finalreport

Project code: **M.723**

Prepared by: **Dr Phillip Spadbery**
Robert S Tozer
XCS Consulting Pty Ltd

Date published: **August 1998**

ISBN: **9781 741 912 180**

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Neem Oil as an Anti-Feedant for Buffalo Fly Control

TABLE OF CONTENTS

FINAL REPORT FOR MEAT RESEARCH CORPORATION:

NEEM OIL AS AN ANTI-FEEDANT FOR BUFFALO FLY CONTROL (M.723)

1. Executive Summary

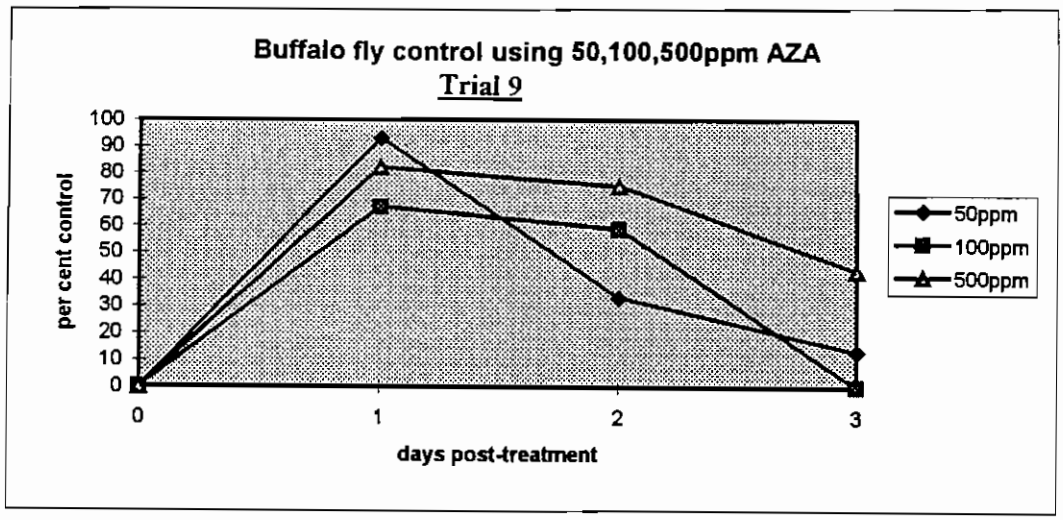
Prior to commencing field evaluations of neem oil, Australian sources of neem were identified and arrangements for supplies put in place. The design of the prototype Self Applicator apparatus ("*Fly Control Rod*") was also finalised. An on-line literature search for neem publications was made and more than 1,480 published research papers were reviewed.

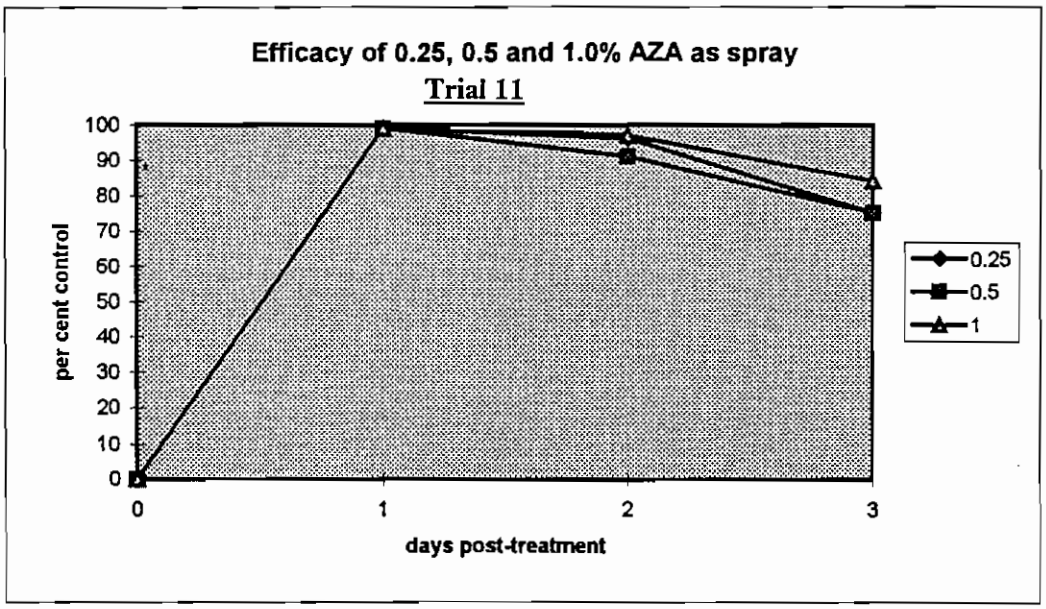
A written submission regarding registration of neem for use as a veterinary chemical in Australia was developed and provided to the National Registration Authority (NRA) as the basis for future discussions on registration and Maximum Residue Limit (MRL) matters. This document provided the inspiration for discussions with senior evaluators at the NRA during the course of the research programme. Such discussions are on going.

The results of the preliminary studies reported here demonstrated that some formulations of neem oil, containing sufficient amounts of the active ingredient, azadirachtin (AZA), are highly effective for control of buffalo fly. This effect was manifested by flies leaving the animals (although remaining flies may well fail to bite the host cattle due to the anti-feeding characteristics of neem). It was also demonstrated that effective fly control products can be dispensed via a proprietary self applicator system based on a reservoir containing a formulated product, which is brushed onto the backs of cattle via polypropylene wicks when animals pass beneath the device. However, the Self Applicator system was not adequately assessed and shown to be effective using neem based products, due to supply and formulation problems.

The studies demonstrated that not all neem based products, even those commercially available overseas and allegedly containing specific and presumably effective amounts of AZA, have an impact on buffalo flies feeding on cattle hosts.

The early field trials were made on cattle by applying neem products, using a syringe or hand sprayer unit to treat the backline or whole body surface of the animals, in a series of titration studies to determine the minimum/ optimum amount of AZA necessary to repel buffalo flies from treated animals. The amount of AZA delivered to individual cattle during this early phase of the study (Trials 1-9, see accompanying Summary Table) was 0.005 - 2.5 mg AZA per animal. Control (based on differences in numbers of flies on treated and untreated control animals expressed as a percentage) was highly variable but did reach 80-93% on some occasions but continued for no longer than 24 hours after treatment (Figure 1, Trial 9). The baseline requirement in these studies was for >80% control for 2 days or more following treatment. The National Registration Authority's (NRA) requirement for buffalo fly control chemical agents is 95% control





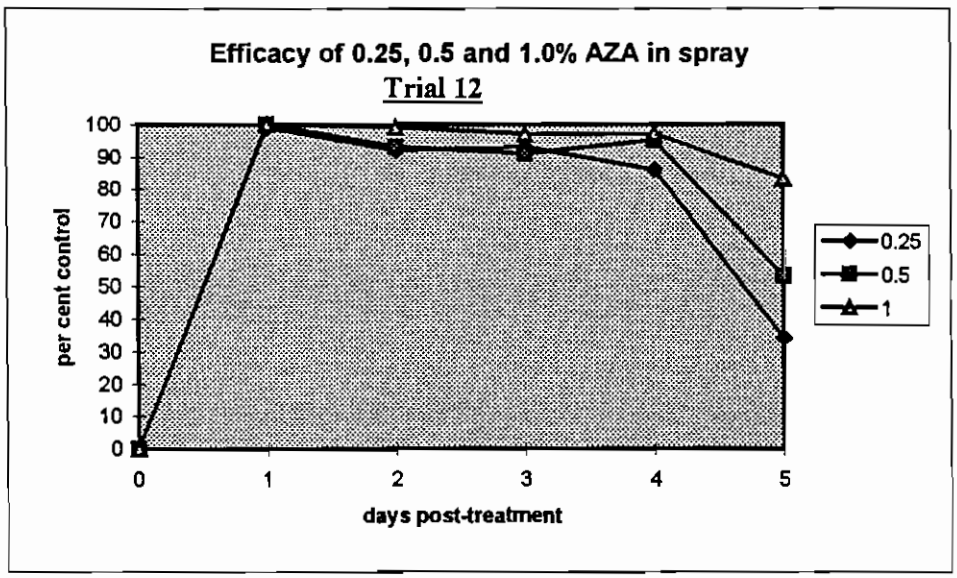


Figure 1. Efficacy of neem oil for buffalo fly control (Trials 9, 11, 12)

By increasing the amount of AZA per animal and ensuring that the whole body of the animal was treated, fly control was spectacular. At >0.75 mg AZA per animal, 100% control of buffalo fly was achieved in several studies one day after treatment and >90% control was maintained for up to 4 days after treatment (Figure 1, Trials 11 and 12). Difficulties with supplies of neem at this time caused a hiatus in the field studies and led to an extension of the project.

After obtaining further supplies of neem and also sourcing supplies from a different company, the field evaluation of neem using the Self Applicator was carried out. A convincing demonstration of the efficacy of the Self Applicator was made, using a commercial but unregistered pour-on product for fly control ("Brute" containing 10% permethrin). After setting up the Self Applicator and charging it with the "Brute" formulation, fly control was 98% within one day and continued at 100% for the following 2 weeks at which time the Self Applicator was removed (Figure 2, Trial 18).

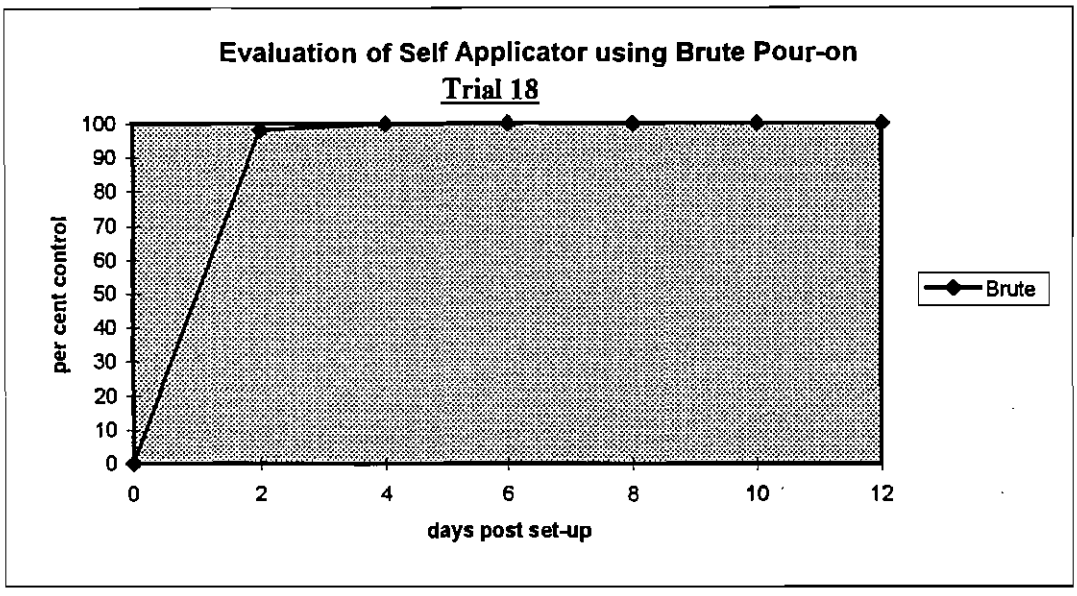


Figure 2. Evaluation of the Self Applicator, using 10% permethrin in Brute™ pour-on formulation

This convincing demonstration of the efficacy of the Self Applicator was followed up with a study using a neem product. However, the level and sustainability of control achieved was not good (Figure 3, Trial 19). It was clear that a formulation of neem that had been used in the earlier studies would most likely have produced good results. The company that processes that very effective formulation of neem no longer produces neem based products and further supplies could not be secured.

This study demonstrated the effectiveness of controlling buffalo fly in the field by deploying the Self Applicator. The study also demonstrated that some formulations of neem (fortified with adequate quantities of AZA) give excellent buffalo fly control. Lack of supplies of neem which had been demonstrated as effective in earlier 'spray studies' were not available for the final phase of this work. Nevertheless, we believe that the marriage of the Self Applicator with an effective organic neem product in a suitable carrier

is essentially a formulation problem but will require extensive research into suitable carrier chemistry.

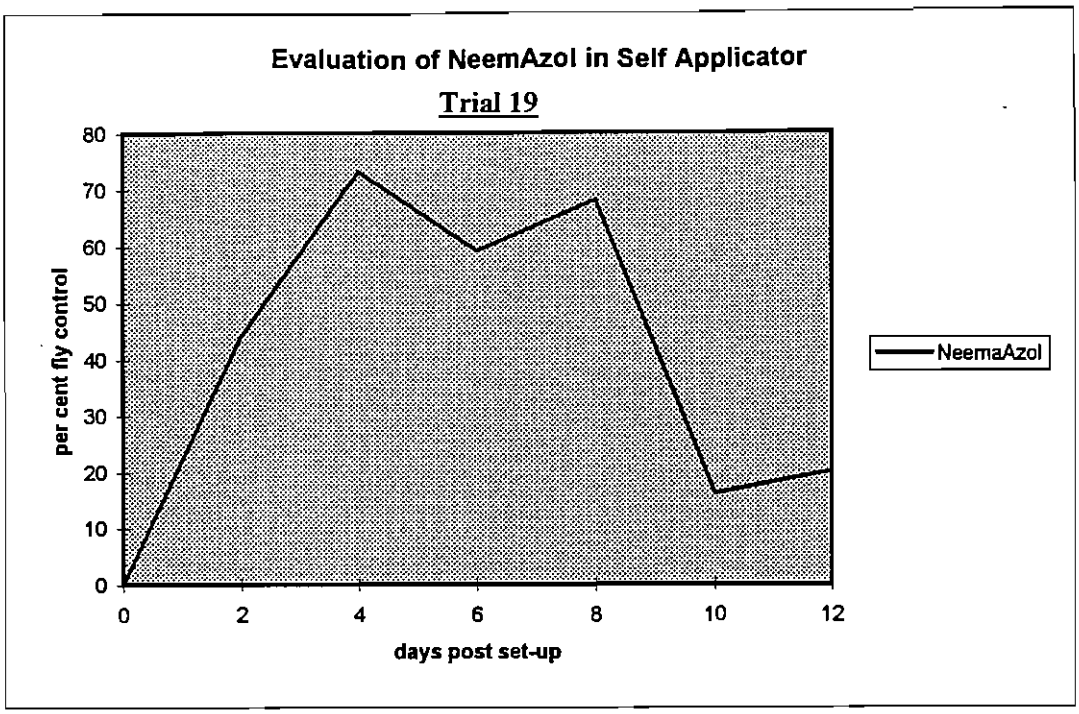


Figure 3. Evaluation of the Self Applicator using NeemAzol (3% AZA)

The further development and future deployment of a neem-based self application system for buffalo fly control in open pasture grazing situations must now await registration of neem for use in Australia. Neem is registered for agricultural use in the USA and many other countries such as Nicaragua, while commercialisation of neem throughout Latin America is imminent. Members of the Australian neem industry are confident that NRA registration of this product cannot be far away. That toxicology packages for neem are now available will certainly assist the registration process. It is hoped that within ten years time, neem and other botanically based insecticides will form a significant part of food crop and animal protection in Australia.

Summary table of results of field studies evaluating neem oil for buffalo fly control

Trial No.	*% AZA*	*Concentration / Dilution*	*Dose/ animal (ml)*	*Amount AZA per animal (mg)*	*Efficacy (% control) +days after treatment 1, 2, 3, 4, 5, 6, 7, ...*
1.	0.13	50% 100%	75	0.049 0.097	11, 26, 25 62, 54, 58
2.	0.13	50% 100%	100	0.065 0.130	84, 73, 16 67, 63, 53
3.	1.0	50%	250	2.5	81, 96, 49
4.	0.03 Nimbitor	4% 10% 20%	100	0.0012 0.003 0.006	57 40 54
5.	0.03	20% 40% 80%	100	0.006 0.012 0.024	72, 29 74, 41
6.	0.03	20% 40% 80%	100	0.006 0.012 0.024	62, 9, 57, 54 62, 0, 37, 0 90, 76, 83, 14
7.	2.0	0.005% 0.01% 0.05%	100	0.005 0.01 0.05	38, 37, 0 39, 0, 0 62, 0, 0
8.	2.0	0.005% 0.01% 0.05%	100	0.005 0.01 0.05	33, 0, 0 45, 0, 0 43, 42, 28
9.	2.0	0.005% 0.01% 0.05%	100	0.005 0.01 0.05	93, 33, 13 67, 59, 0 82, 75, 43
11.	2.0	0.25% 0.5% 1.0%	300	0.75 1.5 3.0	99, 96, 75 99, 91, 75 100, 97, 84
12.	2.0	0.25% 0.5% 1.0%	300	0.75 1.5 3.0	99, 92, 93, 86, 34 100, 93, 91, 95, 53 100, 99, 97, 97, 83

13.	2.0 (new, no sunscreen)	0.25% 0.5% 1.0%	300	0.75 1.5 3.0	99, 94, 84 98, 96, 80 99, 90, 76
14.	2.0	0.25% (in Brute	80 Carrier)	0.8	84, 73
15.	3.0 (NE) 3.0 (OCP)	100% 100%	170 170	5.1 5.1	99, 98, 72, 79 84, 87, 70, 16
16.	3.0 (OCP)	100%	60	1.8	27, 0, 0, 0
17.	3.0 (OCP)	50%	self applicator	-	25, 33, 0
19.	3.0 (OCP)	50%	self applicator	-	14, 44, 73, 59, 42, 70, 68, 16, 20

The results obtained from Trials 9, 11 and 12, using the effective formulation of neem from Neem Extracts Pty Ltd, was subjected to probit analysis, comparing the amount of AZA active per animal with efficacy (equivalent to % control or % mortality) (details of analyses given in Appendix 12). The analyses were made on 1, 2 and 3 day post-treatment data separately to derive response levels after exposure of the one-off neem treatment for 1-3 days under field conditions. To achieve 90% and 95% control at 1, 2 and 3 days after treatment the amount of AZA per animal was estimated to be as follows:

Mean quantity (mg/animal) of AZA required to achieve 90 and 95% control of buffalo flies on cattle after 1, 2 and 3 days after treatment

Control level	*Days post-treatment*		
	1-day	2-day	3-day
90%	0.043	0.460	2.280
95%	0.149	1.398	5.202

It appears that a formulation which provides about 1.4 mg of AZA per animal per day or two will reduce buffalo fly infestations on cattle hosts by 95%. A formulation which results in the even spread of the 1.4 mg of AZA over all or most of the animals body should provide high levels of buffalo fly control within a self applicating system.

