FINAL REPORT

Title: An *in vitro* study to determine the efficacy of an experimental compound to control *Lucilia cuprina* larvae.

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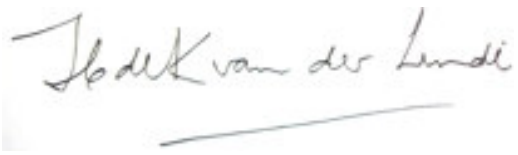
Report compiled by:



1 July 2006

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Study Sponsor:
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1. Objective:

To evaluate the efficacy of two F10 Germicidal formulations with insecticide against *Lucilia cuprina* larvae in an *in vitro* study.

2. Study Design:

The study was performed as a uni-centre controlled efficacy study at the University of the Free State, Bloemfontein.

3. Study Procedures:

3.1 Parasites:

Species: *Lucilia cuprina* (Diptera: Calliphoridae)
Stages: Late second or early third instar larvae.

3.2 Experimental compounds:

F10 Germicidal Wound Spray with Insecticide and F10 Germicidal Barrier Ointment with Insecticide. (Health and Hygiene (Pty) Ltd, P.O. Box 347, Sunninghill, 2154).
Lot#: Spray: 263
Lot#: Ointment: 262
Active ingredients: Quaternary & Biguanide compounds: 0.44%
Cypermethrin: 0.25%
Piperonyl Butoxide: 1.25%

3.3 Experimental set-up:

Thirty experimental units, each consisting of 10 PVC tubes (16mm diam. X 15mm high) were used during the experiment (Fig. 1). Fifteen units were used to evaluate the experimental spray formulation, five units to evaluate the experimental ointment formulation and ten units were used as controls. Treatment took place on the same day for all units. The basic approach was to place liver portions into each tube of the units followed by the release of five late second or early third instar larvae into each tube. The experimental units were maintained at room temperature.

3.4 Treatment procedures:

Spray formulation:

The liver portions in each experimental unit were covered with ~5ml of experimental spray. The spray canister was calibrated prior to the application of the spray.

Ointment formulation:

A 2 – 3mm layer of experimental ointment was applied on top of the liver portions in each tube.

3.5 Assessments:

(i) The larvae in the experimental units treated with the experimental spray were monitored over a 24 hour period. Observations were done at 4 hours, 8 hours and 24 hours post-treatment for each experimental unit and the dead (no movement) or moribund (retarded movement) larvae quantified.

(ii) The larvae in the experimental units treated with the experimental ointment were monitored over a 24 hour period. Observations were done at 4 hours, 8 hours and 24 hours post-treatment and the dead (no movement) and moribund (retarded movement) larvae quantified.

4. Data capture

Assessments on the various experimental units were recorded on data capture forms (see Tables 1 & 2), indicating the larvae as dead or moribund. In the control experimental units, the larvae were indicated as alive or dead (Table 3). A summary of the percentage efficacy of the experimental spray and ointment compared to the controls is represented in Table 4.

5. Analyses of data

The counts for the 15 replicates for the experimental spray, five replicates of the experimental ointment and ten replicates for the control experimental units were totalled for each observation period and the percentage mortality calculated. The Kruskal-Wallis Test (Non-parametric ANOVA) and the Dunn's Multiple Comparisons Test were used to compare the different treatments viz. experimental spray *vs* experimental ointment *vs* controls.

6. Amendments to protocol

(i) The larvae initially migrated from the chicken liver substrates during the 24 hour settling period. The larvae were consequently used in the experiment without a 24 hour settling period.

(ii) The chicken liver puree was not used during the study, because it became very moist during the 24 hour settling period and would probably have diluted the experimental spray, and as such could have affected the results. Application of the experimental ointment would also have been impossible, due to the high viscosity of the ointment and the very moist surface of the chicken liver puree.

(iii) Fifteen replicates of the experiment with the experimental spray were performed over a 24 hour period with assessments at 4 hours, 8 hours and 24 hours post-treatment, instead of three different groups viz. a 4 hour, 8 hour and 24 hour post-treatment assessment as mentioned in the protocol. This was done because it was observed that the majority of the blowfly larvae immediately started migrating from the chicken liver substrates after the application of the experimental spray. It was therefore easy to count the dead and moribund larvae within the container housing the experimental units.

(iv) Only five replicates of the experiment with the experimental ointment were done, because not enough ointment was supplied by the sponsor. Due to the high viscosity of the ointment it was very difficult to apply a layer of even thickness over the tubes with liver portions. Some tubes got too much ointment and others too little.

(v) Although the experiment was terminated after 24 hours, assessments continued daily until all the moribund larvae died (Table 5).

