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EFFICACY OF A NOVEL AREA-REPELLENT AEROSOL FORMULATION AGAINST MOSQUITOES AND FLIES (DIPTERA: CULICIDAE, MUSCIDAE)

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Abstract Field studies of two area repellent aerosol formulations with transfluthrin and permethrin active ingredients were carried out against mixed freshwater mosquitoes near Humpty Doo, Northern Territory and against flies (a mixed population of house flies, *Musca domestica* and bush flies *Musca vetustissima*) in Bell, South West Queensland, Australia. The mosquito test site was adjacent to a breeding area, with a large population of biting mosquitoes. Fly repellency testing was conducted in the milking shed of a dairy farm with a large resident fly population. Non-absorbent (glazed tile) and absorbent (plywood) substrates were sprayed with the formulations and exposed to the ambient conditions. For the mosquito study, substrates were located adjacent to seated human test subjects. The test subjects counted all mosquito landings on the visible areas of their body during a 5 minute period every 2 hours for 6 hours. Fly evaluation was conducted in a similar manner, except that human assessors counted all fly landings on surfaces within a 1 metre radius of where they were seated. There were no significant differences in efficacy between the high and low active level formulations for both mosquitoes and flies. The lower active level formulation (0.5% transfluthrin and 0.05% permethrin) gave a reduction of 96.3% over 6 hours for glazed tiles, compared to the untreated controls, and a reduction of 80.0% for plywood. For flies, the respective reductions for the same rate were 81.4% and 79.0%. These studies demonstrated that both formulations provided effective area protection from mosquitoes and flies for up to 6 hours after application, on both absorbent and non-absorbent surfaces.
Key words Transfluthrin, area repellent, *Musca domestica, Musca vetustissima, Anopheles bancroftii, Culex annulirostris*

INTRODUCTION

An area or spatial repellent has been defined as: 'an inhibiting compound, dispensed into the atmosphere of a three dimensional space which inhibits the ability of mosquitoes to locate and track a target such as a human or livestock' (Nolen et al., 2002). This study addresses the area repellency of a novel aerosol product against biting mosquitoes and nuisance flies.

Outdoor area repellents are a relatively new concept in consumer pest control. Formats include mosquito coils, lanterns, metered aerosols, thermal devices and candles (WHO, 1998). The majority of these products are designed exclusively for mosquito repellency and are generally ineffective in repelling flies. In Australia, the House fly, *Musca domestica* and the Bush fly, *Musca vetustissima* are important nuisance pests (Gerozisis et al., 2008). The bush fly is a particularly annoying pest over much of Australia in the summer months because of the habit of landing on people (Hughes et al., 1972). Therefore, there is a need for an area repellent that is efficacious in repelling both mosquitoes and *Musca* flies.

Treating an inert surface with a vapour-active repellent is an appealing concept. This idea is not new, however. The US Army tried spraying a repellent on the ground in 1943 to repel mosquitoes, with limited success (Moore and Stage, 1943). For the concept to work well a chemical which vapourises at ambient temperatures is needed. The pyrethroid transfluthrin has a vapour pressure of 9 x10^{-4} Pa at 20°C (WHO,2014) and is a good candidate for this use. Ogoma et al. (2012) demonstrated the efficacy of this repellent against *Anopheles arabiensis*, when sprayed onto hessian strips. Permethrin, the second pyrethroid included in our test product, has also been used as a repellent spray on clothing and was shown to be effective (Debboun et al., 2007).

Australian consumers embrace the aerosol format for domestic pest control. This drove the development of an aerosol based area repellent that can be conveniently applied to a variety of substrates to provide area repellency against flies and mosquitoes.

MATERIALS AND METHODS

Aerosol Formulations and Application To Substrates

All aerosol formulations in this study were provided by Pascoe's Pty Ltd, , 40-46 Fairfield Street, Fairfield East, NSW 2165, Australia. Aerosol formulation AS01-37a contained 1.0% transfluthrin and 0.1% permethrin and AS01-37b contained 0.5% transfluthrin and 0.05% permethrin.

For the mosquito study, 30.0g of aerosol formulation AS01-37a was sprayed from a distance of 200mm onto 4 x 500mm x 500mm glazed floor tiles (total area 1.0m^2) and another 30.0g was applied as above to a piece of unpainted plywood 1,200mm x 800mm (total area 0.96m^2). The application was repeated to give 5 sets of approximately 1.0m^2 area of each of the above substrates. All the above was repeated for aerosol formulation AS01-37b. Once the substrates had been treated they were left for 30 minutes to dry outdoors, away from the test site.

