary poisoning from bromadiolone rather than chlorophacinone as can be deduced from this study.

Keywords: *Tyto alba*, secondary poisoning, chlorophacinone, bromadiolone.

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INTRODUCTION

Since the introduction of warfarin in the 1950s, several anti-coagulant compounds have been developed and used for rodent control throughout most parts of the world (Thomas *et al*., 2011; Elmeros *et al.*, 2011). Nowadays, they are the most commonly-used rodenticides for the control of rodent pests in agriculture around the world (Sanchez-Barbudo *et al.*, 2012). In Malaysia, anti-coagulant rodenticides have become a key solution to the threat of rodent pests particularly in oil palm plantations. However, in-line with the Malaysian oil palm industry's commitment to produce sustainable palm oil, through the implementation of the integrated pests management (IPM), effective anti-coagulant rodenticides have been employed in combination with biological control by using barn owls, *Tyto alba javanica* (Lam, 1982; Ducket, 1984; Wood and Chung, 2003; Turner and Gillbanks, 2003).

Initially, researchers proposed integration of barn owls with application of first generation anti-coagulant rodenticide warfarin to control rats; speculating that the poison was not hazardous to barn owls (Ducket, 1984). However, prolonged exposure triggers resistant of warfarin in rats (Lam, 1982). New and more toxic anti-coagulants *e.g.* chlorophacinone, bromadiolone, brodifacoum and flocoumafen have been introduced in the early 1980s and 1990s to deal with rodenticide resistance in oil palm plantation (Khoo *et al*., 1991). The actions of more recent rodenticides are more toxic and exhibit relatively longer biological half-lives in tissues (*e.g*. liver) (Erickson and Urban, 2004). Both characteristics enhance the potential of compounds to cause secondary poisoning to barn owls.

An aviary study by Mendenhall and Pank (1980) reported that barn owls die of haemorrhaging in a week after feeding on four rats that were fed with bromadiolone, brodifacoum, diphacinone and difenacoum. Similarly, in Malaysia, Lee (1994) showed that several anti-coagulants *e.g.* bromadiolone, brodifacoum and flocamafen, including first generation, walfarin exhibit a high degree of toxicity in barn owls. However, the secondary poisoning hazard on barn owls from chlorophacinone exposure was not included in Lee (1994). Chlorophacinone and bromadiolone have been reported as being most widely used in rat control practice in oil palm plantations (Noor Hisham *et al.*, 2007).

In view of the limited studies available in South-east Asia, as well as in-line with moving towards an environmental-friendly practice in the plantations, this study was carried to evaluate the potential secondary poisoning risks to barn owls resulting from the ingestion of rats exposed to chlorophacinone and bromadiolone. A feeding study on captive barn owls was conducted to investigate the toxicity and sub-lethal effects of both anti-coagulants poisoned-rats on the toxicity and sub-lethal effects on Malaysian barn owls, *Tyto alba javanica*. The reliability of a less invasive technique, such as the evaluation of the anti-coagulant rodenticide residue in pellets and blood samples was also evaluated.

METHODOLOGY

Aviary and Feeding Trials

An experiment was conducted at the Tun Razak Agriculture Research Centre (PPPTR), FASSB (N=03°53´, E= 102°31´), Jengka, Pahang, Malaysia. All birds were captured from the FELDA Jengka 24 (N=03°45´, E= 102°25´) oil palm plantation since they were young and beginning to fledge from the occupied nest boxes in the field. The plantation had implemented a rodenticide-free approach, while maintaining a high presence of barn owls, with full or near-full occupancy of nest boxes erected at a high density (one nest box per 15 ha). The fledglings were housed in pairs in an aviary divided in six compartments (each compartment = 10 m^3), each equipped with perches and a nest box. All birds were caged for six months before they were subjected to a feeding experiment throughout which each was fed with a single rat free from rodenticide. The rats were released in a feeding arena (6 m long and 3 m width) inside the aviary to be preyed upon by the owls. The captivity procedures strictly followed the guidelines suggested by The Barn Owl Trust (2010) and Crop Protection, FASSB (2011).

Twelve adult barn owls (approximately six months old after fledging) were selected for the study, weighed and randomly assigned for the control (bird's i.d: 301, 302, 303, 304), bromadiolone treatment group (bird's i.d: 101, 102, 103 and 104) and chlorophacinone treatment group (birds i.d: 201, 202, 203 and 204). The rodenticide treatment groups were offered a single poisoned rat and non-poisoned rats on alternate days over seven days feeding, depicting a 50% exposure to rodenticide in a week's diet. Daily remains of poisoned rat were weighed. Control groups received non-poisoned rats daily throughout the seven-day duration. The wood rat, *Rattus tiomanicus,* the dominant species in oil Malaysian palm plantation was chosen as the prey. Adult rats (90-110 g body weight) were live-trapped in rodenticide-free area in FELDA Jengka 24. The rats were weighed and individually caged in 25 cm^2 stainless steel containers with free access to water; woodchips provided for bedding and substrate for faeces. The rodents were fed with corn and supplied fresh water *ad libitum.* The rats were acclimatised for at least 14 days before being

offered rodenticide baits. Each animal was observed for symptoms of diseases as only healthy ones were chosen for the trial. Sixteen rats were selected and fed with 10 g of bromadiolone block bait (0.005% a.i) each day in a no-choice feeding for two days. Another 16 rats were selected and fed daily with 25 g of fresh chlorophacinone block bait (0.005% a.i) in a similar procedure. In this rodenticide-feeding period, all the rats had free access to water. On Day one after exposure, the remaining baits in the cages and faeces were collected, weighed and recorded. After the cages were cleaned and the woodchips replaced, water and new baits were replaced for each rat. The process of feeding with rodenticide was repeated for the second day exposure before the poisoned-rats were offered to the barn owls. Another 500 rats received water and were fed with corn and were offered as untreated rats to barn owls as control individuals in the study.