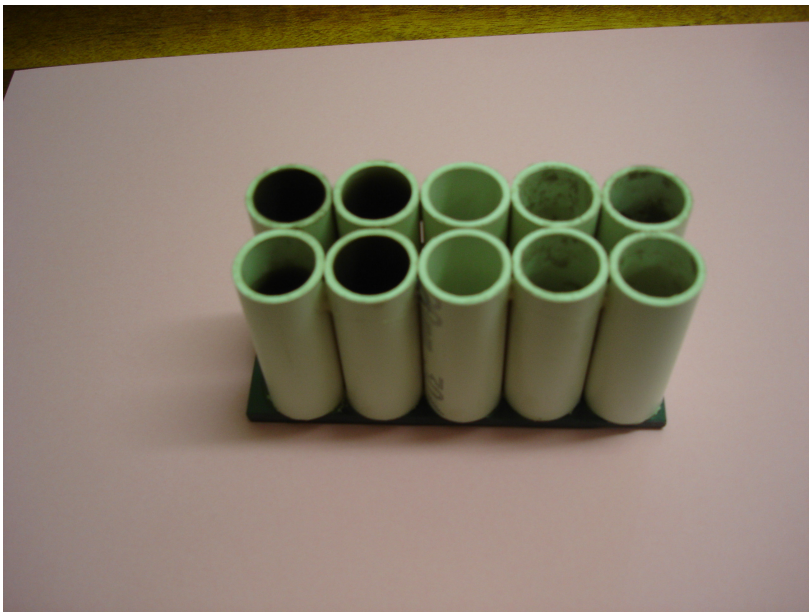

Figure 1: Photograph of the container consisting of ten PVC tubes (16mm diam. X 15mm high), which were used during the trial.

7. Effect of amendments:

It is not envisaged that any amendments would have had an influence on the eventual results of the study. The amendments were necessary because no guidelines exist for a trial of this nature and it was necessary to simplify the study in the set-up phases.

8. Results:

8.1 Experimental spray:

Soon after the experimental units were sprayed with the experimental spray, the blowfly larvae started migrating from the liver. Since the experimental units were placed in plastic basins, the migrating larvae could be easily observed (Fig. 2). Within 30 minutes after spraying, the majority of larvae that migrated into the plastic basins appeared to be dead. The actual assessments were only done at 4, 8, and 24 hours post-treatment. The percentage mortality varied from 70 – 100% (Tables 1) with an average larval mortality of 89,2%, and 10,8% of the larvae being moribund within the first 24

hours after treatment (Tables 1, 4, & 5). No significant differences were found in the percentage mortality during the three observation periods within the first 24 hours post-treatment (Appendix A1).

Figure 2: Blowfly larvae that migrated from the liver containing tubes shortly after the experimental spray was applied.

During the extended assessment period up to 96 hours post-treatment, 100% mortality was reached in the majority of replicates. Only three replicates still had moribund larvae, giving an average mortality of 96,9% over 96 hours. (Table 5). These larvae were still considered moribund, although no voluntary movement were observed. Slight movement or muscle contractions were observed only when the larvae were probed with a blunt pointed object. At 120 hours post-treatment 100% mortality was reached in all 15 replicates.

Since none of the moribund larvae showed any movement during assessment unless being probed, it is suspected that they will not remain on the sheep after treatment with the experimental spray, but will fall to the ground and eventually die.

8.2 Experimental ointment:

Similar results to the experimental spray were obtained with the experimental ointment. The percentage mortality varied from 90 - 100% (Table 2) with an average percentage of 97,6% dead and 2,4% moribund (Tables 2, 4 & 5) within 24 hours post-treatment. However, 100% mortality was reached within 48 hours post-treatment (Table 5). Furthermore, slightly higher percentage mortality occurred with the ointment within 24 hours post-treatment than with the spray. No significant differences were found in the percentage mortality during the three observation periods within the first 24 hours post-treatment (Appendix A2).

8.3 Controls:

The control larvae also migrated from the liver, although at a significantly slower rate. The percentage mortality varied from 2 – 12% (Table 3) with an average mortality of 5,6% and 94,4% of the larvae still alive after 24 hours post-treatment (Tables 3 & 4).

9. Statistical analysis:

The Kruskal-Wallis Test (Nonparametric ANOVA) and the Dunn's Multiple Comparisons Test illustrated that no significant differences occurred in the mortality of the larvae between the experimental spray and experimental ointment treatments. Significantly fewer larvae died in the control groups during each of the observation periods within the first 24 hours post-treatment. (Appendices A3 – A5).

10. Conclusions:

From the results it is clear that both the experimental spray and experimental ointment were effective in killing the blowfly larvae. Although a higher percentage of the blowfly larvae were killed within 24 hours post-treatment by the experimental ointment than by the experimental spray, this difference was not statistically significant. Furthermore, the larvae became moribund shortly after coming into contact with the two experimental formulations. When applying these formulations to sheep, the larvae will probably fall from the sheep and die on the soil surface.

From the viewpoint of the livestock farmer, using the spray formulation will probably be more cost effective and less labour intensive to apply than the viscose ointment. The ointment, due to its viscosity, will probably soil the wool fibres leading to wool of an inferior quality and subsequent financial losses to the farmer.