For the fly study, 60.0g of aerosol formulation AS01-37a was sprayed from a distance of 200mm onto 9 x 450mm x 450mm glazed floor tiles (total area 1.82m^2) and another 60.0g was applied, as above, to 2 pieces of unpainted plywood 1,200mm x 800mm (total area 1.92m^2). The application was repeated to give 5 sets of approximately 1.0m^2 area of each of the above substrates. All the above was repeated for aerosol formulation AS01-37b. Once the substrates had been treated they were left for 30 minutes to dry outdoors, away from the test site.

Mosquito Study

Mosquito species were identified primarily as *Anopheles bancrofti, Culex annulirostris* and *Mansonia uniformis*. A large level cleared section of ground in close proximity to a mosquito breeding area at Thomsen's farm, Thomsen Road, Humpty Doo, NT, Australia, was selected as the study site. Test subjects selected for the study were males and females between the ages of 18 and 70 years. All test subjects were required to wear full body Elite Edition Original Bug Shirt□ mosquito suits (trousers and jackets with hoods) disposable latex gloves and covered shoes for the duration of the study.

There were 5 test subjects, 4 of whom evaluated the product and the other acted as the untreated control. All test subjects sat on folding camping chairs during the pre-count and evaluation periods. To avoid experimental bias, the control was a different subject on every evening of the study. Test subjects sat a minimum of 10m apart from one another, with the control sited at one end of the test area to minimize aerial contamination from the treated subjects.

The study commenced when biting mosquitoes were first noticed each evening (approximately 7pm). All subjects simultaneously conducted a pre-count of 5 minutes duration, counting all mosquito landings of greater than one-second duration on the visible parts of their body (primarily the abdomen, legs and arms). Product evaluation was then started if each subject recorded a minimum of 10 landings during the 5-minute assessment (i.e. 2 per minute).

Non-absorbent (glazed tiles) and absorbent (unpainted plywood) substrates were used to replicate typical domestic outdoor surfaces. These were sprayed with one or other of the coded formulations detailed above. Glazed tiles and plywood for the 4 and 6 hour assessments were aged under ambient conditions (27-35ºC) prior to the study commencing, while substrates for the 0 and 2 hour assessments were aged during the study. These were placed adjacent to the test subjects, 10 minutes prior to the start of each assessment.

For each evaluation, a 5-minute count of mosquitoes landing for more than 1 second was conducted. Landings were defined as the mosquito resting on the subject, not merely touching them. After mosquitoes had settled, movement of the subject disturbed them.

***Musca* Fly Study**

Fly species were the House fly, *Musca domestica* (approximately 80% of the population) and the Bush fly, *Musca vetustissima* (approximately 20% of the population). A large milking shed located on a dairy farm on Walkers Creek Road, Bell, Darling Downs District, Queensland, Australia was selected as the study site. The milking shed provided protection from strong winds and rain, but was open on one side and therefore exposed to the elements (sun and breeze), making it suitable for testing outdoor pest control products. This shed had a very high resident fly population, which was attracted to the cows (which were milked there twice daily) and to the many cowpats (cow faeces) deposited around the shed.

Human assessors were used to count the flies. There were 5 assessors, 4 of whom evaluated the product, while the other counted the untreated control. All assessors sat on folding camping chairs during the pre-count and evaluation periods. To avoid experimental bias, the control was a different subject on every day of the study. Assessors sat a minimum of 5m apart from one another, with the control assessor sited at one end of the test area to minimize aerial contamination from the treated subjects.

The study was commenced when flies were first noticed each morning (approximately 9 am). All assessors simultaneously conducted a pre-count of 5 minutes duration, counting all fly landings of greater than one second within their assessment area (an area with an outer perimeter 1m from the sprayed surfaces placed around the seated assessor). Product evaluation was then started if each subject recorded a minimum of 10 landings during the 5 minute assessment (i.e. 2 per minute).

Non-absorbent (glazed tiles) and absorbent substrates (unpainted plywood) were used to replicate typical domestic outdoor surfaces. These were sprayed with one or other of the coded formulations detailed above. All glazed tiles and plywood were aged *in situ* during the study under ambient conditions (14 - 26ºC). These test substrates were exposed to sunlight and wind during the study, to replicate domestic outdoor conditions. Substrates were placed adjacent to the test subjects, 10 minutes prior to the start of each assessment. For each evaluation, a 5 minute count of flies landing for more than 1 second was conducted. After the flies had settled, movement of the assessor was then used to disturbed them.