Data Collection and Sampling Procedure

Daily behavioural observations were carried out on all birds. The positions and movements of the birds in the aviary and the rats in the feeding arena were monitored 2 hr in the morning (0800-1000) and evening (1600-1800) respectively. Each bird was inspected at pre-treatment, Day 1, Day 3, Day 5, Day 7 during treatment and Day 11, 12, 14, 21 post-treatment for signs of external bleeding, particularly around the eyes, beak, wing tips, shoulders and feet. The weight of each bird was recorded during physical inspection.

Daily fresh regurgitated pellets samples were collected from each treatment cage throughout the study period. The pellets were collected every morning from each nest box. They were weighed, pooled and labelled before being sent to the laboratory. These samples were stored in a deep freezer at -20°C and thawed to the ambient temperature shortly prior to laboratory analysis. The residue content in the regurgitated pellets was then quantified by using high performance liquid chromatography (HPLC).

The procedure for bloodletting followed that of Wong (2005). A small amount of blood was collected from the bracial vein of each barn owl by using 23 gauge needle and 3 ml syringe. In view of the risk of hemorrhaging through blood sampling of birds treated with rodenticides, such sampling was done cautiously. Blood collection (1.0 ml) was done at pre-treatment (three days before treatment) and, 1, 7, 14 and 21 days post-treatment. However, no blood sampling was done on owls 103 and 302 on Day 7 of post-treatment, due to severe hemorrhage observed on the birds. The blood collected was then transferred into an EDTA tube for residue analysis.

Chromatography Condition

Chemical analysis was conducted at the toxicology laboratory, Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The HPLC system (Waters, USA) consisted of a controller (model 600) with a multi-solvent delivery system, plus an auto-sampler (model 717), an ultra violet (UV) detector (model 2996) and a fluorescence detector (model 2475). A reversed-phase C18 column [5 μm particle size, 4.6 mm (ID) x 250 mm (L)] was used for analysis (Nucleosil & Nucleodur® Macherey-Nagel, Strasbourg, France). The mobile phase for chlorophacinone was acetonitrile: ortho phosphoric acid, 0.5% (90:10 for v/v), while the mobile phase for bromadiolone was methanol: distilled water: acetic acid glacial (70: 25: 5 for v/v). The mobile phase flow rate was 1.0 ml min^{-1}. The UV detector was monitored from 240 to 340 nm with quantification done at 285 nm for the detection of chlorophacinone. The fluorescence detector was set at 310 nm excitation and 390 nm emissions for detection of bromadiolone. The injection volume of working standards and samples were set at 20 μl.

Residue Analysis

***Blood samples*.** The analysis of blood samples for bromadiolone and chlorophacinone was based on methods described by Vudhatala *et al.* (2010) and Chalermchaikit *et al.* (1993). One millilitre of blood sample was extracted with 4 ml of acetonitrile and vortex mixed for 2 min, followed by centrifugation (10 min, 2.5 x g) to remove the organic layer. The supernatant was removed and saved. The protein precipitated was re-extracted with 4 ml acetonitrile. The protein wash and the supernatant were combined and passed through a florisil SPE to clean-up the mixture. The column was washed, prior to use with 3 ml methanol, 3 ml water, and then 3 ml acetonitrile. Sample extracts and solvent washes were allowed to flow through the column by gravity at a flow rate of approximately 2 ml min^{-1}. The combined elute was evaporated using rotavapor at 35°C to dryness. The residue was dissolved with 1 ml mobile phase. The solution was then filtered through a Cronus Filter Yellow (13 mm, 0.45 μm) and transferred into vials for analysis in HPLC.

***Regurgitated pellets and faeces*.** The regurgitated pellets of the owls and faeces of rat were fortified according to the procedures described by Adison *et al.* (1982) and Naim (2010). The whole samples were placed in a 100 ml polypropylene tube. For method validation, the pellets samples were spiked at this

point with the standard solution. Forty millilitres of extraction solvent, acetone-chloroform mixture (1:1, v/v) and 2 g anhydrous sodium sulphate were then added to the tube. The mixture was then homogenised by shaking in vortex for 3 min and left to stand for approximately 1 hr. The mixture was filtered with filter paper and re-extracted with the extraction solvent (20 ml) and the combined filtrates evaporated to dryness. The resulting residue was dissolved with 10 ml methyl *tert*-butyl ether. The extract was cleaned up on an aminopropyl column, eluting with 10 ml methyl *tert*-butyl ether-acetic acid glacial (90:10,v/v) after conditioned with 2 ml methyl *tert*-butyl ether. The extract was again evaporated to dryness and reconstituted with 1 ml mobile phase of respective analysis of anticoagulant and filtered by using Cronus Filter Yellow. The solution was then transferred into vials and placed in the auto-sampler for HPLC analysis.