These data should provide a good basis for future studies of neem based formulations for buffalo fly control.

2. Introduction

2.1 Buffalo fly

The buffalo fly, *Haematobia irritans exigua*, is a major pest of cattle in India, Indonesia, Malaysia, China, Papua New Guinea and much of tropical and sub-tropical Australia. The economic impact of buffalo fly in Australia has been estimated as high as AUD$ 150 million per annum with liveweight losses of up to 21% recorded on untreated cattle compared with those kept relatively fly free (Spradbery & Tozer 1996). Buffalo fly is currently controlled by means of chemical pesticides such as organo-phophates and synthetic pyrethroids, administered via dust bags, back rubbers, sprays, and pour-ons, and impregnated ear tags. The broad spectrum chemical pesticides used in agriculture are toxic to many non-target insects and indiscriminately destroy other beneficial insects, including natural enemies of insect pests. There is demonstrable interest among graziers in Australia for more organic and environmentally acceptable ways of controlling buffalo fly such as the walk-through buffalo fly trap that was developed in Australia and USA (Tozer and Sutherst 1998). There is an increasing ground swell of informed opinion supporting the use of organically derived pest control agents such as neem.

2.2 The Indian Neem tree and azadirachtin

Neem oil is a product of the Indian Neem Tree or Indian Lilac, *Azadirachta indica*. The unusual properties of the neem tree have been exploited for centuries and feature in ancient Sanskrit writings. The active ingredient, which is derived primarily from the seed kernel but is also found in the leaves and bark, is azadirachtin, a tetranor-triterpenoid plant liminoid with potent insect anti-feedant and growth disrupting properties (see update review by Mordue and Blackwell 1993). There are several azadirachtins in neem products, but the two which quantitatively dominate are azadirachtin A and to a much lesser extent, azadirachtin B, together with other actives such as salannin and nimbin (see gas chromatogram in Figure 3).

Recent advances in azadirachtin research are related to field trial data, using commercial and semi-commercial preparations of neem. Increasing research on the chemistry of azadirachtin and the development of synthetic analogues are leading to a greater understanding of structure, activity relationships and synthesis (for a review of the chemistry of azadirachtin, see Ley, Denholm and Wood 1993). The interest shown by researchers into neem and its biological effects is apparent from the literature - more than 1,480 publications dealing with neem were found on-line during a recent literature search of the past decade.

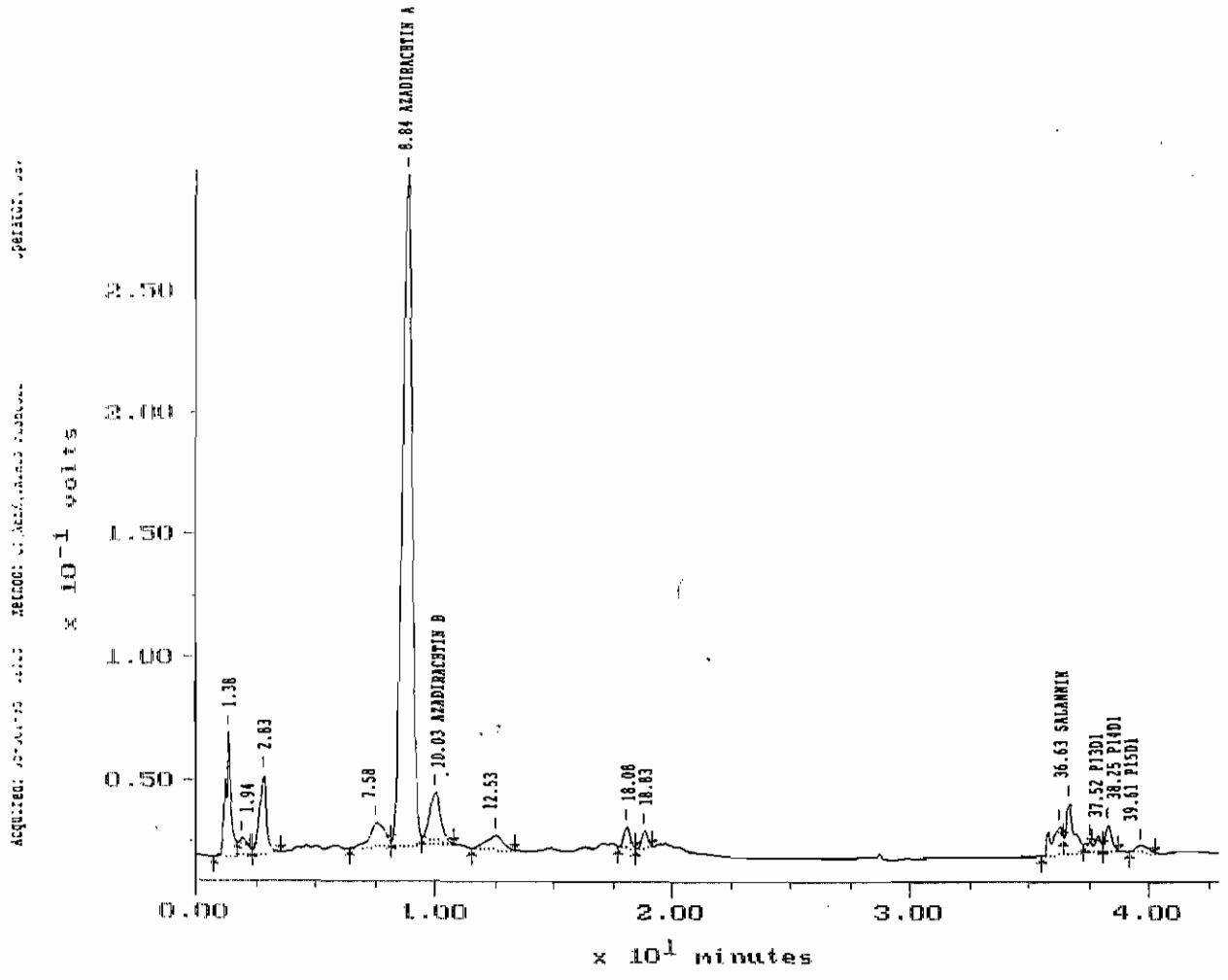


Figure 3. A Gas Chromatogram Profile of Azadiractin and other Actives in Neem Oil (Neem Extracts Pty Ltd, Lismore, NSW)

2.3 The impact of azadirachtin on insects

The major impact of azadirachtin on insects is its anti-feedant effect although it displays other toxic effects which result in insect growth inhibition, malformation, inhibition of ecdysis (= insect growth inhibitor action) and death. More than 200 insects species have been studied during the process of determining anti-feedant effects. As little as 0.01 ppm of AZA caused 100% anti-feeding in locusts (Haskell and Mordue 1969), although some insects required much higher doses to achieve an anti-feedant effect such as a termite species which required >100 ppm AZA (Grace and Yates 1992). Fly larvae such as sheep blowfly, house fly and buffalo fly, can be controlled through insect growth effects when their feeding medium is treated with AZA at 10-20 ppm, while adult sheep blowflies have

been prevented from ovipositing by a dose of 200 ppm AZA in sheep plasma (in Rice 1993).

2.4 Australian neem industry - Quo Vadis?

The neem industry in Australia is embryonic at present, although there appears to be some long term future potential in both growing the neem tree and also in the processing industries (Rice 1993, O'Shea, unpublished communication). Nevertheless, the research project reported here was frequently hampered by lack of sources of neem oil, disruptions to supply, and variations in formulation. Before the local neem industry can claim any share of the pest control market in Australia, there needs be an upgrading of standards in regard to the quantities and quality of neem-based products available for evaluation by research groups. There are also likely to be protracted negotiations to obtain approval for neem to be used for agricultural and veterinary use. Both these issues are related to formulation problems and the need for a technical grade active (or similar) for research and registration purposes. There is a pressing need to further the registration process through the Australian National Registration Authority (see Appendix 11), which requires significant and costly inputs such as toxicological and residue data packages. That neem-based products have the potential to control buffalo fly in a cost effective and environmentally sound manner should provide some stimulus to further the process of registration of neem/ azadirachtin for deployment in Australian agriculture.

2.6 References

Grace JK and Yates JR (1992) Behavioral effects of a neem insecticide on *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Tropical Pest Management* **38**, 176-180.

Haskell PT and Mordue AJ (1969) The role of mouthpart receptors in relatioin to feeding of *Schistocerca gregaria*. *Entomologia Experimentalis et Applicata* **12**, 591-610.

Ley SV, Denholm AA and Wood A (1993) The chemistry of Azadirachtin. *Natural Products Reports* **10**, 109-157.

Mordue AJ and Blackwell A (1993) Azadirachtin: An Update. *Journal of Insect Physiology* **39**, 903-924.

Rice M (1993) Development of neem research and industry in Australia. *Entomology Workshop*, 30 pages.

Sheppard DC and Hinkle NC (1987) A field procedure using disposable materials to evaluate Horn fly insecticide resistance. *Journal of Agricultural Entomology* **4**, 87-89.

Spradbery JP and Tozer RS (1996) The efficacy of diazinon impregnated ear tags against buffalo fly and resulting weight gains and diazinon residues in meat and milk. *Australian Veterinary Journal* **73**, 6-10.

Tozer RS and Sutherst RW (1996) Control of Horn fly (Diptera: Muscidae) in Florida with an Australian Trap. *Journal of Economic Entomology* **89**, 415-420

3. Purpose of Study

The purpose of this study was to determine the efficacy of neem oil products to provide an alternative, 'non-chemical' pesticide for control of the buffalo fly, *Haematobia irritans exigua*, due to neem oil's insect anti-feeding properties. The study also included the refinement and development of a cost-effective self application system for treating cattle with neem products under field conditions in Australia.

In preliminary studies to determine the efficacy of different formulations and concentrations for eventual field use, a bench mark of 80% control of buffalo fly for a period of 2-3 days after a single application was the projected target. These studies were anticipated to evaluate available sources of neem-based products from Australia and also overseas.

Once a suitable formulation(s) of a neem-based product provided the minimum standard of efficacy in the preliminary studies, this formulation would be developed for use in a self applicator. Prior to the commencement of this study, a prototype self applicator, using readily available plumbing equipment, was designed and constructed.

Subject to the results of the field studies using the self-applicator, more widespread field and grazier testing would be undertaken. Commercialization of the system would be sought subject to approval for the use of neem products on food producing animals by the National Registration Authority (NRA). Registration by the NRA of new products for use in agriculture and for veterinary use requires an extensive package of data, from toxicity studies through efficacy and residue studies. Such application packages for registration of a new product typically cost many hundreds of thousands of dollars and is outside the scope of the present study. However, an attempt to progress the registration of neem, through an application for a Maximum Residue Limit (MRL) waiver for neem/ azadirachtin, was initiated with the NRA during the course of this study

4. Materials

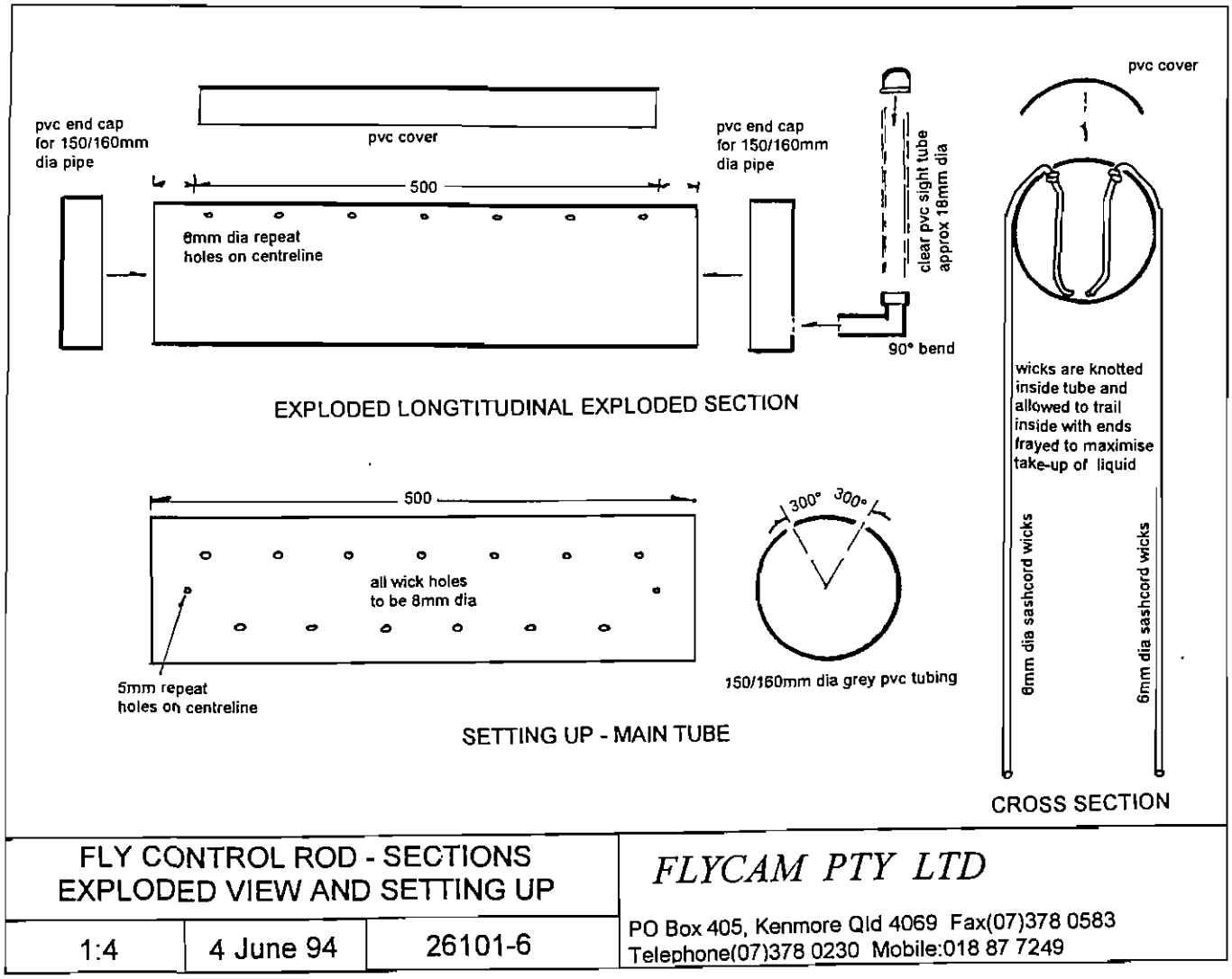
4.1 Experimental materials used

Several different formulations containing neem oil and its derivatives from different sources were used during the course of the study. A typical gas chromatogram profile of the azadirachtins and other actives is given in Figure 3.

Product	*Source*	*Formulation*	Trials used
Neem Oil /I	Neem Extracts Pty Ltd Lismore, NSW	0.31% AZA 2% sunscreen 2% sticking agent 30% emulsifying agent in ethanol	1 and 2
Neem Oil /II	Neem Extracts Pty Ltd Lismore, NSW	1% AZA 2% sunscreen 2% sticking agent 30% emulsifier in ethanol	3
Nimbitor	Zandu Pharmaceutical Works, Bombay, India	0.03% AZA (300 ppm)	4, 5 and 6
Neem Oil / III	Neem Extracts Pty Ltd	2% AZA	7, 8, 9, 10, 11 and 12
Neem Oil / IV	Neem Extracts Pty Ltd	2% AZA (no sunscreen agent included)	13 and 14
NeemAzol	Organic Crop Protectants Lilyfield, NSW	3% AZA	15, 16, 17 and 19
Neem Oil / V	Neem Extracts Pty Ltd	3% AZA	15
Neem Bitters	Neem Extracts Pty Ltd	No AZA	15

4.2 Self Applicator

The Self Applicator or "Fly Rod" was constructed from PVC off-the-shelf plumbing equipment as described in Figure 4, below. Its use in the field is illustrated in Plates 1-5.



4.3 Location of field studies

The neem field study was conducted at "Iwakana", the Peak Crossing (QLD 4306) property of Flycam Pty Ltd (Plate 6) with untreated control animals maintained at Allens Road, Peak Crossing (Plate 7), proprietor, Mr Wayne Bailey, 3 km from the experimental property. The properties were characterized by unimproved open pastures of predominantly native grass species in the Fassifern valley area of southern Queensland. The stocking rates were approximately 1 animal per 1.5 acres.

Treated animals were run in an open paddock of 60 hectares bounded by wire and electric fencing (Plate 8) and incorporating a separate feeding area of 5 hectares in which molasses and water were sometimes provided. The molasses was presented in a drum dispenser (Plate 9). Adjoining the feeding area was a cattle race in which animals could be weighed and treated (Plates 10 and 11).

4.4 Personnel involved

The personnel involved in the study were as follows

Name	*Responsibility*
JP Spradbery	Study Director
RS Tozer	Field trials director and supervision
S Pender	Fly counts and animal maintenance
W Bailey	Property owner

(Appendix 7)

4.5 Animals used

The animals used in both treated and control groups were Brangus and Angus cross steers, all black in colour (Plates 7, 9 and 10).

4.6 Feed and water

Food was grass in the paddocks with supplementary food as hay when required. Water was supplied *ad libitum* as per local practice. Small quantities of lucerne hay were sometimes used to attract animals to facilitate fly counts. Water was supplied from farm dams in each paddock, and the nutritional status of the paddocks was good during the course of the trials.

4.7 Animal identification

Animals in each group were individually identified with numbered ear tags (Y-Tex identification tags)

4.8 Trial Permit

A Trial Permit (TPM0001A) was obtained from the National Registration Authority to enable the need study to be carried out (Appendix 9). The conditions of the permit state that disposal of any produce from animals treated during the trials cannot be done in a manner that can result in direct or indirect consumption of this produce by humans. Any animal treated with neem cannot be put back into the food chain and must be retained for experimental purposes or destroyed and properly disposed.

5. Methods and Procedures

5.1 Weighing animals

The weight of each animal in the treated groups was determined using electronic cattle scales ('TruTest' ±1 kg) on the day of treatment. The amount of material used to treat each animal was determined on the basis of liveweight (see Appendix 2, Treatment Records).