Calculation of Percentage Repellency

The following formula was used to calculate the percentage repellency for each treatment:

$(1 - C_0/C_1 \times T_1/T_0) \times 100$ = Percentage Repellency

C_0 = Control subject count for pre-treatment assessment

C_1 = Control subject count at a given assessment time

T_0 = Test subject count for pre-treatment assessment

T_1 = Test subject count at same given assessment time

Data Analysis
The data were analysed by analysis of variance (ANOVA) general linear model using SPSS® for Windows™ Version 20 (SPSS Inc. 2011). The assumption of normal distribution was checked using P-P plot and homogeneity of variance using Levene's test of equality of error variances.

RESULTS AND DISCUSSION

Mosquito Repellency
Table 1 gives the percentage repellency of each treatment at each of the time points during the study, as well as the average repellency over the six-hour assessment period. Landing inhibition using a glazed tile substrate was 93.6% for AS01-37a and 96.3% for AS01-37b, averaged over the 6 hour study. For plywood, the corresponding figures were 80.4% and 80.0%, respectively.

The mean numbers of mosquito landings at various post treatment times on a glazed tile substrate are presented in Figure 1. Table 2 shows a statistical analysis of the mean number mosquito landings on test subjects at various time point post-treatment on the same substrate. These data show no significant differences between the two treatments at most time points post-treatment.

The mean number of mosquito landings at various post treatment times on a treated plywood substrate is presented in Figure 2. Table 3 shows a statistical analysis of the mean number mosquito landings on test subjects at various time point post-treatment on the same substrate. These data show no significant differences between the two treatments at most time points post-treatment.

The weather conditions prevailing during the mosquito study were: temperature: 24.0 – 31.4°C; relative humidity: 74 – 89%; wind speed: 0 – 9 km/h; rainfall: 0.2 mm.

Table 1. Percentage mosquito landing inhibition of test formulations on two surfaces over 6 hours

Formulation	Surface	Ageing Time (h)	Landing Inhibition (%)	Number of Replicates
AS01-37a	Glazed Tiles	0	81.4	4
		2	96.8	4
		4	97.3	4
		6	99.1	4
		Average	**93.6**	**4**
	Plywood	0	76.1	4
		2	95.4	4
		4	58.2	4
		6	91.9	4
		Average	**80.4**	**4**
AS01-37b	Glazed Tiles	0	98.4	4
		2	98.5	4
		4	90.4	4
		6	98.0	4
		Average	**96.3**	**4**
	Plywood	0	81.5	4
		2	86.2	4
		4	59.8	4
		6	92.3	4
		Average	**80.0**	**4**

Musca Fly Repellency

Table 4 gives the percentage repellency of each treatment at each of the time points during the study, as well as the average repellency over the six-hour assessment period. Landing inhibition using a glazed tile substrate was 85.1% for AS01-37a and 81.4% for AS01-37b, averaged over the 6 hour study. For plywood, the corresponding figures were 80.9% and 79.0%, respectively.

The mean numbers of fly landings on a glazed tile substrate at various post treatment times are presented in Figure 3. Table 5 shows a statistical analysis of the mean number fly landings on test subjects at various time point post-treatment on the same substrate. These data show no significant differences between the two treatments at most time points post-treatment.

Table 2. Summary of the statistical analysis for the average number of mosquito landings at various post treatment times in the presence of a treated glazed tile surface

Treatment	Pre-treatment[1]	F-test	0 hours post treatment	F-test	2 hours post treatment	F-test	4 hours post treatment	F-test	6 hours post treatment	F-test
AS01-37a	42.25* (12.39)	a**	2.25* (0.63)	b**	0.25* (0.25)	a**	0.50* (0.29)	a**	0.50* (0.50)	a**
AS01-37b	31.00 (5.99)	a	0.25 (0.25)	a	0.25 (0.25)	a	1.50 (0.65)	a	0.50 (0.50)	a

* Values are mean number of mosquito landings (n=4). Standard errors of mean are in parentheses. ** Treatments with same letter do not differ significantly from each other. [1]Data were ln(x+1) transformed prior to analysis.

The mean numbers of fly landings on a treated plywood substrate at various post treatment times are presented in Figure 4. Table 6 shows a statistical analysis of the mean number fly landings on test subjects at various time point post-treatment on the same substrate. These data show no significant differences between the two treatments at most time points post-treatment. The weather conditions prevailing during the fly study were: temperature: 13.7 – 26.1°C; relative humidity: 21 – 62%; wind speed: 0 – 19 km/h; rainfall: 0 mm.