Standard and Calibration Curve

Chlorophacinone and bromadiolone standard (Pestanal®) with 98.4% and 97.6% were respectively obtained from Sigma-Aldrich, Germany. A stock standard solution of chlorophacinone was prepared in acetonitrile; while for bromadiolone in methanol – dicholoromethane (60:40, v/v). A stock solution of 100 mg kg^{-1} was prepared by dissolving 5 mg analytical standard in 50 ml of solvents. Working standard, ranging in concentration from 0.01 to 10 mg kg^{-1} were prepared from the stock standard solution. The working standard solutions were then used to prepare standard curve and spiking for the blood samples and regurgitated pellets. The response of both rodenticides was linear for six standard solutions at concentration of 0.01, 0.1, 0.5, 1, 5 and 10 mg kg^{-1}. Linearity of calibration was assessed from a linear regression of response (area) versus concentration of rodenticides, resulting in an r^2 of 0.999. The average retention time of bromadiolone and chlorophacinone was 4.5 min and 6.0 min respectively.

Fortification and Limit of Detection (LOD)

For method validation, the samples of pellets and blood were spiked with three fortification concentration of 1.0, 5.0 and 10.0 mg kg^{-1} of each standard. Recovery rates were assessed from spiked control samples of rodenticides in regurgitated pellets and blood samples with the fortification concentration. It was evident that both rodenticides showed good recoveries at low and high concentration ranged from 83% to 90% respectively. Detection limits for each rodenticide were assessed from calibration standards using statistical regression with three replications. The estimated LOD of the bromadiolone and the chlorophacinone were 0.005 mg kg^{-1} and 0.002 mg kg^{-1} respectively.

Quality Control (QC) Samples

A blank (control) containing purely acetonitrile and methanol was injected between each sample to monitor for any possible contamination due to carry over. Blank samples of pellets and blood were also prepared and extracted similar to samples of the study. All blank solvent and blanks samples were below the analytical LOD for both rodenticides tested.

RESULTS AND DISCUSSION

Residue of Anti-coagulant Rodenticides Offered to Owls

Rats fed with bromadiolone bait in the no-choice feeding test consumed an average of 10.57 ± 0.33 g of baits which corresponds to 0.53 ± 0.01 a.i of compounds ingested. The average amount of bait consumed by rats in the chlorophacinone treatment were 18.44 ± 0.06 g or equivalent to 0.92 ± 0.03 a.i mg of chlorophacinone. Higher amount of the latter consumed by the rats was probably due to differences in bait formulations. The amount of rodenticide compound egested from the body was estimated from the excrement collected. For bromadiolone, the average amount of compound estimated from the faeces was 0.25 ± 0.01 mg a.i or approximately 46.9% from the compound ingested. The amount of residue present in the faeces for chlorophacinone-rat was 0.60 ± 0.02 mg or approximately 64.9% from the residues ingested by the rats after two days of exposure.

Poche (1986) reported over 79.8% of bromadiolone residue in the body of rats was eliminated through the faeces after two days of exposure. Test rats were forced fed with 5 mg kg^{-1} body weight bromadiolone. In a similar procedure, Belleville (1981) orally administered chlorophacinone to rats with a single dose of 1 to 1.26 mg ai per rat (~4 to 6 mg ai kg^{-1}). The study reported 90% of the compound was excreted within two days of exposure. He also reported elimination was almost 99.9% via the faeces of the rodents. The high percentages of residue recovery in faeces from both previous studies were probably due to the dosage administrated in a single oral dose (gavaged technique) thus allowing the rat to excrete the poison more efficiently. In the present study, the rodenticides were naturally consumed by rats during their feeding activities and rodenticides dosage was certainly consumed in several intakes. Askham and Poche (1992) evaluated the amount of rodenticide residue retained in the carcases of voles

fed with 10 g of chlorphacinone bait (0.005% a.i) in a no-choice feeding experiment over a period of nine days or until mortality recorded. They found an average amount of 10% of the residue was retained in the carcasses. While Poche (1988) who subjected *Mus domesticus* and *Rattus norvegicus* to the same analytical procedure reported the rate of recovery of the compound after four days of feeding with bromadiolone bait was 25% and 29% respectively. Besides, the rate of removal of the compound from the body through excrement may depend on the species and size of individual rats tested.

Different species and individuals may have different metabolic rates subjected to the level of activities and even concentration of a.i ingested or consumed. In the current study, by using the mean value of eliminated residue, the average amount of bromadiolone and chlorophacinone retained in the rat's body offered to owls during the seven-day experiment was calculated. The average residue of bromadiolone and chlorophacinone retained in the rats was estimated at 0.280 ± 0.001 mg a.i and 0.323 ± 0.004 mg a.i. respectively. These values were equivalent to 53.07% and 35.68% of the compound consumed. This suggests that the retention rate was higher for bromadiolone than chlorphacinone, as reported by previous studies (Askham and Poche, 1992; Poche, 1988).

Each barn owl consumed a single rat on each of the four feeding days with poisoned-rats. The mean weight of rat consumed by each owl in the bromadiolone and chlorophacinone treatment in no choice feeding test was of 97.50 ± 1.47 g and 107.19 ± 0.64 g respectively. Based on the mean total compound consumed by the four poisoned rats minus excreted residue through faeces and deducted with the uneaten remains, it was estimated that barn owls had secondarily consumed a total of 1.12 ± 0.01 mg a.i of bromadiolone and 1.29 ± 0.04 mg a.i of chlorophacinone (*Table 1*).