5.2 Buffalo fly counts

The number of buffalo fly on the animals was estimated by counting flies on both sides of each animal (whole body counts) and recording on a pro-forma (Plates 12 and 13) (Appendix 3 - Fly Counts). When numbers of buffalo fly were >20 and <100, they were counted in groups of 10s; when >100 and <200, in groups of 20s; and when >200 per animal, flies were counted in groups of 50s. In some trials, the numbers of flies on upper or lower body were distinguished, and also upper, mid, lower body and head were distinguished.

5.3 Timetable

The different field studies were carried out as follows:

Number	*Experimental design*	*Dose (ml/animal)*	*Date of treatment*	*No. days*
1.	Comparison of 50% and 100% neem	55-75	24/5/96	4
2.	Comparison of 50% and 100% neem	75-100	28/5/96	5
3.	Ethanolic neem (1%): compare spray and re-chargeable ear tag applications	250	1/6/96	5
4.	Compare 4, 10, 20% "Nimbitor"	100	26/11/96	6
5.	Compare 20, 40, 80% "Nimbitor"	100	3/12/96	3
6.	Compare 20, 40, 80% "Nimbitor"	100	13/12/96	6
7.	Compare 50, 100, 500 ppm AZA in canola	100	29/12/96	6
8.	Compare 50, 100, 500 ppm AZA in canola	100	8/1/97	5
9.	Compare 50, 100, 500 ppm AZA in canola	100	19/1/97	4
10.	0.05, 0.1, 0.5% AZA in Brute carrier	50	31/1/97	6
11.	0.25, 0.5, 1.0 % AZA in water spray	300	22/2/97	4
12.	0.25, 0.5, 1.0% AZA in water (2% AZA)	300	1/3/97	6
13.	0.25, 0.5, 1.0% AZA in water (2% AZA)	300	8/3/97	4
14.	0.25% AZA (2,500 ppm) in Brute carrier	80	25/3/97	3
15.	OCP neem/Neem Extracts neem/Bitters	50-190	21/1/98	13
16.	Neemazal (3% AZA, 10g/L AZA) OCP	60	19/3/98	5
17.	50% Neemazal in Self Applicator	-	26/3/98	7
18.	Brute pour-on in Self Applicator	-	4/4/98	13
19.	50% OCP Neem in Dctrate	-	6/5/98	13
Total				*118*

5.4 Observations

Recordings and observations were made on the following:

- Weight of treated animals (Appendix 1 - Treatment Records)
- Dose amounts of pour-on per animal (Appendix 1 - Treatment records)
- Observations on animal health (Appendix 2 - Animal Health Observations)
- Buffalo fly counts (Appendix 3 - Fly Counts)
- Activities associated with trial (Appendix 4 - Farm Diary)
- Resistance status of buffalo fly populations (Appendix - 5 - Resistance Test Data)
- Weather conditions at the experimental locality (Appendix 4)

5.5 Statistical treatment

The per cent reduction in fly numbers on treated cattle relative to the respective untreated control group was calculated as per the NRA's *"Guidelines for the Establishment of Efficacy and Management Data in Support of Applications for the Registration of Products to be used in Control of Buffalo Fly"*

$$\% \text{ control} = \frac{\text{mean no. flies on controls - mean no. flies on treated}}{\text{mean no. flies on controls}} \times 100$$

5.6 Resistance tests

Resistance tests were carried out at the experimental study site at "Ikawana", using a series of dilutions of fenvalerate (for synthetic pyrethroid resistance) and diazinon (for organophosphate resistance), according to the method of Sheppard and Hinkle (1987), *J.Agric Entomol* 4, 87-89. The filter papers were prepared and supplied by the Sheppard laboratory at the University of Georgia, USA. The results (Appendix 5 - Resistance Test Data) were analyzed by probit analysis (Figures 5 and 6).

Trans dose	dose	Obs Number used	Est Number respond	Contrib Number respond	diff	to chi2	
0.310-0 509		58	1	0.2			
0.470-0.328		64	0	2.4	-2.9	0.43	Pooled
0.700-0 155		77	20	15.5			
1.050 0 021		64	32	35.1	3.1	0.59	
1.580 0 199		85	71	73.1			
2.370 0.375		70	70	68.5			
3.560 0.551		93	93	92.9	-0.6	0.02	Pooled
5.330 0.727		77	77	77.0			
8.000 0.903		93	93	93.0			

Snw = 137.0 Snwx = 1.2152 Snwy = 692.142
1/Snw = 0.007300599 Mean x = 0.2545 Mean y = 6.6124
Snwx2 = 177.15909 Snwxy = 30.6733 Snwy2 = 177.1591

Chi Squared based on pooling = 1.04 with 4 degrees of freedom P = 0.
Chi Squared based on all data = 10.81 with 7 degrees of freedom P = 0.

Equation is b = 5.4232 Y = 5.0049 + 5.4232x

Variance of b V(b) = 0.273 Therefor b = 5.423± 0.523
Heterogeneity factor = 1.5444
Heterogeneity factor exceeds 1, Variances corrected
!! Warning !! this is only valid if there is no systematic variation

Response level	lower CI 99%	lower CI 95%	EDx	upperCI 95%	upperCI 99%	
ED .00001	0.026	0.048	0.110	0.186	0.225	Extrapolated
ED .0001	0.035	0.061	0.133	0.217	0.260	Extrapolated
ED .001	0.047	0.080	0.163	0.257	0.303	Extrapolated
ED .01	0.067	0.107	0.206	0.311	0.361	Extrapolated
ED .1	0.100	0.151	0.269	0.387	0.442	Extrapolated
ED .5	0.139	0.201	0.334	0.463	0.522	
ED 1	0.163	0.230	0.372	0.506	0.566	
ED 5	0.251	0.333	0.496	0.642	0.706	
ED 10	0.315	0.406	0.579	0.730	0.795	
ED 25	0.461	0.564	0.750	0.905	0.972	
ED 50	0.699	0.808	0.998	1.155	1.224	
ED 75	1.043	1.146	1.329	1.490	1.566	
ED 90	1.445	1.537	1.720	1.915	2.024	
ED 95	1.712	1.804	2.007	2.260	2.419	
ED 99	2.249	2.368	2.680	3.169	3.538	
ED 99.5	2.463	2.601	2.979	3.608	4.104	
ED 99.9	2.946	3.140	3.706	4.737	5.617	
ED 99.99	3.638	3.932	4.840	6.642	8.308	
ED 99.999	4.353	4.768	6.102	8.928	11.712	
ED 99.9999	5.104	5.661	7.508	11.647	15.952	
ED 99.99999	5.897	6.617	9.073	14.855	21.166	Extrapolated

CI: Confidence limits ED: Effective dose (=Lethal dose LD)

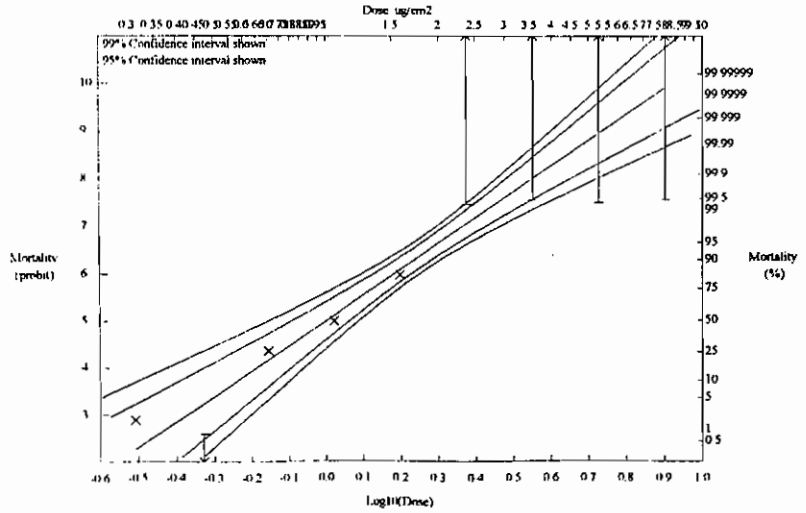


Figure 5. Probit analysis for diazinon using the buffalo fly population at "Ikawana"

Trans dose	dose	Obs Number used	Est Number respond	Contrib Number respond	diff	to chi2	
0 195-0 710		64	0	0.0 |			
0 390-0 409		70	0	0.0 |			
0.780-0 108		66	0	0.0 |	-1.2	0.08	Pooled
1.560 0.193		76	0	0.1 |			
3.125 0 495		72	5	1.9 |			
6.250 0 796		82	13	14.8 |			
12.500 1 097		89	40	48 0	8.0	2.92	
25.000 1 398		64	60	55.5 |			
50.000 1 699		72	71	70.8 |	4.9	4.64	Pooled
100.000 2.000		91	91	90.9 |			

Snw = 141 2 Snwx = 148 7115 Snwy = 699.289
1/Snw = 0 0070814 Mean x = 0.7048 Mean y = 3.7984
Snwx2 = 171.22986 Snwxy = 47.5440 Shwy2 = 171.2299

Chi Squared based on pooling = 7.64 with 7 degrees of freedom P = 0.
Chi Squared based on all data = 11.02 with 8 degrees of freedom P = 0.

Equation is b = 3.3698 Y = 1.4033 + 3.3698x

Variance of b V(b) = 0.098 Therefor b = 3.370± 0.312
Heterogeneity factor = 1.3770
Heterogeneity factor exceeds 1, Variances corrected

Response level	lower CI 99%	lower CI 95%	EDx	upperCI 95%	upperCI 99%	
ED .00001	0.095	0.157	0.335	0.551	0.657	
ED .0001	0.147	0.230	0.454	0.710	0.832	
ED .001	0.238	0.351	0.633	0.938	1.079	
ED .01	0.406	0.560	0.920	1.282	1.445	
ED .1	0.747	0.958	1.413	1.844	2.033	
ED .5	1.221	1.479	2.008	2.495	2.708	
ED 1	1.543	1.821	2.382	2.898	3.126	
ED 5	2.840	3.151	3.794	4.444	4.763	
ED 10	3.814	4.139	4.864	5.694	6.149	
ED 25	5.848	6.265	7.367	8.979	10.056	
ED 50	8.789	9.507	11.677	15.537	18.559	
ED 75	12.821	14.145	18.510	27.423	35.291	
ED 90	17.831	20.094	28.035	46.078	63.635	
ED 95	21.669	24.750	35.939	62.957	90.766	
ED 99	31.142	36.512	57.257	113.258	177.157	
ED 99.5	35.540	42.078	67.898	140.473	226.429	
ED 99.9	46.630	56.347	96.488	219.093	375.814	
ED 99.99	64.925	80.460	148.256	377.454	698.801	Extrapolated
ED 99.999	86.484	109.574	215.256	605.533	1198.079	Extrapolated
ED 99.9999	111.744	144.425	300.535	924.617	1941.718	Extrapolated
ED 99.99999	141.160	185.802	407.563	1360.893	3017.867	Extrapolated

CI: Confidence limits ED: Effective dose (=Lethal dose LD)

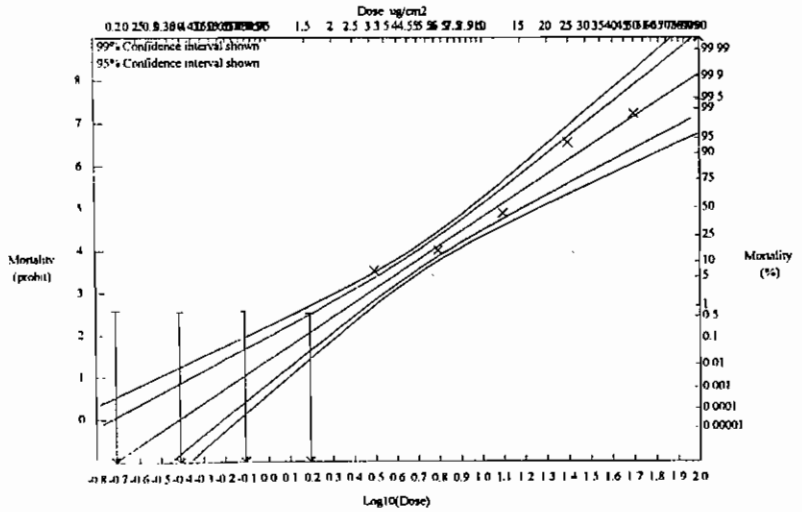


Figure 6. Probit analysis for fenvalerate, using buffalo flies from "Ikawana"

6. Results

6.1 Animal health observations

No adverse animal health observations were noted during the study. No treatments caused adverse reactions and, except for circumstances which were not associated with the study such as 3-day sickness, the animals remained in good health throughout the two year study period (Appendix 2 - Animal Health Observations).

6.2 Resistance status

The results of resistance tests carried out at "Iwakana", Peak Crossing on 26 February 1997 are presented in Figures 5 and 6 and summarised below and .

Fly source	*Compound tested/ Strain*	LD_{50}	RR_{50}
CSIRO/LPL	diazinon / susceptible lab strain	1.128	-
Peak Crossing	diazinon / field population	0.998	0.885
CSIRO / LPL	fenvalerate / susceptible lab strain	0.392	-
Peak Crossing	fenvalerate / field population	11.677	29.788

The results indicate that resistance in the field population of buffalo fly at Peak Crossing to the organophosphate (OP), diazinon, was negligible, while resistance to the synthetic pyrethroid (SP), fenvalerate, indicated significant resistance to this class of chemical pesticide. It is considered unlikely that such SP resistance would impact on the response of buffalo flies to neem and associated products, which are an entirely different class of chemical to which this fly species has not been previously exposed.

6.3 Efficacy studies

6.3.1 Trial 1

Experimental design: The first series of trials was made using a 0.31% azadirachtin/AZA (=31,000 ppm AZA) formulation containing in addition: 2% sunscreen, 2% sticking agent, 30% emulsifying agent, in alcohol (ethanol), produced by Neem Extracts of Lismore, NSW. The formulation can be diluted with water or other carriers such as canola (rape) oil.

The first study compared the full strength formulation with one diluted by a half using canola oil as a solvent. The dose rate was determined on weight of animals and was 55-75ml per animal, applied via spray mister bottles along the back of the animals. Four animals were treated with the 100% formulation, and 4 cattle were treated with the 50% formulation, 6 animals were treated with canola oil only as a placebo and there were 4 untreated controls.

Results: The results of the trial are given below. The numbers of buffalo fly on untreated controls and the per cent reduction in fly numbers resulting from the different treatments compared with the controls (see *4.5 Statistical treatment*, above, for details) is as follows:-

Per cent control of buffalo fly using 50 and 100% formulations of 0.13% AZA

Group	*Pre-treat.*	*Post-treatment*				
	24/5/96	24/5/96 *(+ 2 hours)*	24/5/96 *(+ 4 hours)*	25/5/96 *(+24 hours)*	26/5/96 *(+2 days)*	27/5/96 *(+3 days)*
Controls (x no.flies)	72.5	60.8	53.0	44.0	65.3	111.3
Placebo	-	0	3.2%	0	0	32.6%
50% neem	-	20.2%	56.6%	11.4%	26.0%	24.7%
100% neem	-	44.3%	42.5%	61.8%	55.9%	58.4%

Comment: The full strength formulation gave a maximum of 62% control of flies one day after treatment and this was apparently sustained for a further day or two. The half strength formulation peaked at 57% control 4 hours after treatment but efficacy fell away sharply one day after treatment. The placebo canola oil did not impact on buffalo fly control, although after 3 days, a sharp increase in buffalo fly numbers on the untreated controls gave an apparent but spurious control effect.

6.3.2 Trial 2

Experimental design: This study was a continuation of the previous study, using the same animals in the control group, without a placebo group. The quantities of 50 and 100% neem in synertrol diluent were increased by raising the dose rate to 75-100 ml per animal, treated by applicator mist sprayer to the entire body surface.

Results: The results of this study are given below.

Per cent control of buffalo fly using 50 and 100% dilutions of 0.13 % AZA

Group	*Pre-treat.*	*Post-treatment*			
	28/5/96	28/5/96 *(+2 hours)*	29/5/96 *(+1 day)*	30/5/96 *(+2 day)*	31/5/96 *(+3 day)*
Control (x no. flies)	100.3	98.8	76.9	83.5	56.6
50% neem	-	88.6%	84.4%	72.7%	16.4%
100% neem	-	95.6%	67.1%	62.5	52.7%

Comment: Although almost 100 per cent control was achieved with the full strength formulation 2 hours after treatment, this level of efficacy was not maintained consistently over the following days. The 50 per cent formulation provided more than 84 per cent fly control for 24 hours.

6.3.3 Trial 3

Experimental design: This study compared a 50% dilution in water of a 1% AZA formulation (in an ethanolic extract of neem oil from Neem Extracts Pty Ltd) and the undiluted formulation in a re-chargeable cattle ear tag. The diluted neem was sprayed over the whole body of the animal, and the undiluted formulation in ear tags applied at 2 tags per animal.

Results: The results of this trial are summarised below.

Per cent control of buffalo fly using a 50% formulation of 1% AZA as a spray and 100% formulation in two ear tags per animal.

Group	*Pre-treat.*	*Post-treatment*			
	1/6/96	1/6/96 *(+2 hours)*	2/6/96 *(+1 day)*	3/6/96 *(+2 day)*	4/6/96 *(+3 day)*
Control (x no. flies)	79.0	68.3	62.9	87.5	88.5
50% dilution	-	52.4%	80.9%	96.2%	49.2
AZA impregnated ear tags	-	0	0	0	12.4%

Comment: The diluted neem formulation sprayed over the animal reduced fly populations by 80-96% over 2 days, but the neem oil failed to be discharged from the re-fillable ear tag and there was thus no control via this method of application. Should re-chargeable ear tag design change in the future to allow a more viscous material to be discharged, an ear tag with a suitably formulated neem may well prove effective.

6.3.4 Trial 4

Experimental design: The next three trials (Trials 4, 5 and 6) were made to evaluate a neem based product from India called, "Nimbitor". This product was described at the 5th International Neem Conference in 1996 (Gatton, Australia) by AC Desai and KM Parikh of Zandu Pharmaceutical Works in Bombay, India. These authors claimed that "Nimbitor" showed excellent bio-effectiveness against ectoparasites of cattle such as flies, lice, and larval ticks (Desai & Parikh [1996] *Abstracts 5th International Neem Conference, Gatton*, p 36).

Concentration of Nimbitor (%)	*Amount of AZAdirachtin*		*Dilution for 100ml aliquots N : water*
	Per cent	ppm	
1.0	0.0003	3	1:99
2.0	0.0006	6	2:98
4.0	0.0012	12	4:96
8.0	0.0024	24	8:92
10.0	0.003	30	10:90
20.0	0.006	60	20:80
40.0	0.012	120	40:60
80.0	0.024	240	80:20
100	0.03	300	100:0

For the first trial, 100ml aliquots of 4, 10 and 20% formulations of "Nimbitor" in water were sprayed over the bodies of the treated cattle. Three cattle were used in each group, with 3 cattle used as untreated controls.