Table 3. Summary of statistical analysis for average number of mosquito landings at various post treatment times in the presence of a treated plywood surface

Treatment	Pre-treatment[1]	F-test	0 hours post treatment[1]	F-test	2 hours post treatment	F-test	4 hours post treatment	F-test	6 hours post treatment	F-test
AS01-37a	31.00* (5.99)	a**	12.25* (4.11)	b**	1.00* (0.41)	a**	5.75* (0.85)	a**	1.50* (0.87)	a**
AS01-37b	31.50 (6.89)	a	2.25 (0.48)	a	2.00 (0.71)	a	7.00 (0.71)	a	1.50 (0.96)	a

* Values are the mean number of mosquito landings (n=4). Standard errors of mean are given in parentheses.** Treatments with the same letter do not differ significantly from each other.[1]Data were ln(x+1) transformed prior to analysis.

Table 4. Percentage fly landing inhibition of test formulations on two surfaces over 6 hours

Formulation	Surface	Ageing Time (h)	Landing Inhibition (%)	Number of Replicates
AS01-37a	Glazed Tiles	0	81.7	4
		2	88.0	4
		4	87.7	4
		6	80.9	4
		Average	**85.1**	**4**
	Plywood	0	79.7	4
		2	85.0	4
		4	82.8	4
		6	76.2	4
		Average	**80.9**	**4**
AS01-37b	Glazed Tiles	0	65.2	4
		2	80.1	4
		4	90.0	4
		6	90.4	4
		Average	**81.4**	**4**
	Plywood	0	66.9	4
		2	81.1	4
		4	91.2	4
		6	76.9	4
		Average	**79.0**	**4**

Table 5. Summary of the statistical analysis for the average number of fly landings at various post treatment times in the presence of a treated glazed tile surface

Treatment	**Pre-treatment**	**F-test**	**0 hours post treatment**	**F-test**	**2 hours post treatment**	**F-test**	**4 hours post treatment**	**F-test**	**6 hours post treatment**	**F-test**
AS01-37a	23.50* (2.26)	**a****	7.00* (3.00)	**a****	2.50* (0.65)	**a****	5.50* (0.87)	**a****	4.25* (1.70)	**a****
AS01-37b	51.00 (12.80)	**a**	11.50 (4.09)	**a**	6.75 (1.32)	**b**	3.25 (0.63)	**a**	3.25 (0.95)	**a**

* Values are mean number of fly landings (n=4). Standard errors of mean are given in parentheses.
** Treatments with the same letter do not differ significantly from each other.

Table 6. Summary of the statistical analysis for average number of fly landings at various post treatment times in the presence of a treated painted plywood surface

Treatment	Pre-treatment	F-test	0 hours post treatment[1]	F-test	2 hours post treatment	F-test	4 hours post treatment	F-test	6 hours post treatment	F-test
AS01-37a	23.50* (2.26)	a**	4.50* (1.04)	a**	4.50* (1.71)	a**	7.00* (2.52)	a**	6.25* (2.02)	a**
AS01-37b	51.00 (12.80)	a	17.50 (5.72)	b	4.00 (1.23)	a	3.25 (1.38)	a	7.75 (3.61)	a

* Values are the mean number of fly landings (n=4). Standard errors of mean are given in parentheses. ** Treatments with same letter do not differ significantly.[1]Data were ln(x+1) transformed prior to analysis.

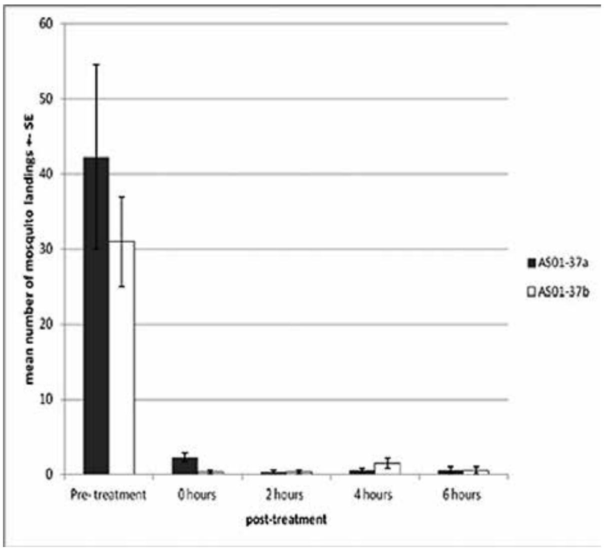

Figure 1. The average number of mosquito landings at various post treatment times in the presence of a treated glazed tile surface (bars) with their standard errors of mean