Effects on Owls

Barn owls fed with poisoned-rats showed behavioural aberrations such as coarse breathing, frequent closing of the eyes and reduced flying activity as early as Day 5 after consuming three poisoned-rats. The control birds stayed healthy and active from the start of the experiment and remain so until the end of the study. The weight recorded at Day 7 after treatment showed three out of four birds in chlorophacinone treatment showed reduction in weight at an average of 11.25 g. Similarly, all bromadiolone treated birds reduced in weight at an average of 35 g after at Day 7 of exposure. On the contrary, the birds in control group gained weight at an average of 36 g compared to the initial weight at the start of the experiment (*Table 2*).

Physical assessment at Day 1 post-treatment showed one bird fed with bromadiolone poisoned-rat had signs of haemorrhaging in her beak. The bird stayed on the ground unable to fly. A hematoma symptom was detected in both wings. The bird lost almost 91 g of her weight compared to its initial weight. Similarly, at Day 7 (a total of four poisoned-rats consumed), one bird fed with chlorophacinone poisoned-rats showed signs of haemorrhaging in her beak. The bird lost up to 25 g in weight compared to her initial weight; however no hematoma

TABLE 1. CALCULATED MAXIMUM AMOUNT OF RODENTICIDES CONSUMED BY PAIRED CAGED BARN OWL OFFERED BROMADIOLONE AND CHLROPHACINONE-FED FOR TWO DAYS

Treatments	Barn owl		No. rat consumed	Mean weight rat (g)	Mean possible remains (g)	Total a.i [consumed – (excreted +remains) mg]	% a.i from original dose eaten by rats
	Code (sex)	Weight (g)					
Bromadiolone	101(♂)	474	4	98.13 ± 2.02	7.36 ± 0.31	1.14 ± 0.02	54.49
	102(♀)	489	4				
	103(♀)	493	4	96.88 ± 1.25	5.76 ± 0.45	0.97 ± 0.03	42.28
	104 (♂)	487	4				
Mean	**-**	**485.75**	**16**	**97.50 ± 0.73**	**5.38 ± 2.93**	**1.12 ± 0.01**	**48.39** [a]
Chlorophacinone	301 (♂)	452	4	106.88 ± 1.50	10.84 ± 0.78	1.25 ± 0.03	34.92
	302 (♀)	512	4				
	303(♀)	508	4	107.50 ± 2.48	2.79 ± 0.33	1.13 ± 0.10	28.07
	304 (♂)	490	4				
Mean	**-**	**490.5**	**16**	**107.19 ± 0.32**	**6.81 ± 1.29**	**1.29 ± 0.04**	**31.50** [a]

Note: Values in columns with different letters are significantly different (P<0.05) by non-parametric two-sample test (Mann-Whitney Wilcoxon test).

TABLE 2. TOXICITY EFFECTS OF ANTICOAGULANT RODENTICIDES TO BARN OWLS FED WITH 4 POISONED-RATS IN SEVEN-DAY PERIOD

Treatments	Owls code (sex)	Initial weight	Weight at Day 7 (g)	Weight change after 1 week (g)	Fate after 1 month	Toxicity symptoms
	101(♂)	474	460	-14	S	
Bromadiolone	102(♀)	489	398	-91	HR	Haemorrhage (Day 6/7)
	103(♀)	493	473	-20	S	
	104(♂)	487	470	-17	S	
Mean	**-**	**485.75**	**450.25**	**-35.5****	-	-
Chlorophacinone	301(♂)	452	452	0	S	
	302 (♀)	512	497	-15	S	
	303(♀)	508	483	-25	HR	Haemorrhage (Day 7)
	304(♂)	490	489	-1	S	
Mean	-	490.5	487.25	-10.25**	-	-
Untreated	201(♂)	455	517	62	S	
	202(♀)	490	509	19	S	
	203(♂)	452	500	48	S	
	204(♀)	481	497	16	S	
Mean	**-**	**469.5**	**505.8**	**36.3****	-	-

Note: ** Significant ($P<0.05$) according to non-parametric test (Kruskal-Wallis). S- survived, HR – recovered after showing signs of haemorrhaging.

symptom was detected in both her wings. Both birds showing haemorrhaging symptoms fully recovered physically and started to gain weight after one week of feeding with non-poisoned rats. In general, both bromadiolone and chlorophacinone were toxic to barn owls as indicated by the signs of haemorrhaging symptoms in one barn owl in each treatment. Haemorrhaging occurred in barn owls after being fed with four poisoned-rats over one week. This finding is important, since barn owls could consume up to three rats per night (Lenton, 1984) and thus increased the vulnerability to lethal exposure and secondary poisoning.

The high toxicity of the anti-coagulant rodenticide against barn owls was previously acknowledged by Lee (1994). He found three out of four barn owls fed with four poisoned rats (exposed to rodenticides in four-day no-choice feeding) of bromadiolone, brodifacoum and flacomafen died in about two weeks after exposure. In fact, two barn owls fed with first generation anti-coagulant rodenticide, warfarin died three weeks after exposure. A multiple choice-feeding study with poisoned-rat (fed in a five-day period) has substantiated that bromadiolone can cause secondary poisoning to barn owls; however chlorophacinone did not exhibit any secondary poisoning symptoms during the subsequent 30-day period of study (Mendenhall and Pank, 1980). The barn owls fed with chlorophacinone poisoned-rats survived without apparent intoxicity. Similarly, Wyllie (1995) fed six barn owls (*T. alba*) with bromadiolone poisoned-mice for either one-day, three-days, or six-days. Mice were allowed to feed on commercially available bromadiolone bait (product and percent a.i. were not reported) for one day and then allowed to die (six to eight days). The study reported that none of the owls died or exhibited signs of haemorrhaging. The possible reasons for these contradictions could be attributed to the varying number of days the prey was exposed to the poisoned rat. Multiple choice-feeding in Mendenhall and Pank (1980) allowed rats to feed on other food thus may lead to less residue of chlorophacinone in the rat's body.