Results: The results of this trial are given below:-

Number of buffalo flies and (per cent control) using different concentrations of "Nimbitor"

Group	*Pre-treatment fly counts*		*Post-treatment*
	24/11/96	26/11/96	27/11/96 (+ 1 day)
Control	65	73	70
4% Nimbitor	73	58	30 (57.1%)
10% Nimbitor	77	112	42 (40.0%)
20% Nimbitor	72	80	32 (54.3%)

Comment: These concentrations of "Nimbitor" clearly did not effectively reduce buffalo fly populations. If control was estimated on the basis of fly reduction on the treated group before and after treatment, the control was 48-63%, still below the benchmark.

6.3.5 Trial 5

Experimental design: A similar trail as above was made, using 20, 40 and 80% concentrations of "Nimbitor" on 3 animals in each group, plus 4 controls. The day of treatment was hot (+39°C) and the 20% and 40% mixes were relatively easy to apply via a hand-sprayer. However the 80% was the consistency of a heavy glue and difficult to apply despite the heat and this concentration was pored onto the animal and then smeared over the body by (gloved) hand. At 4.30am the following morning, a storm dumped 39ml of rain at the experimental site and this rain probably washed off much of the "Nimbitor" applied the previous day.

Results: The results of this trial as below:-

Number of buffalo fly (and per cent control) using different concentrations of "Nimbitor"

Group	*Pre-treatment counts*	*Post-treatment*	
	3/12/96	4/12/96 (+1 day)	5/12/96 (+2 day)
Control	53	93	42
20% Nimbitor	45	26 (72.0%)	30 (28.6%)
40% Nimbitor	47	24 (74.2%)	25 (40.5%)
80% Nimbitor	43	15 (83.9%)	24 (42.9%)

Comment: The higher values for control after one day following treatment were partly due to a high number of buffalo flies on the control group on that day. Nevertheless, >80% control was only achieved for a single day at the highest concentration of 80% "Nimbitor".

6.3.6 Trial 6

Experimental design: The final study of "Nimbitor" was a repeat of the Trial 5. At 80% "Nimbitor", the resulting formulation was very difficult to apply because of its viscosity, and any higher concentrations would be impracticable to apply. Three animals were treated at each concentration, with 3 controls.

Results: The results of this trial are given below:-

Number of buffalo fly and (per cent control) using different concentrations of "Nimbitor"

Group	*Pre-treatment fly counts**		*Post-treatment fly counts*			
	10/11/96	13/11/96	14/12/96 (+1 day)	15/12/96 (+2 day)	16/12/96 (+3 day)	17/12/96 (+4 day)
Control	58	65	73	55	132	95
20% Nimbitor	47	48	28 (61.6%)	50 (9.0%)	57 (56.8%)	44 (53.7%)
40% Nimbitor	88	72	28 (61.6%)	58 (0%)	83 (37.1%)	108 (0%)
80% Nimbitor	55	88	7 (90.4%)	13 (76.4%)	22 (83.3%)	82 (13.7%)

* Single side counts

Comment: Control of buffalo fly was achieved for 1-3 days at the highest concentration of "Nimbitor". It is considered that this concentration would be impractical, would not be suitable for administration via a self-applicator, and would be too expensive.

6.3.7 - Trial 7

Experimental design: A quantity of a **2% AZA** neem formulation was supplied by Neem Extracts Pty Ltd. This neem formulation contained 2% AZA, 2% sunscreen, 2% sticking agent and 30% emulsifying agent in an ethanolic base. The first few studies were titration experiments in which suitable dilutions of the stock formulation were made up and evaluated in the field to determine the quantity of AZA necessary to achieve control objectives:

% AZA	*ppm AZA*	*Quantity for 100ml aliquots**	
		Stock neem solution	*Solvent*
2.0	20,000	100	0
1.0	10,000	50 (150)	50 (150)
0.5	5,000	25 (75)	75 (225)
0.1	1,000	5.0 (15.0)	95.0 (285)
0.05	500	2.5 (7.5)	97.5 (292.5)
0.001	100	0.5 (1.5)	99.5 (298.5)
0.0005	50	0.25 (0.75)	99.75 (299.25)
0.00001	10	0.05 (0.15)	99.95 (299.85)

* Amount for treating 3 animals in parentheses

The dilutions of the 2% AZA formulation in relation to the absolute quantity of AZA received per animal is shown in the following table:

% AZA	*ppm AZA*	*Neem / animal (ml)**	*Quantity of solvent (ml)**	*Total AZA / animal (mg)*
2.0	20,000	300	0	0.6
1.0	10,000	150	150	0.3
0.5	5,000	75	225	0.15
0.25	2,000	40	260	0.175
0.1	1,000	20	280	0.038
0.05	500	7.5	292.5	0.015
0.001	100	0.2	299.8	0.00038
0.0005	50	0.75	299.25	0.00015

*Based on a dose of 300 ml per animal

The first trial evaluated 50, 100 and 500 ppm AZA formulations in canola oil solvent. Three animals were used at each concentration with 3 untreated controls. The formulations were applied with a 10ml syringe, 50 ml to all upper parts of each animal. This method of application resulted in good dispersion over the treated body, except for the lower belly area.

Results: The results of the trial are summarised below:-

Numbers of buffalo fly and (per cent control) using different concentrations of AZA

Group	*Pre-treatment fly counts*			*Post-treatment counts (% control)*		
	22/12/96	28/12/96	29/12/96	30/12/96 (+1 day)	31/12/96 (+2 day)	1/1/97 (+3 day)
Control	138.3	123.3	176.7	188.3	81.7	81.7
50 ppm AZA	128.3	143.3	178.3	116.7 (38%)	51.7 (37%)	150.3 (0%)
100 ppm AZA	191.7	180.0	218.3	115.3 (39%)	83.7 (0%)	121.7 (0%)
500 ppm AZA	108.3	161.7	203.3	70.7 (62%)	101.7 (0%)	125.0 (0%)

Comment: Although the results above do not indicate that the treatment was working, nearly all the flies on the treated animals were confined to the belly area which had failed to be adequately treated during application. Where the neem was physically applied, there were virtually no buffalo flies.

This observation underlines the necessity to either coat the animal overall with a spray/ dip application, or use a solvent carrier which itself spreads the active ingredient thoroughly over the hair-coat of the animal. Effective carriers essential for the spreading of AZA over treated animals are used in modern pour-on or back-line formulations.

6.3.8 - Trial 8

Experimental design: The same number of cattle and method of application of formulations of 50, 100 and 500ppm AZA neem were used as in Trial 7. The numbers of flies on upper and lower parts of the body were distinguished during fly counts on treated animals.
Results: The results of trial 8 are summarised below:-

Numbers of buffalo fly and (per cent control) using different concentrations of AZA

Group	*Pre-treatment fly counts**		*Post-treatment fly counts*		
	5/1/97	8/1/97	9/1/97 (+1 day)	10/1/97 (+2 day)	11/1/97 (+3 day)
Control	-	221.7	193.3	178.3	223.3
50 ppm AZA	128.3	236.7	130.0 (33%)	204.3 (0%)	140.0 (37%)
100 ppm AZA	206.7	243.3	106.0 (45%)	183.3 (0%)	225.0 (0%)
500 ppm AZA	213.3	193.3	110.0 (43%)	104.0 (42%)	159.7 (28%)

The number of buffalo fly on the upper and lower parts of the body of treated cattle were as follows:

Number of buffalo flies on upper and lower parts of the body of individual AZA treated cattle

Group	*10/1/97 (+2 day)*		*11/1/97 (+3 day)*	
	Upper	Lower	Upper	Lower
50 ppm AZA	6 25 0	160 380 42	15 0 0	180 42 65
100 ppm AZA	15 0 0	260 180 95	5 0 0	320 130 220
500 ppm AZA	4 25 5	23 166 95	12 5 0	250 27 185
Mean	8.9 (5.4%)	155.7	4.1 (2.5%)	157.7

It is clear that those parts of the animals body which receive a sufficient dose of AZA result in very few flies remaining on treated areas. Of the total number of flies on the treated animals only 5.4% and 2.5% were observed on the upper (treated) part of the animals one and two days after treatment.

6.3.9 - Trial 9

Experimental design: A repeat of the earlier experiments using 50, 100 and 500 ppm AZA in a neem oil base mixed with canola oil. On this occasion there was an emphasis on counting flies on different parts of the treated animal's body to determine the impact of treated parts of the body on fly activity. There were three animals in each treated group and three control animals.

Results: The results of the study are summarised below:-

Buffalo fly counts and (per cent control) after application of different concentrations of AZA

Group	*Pre-treatment*	*Post-treatment*		
	19/1/97	20/1/97 (+1 day)	21/1/97 (+2 day)	22/1/97 (+3 day)
Control	263.3	250.0	180.0	126.7
50ppm AZA	190.0	18.0 (93%)	120.3 (33%)	110.3 (13%)
100ppm AZA	251.7	82.7 (67%)	74.0 (59%)	139.0 (0%)
500ppm AZA	211.7	44.7 (82%)	45.0 (75%)	72.7 (43%)

Comment: Although two treatments gave >80% fly control after one day, the overall impact on fly control did not appear successful. However, where the neem formulation had been applied to parts of the animals body, those parts remained relatively fly free for up to two days after treatment. The numbers of flies on upper and lower parts of their treated animals are given below:

Number of buffalo flies on upper and lower parts of AZA treated cattle

Group	*20/1/97 (+1 day)*		*21/1/97 (+2 day)*		*22/1/97 (+3 day)*	
	Upper	Lower	Upper	Lower	Upper	Lower
50 ppm AZA	2	14	30	150	65	140
	2	19	2	155	25	80
	0	17	2	22	6	15
100 ppm AZA	7	7	1	12	19	25
	35	180	3	65	3	150
	0	19	1	140	35	185
500 ppm AZA	15	95	2	13	12	45
	3	17	2	70	35	80
	2	2	8	40	12	34
Mean	66 (17.8%)	370	51 (7.6%)	667	212 (28.1%)	754

Again, the above data show that where an animal receives sufficient quantity of AZA on the body, buffalo fly activity is substantially reduced. In this study, the proportion of flies on the upper (treated) part of the body was 18% and 8% of total flies per animal on the first two days after treatment.

6.3.10 - Trial 10

Experimental design: This study used the 2% AZA neem formulation used in the preceding studies, but at considerably higher concentrations of AZA as part of the series of titration studies. The concentrations selected were 0.05% AZA (=500 ppm), 0.1% (=1,000 ppm), and 0.5% (=5,000 ppm) in a commercial carrier (used for the pour-on, "Brute"), called here *Brute Carrier*.

% AZA	*ppm AZA*	*Neem oil per animal (ml)*	*Total neem for 3 animals*	*Total AZA per animal (mg)*
2.0	20,000	100	300	0.2
1.0	10,000	50	150	0.1
0.5	5,000	25	75	0.05
0.25	2,000	10	30	0.02
0.1	1,000	5	15	0.01
0.15	500	2.5	7.5	0.005
0.001	100	0.5	1.5	0.001

The material was applied at 50 ml per animal using a 10 ml syringe at one 'swipe' along the backline and two 'swipes' along body on each side.

Results: The results of this study are summarised below:

Numbers of buffalo fly using 0.05%, 0.1% and 0.5% AZA in Brute Carrier

Group	*Pre-treatment*		*Post-treatment*			
	31/1/97	1/2/97	2/2/97 (+1 day)	3/2/97 (+2 day)	4/2/97 (+3 day)	5/2/97 (+4 day)
Control	238.3	233.3	173.3	273.3	280.0	216.7
0.05 % AZA	65.0	27.0	27.0	18.7	22.3	117.3
0.1 % AZA	58.3	76.7	17.0	23.7	28.7	121.7
0.5 % AZA	30.0	56.3	22.3	33.7	16.7	72.3

Comment: Because the numbers of buffalo fly on the treatment groups were very low prior to treatment compared with the untreated controls, it was not possible to estimate per cent control. There was also rain recorded on 2 /2/97 which may have adversely affected the applied neem formulations. The fly numbers before and after treatment suggest the formulations under the prevailing conditions were ineffective in this study.

6.3.11 - Trial 11

Experimental design: Using the 2% AZA stock solution, concentrations of 0.25%, 0.5% and 1.0% AZA in water were made up in 300 ml aliquots per animal and applied with a manual air pressure spray applicator to all parts of the animals body, including the lower belly.

Results: The results of this study are summarised below:-

Number of buffalo fly and (per cent control) using 0.25, 0.5 and 1.0% AZA

Group	*Pre-treatment*	*Post-treatment*		
	22/2/97	23/2/97 (+1 day)	24/2/97 (+2 day)	25/2/97 (+3 day)
Control	200.0	163.3	210.0	281.7
0.25% AZA	190.0	1.3 (99.2%)	9.0 (95.7%)	71.0 (74.8%)
0.5% AZA	220.0	2.0 (98.9%)	19.7 (90.6%)	70.7 (74.9%)
1.0% AZA	280.0	0.3 (99.8%)	6.7 (96.8%)	44.0 (84.4%)

Comment: This study gave the most dramatic results to date. There was nearly 100% control for 24 hours, and 91-97% after 2 days. The buffalo fly numbers before treatment were high and thus provided a good fly challenge during the course of the study.

It is clear that with the right formulation using an effective solvent/carrier and with sufficient AZA active applied over the whole animal, buffalo fly control with neem would be assured.

6.3.12 - Trial 12

Experimental design: This study was a repeat of Trial 11 to confirm the excellent results using the same concentrations and method of application. The study was continued for 5 days, 2 days longer than the pervious study.

Results: The results of this study are summarised below:-

Number of buffalo fly and (per cent control) after treatment with 0.25, 0.5 and 1.0% AZA

Group	*Pre-treat.*	*Post-treatment*				
	1/3/97	2/3/97 (+1 day)	3/3/97 (+2 day)	4/3/97 (+3 day)	5/3/97 (+4 day)	6/3/97 (+5 day)
Control	290.0	280.0	210.0	203.3	183.3	198.3
0.25% AZA	118.3	2.0 (99.3%)	16.3 (92.2%)	14.0 (93.1%)	25.7 (86.0%)	131.7 (33.6%)
0.5% AZA	176.7	0.3 (99.9%)	15.3 (92.7%)	18.7 (90.8%)	8.7 (95.3%)	94.0 (52.6%)
1.0% AZA	88.3	0 (100%)	3.0 (98.6%)	6.3 (96.9%)	4.7 (97.4%)	34.3 (82.7%)

Comment: The results of this study confirmed Trial 11. Efficacy of all treatments was 100% after one day, 92-99% after 2 days, 91-97% after 3 days and 86-97% after 4 days. After 5 days, the highest concentration achieved 83% control of buffalo fly compared with the untreated controls.

6.3.13 - Trial 13

Experimental design: Supplies of the original **2% AZA** formulation had been depleted during the preceding studies. A new batch of **2% AZA(#2)** was supplied by Neem Extracts Pty Ltd for the continuing studies. This formulation was lighter in colour and more miscible in water but did not apparently contain a sunscreen agent. The same concentrations of AZA and methods of application etc. were used as in Trials 11 and 12 to confirm the efficacy of the new batch of neem. The weather throughout the study was cloudy/overcast.

Results: The results of this study are summarised below:-

Number of buffalo flies and (per cent control) after treating cattle with 0.25, 0.5 and 1.0% AZA using 2% AZA (#2) formulation

Group	*Pre-treatment*	*Post-treatment*		
	8/3/97	9/3/97 (+1 day)	10/3/97 (+2 day)	11/3/97 (+3 day)
Control	260.0	311.7	263.3	243.3
0.25% AZA	141.7	4.7 (98.5%)	16.7 (93.7%)	40.0 (83.6%)
0.5% AZA	121.7	5.0 (98.4%)	9.7 (96.3%)	49.3 (79.7%)
1.0% AZA	88.3	4.0 (98.7%)	25.3 (90.4%)	58.3 (76.0%)

Comment: Excellent results were again recorded using the new formulation. Although fly numbers on the control group were considerably higher than the pre-treatment fly counts on the neem groups. Allowing for fly numbers similar to the pre-treatment counts (mean of 117 flies per animal), control was >96% for all AZA concentrations after one day, 78-92% after 2 days and 50-66% after 3 days.

6.3.14 - Trial 14

Experimental design: The 2% AZA (#2) was used at a concentration of 0.25% AZA with Brute Carrier and applied with a 40ml syringe in strips along both sides of 9 treated animals.

% AZA	*ppm AZA*	*2% Neem #2 / animal*	*Quantity of Brute Carrier (ml)*	*Total AZA per animal (mg)*
0.5	5,000	75	40	1.5
0.25	2,500	40	40	0.8
0.1	1,000	20	40	0.4

The study was carried out under hot, dry and sunny conditions.

Results: The results of the study are summarised below:-

Number of buffalo fly and (per cent control) using 0.25% AZA in Brute carrier

Group	*Pre-treatment*	*Post-treatment*	
	25/3/97	26/3/97 (+1 day)	27/3/97 (+2 day)
Control	313.3	436.7	370.0
0.25% AZA/Brute Carrier	266.1	71.7 (83.6%)	101.4 (72.6%)

Comment: In contrast to the previous studies with the #2 batch of 2% neem, this study gave mediocre results for fly control. One reason was the method of application, using a syringe and "pour-on" technique, rather than whole body application as with the spray gun. Fly control on those parts (mid-body) of the animals which had received a dose of neem was generally better than the lower body which was not directly treated (see below). However, it was anticipated that the Brute Carrier would have ensured overall body coverage.

Number of buffalo flies on different parts of the body of treated and untreated cattle

Group	*+1 day post-treatment*				*+2 days post-treatment*		
	upper	mid	lower	head	upper	mid	lower
Treated (n=9)	7.4 (10.3%)	2.1 (2.9%)	54.7 (76.5%)	7.3 (10.2%)	26.1 (25.7%)	4.8 (4.7%)	70.6 (69.6%)
Control (n=3)	106.7 (24.4%)	96.7 (22.1%)	233.3 (53.4%)	-	126.7 (34.2%)	90.0 (24.3%)	153.3 (41.4%)

In contrast to the previous trial when it was cloudy and overcast the weather during this trial was hot and sunny. The decreased efficacy could have been due to the lack of a sunscreen agent in the formulation.