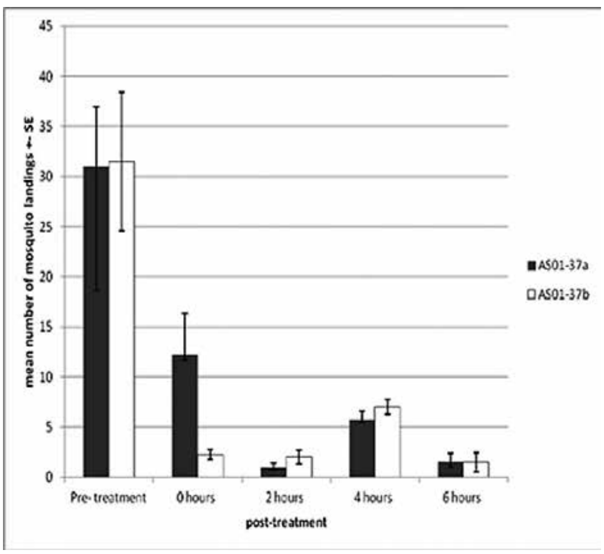

Figure 2. The average number of mosquito landings at various post treatment times in the presence of a treated plywood surface (bars) with their standard errors of mean

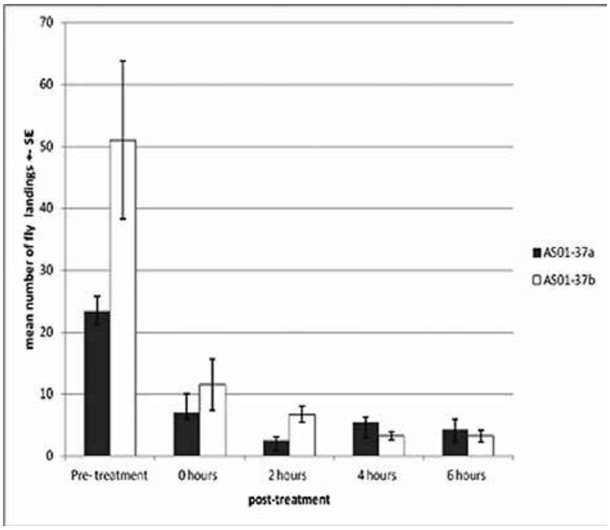

Figure 3. The average number of fly landings at various post treatment times in the presence of a treated glazed tile (bars) with standard errors of mean

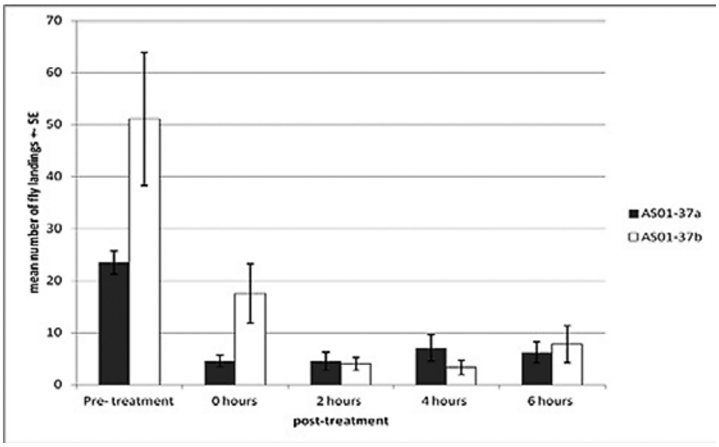

Figure 4. Average number of fly landings at post treatment times in the presence of treated plywood (bars) with standard errors of mean.

CONCLUSIONS

The mosquito and fly field studies showed that there were no significant differences in efficacy between the high active rate and the low active rate. Both formulations provided effective protection from biting mosquitoes and nuisance flies on both absorbent and non-absorbent substrates. The low active rate can be used, without compromising efficacy. A sprayed surface area of $1m^2$ was sufficient to provide continuous protection for at least 6 hours against biting mosquitoes, $2m^2$ was required for adequate fly protection. This product functions by vapour action from sprayed substrate, and is dependent on ambient conditions for emanation and consequently for mosquito and fly efficacy. The product remained effective over a range temperature and humidity and both still and windy conditions. The product is suitable for use in repelling flying nuisance insects in domestic environments.

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