Wyllie (1995) fed rats (no-choice feeding) for just a single day over two to 11 days of feeding period. Thus the rats could have possibly removed the residue before they were offered to the barn owls. Bellville (1981) found that approximately 90% and almost 99% of chlorophacinone administered orally in rats were removed after 48 hr and within four days respectively. Chlorophacinone being less persistent in rodents has been acknowledged by Asham and Poche (1992). They reported that only 10% of residue was recovered from rat carcasses after nine days of being continuously exposed to chlrophacinone baits (0.005% a.i.). Thus, the small amounts of chlrophacinone retained by the rats at Day 5 were not sufficient to cause injury or death to barn owls. While, Poche (1986) reported that

within four days after ingestion by rats, over 89% of bromadiolone residues was eliminated through faeces and only about 10% was retained in the liver. However, bromadiolone was reported to be more persistent in the liver of mammals and rodents compared to chlorophacinone. Bromadiolone was detected after 256 days in the liver of sheep that received a sub-lethal dose of 2 mg kg^{-1} (Nelson and Hicking, 1994). The retention period of bromadiolone in the liver of rats were 170 days and 318 days when administrated with 0.93 mg kg^{-1} of the compound as reported by Hawkins *et al.* (1991) and Parmar *et al.* (1987) respectively.

Bromadiolone was hazardous to mammals such as mongoose, *Galidictis* sp. (Pank and Hirata, 1976), coyote, *Canis latrans* (Marsh and Howard, 1986) and red-fox, *Vulpes vulpes* (Sage *et al.*, 2010). Pank and Hirata (1976) offered Mongoose with bromadiolone-poisoned rats. The rats were fed with bromadiolone bait (0.005% a.i) for five days. Mongoose were fed a single poisoned-rats for three days, five days, six days. All mongooses died in the final day of the experiment. Marsh and Howard (1986) fed seven coyotes daily with single poisoned-ground squirrels over a five-day experiment. Before that, ground squirrels were fed 15 g of bait (0.005% a.i) for six days. Three of the coyotes died at the end of the study. Sage *et al*. (2010) reported a high toxicity of bromadilone to the red fox (*Vulpes vulpes*). Four captive foxes were fed for two or five days with water voles (*Arvicola terrestris*), which had been fed with bromadiolone at concentrations close to those measured in the field. Two foxes were to have severe signs of external haemorrhaging

Several laboratory studies have been conducted to test the toxicity of chlorophacinone to predatory and scavenger birds (Radvanyi *et al*. 1988; Riedel *et al*., 1991) and mammals (Fisher and Timm, 1987; Ahmed *et al.*, 1996). Radvanyi *et al*., (1988) fed the American kestrel, *F. sparverius* with one or three poisoned-voles in 21 and 61 days experiments respectively. Voles fed with baits (0.01%) daily until they succumbed, which took six to eight days; were offered to the birds. All kestrels had shown a high degree of intoxicity; such as external bleeding and internal hematoma symptoms. Riedel *et al.* (1991) fed the Eurasian buzzard, *B. buteo* and the Tawny owl, *S. aluco* with a single rat previously exposed to chlorophacinone bait (0.0075% a.i) daily. They found that after seven days of exposure all animals tested showed signs of intoxicity, *i.e* internal haemorrhaging and extended coagulation time of the blood. Fisher and Timm (1987) found that five out of six Black–footed ferrets, *Mustela nicripes* died after consuming Prairie dogs (*Cynomys* sp.) that were fed with 25 g of chlorophacinone bait at concentration of 0.0025% a.i. Ahmed *et al.* (1996) fed 20 European ferrets, *M. putorius ad libitium* with rats exposed to chlorophacinone (0.005% a.i) for five days, killing 11 of them.

Residue Analysis

Regurgitated pellets. Since barn owls were caged in pairs, the pellets were pooled for a single analysis. Due to large samples, only pellets collected in pre-treatment and at Day 1, 2, 3, 4, 5, 6, 7, 11, 12, 21 and 30 after treatment were analysed in HPLC. Bromadiolone residues in the pellets ranged from an average of 72.24 to 94.07 μg per day. These values were in the range of 27.2% to 34.5% of the bromadiolone bait consumed by the rats that was fed to the barn owls. At Day 11 to Day 12 after treatment, the residues detected drastically decreased to 2.763 – 5.022 μg per day. After 37 days, the concentration of the residue decreased to below minimum detection limit (MDL).

Similarly, chlorophacinone residue in the pellets ranged from an average of 130.59 to 157.23 μg per day. These values were equivalent to 17.2% to 27.4% of the chlorophacinone bait consumed by the rats in two days feeding period before being offered to the barn owls. At Day 11 and Day 12 after treatment, the residue detected rapidly declined to 2.34 – 5.28 μg per day and gradually to below MDL at day 30 after treatment (*Table 3*). The residue detected in pellets regurgitated by the barn owls revealed that bromadiolone and chlorophacinone were consumed and excreted unchanged in the pellet samples. Similar findings were shown by Newton *et al*. (1994) where they detected anti-coagulant rodenticides in the pellets of barn owls. Gray *et al*. (1994) estimated that pellets regurgitated by barn owls that preyed on rats fed with brodifacoum, difenacoum and flocoumafen bait contained between 21% to 29% of the ingested rodenticide compounds.