6.3.15 - Trial 15

Experimental design: Several new neem based products were evaluated in this study:

1. A new (June 1997) batch of neem oil from Neem Extracts (**3% AZA #3**, 10% emulsifier, 3% stabilizer/sunscreen, in a 40% neem oil base, in ethanol).
2. A **3% AZA "NeemAzal"** neem product from Organic Crop Protectants (OCP) which was a thick, brown, viscous formulation similar to car engine oil in consistency.
3. **Bitters** from Neem Extracts Pty Ltd, a by-product of neem processing which contains little or no AZA, but has been reported to have insecticidal properties.

The products were applied to the backline and sides of animals using a hand mist sprayer unit, applying 150-190 ml of product per animal. There were 3 animals in each experimental group and three controls.

Results: The results of this study are summarised below:-

Number of buffalo fly and (per cent control) on neem treated cattle compared with untreated control animals

Treatment group	*Pre-treatment fly counts*			*Post-treatment fly counts*							
	17/1	20/1	21/1	21/1 +2h	21/1 +5h	22/1 +24h	22/1 +26h	22/1 +28h	23/1 +2d	24/1 +3d	25/1 +4d
Control	167	330	192	-	-	340	-	-	493	430	368
3% AZA *OCP* NeemAzal	193	307	205	57 70%	32 83%	54 84%	23 93%	46 86%	63 87%	127 70%	310 16%
3% AZA *Neem Extracts*	102	287	143	0 100%	0 100%	2 99%	0.7 99%	0.3 99%	12 98%	120 72%	77 79%
Bitters *Neem Extracts*	93	200	143	40 79%	11 94%	35 87%	13 96%	47 86%	98 80%	138 68%	67 82%

Comment: the 3% Neem Extracts product provided 98-100% fly control for up to 2 days, and 72-79% up to 4 days after treatment.
The OCP product, NeemAzal, was more variable providing fly control at levels of 84-93% during the first 2 days after treatment decreasing to 72% after 3 days and negligible control at 4 days post-treatment.
The Bitters was the least active formulation but still provided buffalo fly control of >80% for 2 days after treatment.

6.3.16 - Trial 16

Experimental design: This study evaluated the OCP NeemAzal product which contained 3% AZA. The full strength formulation was used, applying 60 ml per animal via the hand operated mist sprayer. Nine animals were treated with 3 untreated controls. The amount of active per animal was approximately 0.18 mg AZA.

Results: The results of this study are summarised below:-

Number of buffalo fly and (per cent control) after application of NeemAzal to cattle

Group	*Pre-treat.*	*Post-treatment*			
	19/3/98	20/3/98 (+1 day)	21/3/98 (+2 day)	22/3/98 (+3 day)	23/3/98 (+4 day)
Control	293.3	120.0	100.0	80.0	153.3
NeemAzal 3%	668.9	87.2 (27.3%)	111.1 (0%)	163.0 (0%)	234.4 (0%)

Comment: Number of buffalo fly on the treated group were far greater than the control group. If per cent control was estimated on the basis of fly reduction in the treated group, fly control was 87% (668.9-87.2 ÷ 668.9 x 100) after one day, 83% after 2 days and 76% after 3 days.

6.3.17 - Trial 17

Experimental design: This study was a continuation of Trial 16, but with the NeemAzal applied via the Self Applicator at 50% dilution with Synetrol. There were showers on days 4-6 after setting up the self applicator.
Results: The results of this study are summarised below:-

Number of buffalo fly and (per cent control) using 3% AZA *NeemAzol* at 50% dilution in the Self Applicator

Group	*Pre-treatment*	*Post-treatment*		
	28/3/98	30/3/98 (+2 day)	1/4/98 (+4 day)	3/4/98 (+6 day)
Control	270.0	253.4	221.7	195.4
NeemAzal	182.8	190.0 (25%)	149.4 (32.6%)	236.0 (0%)

Comment: This trial of the Self Applicator using NeemAzal diluted with Synetrol, was not successful because the formulation did not pass down the wick to reach the animals.

6.3.18 - Trial 18

Experimental design: To determine the practicality and usefulness of the Self Applicator, the reservoir was charged with the experimental pour-on, **Brute** (containing 10% permethrin in a solvent carrier designed to spread rapidly over a treated animal's body).

Results: The results of this study are summarised below:-

Number of buffalo fly and (per cent control) after cattle used a Self Applicator charged with full strength Brute (10% permethrin)

Group	*Pre-treat.*	*Post-treatment*					
	4/4/98	5/4/98 (+1 day)	6/4/98 (+2 day)	7/4/98 (+3 day)	10/4/98 (+6 day)	14/4/98 (+10 day)	17/4/98 (+13 day)
Control	193.3	233.3	250.0	170.0	220.0	251.6	188.3
Self Applicator	298.9	4.4 (98.1%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)	0 (100%)

About 80% knock down of buffalo fly was achieved within one hour of the cattle passing through the Self Applicator. The Brute pour-on was passing down the wicks of the apparatus very well, in contrast to most previously tested materials which tended to clog the wicks and prevent material reaching the ends of the wick and thus failing to be applied to cattle passing through the apparatus.

The Self Applicator was removed on 18/4/98. By 5/5/98 (17 days after removal of the Self Applicator), buffalo flies were beginning to return to the treated animals in small numbers.

Comment: This study provided a vivid example of how successful the Self Applicator could be with an appropriate active ingredient and carrier. This convincing demonstration of the efficacy of the Self Applicator should encourage continued development work on organically based actives for self administration to enable economic and environmentally preferable buffalo fly control.

6.3.19 - Trial 19

Experimental design: This study was made to evaluate a neem based product in the Self Applicator. The OCP **NeemAzol 3%** AZA diluted 1:1 with DCtrate was used. The product wicked well. Nine animals were in the treated group and there were 3 control animals. The study commenced on 6/5/98 and terminated on 18/5/98, 12 days later.

Results: The results of this study are summarised below:

Number of buffalo fly (and per cent control) after using NeemAzol 3% AZA diluted 1:1 in a Self Applicator

Group	*Pre-treat*	*Post-treatment*								
	6/5/98	7/5/98 (+1 day)	9/5/98 (+3 day)	10/5/98 (+4 day)	11/5/98 (+5 day)	12/5/98 (+6 day)	13/5/98 (+7 day)	14/5/98 (+8 day)	16/5/98 (10 day)	18/5/98 (+12day)
Control	161.7	186.7	181.7	218.3	285.0	150.0	193.3	190.0	94.0	88.3
NeemAzol (self-applic)	203.9	160.6 (14.0%)	102.2 (43.8%)	59.9 (72.6%)	117.2 (58.9%)	86.7 (42.2%)	58.4 (69.8%)	61.4 (67.7%)	79.3 (15.6%)	70.7 (19.9%)

Comment: This study showed how a Self Applicator containing a neem based product reduced buffalo fly numbers to an almost sub-economic level. Buffalo fly populations towards the end of the study (16-18/5/98) were low, resulting in a decrease in apparent efficacy (per cent control). During days 4-8 post-treatment, buffalo fly control was 60-73%.

It is clear that with a re-formulated product containing sufficient AZA active (and possibly a sunscreen to prolong its activity in the field), together with a solvent carrier as efficient as the Brute carrier, the Self Applicator system for administering neem would be a viable method for control of buffalo fly.

VOLUME 2

FINAL REPORT TO THE MEAT RESEARCH CORPORATION

NEEM OIL AS AN ANTI-FEEDANT FOR BUFFALO FLY CONTROL

PROJECT NUMBER: M.723

PERFORMING ORGANISATION:
XCS CONSULTING PTY LTD

STUDY DIRECTOR:
DR PHILIP SPRADBERY

FIELD TRIALS DIRECTOR:
ROBERT S TOZER

DATE OF REPORT:
10 AUGUST 1998

TRIAL IDENTIFICATION:
XCS/BF:25/95

APPENDIX 1

TREATMENT RECORDS

TREATMENT #1

(1)

TREATMENT RECORD

Trial Number: XCSSP Bf25/95 **Date:** 24/05/96

Ident./Batch Number of chemical: 100% Neem Oil + 50% Neem Oil

Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
11	328	100%	75 ml	
14	304	Neem	75 ml	
21	295	Oil	55 ml	
22	331	"	75 ml.	
9	347	50%	75 ml.	
13	299	Neem Oil	55 ml	
19	310	"	75 ml.	
23	245	"	55 ml.	

TREATMENT #1

U

* CONTROLS - PLACEBO

TREATMENT RECORD

Trial Number: XCSSP BF 25/95 **Date:** 24/05/96

Ident./Batch Number of chemical: 100% NEEM OIL 0.13% Azadirachtin 100% Canola Oil Placebo

Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
5	318	Placebo	75 ml	
3	291	(Canola	56 ml.	
6	299	Oil)	56 ml.	
16	276	"	56 ml.	
17	310	"	75 ml.	
18	308	"	75 ml.	
2	369	NO	—	
15	271	TREATMENT	—	
24	308		—	
20	277		—	

* CONTROLS NO TREATMENT

TREATMENT #2 – NEEM (2)

TREATMENT RECORD

Trial Number: XCSSP 6F 25/95 **Date:** 28/04/96

Ident./Batch Number of chemical: 0.3% AZAD - NEEM OIL + SYNERTROL

Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
11	328	100% NEEM OIL	100 ml	Applied in
14	304	"	100ml	mister bottle
21	295	100% NEEM ~~OIL~~	75ml	to entire body
22	331	"	100ml.	surface
9	347	50% NEEM	100 ml	Applied in
13	299	OIL	75 ml	mister bottle to
19	310	DILUTED	100ml	entire body
23	295	SYNERTROL	75 ml	surface
2		NO TREATMENT		
15		"	"	
24		"	"	
20		"	"	
3		"	"	
5		"	"	
6		"	"	
16		"	"	
17		"	"	
18		"	"	

TREATMENT #3 - NEEM (5)

TREATMENT RECORD

Trial Number: XCSSP BF 25/95 **Date:** 01/06/96

Ident./Batch Number of chemical: 100% NEEM OIL IMPREGNATED TO PLASTIC EAR TAG + 1% AZA-NEEM IN ETHANOL

Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
11	328	Neem	250 ml	Preparation mist
14	304	in ethanol + 1% Aza.	250 ml	sprayed to
21	295	diluted	250 ml	whole body
22	331	1:1 with	250 ml	surface
		H2O		
9		100% Neem	2 taps	
13		Oil with	per animal	
19		0.13% aza	"	
23		impregnated	"	
		to prototype	"	
		ear tag.	"	

Neem

FARM DIARY/DAILY LOG

Experiment number: XCSSP BF 25/95 **Page:** 3

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
01/06/96	OVERCAST	Third Treatment applied to	
		both treatment groups	
		viz - neem impregnated plastic prototype	
		ear tags applied to group II.	
		Neem in ethanol containg	
		1% Azadirachtin applied to	
		whole body surface in mist	
		sprayer to group #I.	
		Fly counts pre-treatment	
		& 2 hours post-treatment	
02/06/96	FINE, SUNNY	Small shower since last count.	
		Fly counts on all groups	
03/06/96	FINE SUNNY.	Shower overnight	
		Fly counts on all groups	
04/06/96	FINE SUNNY	Fly counts on all groups	
		Shower late pm	
		STUDY TERMINATED	

TREATMENT RECORD

Trial Number: Bf 25/95 **Date:** 26/11/96

Ident./Batch Number of chemical: NIMBITOR

Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
19	✓	10% NIMBITOR	100ml	Time of application all species of fly were dislodged and were transferred to remaining animals yet to be treated
11	✓	IN		
63	✓	H_2O		
6	✓	4% NIMBITOR	100 ml	
14	✓	IN		
15	✓	H_2O		
22	✓	20% NIMBITOR	100 ml	
20	✓	IN		
12	✓	H_2O		

(5)

TREATMENT RECORD

~~RE TREAT~~

Trial Number: BF 26/95 Date: 03/12/96 ~~12/12/95~~ 12:00

Ident./Batch Number of chemical: NIMBITOR

~~SUNNY~~

	Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
	19	✓		100mL	12-30
(1)	11	✓	40%	"	SUNNY
	G3	✓		"	WITH SOME CLOUD
	6	✓		100mL	APPROX 38°
(2)	14	✓	20%	"	
	15	✓		"	
					AT 80% NIMBITOR
					TREATMENT
	22	✓		100mL	HAS GONE
(3)	20	✓	80%	"	GLUE LIKE
	12	✓		"	

(6)

TREATMENT RECORD

Trial Number: BF 25/95 **Date:** 13/12/96

Ident./Batch Number of chemical: NIMBITOR

	Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
	19		40%	100 ml	
(1)	11		40%	100 ml	
	93		40%	100 ml	
	6		20%	100 ml	
(2)	14		20%	100 ml	
	15		20%	100 ml	
	22		80%	100 ml	80% NIMBITOR
(3)	20		80%	100 ml	HAS THE
	12		80%	100 ml	CONSISTENCY OF GLUE

(7)

TREATMENT RECORD

Trial Number: AZA **Date:** 29/12/96

Ident./Batch Number of chemical: _____

Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
19		AZA	0.5ml AZA	APPLIED TREATMENT
11		"	99.5ml CANOLA OIL	WITH 10ml SYRINGE
63		"	PER ANIMAL	50ml PER SIDE TO
			100ppm	ALL UPPER PARTS
				OF EACH ANIMALS
6		AZA	2.5ml AZA	BODY. TREATMENT
14		"	97.5ml CANOLA OIL	DISPERSED
15		"	PER ANIMAL	WELL EXCEPT
			500 ppm	FOR THE LOWER
				BELLY AREA.
22		AZA	0.25ml AZA	
20		"	99.75ml CANOLA OIL	
12		"	PER ANIMAL	
			50ppm	

(1) (2) (3)

TREATMENT RECORD

Trial Number: AZA #2 Date: 08/01/97

Ident./Batch Number of chemical: AZA

	Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
①	19		AZA		
	11		"	500 PPM	MIXED WITH CANOLA OIL TO MAKE UP TO 100ML/ANIMAL TREATMENT APPLIED TO ALL UPPER PARTS OF EACH ANIMAL AT 50ML/GPE
	63				
②	6		AZA		
	14		"	50 PPM	
	15		"		
③	22		AZA		
	20		"	100 PPM	
	12		"		

(9)

TREATMENT RECORD

Trial Number: AZA #3 Date: 17/01/97

Ident./Batch Number of chemical: _____

	Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
(1)	19		AZA	50 ppm	APPLIED MIXED
	11		"		WITH CANOLA OIL
	G3		"		USING 50ml SYRINGE
					~~TOOK~~ 50ml/SIDE
					100ml PER ANIMAL
(2)	6		AZA	100 ppm	
	14		"		
	15		"		
(3)	22		AZA	500 ppm	
	20		"		
	12		"		

TRIAL 4. (10)

TREATMENT RECORD

Trial Number: XCS NEEM 25/95 Date: 31/01/97

Ident./Batch Number of chemical: NEEM WITH BRUTE CARRIER

Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
① 19		0.05% AIA	50ml	1.25 + 48.75ml carrier
① 11			"	
① G3			"	
② 6		0.1% AIA	50ml	2.5ml + 47.5 carrier
② 14			"	
② 15			"	
③ 22		0.5% AIA	50ml	12.5ml + 37.5 carrier
③ 20			"	
③ 12			"	
		* 10ml Syringe used for		
		- one swipe along backline		
		- two swipes along body on each side		

APPLIED IN 50 ML APPLICATION

RUN #1

(II)

TREATMENT RECORD

Trial Number: BF 25/95 **Date:** 22/02/97

Ident./Batch Number of chemical: 2% AZA EC ex Neem Extracts

	Ident. Number	Weight (kg)	Treatment	Dose Amount (ml)	Comments
	19	/	0.5%	300 ml	PREPARATION
(1)	11	/	AZA	"	APPLIED IN
	G3	/		"	1 litre manually
					air pressurized
(2)	6	/	1.0%	300 ml	spray applicator
	14	/	AZA	"	applied to
	15	/		"	all parts of
					animals body
(3)	22	/	0.25%	300 ml	including
	20	/	AZA	"	lower belly.
	12	/		"	

Run 2 (12)

TREATMENT RECORD

Trial Number: BF 25/95 Date: 01/03/97

Ident./Batch Number of chemical: 2% AZA EC ex Neem Extracts

Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
19			300 ml	
11	/	0.25%	"	Applied via
03		AZA	"	air pressurized
				spray applicatn
6			"	
14	/	0.5%	300 ml	as per Run I
15		AZA	"	
22			300 ml	
20	/	1.0% AZA	"	
12			"	

Gp 1 Gp 2 Gp 3

RUN III (13)

TREATMENT RECORD

Trial Number: BF 25/95 Date: 08/03/97

Ident./Batch Number of chemical: 2000 ppm AZA

	Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
	19		1.0 %	300 ml	PREPARATION
①	11	/	AZA	"	APPLIED
	43			"	IN AIR
					PRESURIZED
	6			"	SPRAY
	14	/	0.25 %	"	APPLICATOR
②	15		AZA	"	
					AS PER
	22			"	I & II
③	20	/	0.5 %	"	
	12		AZA	"	

(14)

TREATMENT RECORD

Trial Number: XCSSP BF 25/95 Date: _____

Ident./Batch Number of chemical: "BUFFALO FLY TREATMENT" @ 2% AZA

Ident. Number	Weight (kg)	Treatment	Dose Amount	Comments
ALL TREATED ANIMALS	/	80 ml Preparation Neem Oil	80 ml	40 ml applied on each side of all animals. Applied via a 40 ml syringe 5 strips / side

(15)

TREATMENT RECORD

[treatrec.doc]

Trial Number: #15 **Date:** 21/1/97

Ident./Batch Number of chemical: _____

Ident. Number	Weight (kg)	Treatment	Dose Amount (ml)	Comments
1 22		OCP	150	
2 15		Neem X	190	
3 19		OCP	190	
4 11		OCP	150	
5 15		Neem X	150	
6 6		Neem X	150	
7 12		Bitters	190	
8 14		Bitters	150	
9 7		Bitters	150	
10				
11				
12 · (A) OCP 3% AZA/NeemAzal – Thick brown, viscous/Gel				
13				
14				
15				
16				
17 (B) Neem Extracts (Acetone) 3% AZA [Jan 98]				3% AZA w/v 40% Neem oil 10% emulsifier 3% Stabilizer (Sunscreen)
18				
19				
20				
21 (C) Bitters (Neem Extracts P.C).				
22				
23				
24		Pre-treatment Fly Counts:		
25				
26				
27		Small	Medium	Large
28		(A) 22	4	19
29		(B) 15	20	6
30		(C) 7	12	14
31				
32				
33				
34		Animals sprayed thoroughly over entire body		
35				
36		using a 500 ml Hand-Sprayer		
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				
50				

APPENDIX 2

ANIMAL HEALTH OBSERVATIONS

(1-3)

~~PRE~~/POST* - TREATMENT ANIMAL HEALTH OBSERVATIONS
[*delete one]
Trial number: XCSSP BF 25/95

Date: 24/05/96 Time of observations: 1400 hrs. ie 2 hrs post treatment application

Anim. Ident.	Observations
ALL	NO ADVERSE REACTIONS TO
ANIMALS	TREATMENT EVIDENT. OIL
	HAD DISPERSED WELL DOWN
	BODY. GREATER DISPERSION
	EVIDENT IN ANIMALS WITH
	SMOOTHER BODY COAT.