Table 1: Percentage dead or moribund late second or early third instar *Lucilia cuprina* larvae after exposure to insecticide treated chicken liver portions for 24 hours.

Experimental Spray on Chicken Liver Portions									
Replicates	Number Larvae	Assessment times							
		4 hours		8 hours		24 hours		% Dead or Moribund	
		D*	M**	D*	M**	D*	M**	D*	M**
1	50	41	9	45	5	47	3	94	6
2	50	39	11	46	4	50	0	100	0
3	50	44	6	45	5	48	2	96	4
4	50	29	21	30	20	31	19	62	38
5	50	40	10	43	7	45	5	90	10
6	50	47	3	48	2	48	2	96	4
7	50	39	11	41	9	42	8	84	16
8	50	31	19	37	13	39	11	78	22
9	50	29	21	36	14	37	13	74	26
10	50	32	18	34	16	35	15	70	30
11	50	49	1	49	1	50	0	100	0
12	50	49	1	50	0	50	0	100	0
13	50	49	1	50	0	50	0	100	0
14	50	48	2	49	1	50	0	100	0
15	50	40	10	44	6	47	3	94	6
Total number dead / moribund		606	144	647	103	669	81		
Average number dead / moribund		40,4	9,6	43,1	6.9	44,6	5,4		
Percentage dead / moribund		80,8	19,2	86,3	13,7	89,2	10,8	89,2	10,8

*D = Dead larvae (no movement)

**M = Moribund larvae (retarded movement)

Table 2: Percentage dead or moribund early second and late third instar *Lucilia cuprina* larvae after exposure to insecticide treated chicken liver portions for 24 hours

Experimental Ointment on Chicken Liver Portions									
Replicates	Number Larvae	Assessment times							
		4 hours		8 hours		24 hours		(%) Dead or Moribund	
		D*	M**	D*	M**	D*	M**	D**	M**
1	50	37	13	41	9	45	5	90	10
2	50	47	3	48	2	49	1	98	2
3	50	50	0	50	0	50	0	100	0
4	50	50	0	50	0	50	0	100	0
5	50	50	0	50	0	50	0	100	0
Total number dead / moribund		234	16	239	11	244	6		
Average number dead / moribund		46,8	3,2	47,8	2,2	48,8	1,2		
Percentage dead / moribund		93,6	6,4	95,6	4,4	97,6	2,4	97,6	2,4

*D = Dead larvae (no movement)

**M = Moribund larvae (retarded movement)

Table 3: Percentage alive or dead late second or early third instar *Lucilia cuprina* larvae after exposure to insecticide free chicken liver portions. **(Control for spray and ointment formulations).**

Chicken Liver Portions free from experimental product									
Replicates	Number Larvae	Assessment times							
		4 hours		8 hours		24 hours		(%) Alive or Dead	
		L*	D**	L*	D**	L*	D**	L*	D**
1	50	50	0	50	0	48	2	96	4
2	50	48	2	47	3	47	3	94	6
3	50	50	0	50	0	49	1	98	2
4	50	49	1	49	1	46	4	92	8
5	50	50	0	49	1	48	2	96	4
6	50	50	0	48	2	47	3	94	6
7	50	50	0	47	3	46	4	92	8
8	50	49	1	49	1	48	2	96	4
9	50	50	0	49	1	49	1	98	2
10	50	48	2	47	3	44	6	88	12
Total number alive / dead		494	6	486	14	472	28		
Average alive / dead		49,4	0,6	48,6	1,4	47,2	2,8		
Percentage alive / dead		98,8	1,2	97,2	2,8	94,4	5,6	94,4	5,6

*L = Alive larvae (active movement)

**D = Dead larvae (no movement)

Table 4: Summary of the percentage efficacy of the experimental spray and experimental ointment compared to the controls on late second or early third instar *Lucilia cuprina* larvae, 24 hours post-treatment.

Treatments	Average % Treatments		Average % Controls	
	D*	M**	D*	L***
Spray	89,2	10,8		
Ointment	97,6	2,4		
Controls			5,6	94,4

D* = Dead larvae (no movement)
M** = Moribund larvae (retarded movement)
L*** = Alive larvae (active movement)

Table 5: Percentage dead and moribund late second or early third instar *Lucilia cuprina* larvae 96 hours after exposure to insecticide treated chicken liver portions.

Treatments	Assessment times and dates							
	24 hours 22/4/2006		48 hours 23/5/2006		72hours 24/5/2006		96 hours 25/5/2006	
	D*	M**	D*	M**	D*	M**	D*	M**
Spray								
Percentage (%)	89,2	10,8	92,3	7,7	94,0	6,0	96,9	3,1
Ointment								
Percentage (%)	97,6	2,4	100	0				

D* = Dead larvae (no movement)
M** = Moribund larvae (retarded movement)