Blood Samples

Bromadiolone residue was detected in blood samples on Day 1 post-treatment with a mean residue of 0.58 μg $litre^{-1}$. At Day 7 post-treatment, a very small amount was detected *i.e*. mean residue of 0.02 μg $litre^{-1}$. The residue declined to below detectable concentration by Day 14. Although chlorophacinone concentration on Day 1 (mean = 0.73 ± 0.01 μg $litre^{-1}$) was comparable to bromadiolone residue, the former declined drastically to below MDL after Day 4 (*Table 4*). These values were equivalent to only 0.06% of the bromadiolone and chlorophacinone bait consumed by the rats in a two days feeding period before being offered to the barn owls.

TABLE 3. RODENTICIDE RESIDUE IN REGURGITATED PELLETS IN CAPTIVATE PAIRED OWLS

Treatment	Owl No.	Rodenticides content (ug per day) in collected regurgitated pellets												
		Pre	PR 1	R 1	PR 2	R 1	PR 3	R 1	PR 4	Post-treatment				
		0	1	2	3	4	5	6	7	11	12	14	21	30
Broma-diolone	101 102	0.000	86.433	11.450	70.896	7.391	104.421	7.877	85.651	6.253	2.998	1.799	0.456	0.000
	103 104	0.000	85.118	12.053	73.593	6.932	83.711	7.074	84.256	3.792	2.528	0.843	0.354	0.000
	Mean	0.000 a	85.776 a	11.751 a	72.244 a	7.162 a	94.066 a	7.475 a	84.954 a	5.022 a	2.763 a	1.321 a	0.405 a	0.000 a
Chloro-phacinone	301 302	0.000	60.107	5.410	69.931	4.895	84.159	4.309	80.127	5.328	3.373	1.514	0.361	0.000
	303 304	0.000	79.886	6.060	73.287	3.914	66.637	3.167	83.076	2.933	2.314	1.014	0.296	0.000
	Mean	0.000 a	69.996 a	5.735 a	71.609 a	4.404 a	75.398 a	3.738 a	81.601 a	4.131 a	2.844 a	1.264 a	0.328 a	0.000 a
Control	201 202	na	na	na	0.000	0.000	0.000	0.000	0.000	na	na	na	na	0.000
	203 204	0.000	0.000	0.000	na	na	na	na	na	0.000	0.000	0.000	0.000	na
	Mean	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: [a]PR = Poisoned- rat 1- 4, [b]R = interval non-poisoned-rat.
Values in coloumn with same letters are not significantly different (P<0.05) by non-parametric two-sample test (Mann-Whitney Wilcoxon test), na = no analysis.

Due to the less persistence in blood samples, it is suggested that blood samples were not suitable to be used for the monitoring of rodenticide residue toxicity of barn owls in the field. For comparison, Kamil (1987) reported half life of bromadiolone residue in blood samples of rats administered with 3.0 mg kg^{-1} of rodenticide by single oral dosage was 2.4 days and residue remained over five days after treatment. While Belleville (1981) orally administrated rats with chlorophacinone at a dosage of 4.5 mg kg^{-1} and determine the half life of the compound to be 0.4 days and residue did not persist more than one day in the blood.

CONCLUSION

Regardless of their classification, bromadiolone and chlorophacinone were found to have a high degree of toxicity to captive barn owls. The signs of intoxicity such as haemorrhages were found to occur after feeding on poisoned-rats at a low consumption rate of four rats per week. This finding is very crucial since application of rodenticides in oil palm plantations takes about one week to complete for a plantation of the size at least 100 ha. It has been reported that barn owls could consume up to three rats per night and this would certainly increased the potential for exposure to lethal secondary poisoning. Moreover, it was reported that barn owls are highly dependent on rats in their diet. Lenton (1984) and Hafidzi *et al.* (1999) estimated that 99.8% of barn owls prey in oil palm plantations constituted of rats. Study on rodenticides residue in pellets regurgitated by barn owls is very useful and can be used to indicate exposure of barn owls to bromadiolone and chlorophacinone. However, blood residue method is limited to duration of exposure as the technique can only detect recent exposure of bromadiolone and chlorophacinone. The amount of residue detected in the pellet samples for chlorophacinone was 17.2% to 27.4% of the compound consumed. The corresponding value for bromadiolone was 27.2% to 34.5%; indicating that bromadiolone has a higher retention than chlorophacinone in the rat tissue. Thus, the risk of exposure of bromadiolone in barn owls is higher than chlorophacinone; suggesting a greater potential for secondary poisoning from bromadiolone than chlorophacinone.