(1-3)

PRE/~~POST~~* - TREATMENT ANIMAL HEALTH OBSERVATIONS

[*delete one]

Trial number: XCSSP BF 25/95

Date: 24/05/96 **Time of observations:** 1030 hrs.

Anim. Ident.	Observations
ALL	ALL IN GOOD HEALTH & PHYSICAL
ANIMALS	APPEARANCE.

(5)

~~PRE~~/POST* - TREATMENT ANIMAL HEALTH OBSERVATIONS

[*delete one]

Trial number: BF 26/95 UNTREATED CONTROLS

Date: 04/12/96 **Time of observations:** 10-30

Anim. Ident.	Observations
9	ANIMAL AWAY FROM GROUP & IN SHADY
	GULLY. APPEARS PHOTO SENSITIVE EARS
	NOSE EYES. POSSIBLE LANTANA POISONING

~~PRE~~/POST* - TREATMENT ANIMAL HEALTH OBSERVATIONS
[*delete one]
Trial number: BF 26/95 UNTREATED CONTROLS

Date:_____ **Time of observations:**_____

Anim. Ident.	Observations
9	ANIMAL NUMBER 9 HAS BEEN WITHDRAWN
3	DUE TO ILL HEALTH AND ANIMAL NUMBER 3
	SUBSTITUTED.

(7)

PRE/~~POST~~* - TREATMENT ANIMAL HEALTH OBSERVATIONS

[*delete one]

Trial number: AZA

Date: 24/12/96 **Time of observations:** 10-30

Anim. Ident.	Observations
19	3 DAY SICKNESS

(7)

PRE/~~POST~~* - TREATMENT ANIMAL HEALTH OBSERVATIONS

[*delete one]

Trial number: AZA

Date: 28/12/96 **Time of observations:** 11-00

Anim. Ident.	Observations
20	THREE DAY SICKNESS
11	THREE DAY SICKNESS

(7)

~~PRE~~/POST* - TREATMENT ANIMAL HEALTH OBSERVATIONS
[*delete one]
Trial number: AZA

Date: 31/12/96 **Time of observations:** 12-30

Anim. Ident.	Observations
6	3 DAY SICKNESS

~~PRE~~/POST* - TREATMENT ANIMAL HEALTH OBSERVATIONS
[*delete one]
Trial number: BF 25/95

Date: 22/02/97 **Time of observations:** 2 hours post treatment.

Anim. Ident.	Observations
All	No adverse reactions
Animals	elicited.

APPENDIX 3

FLY COUNTS
(PRO-FORMAS)

NEEM OIL - TREATED (1-3)

Experiment: XCSSP BF 25/95

Location: PEAK CROSSING

Property name: BAILEY

Group name: TREATED GROUP

FLY COUNTS (~~TOTAL~~) T #1 T #2

Animal number		Pre-treat 23/05/96	+2hrs 24/05/96	+4hrs 24/05/96	+24. 25/05/96	Date 26/05/96	27/05/96	28/05/96	28/05/96	29/05/96	30/05/96
1											
2	11	55	30	12	8	10	20	90	8	25	10
3	14	135	46	33	6	45	30	120	4	10	60
4	21	20	10	26	4	5	40	65	0	6	10
5	22	130	22	51	49	55	95	290	5	60	45
6											
7	$\bar{x}$	85.0	27.0	30.5	16.8	31.8	46.8	141.2	4.2	21.8	31.2
8											
9											
10											
11											
12	9	50	40	18	12	32	65	110	4	6	15
13	13	30	45	29	6	56	50	60	4	6	22
14	19	145	90	35	130	85	180	360	27	28	34
15	23	25	19	10	8	20	40	90	10	8	20
16											
17	$\bar{x}$	62.5	48.5	23.0	39.0	48.3	83.8	155.0	11.2	12.0	22.5
18											
19											
20											
		1200	1400	1600	1200	1120	1130	1400	1600	1130	1200

1.0% NEEM

5.0% NEEM

R

(1-3)

BUFFALO FLY COUNTS - (~~SINGLE SIDE~~ TOTAL COUNTS)

Experiment..... NEEM OIL XL88P BF 25/95

Location..... PEAK CROSSING

Property Name..... BAILEY.

Group/ Herd Name..... TREATED

FLY COUNTS

T #3 +2hrs

Animal number		Date 31/05/96	01/06/96	01/06/96	02/06/96	03/06/96	04/06/96						
1	11	48	30	12	17	2	35						
2	14	29	75	20	22	4	50						
3	21	5	20	8	1	3	20						
4	22	25	120	90	8	4	75						
5													
6	SC	26.8	61.2	32.5	12.0	3.3	45.0						
7													
8													
9													
10													
11													
12	9	15	110	50	50	45	55						
13	13	18	100	90	45	50	40						
14	19	145	150	110	260	290	180						
15	23	11	55	50	25	20	35						
16													
17	SC	47.3	103.8	75.0	95.0	101.3	77.5						
18													
19													
20													
		1240	1200	1400	1140	1045	1030						

flycount.doc

NEEM OIL - CONTROLS (1-3)

Experiment: XCSSP BF 25/95

Location: PEAK CROSSING.

Property name: DWYER / BAILEY.

Group name: * PLACEBO CONTROLS & UNTREATED CONTROLS.

FLY COUNTS (TOTAL)

	Animal number	Pre-treat 24/05/96	+2hrs 24/05/96	+4hrs 24/05/96	+24 25/05/96	Date 26/05/96	27/05/96	28/05/96	28/05/96	29/05/96	30/05/96
PLACEBO CONTROLS	3	60	170	140	135	130	70	190	150	130	110
	5	95	45	35	44	75	140	230	200	105	120
	6	40	70	39	65	110	85	95	80	40	40
	16	55	75	30	38	25	20	80	55	35	20
	17	110	40	35	42	45	55	40	30	45	20
	18	55	30	29	25	60	80	110	145	80	55
	$\bar{x}$	67.2	71.7	51.3	58.2	74.2	75.0	124.2	110.0	72.5	
	10										
UNTREATED CONTROLS	2	70	35	45	29	55	70	110	80	125	150
	15	20	18	12	19	21	20	25	35	20	45
	20	155	170	140	95	160	250	130	190	160	195
	24	45	20	15	33	25	55	40	45	20	80
	15										
	$\bar{x}$	72.5	60.8	53.0	44.0	65.3	111.3	76.3	87.5	81.3	8
	17										
	SD_1							100.3	99.8	76.9	83.5
	19										
	20										
		1200	1400	1620	1200	1104	1140	1360	1640	1200	1130

(1-3)

TOTAL

BUFFALO FLY COUNTS - (~~SINGLE SIDE~~ COUNTS)

Experiment.....NEEM OIL XCSSP BF 25/95

Location.....PEAK CROSSING

Property Name.....DWYER/BAILEY

Group/ Herd Name.....CONTROLS

FLY COUNTS

Animal number		Date 31/05/96	01/06/96	01/06/96	02/06/96	03/06/96	04/06/96						
1	3	95	90	110	60	50	50						
2	5	85	150	95	80	110	220						
3	6	30	50	90	20	125	65						
4	16	20	60	15	20	35	30						
5	17	40	60	30	75	40	65						
6	18	55	45	45	50	90	60						
7													
8													
9													
10													
11	2	90	120	110	95	125	130						
12	15	21	20	18	14	20	40						
13	20	75	130	110	125	210	160						
14	24	55	65	60	90	70	65						
15													
16	5	56.6	79.0	68.2	62.9	87.5	77.5						
17													
18													
19													
20													
		1140	1210	1440	1210	1030	1150						

flycount.doc

2. 12 15 7248 20

G3 G5 G7 G4

(4)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment.....NIMBITOR.....BF.26/95

Location.....PEAK CROSSING.....

Property Name.....IWAKANA.....

Group/ Herd Name.....TREATED.....

FLY COUNTS

			12-30	11-00	11-00										
		Animal number	**Date** 24/11/96	26/11/96	27/11/96										
	1	19	80	140	55										
(1)	2	11	110	130	60										
	3	63	40	65	10										
	4														
	5	6	120	40	35										
(2)	6	14	80	110	35										
	7	15	20	25	20										
	8														
	9	22	60	90	40										
(3)	10	20	90	90	40										
	11	12	65	60	15										
	12														
	13														
	14														
	15														
	16														
	17														
	18														
	19														
	20														

flycount.doc

(4)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment..... NIMBITOR (NEEM) .....

Location..... PEAK CROSSING .....

Property Name..... IWAKANA .....

Group/ Herd Name..... TREATED .....

FLY COUNTS

39mL

	Animal number	24/10/96 1630 hrs	26/11/96 1200	27/11/96 11:00	03/12/96 Date	04/12/96	05/12/96						
(1)	1 19	80	140	55	60	30	30						
	2 11	110	130	60	35	35	20						
	3 93	40	65	10	45	6	15						
	4												
(2)	5 6	120	40	35	50	4	45						
	6 14	80	110	35	45	55	25						
	7 15	20	25	20	40	20	20						
	8												
(3)	9 22	60	90	40	45	6	40						
	10 20	90	90	40	50	25	20						
	11 12	65	60	15	35	15	12						
	12												
	13												
	14												
	15												
	16												
	17												
	18												
	19												
	20												

flycount.doc

(1) 10% NIMBITOR — (1) 40%

(2) 4% NIMBITOR — (2) 20%

(3) 20% NIMBITOR — (3) 80%

This

06288 3325

(4)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment.....NIMBITOR.....BF.26/95

Location.....PEAK CROSSING.....

Property Name.....W. BAILEY.....

Group/ Herd Name.....UNTREATED CONTROLS

FLY COUNTS

	11-30	10-00	10-00									
	Date											
Animal number	24/11/96	26/11/96	27/11/96									
1 G7	55	60	70									
2 16	30	50	65									
3 9	110	110	75									
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												

flycount.doc

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment..... NEEM .....

Location..... PEAK CROSSING .....

Property Name..... W. BAILEY .....

Group/ Herd Name..... UNTREATED CONTROLS .....

FLY COUNTS

Animal number	24/11/96	26/11/96	27/11/96	03/12/96	04/12/96	05/12/96						
				11.00	10-30	Date						
1 97	55	60	70	65	120	60						
2 16	30	50	65	50	70	35						
3 9	110	110	75	45	90	30						
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												

flycount.doc 04/12/96

ANIMAL No 7 HOLDING NEAR FRONT LEG OFF GROUND

ANIMAL No 9 AWAY FROM GROUP APPEARS TO BE PHOTOSENSITIVE EARS/NOSE/EYES (POSSIBLE LANTANA POISONING)

(5)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment.....NIMBITOR.....BF 26/95.....

Location.....PEAK GROSSING.....

Property Name.....IWAKANA.....

Group/ Herd Name.....TREATED.....

FLY COUNTS

		12:00	11:30	11:00									
	Animal number	03/12/96	04/12/96	05/12/96									
1. (1)	1 19	60	30	30									
	2 11	35	35	30									
	3 63	45	6	15									
	4												
2. (2)	5 6	50	4	45									
	6 14	45	55	25									
	7 15	40	20	20									
	8												
3. (3)	9 22	45	6	40									
	10 20	50	25	20									
	11 12	35	15	12									
	12												
	13												
	14												
	15												
	16												
	17												
	18												
	19												
	20												

flycount.doc

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment.....NIMBITOR.....

Location.....PEAK CROSSING.....

Property Name.....W. BAILEY.....

Group/ Herd Name.....UNTREATED CONTROLS

FLY COUNTS

	11-00	10-30	10-00									
Animal number	**Date** 03/12/96	04/12/96	05/12/96									
1 G7	65	120	60									
2 16	50	70	35									
3 9	45	90	30									
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												

flycount.doc

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment.....NIMBITOR.....BE 26/95

Location.....PEAK GROSSING.....

Property Name.....IWAKANA.....

Group/ Herd Name.....TREATED.....

FLY COUNTS

		12:30	12:00	11:30	11:00	10:00	Date 11:00						
	Animal number	10/12/96	13/12/96	14/12/96	15/12/96	16/12/96	17/12/96						
(1)	1 19	90	70	35	55	130	180						
	2 11	140	120	10	80	110	130						
	3 G3	35	25	40	40	8	15						
	4												
(2)	5 6	40	45	5	15	95	22						
	6 14	75	75	45	60	65	70						
	7 15	25	25	34	75	10	40						
	8												
(3)	9 22	60	140	4	5	26	75						
	10 20	45	75	7	8	12	110						
	11 12	60	50	10	25	29	60						
	12												
	13												
	14												
	15												
	16												
	17												
	18												
	19												
	20												

flycount.doc

(6)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment.....NIMBITOR.....

Location.....PEAK CROSSING.....

Property Name.....W. BAILEY.....

Group/ Herd Name..UNTREATED CONTROLS

FLY COUNTS

	11·30	11·00	10·00	10·00	10·00 Date 10·00							
Animal number	10/12/96	13/12/96	14/12/96	15/12/96	16/12/96	17/12/96						
1 G7	65	70	75	90	230	140						
2 16	70	80	60	45	90	80						
3 9	40	45										
4 3			85	30	75	65						
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												

flycount.doc

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment.....AZA.....

Location.....PEAK GROSSING.....

Property Name.....IWAKANA.....

Group/ Herd Name.....TREATED (29/12).....

FLY COUNTS

		11-30	11-30	12-30 ↓	13-00	12-30	Date 12-00						
	Animal number	22/12/96	28/12/96	29/12/96	30/12/96	31/12/96	01/01/97						
(1)	1 19	280	140	350	210	220	240						
	2 11	220	240	175	46	6	65						
	3 G3	75	160	130	90	25	60						
	4												
(2)	5 6	140	180	180	115	130	155						
	6 14	140	250	295	70	140	80						
	7 15	45	55	135	27	35	140						
	8												
(3)	9 22	160	220	190	160	55	320						
	10 20	170	100	135	125	75	76						
	11 12	55	110	210	65	25	55						
	12												
	13												
	14												
	15												
	16												
	17												
	18												
	19												
	20												

flycount.doc

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment.....AZA.....

Location.....PEAK CROSSING.....

Property Name.....W. BAILEY.....

Group/ Herd Name...UNTREATED CONTROLS

FLY COUNTS

	10-30	10-00	13-00	12-30	11-30	Date 11-00						
Animal number	22/12/96	28/12/96	29/12/96	30/12/96	31/12/96	01/01/97						
1 G7	95	120	220	290	120	140						
2 16	180	160	190	180	80	50						
3 3	140	90	120	95	45	55						
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												

flycount.doc

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment: AZA #2

Location: ~~IWAKANA~~ PEAK CROSSING

Property name: IWAKANA

Group name: TREATED

FLY COUNTS

15-30 15:00 14-30 15-00 Date 14-30

Animal number	05/01/97	08/01/97	09/01/97	10/01/97	11/01/97
19	320	320	190	185	262
11	150	150	110	100	185
G3	170	110	30	27	32
6	180	250	200	166	65
14	120	350	125	405	195
15	85	110	65	42	160
22	190	210	220	275	325
20	350	360	80	180	220
12	80	160	18	95	130

11/01/97

#15 10 FLIES ON UPPER
#14 15 " " "
#G3 5 " " "
#6 6 " " "
#11 10 " " "
#22 5 " " "
#12 0 " " "

#20 0 FLIES ON UPPER
#19 12 " " "

10/01/97

#6 6 UPPER 160 LOWER
#G3 4 UPPER 23 LOWER
#19 25 UPPER 160 LOWER
#22 15 UPPER 260 LOWER
#11 5 UPPER 95 LOWER
#14 25 UPPER 320 LOWER

88

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment: AZA #2

Location: PEAK CROSSING

Property name: W. BAILEY

Group name: UNTREATED CONTROLS

FLY COUNTS

	14-30	14-00	14:30	11-00						
Animal number	08/01/97	09/01/97	10/01/97	11/01/97						
~~3~~ 16	170	110	55	140						
~~2~~ G7	250	260	350	310						
~~1~~ 3	245	280	130	220						
~~4~~										
~~5~~										
~~6~~										
~~7~~										
~~8~~										
~~9~~										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										

(9)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment: AZA #3

Location: PEAK CROSSING

Property name: IWAKANA

Group name: TREATED

FLY COUNTS

	11-00	11-30	11-00	Date						
Animal number	19/01/97	20/01/97	21/01/97	22/01/97						
19	200	16	180	205						
11	180	21	157	105						
G3	170	17	24	21						
6	310	14	13	44						
14	350	215	68	153						
15	95	19	141	220						
22	160	110	15	57						
20	300	20	72	115						
12	175	4	48	46						

20/01/97

22	15 UPPER	95 LOWER
	0 "	19 "
20	3 "	17 "
	35 "	180 "
	2 "	14 "
	2 "	19 "
	2 "	2 "
	7 "	7 "
G3	0 "	17 "

21/01/97

#6	1 UPPER	12 LOWER
G3	2 "	22 "
22	2 "	13 "
11	2 "	155 "
12	8 "	40 "
15	1 "	140 "
14	3 "	65 "
20	2 "	70 "
19	30 "	150 "

22/01/97

#19	65 UPPER	140 LOWER
11	25 "	80 "
G3	6 "	15 "
6	19 "	25 "
14	3 "	150 "
15	35 "	185 "
22	12 "	45 "
20	35 "	80 "
12	12 "	34 "

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment: AZA #3

Location: PEAK CROSSING

Property name: W. BAILEY

Group name: UNTREATED CONTROLS

FLY COUNTS

	12-00	11-00								
	Date									
Animal number	19/01/97	20/01/97	21/01/97	22/01/97						
G7	420	410	280	150						
16	150	210	110	70						
3	220	130	150	160						