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TABLE 4. CONCENTRATION OF ANTICOAGULANT ($\mu g\ g^{-1}$) IN BLOOD SAMPLES OF BARN OWL

Treatment	Owl code	Day of sampling after owl fed 4 poisoned-rat in a week			
		Pre-treatment	1	7	14
		residue (ug litre^{-1})	residue (ug litre^{-1})	residue (ug litre^{-1})	residue (ug litre^{-1})
Bromadiolone	101	nd	15.46	0.01	< MDL
	102	nd	na (HR)	0.04	< MDL
	103	nd	13.83	0.01	< MDL
	104	nd	10.45	0.02	< MDL
	Mean	nd	**13.25**	0.02	< MDL
Chlorophacinone	301	nd	12.44	< MDL	< MDL
	302	nd	9.65	0.01	< MDL
	303	nd	na (HR)	< MDL	< MDL
	304	nd	8.32	< MDL	< MDL
	Mean	nd	**10.14**	< MDL	< MDL
Control	201	nd	nd	nd	nd
	202	nd	nd	nd	nd
	203	nd	nd	nd	nd
	204	nd	nd	nd	nd
	Mean	**nd**	**nd**	**nd**	**nd**

Note: na (HR)= sample not avaiable due to haemorrhage of birds.
nd= not detected.
MDL = method detection limit, > = more than. < = less than.

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REFERENCES

ADISON, J B (1982). Improved method for high pressure liquid chromatography determination of chlorophacinone in mouse tissue. *J. Association of Anal. Chem., 65*: 1299-1301.

AHMED, M S; BAROCH, J; CARLET, L and WHALEY, D (1996). Secondary hazard study using chlorophacinone-killed laboratory rats fed to domestic ferrets (*Mustela putorius furo).* Report submitted to EPA by LiphaTech, Inc., Milwaukee. Assessing the environmental risk for primary and secondary poisoning in birds and mammals of the rodenticide chlorophacinone. *Annex to the Chlorophacinone Environmental Risk Assessment Report* [Suárez, E and Fernández, C *et al.* (2008)]. 18 pp.

ASKHAM, L R and POCHE, R M (1992). Biodeterioration of chlorophacinone in voles, hawks and an owl. *Mammalia, 56*: 145-150.

BELLEVILLE, M J (1981). Absorption, distribution, metabolism and excretion studies in the rat using 14C-labeled chlorophacinone. *Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: A Comparative Approach* [Erickson,W and Urban, D (2004)]. Washington, DC, USA, United States Enviromental Protection Agency, Office of Pesticides Programs Environmental Fate and Effects Division. 225 pp.

CHALERMCHAIKIT, T; FELICE, L J and MURPHY, M J (1993). Simultaneous determination of eight anticoagulant rodenticides in blood serum and liver. *J. Analytical Toxicology, 17*: 56-61.

DUCKETT, D E (1984). Barn owls (*Tyto alba*) and the 'second generation' rat-baits utilised in oil palm plantations in Peninsular Malaysia. *The Planters, 60*: 3-11.

ELMEROS, M; CHRISTENSEN, T K and LASSEN, P (2011). Concentrations of anticoagulant rodenticides in stoats *Mustela erminea* and weasels *Mustela nivalis* from Denmark. *Science of the Total Environment, 409*: 2373-2378.

ERICKSON, W and URBAN, D (2004). *Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: A Comparative Approach*. Washington, DC, USA, United States Enviromental Protection Agency, Office of Pesticides Programs Environmental Fate and Effects Division. 225 pp. Available at http://www.fluoridealert.org/pesticides/EPA-HQ-OPP-2006-0955-0005.pdf

FELDA AGRICULTURE SERVICES SDN BHD (2011). *Prosedur dan Kaedah Memelihara Burung Hantu dalam Sangkar*. Crop Protection Division, Felda Agriculture Services Sdn Bhd. 12 pp.

FISHER, D D and TIMM, R M (1987). *Laboratory Trial of Chlorophacinone as a Prairie Dog Toxicant*. Wildlife Damage Management, Internet Center for Great Plains Wildlife Damage Control Workshop Proceedings.

GRAY, A; EADSFORTH, C V; DUTTON, A J and VAUGHAN, J A (1994). Toxicity of three second generation rodenticides to barn owls. *Pesticide Science*, *42*: 179-184.

HAFIDZI, M N; ZULKIFLI, A and KAMARUDDIN, A A (1999). Barn owl as a biological control agent of rats in paddy fields. *Symposium on Biological Control in the Tropics*. Mardi Training Centre. Serdang. Malaysia. p. 85-88.

HAWKINS, D R; BRODIE, R R; CLARKE, D and BRINDLEY, C (1991). Determination of the residues and half-life of the rodenticides brodifacoum, bromadiolone, and flocoumafen in the livers of rats during 200 days after a single oral dose of each at a dose level of 0.2 mg/kg. *Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: A Comparative Approach* [Erickson,W and Urban, D (2004)]. Washington, DC, USA, United States Enviromental Protection Agency, Office of Pesticides Programs Environmental Fate and Effects Division. 225 pp.

KAMIL, N (1987). Kinetics of bromodialone, anticoagulant rodenticide, in the Norway rat (*Rattus norvegicus*). *Pharmacology Research Communications, 19*: 767-775.

KHOO, K C; PETER, A C O and HO, C T (1991). *Crop Pests and their Management in Malaysia*. Tropical Press Sdn Bhd, Kuala Lumpur. 242 pp.

LAM, Y M (1982). Chemical control of rodents. *Rodent Pests of Agricultural Crops in Malaysia* (Khoo, K C; Lam, Y M; Teoh, C H; Lim, W H and Mohammad, B M eds.). The Malaysian Plant Protection Society. p. 33-56.