(19)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment: NEEM

Location: PEAK CROSSING

Property name: IWAKANA

Group name: TREATED

FLY COUNTS

T

Animal number	31/01/97	01/02/97	02/02/97	03/02/97	04/02/97	05/02/97				
				Date						
1 19	35	38	36	15	16	170				
2 11	110	21	17	17	29	110				
3 63	50	22	28	24	22	72				
4	65.0	27.0	27.0	16.7	22.7	47.3				
5 6	25	42	14	12	25	70				
6 14	70	135	23	27	47	190				
7 15	80	11	14	32	14	105				
8	58.3	76.7	17.0	23.7	28.7	121.7				
9 22	35	120	40	55	21	140				
10 20	26	34	16	28	12	22				
11 12	30	15	11	18	17	55				
12	30.3	50.7	22.3	32.7	16.7	72.3				
13										
14										
15										
16										
17										
18										
19										
20										

0.05 (1) (2) (3) 0.5

(10)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment: NEEM

Location: PEAK CROSSING

Property name: W. BAILEY

Group name: UNTREATED

FLY COUNTS

Animal number	Date 31/01/97	01/02/97	02/02/97	03/02/97	04/02/97	05/02/97				
1 3	250	310	160	240	280	260				
2 G7	370	280	280	420	380	250				
3 16	95	110	80	160	180	140				
4										
5	238·3	233·3	173·3	273·3	280·0	216·7				
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										

RUN #1

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment..... NEEM EVALUATIONS - AIR PRESSURIZED SPRAYER WITH WATER

Location..... PEAK CROSSING

Property Name..... IWAKANA

Group/ Herd Name..... NEEM TREATED

FLY COUNTS

Date

Animal number		22/06/17	23/06/17	24/06/17	25/06/17								
1	19	410	6/11	45	110								
2	11	120	0	5	80								
3	G3	130	0	6	22								
4													
5	6	340	0	5	11								
6	14	360	1	15	95								
7	15	140	0	0	26								
8													
9	22	130	4	17	60								
10	20	290	0	10	93								
11	12	150	0	0	58								
12													
13													
14		CONTROL											
15													
16	3	180	110	260	290								
17	16	90	90	180	370								
18	7	330	290	190	185								
19													
20													

flycount.doc

RUN II (12)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment..... NEEM EVALUATIONS

Location..... PEAK CROSSING

Property Name..... IWAKANA

Group/ Herd Name..... NEEM TREATED + CONTROLS

FLY COUNTS

	Animal number		Date 01/03/97	02/04/97	03/13/97	04/03/97	05/03/97	06/04/97						
0.25%	1	19	95	4	35	27	55	210						
	2	11	160	1	12	10	9	170						
	3	G3	100	1	2	5	13	15						
	4													
0.5%	5	6	170	0	15	9	5	31						
	6	14	190	1	29	43	16	210						
	7	15	170	0	2	4	5	41						
	8													
1.0%	9	22	110	0	4	4	6	26						
	10	20	85	0	3	12	5	65						
	11	12	70	0	2	3	3	12						
	12													
	13													
CONTROLS	14	3	390	430	310	120	190	210						
	15	16	360	290	120	90	110	190						
	16	G7	120	120	200	400	250	195						
	17													
	18													
	19													
	20													

flycount.doc

RUN II (15)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment..... NEEM EVALUATIONS (NEW BATCH 2% AZA)

Location..... PEAK CROSSING

Property Name..... IWAKANA

Group/ Herd Name..... NEEM TREATED + UNTREATED CONTROLS

FLY COUNTS

	Animal number		PRE-TREAT 08/03/97	09/03/97	10/03/97	11/07/97								
1.0	1	19	145	8	47	130								
	2	11	80	2	19	40								
	3	G3	40	2	10	5								
	4		283											
0.25	5	6	170	8	9	10								
	6	14	145	5	26	105								
	7	15	110	1	15	5								
	8		141.7											
0.5	9	22	190	4	1	89								
	10	20	140	10	18	35								
	11	12	35	1	10	26								
	12		121.7											
	13													
CONTROLS	14	3	250	485	340	310								
	15	16	180	290	210	120								
	16	G7	350	160	240	300								
	17													
	18													
	19													
	20													

flycount.doc

14

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment..... NEEM EVALUATIONS

Location..... PEAK CROSSING

Property Name..... IWAKANA

Group/ Herd Name..... BOTH GROUPS

FLY COUNTS (SINGLE SIDE)

Pre-Treat 26/03/97 Date 27/03/97

	Animal number		25/03/97	UPPER BODY	MID BODY	LOW BODY	HEAD	UPPER BODY	MID BODY	LOW BODY				
TREATED	1	20	210	2	0	25	2	25	2	60				
	2	14	400	16	4	75	15	100	30	140				
	3	G3	140	2	0	18	8	2	0	40				
	4	11	295	3	5	25	7	6	0	35				
	5	15	150	2	0	20	9	30	2	65				
	6	6	210	4	6	30	2	5	0	120				
	7	12	200	1	0	65	6	20	4	25				
	8	19	460	15	2	70	15	40	5	100				
	9	22	330	22	2	165	2	7	0	50				
	10	5	246·1	7·4	2·1	45·7	7·2	16·1	4·7	70·6				
	11													
	12													
CONTROLS	13	3	400	150	200	250		250	150	250				
	14	16	320	70	60	250		50	20	120				
	15	G7	220	100	30	200		80	100	90				
	16			100·7	26·7	222·3		126·7	93·0	153·3				
	17													
	18													
	19													
	20													

flycount.doc

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment.....NEEM SELF APPLICATOR

Location.....TEAK GROSSING

Property Name.....IWAKANA

Group/ Herd Name....TREATED / UNTREATED CONTROLS

FLY COUNTS

Animal number		18/12/97	24/12/97	03/01/98	06/01/98	11/01/98	15/01/98	17/01/98	20/01/98	21/01/98 T	21/01 +2HR	21/01 +5h	22/01 +24h
1	19	—	—	230	350	260	220	240	400	420	90	34	85
2	14	110	80	140	180	155	180	150	260	230	70	25	70
3	11	135	160	110	100	170	130	210	220	125	62	28	25
4	20	100	180	260	185	120	60	95	180	110	0	0	2
5	22	180	230	275	320	130	80	130	300	70	20	35	53
6	12	50	65	190	95	135	180	90	160	110	18	4	20
7	6	110	110	180	90	135	180	100	450	240	0	0	2
8	15	30	45	90	80	50	50	110	230	60	0	0	3
9	7	30	60	110	50	90	25	40	180	90	32	3	15
10													
11	3	50	90	130	120	85	90	90	300	95	-	-	440
12	16	110	85	40	60	125	110	190	400	200	-	-	260
13	9	110	180	110	340	195	120	220	290	280	-	-	320
14													
15													
16										200			
17													
18													
19													
20													

1.)

flycount.doc

1.) Most flies on belly

19/11/22 = (A) OCP

20/6/15 = (B) NXTS.

= (C) BITTERS.

17 - 19

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment..NEEM.....

Location..PEAK CROSSING.....

Property Name..IWAKANA.....

Group/ Herd Name.....

FLY COUNTS

Date

Animal number	22/01 +24h	22/01 +28h	23/01 +48h	24/01 +72 h	25/01 4-d							
1 19	24	45	95	170	350*							
2 14	25	105	210	260	110							
3 11	18	42	45	100	290*							
4 20	0	0	12	190	160							
5 22	28	50	50	110	290							
6 12	3	20	3	35	50							
7 6	1	1	18	75	50							
8 15	1	0	5	95	20							
9 7	11	15	80	120	40							
10												
11 3			380	290	320							
12 16			500	450	295							
13 9			600	550	490							
14												
15												
16												
17												
18												
19												
20												

flycount.doc

2.) on top.

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment..NEEMAZAL T.S 10g/litre AZADIRACTIN

Location.....PEAK CROSSING.....

Property Name.....IWAKANA.....

Group/ Herd Name.....TREATED / CONTROL

FLY COUNTS

T ↓

Date

Animal number	19/03/98	20/03/98	21/03/98	22/03/98	26/03/98							
1 15	450	120	130	80	90							
2 7	470	15	15	40	120							
3 11	500	70	90	210	290							
4 19	1200	70	180	300	300							
5 6	800	130	180	280	400							
6 14	750	120	150	230	260							
7 12	550	90	25	60	280							
8 22	450	100	90	160	240							
9 20	850	70	140	110	130							
10												
11 SC	668·9	87·2	111·1	167·0	234·4							
12												
13												
14												
15												
16												
17												
18 3	230	70	60	50	60							
19 16	250	90	90	110	190							
20 9	400	200	150	80	210							
$\bar{x}$	298·3	120	150	80	213							

flycount.doc

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment..

Location.....PEAK GROSSING...............

Property Name..........IWAKANA..........................

Group/ Herd Name.......TREATED /CONTROL

FLY COUNTS

Date

Animal number	28/03/98	30/03/98	01/04/98	03/04/98	Top of body / Lower body							
1 15	180	30/60	6/100	80/110								
2 7	55	5/40	0/80	60/100								
3 11	200	15/200	3/160	50/120								
4 19	350	40/140	20/190	230/350								
5 6	250	70/250	20/200	80/210								
6 14	190	50/250	80/160	90/140								
7 12	160	90/160	10/70	140/150								
8 22	180	60/80	10/100	4/80								
9 20	80	40/110	25/170	130/110								
10												
11 x	152.2	139.3/143.3	12.7/136.7	94.9/141.1								
12 Σ		149090	149.4	236.0								
13												
14												
15												
16												
17												
18 3	180	20/40	30/25	70/6								
19 16	280	100/200	120/180	150/60								
20 9	350	50/350	20/200	100/200								

270.0 56.7/195.7 22.7/135 32.?/88.7

252.4 221.7 145.4

flycount.doc

(18)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment: BRUTE SELF APPLICATOR

Location: PEAK CROSSING

Property name: INAKANA

Group name: TREATED / CONTROL

FLY COUNTS

Animal number	Date 04/04/98	05/04/98	06/04/98	07/04/98	10/04/98	14/04/98	17/04/98			
1 15	240	2	0	0	0	0	0			
2 7	210	5	0	0	0	0	0			
3 11	180	2	0	0	0	0	0			
4 19	420	4	0	0	0	0	0			
5 6	270	1	0	0	0	0	0			
6 14	500	17	0	0	0	0	0			
7 12	250	0	0	0	0	0	0			
8 22	380	3	0	0	0	0	0			
9 20	240	6	0	0	0	0	0			
10							6			
11	198·9	4·4								
12										
13										
14										
15										
16										
17										
18 3	70	130	110	90	260	185	110			
19 16	260	270	280	140	190	210	260			
20 9	250	300	360	280	210	360	195			
	193·3	212·2	215	170	220	177·6	188·3			

(19)

BUFFALO FLY COUNTS - (SINGLE-SIDE COUNTS)

Experiment..SELF APPLICATOR 50% OF NEEM 50% DC TRATE

Location..PEAK CROSSING..

Property Name..IWAKANA..

Group/ Herd Name..TREATED + CONTROL

FLY COUNTS

T ↓

Animal number.		Date 06/05/98	07/05/98	09/05/98	10/05/98	11/05/98	12/05/98	13/05/98	14/05/98	16/05/98	18/05/98		
1	6	285	210	195	35	90	85	32	80	23	50		
2	7	45	30	15	15	110	40	21	32	65	36		
3	11	240	190	65	29	140	160	75	60	70	70		
4	12	180	65	55	35	60	25	20	31	35	45		
5	14	165	110	85	90	70	110	98	120	150	95		
6	15	90	40	60	28	85	30	55	15	19	45		
7	19	390	390	330	185	265	180	95	160	290	190		
8	20	130	180	50	60	110	90	60	15	32	65		
9	22	310	230	65	62	125	60	70	40	30	40		
10													
11	x̄	203.9	160.6	102.2	59.9	117.2	86.7	58.4	61.4	79.7	78.7		
12													
13	%C		12.0	13.8	72.6	58.9	42.2	69.8	67.7	15.6	19.9		
14													
15													
16													
17													
18	3	110	80	130	280	320	90	95	120	72	60		
19	9	190	260	220	165	290	110	210	260	90	95		
20	16	185	220	195	210	245	250	275	190	120	110		
	x̄	161.7	186.7	18.7	218.2	265.0	150	193.3	190	94	88.3		

TREATED

CONTROL

flycount.doc

DINGOES RAN CATTLE THROUGH FENCE

APPENDIX 4

FARM DIARY / DAILY LOG

RUN #1 (4)

FARM DIARY/DAILY LOG

Experiment number: BF 26/95 **Page:** 1

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
24/11/96	SUNNY CLOUDY PERIODS	PRE-TREATMENT FLY COUNTS TREATMENT ABANDONED DUE	SP
		TO STORM ACTIVITY	
26/11/96	FINE SUNNY	PRE-TREATMENT FLY COUNTS	
		TREATMENT APPLICATION	SP
27/11/96	SUNNY	POST TREATMENT FLY COUNTS	SP

MRC NEEM OIL PROJECT (1-3)

FARM DIARY/DAILY LOG

Experiment number: XCSSP BF25/95 **Page:** 1

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
24/05/96	FINE, MILD, SUNNY.	STUDY SET UP.	
		Pre-treatment fly counts on all	
		animals.	
		All animals mustered, yarded	
		& weighed, then allocated	
		to treatment groups.	
		Two treatments. 1) 100% Neem	
		Oil (0.13% Azadirachtin) 2) 50%	
		Neem oil diluted with Canola	
		oil. Two Control groups	
		1) Placebo administered control	
		2) No treatment.	
		Application of respective treatment	
		Animals returned to allocated	
		paddock & monitored at	
		two hourly intervals.	
25/05/96	FINE, MILD. SUNNY	Fly counts on all animals	

(1-3)

NEEM O/L

FARM DIARY/DAILY LOG

Experiment number: XCS8P BF 25/95 **Page:** 2

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
26/05/96	FINE, SUNNY	Fly counts on all animals.	
		Still majority of flies on	
	MILD	heads of animals.	
	OVERNIGHT	need to consider application	
		Technique - MAX 24°	
27/05/96	FINE MILD	Fly counts on all groups	
28/05/96	FINE MILD	RE-TREATMENT -	
		100% NEEM - 100ml (except #21 = 75ml)	
		applied in a mister bottle to	
		entire body surface.	
		50% NEEM - 100ml (except 75ml #13 & #23)	
		diluted with SYNERTROL &	
		applied in mister bottle to entire	
		body surface. Very evident	
		that flies move to side of	
		body where oil not applied	
29/05/96	FINE MILD	Fly counts on all groups	
30/05/96	" "	Fly counts on all groups	
31/05/96	" "	Fly counts on all groups	

APPLICATION RATE (AS PER BAYTICOL)

151 - 300 kg = 55 ml.
301 - 500 kg = 75 ml.

APPLICATION METHOD

ADJUSTABLE NICVERM APPLICATOR - PHILIPS.
- APPLIED CALCULATED DOSE OVER BACKLINE
- SPOT APPLICATION TO ALLOW DISPERSION OVER MAJORITY OF ANIMALS BODY.

NEEM OIL. XCSSK BF 25/95

OBSERVATIONS

HALF DOSE

5 mins post applic - little or no repellency evident
+2hrs - flies restless - move to belly

FULL DOSE

5 mins post applic - animals still agitated due to fly annoyance
+1hrs - flies definitely moving to lower legs + belly - animals stomping - flies still restless.

+ 4 hrs - a definite concentration of flies on head - agitated
- oil well dispersed down side of body

PLACEBO CONTROL

+4 hrs - #5 has smooth coat - oil well dispersed + flies don't like landing

RUN #2 (5)

FARM DIARY/DAILY LOG

Experiment number: BF 26/95 **Page:** 1

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
03/2/96	VERY HOT 39°C	PRE-TREATMENT FLY COUNTS	
	SUNNY SOME CLOUD	TREATMENT APPLICATION	AP
04/2/96	STORM OVERNIGHT	POST TREATMENT	
	39mi IN RAINGAUGE	FLY COUNTS	AP
	SHOWERS BECOMING FINE		AP
05/2/96	CLOUDY NO RAIN	POST TREATMENT FLY COUNTS	AP

Dear Phil,

Last Tuesday I treated the cattle as arranged with 20%, 40% + 80% Nimbitor. The day turned out as promised to be very hot 39°C+. The 20% + 40% mixed + applied very well, however the 80% at the time of application had the consistency of glue. I had to pour it on and rub it over the animal myself. To top off a stinking day, at approximately 4-30AM the next morning we had a huge storm which dumped 39 m/l at the treatment property. As a result we don't have any useable data. Do you want me to do anything different in the preparation of 80% Nimbitor

Regards Sue

09/12/96

07 3281 7007

RUN #3 (6)

FARM DIARY/DAILY LOG

Experiment number: BF 26/95 **Page:** 1

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
10/12/96	SUNNY + HOT SOME CLOUD	PRE-TREATMENT FLY COUNTS	
		TREATMENT ABANDONED DUE TO IMMINENT STORMS	SP
	41 MI SINCE 10/12/96		
13/12/96	FINE SUNNY	PRE-TREATMENT FLY COUNTS	
		TREATMENT APPLICATION	SP
14/12/96	SUNNY SOME CLOUD	POST TREATMENT FLY COUNTS	SP
15/12/96	SUNNY	POST TREATMENT FLY COUNTS	SP
16/12/96	FINE	POST TREATMENT FLY COUNTS	SP
17/12/96	FINE SUNNY	POST TREATMENT FLY COUNTS	SP

RUN # 1 (1)