LEE, C H (1994). *Secondary Toxicity of Some Rodenticides to Barn Owls*. 4th International Conference of Plant Protection in the Tropics, 28-31 March, Kuala Lumpur, Malaysia. p. 161-163.

LENTON, G M (1984). The feeding and breeding ecology of barn owl, *Tyto alba* in Peninsular Malaysia. *Ibis, 126*: 551-575.

MARSH, R E and HOWARD, W E (1986). Ground squirrel--coyote secondary toxicity studies with chlorophacinone and bromadiolone (an administrative report of laboratory findings). *Potential Risks of Nine Rodenticides to Birds and Nontarget Mammals: A Comparative Approach* (Erickson,W and Urban, D (2004). Washington, DC, USA, United States Enviromental Protection Agency, Office of Pesticides Programs Environmental Fate and Effects Division. 225 pp.

MENDENHALL, V M and PANK, L F (1980). Secondary poisoning of owls by anticoagulant rodenticides. *Wildlife Society Bulletin, 8*: 311-315.

NAIM (2010). *Effects of Secondary Poisoning from Anticoagulants and Biological Rodenticides on Barn Owls,* Tyto alba javanica *in an Immature Oil Palm Plantation*. Universiti Putra Malaysia. Ph.D thesis. Unpublished. 167 pp.

NELSON, P C and HICKLING, G J (1994). Pindone for rabbit control: efficacy, residue, and costs. *Proc. of the Vertebrate Pest Conference, 16*: 217-222.

NEWTON, I; WYLLIE, I; GRAY, A and EADSFORTH, C V (1994). The toxicity of the rodenticide flocoumafen to barn owls and its elimination via pellets. *Pesticide Science, 41*: 187-193.

NOOR HISHAM, H; HASBER, S; SUKRI, T I and SAMSUDIN, A (2007). *Integrated Approach and Effective Control Measures of Major Pests and Diseases in Oil Palm Cultivation in FELDA-FASSB Experiences*. Seminar APS Kumpulan Perladangan FELDA, Jerantut, Pahang. p. 45-57.

PANK, L F and HIRATA, D N (1976). Primary and secondary toxicity of anticoagulant rodenticides. A review of secondary-poisoning studies with rodenticides [in Joermann (1998)]. *Bulletin OEPP/ EPPO Bulletin, 28*: 157-176.

PARMAR, G; BRATT, H; MOORE, R and BATTEN, P L (1987). Evidence for a common binding site *in vivo* for the retention of anticoagulants in rat liver. *Human Toxicol*, *6*: 431-432.

POCHÉ, R M (1988). Rodent tissue residue and secondary hazard studies with bromadiolone. *Bulletin OEPP/EPPO Bulletin, 18*: 323-330.

POCHE, R M (1986). The status of bromadiolone in the United States. *Proc. of the Vertebrate Pest Conference, 12*: 6-15.

RADVANYI, A; WEAVER, P; MASSARI, C; BIRDT, D and BROUGHTON, E (1988). Effects

of chlorophacinone on captive kestrels. *Bulletin Environment Contaminant Toxicology, 41*: 441-448.

RIEDEL, M; RIEDEL, B and SCHLEGELMILCH, H (1991). Risk of secondary intoxication for birds of prey and owls following use of chlorophacinone baits against common voles. A review of secondary-poisoning studies with rodenticides [in Joermann (1998)]. *Bulletin OEPP/EPPO Bulletin, 28*: 157-176.

SAGE, M E; FOUREL, I; COEURDASSIERA, M E; BARRAT, J; BERNY, P and GIRAUDOUX, P (2010). Determination of bromadiolone residues in fox faeces by LC/ESI-MS in relationship with toxicological data and clinical signs after repeated exposure. *Environmental Research, 110*: 664-674.

SÁNCHEZ-BARBUDO, I S; CAMARERO, P R and MATEO, R (2012). Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. *Science of the Total Environment, 420*: 280-288.

THE BARN OWL TRUST (2010). Thinking of having a captive barn owl? Available at http://www.barnowltrust.org.uk/infopage.html?Id=51

THOMAS, P J; PIERRE MINEAU, P; SHORE, R F; CHAMPOUX, L; MARTIN, P A; WILSON, L K; FITZGERALD, G and ELLIOTT, J E (2011). Second generation anticoagulant rodenticides in predatory birds: probabilistic characterization of toxic liver concentrations and implications for predatory bird populations in Canada. *Environment International, 37*: 914-920.

TURNER, P D and GILLBANKS, R A (2003). *Oil Palm Cultivation and Management.* Second edition. The Incorporated of Planters, Kuala Lumpur, Malaysia. 915 pp.

VUDATHALA, D; CUMMINGS, M and MURPHY, L (2010). Analysis of multiple anticoagulant rodenticides in animal blood and liver tissue using principles of QuEChERS method. *J. Analytical Toxicology, 34*: 273-279.

WONG, Y L J (2005). *Screening on Suspected Secondary Rodenticide Poisoning by Determination of Coagulation Times in Barn Owls* (Tyto alba) *in Tanjung Karang, Selangor*. Universiti Putra Malaysia. Final year project of Doctor of Veterinary Medinie (D.V. M).

WOOD, B J and CHUNG, G F (2003). A critical review of the development of rat control in Malaysian agriculture since the 1960's. *Crop Protection, 22*: 445-461.

WYLLIE, I (1995). Potential secondary poisoning of barn owls by rodenticides. *Pesticide Outlook, 6*: 19-25.