FARM DIARY/DAILY LOG

Experiment number: AZA **Page:** 1

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
22/2/96	CLOUDY	PRE-TREATMENT FLY COUNTS	SP
28/2/96	FINE SOME CLOUD	PRE-TREATMENT FLY COUNTS	SP
29/2/96	FINE+HOT 34°	PRE-TREATMENT FLY COUNTS	
		TREATMENT APPLICATION	
		APPLIED WITH CANOLA OIL AT SPECIFIED CONCENTRATIONS USING	
		10ml SYRINGE. APPLIED 50ml/SIDE TO ALL UPPER AREAS OF BODY.	APPLIED IN LONG SWEEPING ARM MOTIONS.
		APPLICATION WAS WELL DISPERSED EXCEPT FOR LOWER BELLY ON	
		EACH ANIMAL.	
		GROUP # 1 100PPM AZA	
		" # 2 500PPM AZA	
		" #3 50PPM AZA	SP
30/2/96	FINE+ SUNNY	POST TREATMENT FLY COUNTS	
		ALL TREATED ANIMALS HAVE FLY ON THEM BUT THEY ARE ALL	
		CONFINED TO THE LOWER BELLY AREA	SP
31/2/96	FINE SHOWERS OVERNIGHT	FLIES CONTINUE TO BE CONFINED TO BELLY AREA	SP
~~01/3/96~~	~~CLOUDY BREEZY~~	EXCEPTING ANIMALS 15 - 8 ON UPPER BODY	
		14 - 7 ON UPPER "	
		6 - 5 ON UPPER "	SP
01/6/97		FLIES DISPERSED OVER THE BODY OF ALL ANIMALS EXCEPT 20-6 ON UPPER BODY	
		19-10 ON UPPER "	SP

RUN #2 (8)

FARM DIARY/DAILY LOG

Experiment number: AZA #2 **Page:** 1

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
05/01/97	SUNNY 30° BREEZE	PRE-TREATMENT FLY COUNTS	JP
08/01/97	FINE 35° SOME CLOUD	PRE-TREATMENT FLY COUNTS	JP
[redacted]	[redacted]	TREATMENT APPLICATION	
		APPLIED WITH CANOLA OIL AT SPECIFIED CONCENTRATIONS	APPLIED WITH LONG SWEEPING ARM MOTIONS
		USING 50ml SYRINGE. 50ML WAS APPLIED TO EACH SIDE ON ALL	
		UPPER AREAS OF THE BODY. APPLICATION DISPERSED WELL	
		EXCEPT FOR THE LOWER BELLY	
		OF EACH ANIMAL	
		GROUP #1 500 PPM AZA	
		" #2 50 PPM "	
		" #3 100 PPM "	JP
09/01/97	FINE HOT 35°	POST TREATMENT FLY COUNTS	
		ALL FLIES ARE CONFINED TO	
		THE LOWER BELLY OF EACH	
		ANIMAL.	JP
10/01/97	FINE HOT 35°	THE MAJORITY OF FLIES ARE CONFINED TO THE LOWER BELLY AREA	
		#6 6 UPPER 160 LOWER # 14 25 UPPER 380 LOWER	
		#G3 4 UPPER 23 LOWER # 11 5 UPPER 95 LOWER	
		#19 25 UPPER 160 LOWER #22 15 UPPER 260 LOWER	
		THE REMAINING [redacted] 3 ANIMALS HAD FLIES ONLY ON THE LOWER BODY 12.20.15	JP
11/01/97	FINE HOT 35° SOME CLOUD		

FARM DIARY/DAILY LOG

Experiment number: AZA #2 Page: 2

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
11/01/97	FINE 35° SOMEGAID	POST TREATMENT FLY COUNTS ANIMAL NUMBERS 15+11+12+20	
		HAD 0 FLIES ON THE UPPER BODY	
		#14 HAD 15 FLIES ON UPPER BODY	
		~~#~~ #63 " 4 " " " "	
		#6 " 6 " " " "	
		#22 " 5 " " " "	
		#19 " 12 " " " "	JP

RUN #3 (9)

FARM DIARY/DAILY LOG

Experiment number: AZA #3 **Page:** 1

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
19/01/97	FINE 29° SOME CLOUD	PRE TREATMENT FLY COUNTS TREATMENT APPLICATION	
		APPLIED WITH CANOLA OIL AT SPECIFIED CONCENTRATIONS	
		USING 50ML SYRINGE. 50ML/SIDE 100ML PER ANIMAL. APPLIED WITH	
		LONG SWEEPING ARM MOTIONS ALL OVER ANIMAL + UP UNDER	
		BELLY AREA. APPLICATION WAS WELL DISPERSED	
		GROUP#1 500 PPM	
		#2 100 PPM	
		#3 50 PPM	
		15 MINUTES AFTER APPLICATION	
		THE MAJORITY OF FLIES HAD ACCUMULATED ON THE FORELEGS	SP
20/01/97	CLOUDY 29° SOME SUNNY PATCHES BREEZY	POST TREATMENT FLY COUNTS	
		# 22-15 UPPER 95 LOWER	
		# 15-0 " 19 "	
		# 20-3 " 17 "	
		# 14-35 " 180 "	
		# 19-2 " 14 "	
		# 11-2 " 19 "	
		# 12-2 " 2 "	
		# 6-7 " 7 "	
		# 63-0 " 17 "	SP
21/01/97	FINE SOME CLOUD 28°	POST TREATMENT FLY COUNTS	
		6 1 UPPER 12 LOWER	
		63 2 " 22 "	
		22 2 " 13 "	
		11 2 " 155 "	SP
		12 8 " 4 "	
		15 1 " 140 "	
		14 3 " 65 "	
		20 2 " 70 "	
		19 3 " 150 "	

FARM DIARY/DAILY LOG

Experiment number: NEEM **Page:**

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
31/01/97	FINE CLOUDY	PRETREATMENT FLY COUNTS TREATMENT APPLICATION	
01/02/97	HOT + HUMID SOME CLOUD	HIGH DUNG BEETLE ACTIVITY POST TREATMENT FLY COUNTS	
		FLIES STILL ON ANIMALS BUT NUMBERS HAVE REDUCED	
		FLIES ARE DISPERSED IN NATURAL POPULATIONS OVER THE BODY	SP
02/02/97	RAIN	DUE TO THE RAIN THE FLIES ARE MOSTLY UNDER THE BELLY,	
		NECK + DEWLAP ON BOTH TREATED + UNTREATED ANIMALS	SP
03/02/97	FINE CLOUDY	LEN ALLEN TREATED HIS CATTLE 02/02 BARRICADE POST TREATMENT FLY COUNTS	SP
04/02/97	FINE SUNNY	POST TREATMENT FLY COUNTS	SP
05/02	FINE SUNNY	POST TREATMENT FLY COUNTS	SP

FARM DIARY/DAILY LOG

Experiment number: BF 25/95 **Page:** 1

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
22/02/95	FINE HOT	Pre-treatment fly counts.	
		Preparation diluted in	
		Water to required dilutions	
		& applied with air pressurised	
		applicator to all parts	
		of animals body.	
23/02/95	FINE HOT.	Fly counts on both	
		groups.	
24/02/95	FINE HOT	Fly counts on both groups	
25/02/95	FINE HUMID	Fly counts on both groups	

Run II (12)

FARM DIARY/DAILY LOG

Experiment number: BF 25/95 Page: 1

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
01/03/97	FINE HOT	Pre-treatment fly counts.	
		Applied Neem.	
02/03/97	FINE SUNNY	Fly counts	
03/03/97	HOT HUMID	Fly counts on both groups	
04/03/97	FINE	Fly counts on both groups	
05/03/97	Overcast	Fly counts on both groups	
06/03/97	"	Fly counts on both groups	

Run III (13)

FARM DIARY/DAILY LOG

Experiment number: BF 25/95 Page:____

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
08/03/97	CLOUDY	Pretreatment fly counts both groups	
		Treatment application of Run III	
		* New Neem formulation not as "dark" as previous formulation. Seems much more miscible in water.	
09/03/97	CLOUDY PERIODS WIND	Day +1 Fly counts on both groups.	
10/03/97	SUNNY PERIODS	Fly counts on both groups - Trace shower o/n	
11/03/97	CLOUDY	Fly counts on both groups	
		STUDY TERMINATED	

(14)

FARM DIARY/DAILY LOG

Experiment number: XCS BF 25/95 Page: 1

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
25/03/97	SUNNY WARM	Pre-treatment fly counts on both groups.	
		Used 2500 ppm Ifn a Buffalo fly Treatment on Bulls	
		Carrier (40ml + 40ml) applied with 40 ml Syringe on strips along both sides of treated animals.	
		Post-treatment observations:	
		Flies landing to congregate on belly underside.	
26/03/97	HOT DRY	Fly counts on both groups	
27/03/97	HOT DRY	Fly counts on both groups	

(16-17)

FARM DIARY

Experiment Number: NEEM **Page:** 1

List all activities related to the study. Include unforeseen circumstances and any responses

Month: MARCH/APRIL

Date	Weather Conditions	Activity,unforeseen cicumstance, etc.	Initials
19/03/98	CLOUDY HUMID	PRE-TREATMENT FLY COUNTS	SP
		TREATED EACH ANIMAL WITH	
		60ML NEEMAZAL 100% FORMULATION	
20/03/98	HOT FINE	FLY COUNTS TREATED/UNTREATED	
21/03/98	" "	" " " "	
22/03/98	HOT FINE	" " " "	
23/03/98	HOT FINE	FLY COUNTS TREATED/CONTROL	
~~24/03/98~~	~~HOT FINE~~	CHARGED SELF APPLICATOR	
		LIQUIDISER WITH 50% NEEMAZAL	
		50% SYNTROL	
28/03/98	FINE HOT	FLY COUNTS TREATED/CONTROL	
30/03/98	" "	" " " "	
01/04/98	CLOUDY SHOWERS	FLY COUNTS TREATED/CONTROL	
03/04/98	CLOUDY CLEARING	" " " "	
		RAINFALL 12½ ML	SP
04/04/98			

FARM DIARY

Experiment Number: Neem **Page:** 2

List all activities related to the study. Include unforeseen circumstances and any responses

Month: APRIL

Date	Weather Conditions	Activity,unforeseen cicumstance, etc.	Initials
04/04/98	CLOUDY SOME RAIN	PRE TREATMENT FLY COUNTS	
	CLEARING 3ML 30°		
~~05/04/98~~	~~FINE WARM 30°~~	SELF APPLICATOR PLACED IN	
		GATEWAY WITH BRUT FULL STRENGTH	SP
05/04/98	FINE WARM 30°	FLY COUNTS	SP
06/04/98	FINE WARM 29°	FLY COUNTS	SP
07/04/98	FINE WARM 29°	FLY COUNTS	SP

(18)

FARM DIARY/DAILY LOG

Experiment number: BRUTE SELF APPLICATOR Page:____

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
04/04/98		PRE-TREATMENT FLY COUNTS SET UP SELF APPLICATOR	
		WITH 100% BRUTE APPROXIMATELY	
		80% FLY KNOCKDOWN AFTER 1 HOUR	SP
05/04/98		FLY COUNTS	SP
06/04/98		FLY COUNTS	SP
07/04/98		FLY COUNTS APPLICATOR WICKING WELL	SP
10/04/98		FLY COUNTS	SP
14/04/98		FLY COUNTS	SP
17/04/98		FLY COUNTS	SP
18/04/98		SELF APPLICATOR REMOVED	
		BIOPSIES PERFORMED ON	
		7 TREATED ANIMALS	SP
18/04/98	- 05/05/98	FLIES SLOW TO RETURN TO TREATED	
		ANIMALS	SP

FARM DIARY/DAILY LOG

Experiment number: SELF APPLICATOR 50% NEEM 50% DC.TRATE Page:____

List all activities related to the study. Include unforseen circumstances and any responses.

Date	Weather conditions	Activity, unforseen circumstances etc	Initials
06/05/98		PRE-TREATMENT FLY COUNTS	
		SET UP SELF APPLICATOR IN FENCE	
		LINE ONTO WATER	SP
07/05/98		FLY COUNTS APPLICATOR WICKING WELL	SP
09/05/98		DINGOES PUT TREATED CATTLE THROUGH	
		FENCE OVERNIGHT. FLY COUNTS	SP
10/05/98		FLY COUNTS GOOD REDUCTION IN	
		FLY NUMBERS ON TREATED GROUP	SP
11/05/98		FLY COUNTS	SP
12/05/98		FLY COUNTS	SP
13/05/98		FLY COUNTS APPLICATOR WICKING WELL	SP
14/05/98		FLY COUNTS	SP
16/05/98		FLY COUNTS	SP
18/05/98		FLY COUNTS SELF APPLICATOR REMOVED	SP

APPENDIX 5

RESISTANCE TESTS

RESISTANCE TEST

Date: 26/02/97

Location: "Inakana" via Peak Crossing

Toxicant: DIAZINON

Observation time: +2hrs.

Comments: 27°C
Papers constructed 05/02/97

DILUTION	REPLICATE #1		REPLICATE #2		REPLICATE #3	
	No set	No dead	No set	No dead	No set	No dead
CHECK	24	0	29	0	19	0
0.31	20	0	21	1	17	0
0.47	25	0	17	0	22	0
0.70	18	4	31	3	28	13
1.05	17	4	23	12	24	16
1.58	33	26	32	29	20	16
2.37	29	29	25	25	16	16
3.56	28	28	22	22	43	43
5.33	31	31	25	25	21	21
8.00	46	46	27	27	20	20

RESISTANCE TEST

Date: 26/02/97

Location: "WAKANA" VIA PEAK CROSSING

Toxicant: Fenvalerate

Observation time: +2 hrs.

Ambient temp: 27°C

Comments: Papers constructed 31/01/97

DILUTION	REPLICATE #1		REPLICATE #2		REPLICATE #3	
	No set	No dead	No set	No dead	No set	No dead
CHECK	24	0	29	0	19	0
0.1953	25	0	21	0	18	0
0.39	20	0	14	0	36	0
0.78	11	0	24	0	51	0
1.56	28	0	29	0	19	0
3.125	32	0	24	3	16	2
6.25	41	2	26	7	15	4
12.5	36	12	28	12	27	16
25.0	19	17	27	25	18	18
50.0	26	26	23	23	23	22
100.0	26	26	39	39	26	26

3 0

2 0

1 0

APPENDIX 6

WEATHER CONDITIONS

WEATHER CONDITIONS

Experiment number: BF 26/95

Date	Weather conditions (t°C early morning, noon, evening etc)	Rainfall (mm)	Initials
24/11/96	SUNNY CLOUDY PERIODS 26°C	–	SP
26/11/96	SUNNY 30°C	–	SP
27/11/96	SUNNY 32°C	–	SP

WEATHER CONDITIONS

Experiment number: XCSSP BF 25/95

Date	Weather conditions (t°C early morning, noon, evening etc)	Rainfall (mm)	Initials
24/6/96	FINE, MILD, SUNNY MAX 24°C	0	
25/6/96	FINE MILD, SUNNY MAX 24°C	0	
26/6/96	" " " MIN 11° "	0	
27/6/96	" " " MIN 10° "	0	
28/6/96	" " " " 10° 23°	0	
29/6/96	" " " " 8° 24°	0	
30/6/96	" " " " 7° 23°	0	
31/6/96	" " " " 10° 24°	0	
01/6/96	SHOWERY 'O CAST 13° 20°	trace	
02/6/96	FINE SUNNY " 13° 22°	1·5ml	
03/06/06	" " " 12 22°	4·5ml	
04/06/06	" " " 12 23	0·5	

WEATHER CONDITIONS

Experiment number: ____________________

Date	Weather conditions (t°C early morning, noon, evening etc)	Rainfall (mm)	Initials
03/12/96	VERY HOT SUNNY SOME CLOUD 39°C	—	SP
04/12/96	SHOWERS BECOMING FINE STORM OVERNIGHT 28°C	38mm	SP
05/12/96	CLOUDY NO RAIN 30°C		SP

metdata.doc xcs

WEATHER CONDITIONS

Experiment number: BF 26/95

Date	Weather conditions (t°C early morning, noon, evening etc)	Rainfall (mm)	Initials
10/12/96	SUNNY SOME CLOUD STORMS DEVELOPING 36°C		AP
13/12/96	SUNNY SOME CLOUD FORECAST FINE NEXT 3 DAYS 30°C	41 SINCE 10/12	AP
14/12/96	SUNNY SOME CLOUD 30°C	—	AP
15/12/96	SUNNY FINE 29°	—	AP
16/12/96	FINE SUNNY 28°	—	AP
17/12/96	FINE SUNNY 29°	—	AP

WEATHER CONDITIONS

Experiment number: AZA

Date	Weather conditions (t°C early morning, noon, evening etc)		Rainfall (mm)	Initials
23/12/96	CLOUDY SUNNY PATCHES	30°C	4.2 SINCE 17/12	SP
24/12/96	FINE SUNNY	34°C	—	SP
28/12/96	FINE	32	-	SP
29/12/96	FINE SUNNY	35	—	SP
30/12/96	FINE SUNNY	32°		SP
31/12/96	FINE SUNNY OVERNIGHT SHOWERS	32°	8.5m	SP
01/01/97	CLOUDY LIGHT TO MEDIUM BREEZE	28°	—	SP

WEATHER CONDITIONS

Experiment number: AZA #2

Date	Weather conditions (t°C early morning, noon, evening etc)	Rainfall (mm)	Initials
05/01/97	FINE + SUNNY FRESH BREEZE	—	AP
08/01/97	FINE SOME CLOUD 35°	—	AP
09/01/97	FINE + SUNNY 35°	—	AP
10/01/97	FINE + SUNNY 35°	—	AP
11/01/97	FINE SOME CLOUD 35°	—	AP

(9)

WEATHER CONDITIONS

Experiment number: AZA #3

Date	Weather conditions (t°C early morning, noon, evening etc)	Rainfall (mm)	Initials
19/01/97	FINE SOME CLOUD 29°	—	SP
20/01/97	CLOUDY SOME SUNNY PATCHES BREEZY 29°	—	SP
21/01/97	FINE SOME CLOUD 28	0.5mm	SP

metdata.doc xcs

WEATHER CONDITIONS

Experiment number: BF 25/95

Date	Weather conditions (t°C early morning, noon, evening etc)	Rainfall (mm)	Initials
22/02/97	Very hot & humid 33°C Max	0	
23/02/97	Extremely hot 30° Max	0	
24/02/97	Fine hot 32°C	0	
25/02/97	Hot humid. 29°C	0	

Run II (12)

WEATHER CONDITIONS

Experiment number: BF 25/95

Date	Weather conditions (t°C early morning, noon, evening etc)	Rainfall (mm)	Initials
01/03/97	HOT SUNNY 31°c	0	
02/03/97	SUNNY CLEAR 30°C	0	
03/03/97	HOT HUMID - CLOUDY PERIODS 29°C	0	
04/03/97	FINE, SUNNY 28°C	0	
05/03/97	OVERCAST, HUMID 28°C Shower.	trace	
06/03/97	Overcast 25° Sunny periods	0	

Run III

(15)

WEATHER CONDITIONS

Experiment number: BF 25/95

Date	Weather conditions (t°C early morning, noon, evening etc)	Rainfall (mm)	Initials
08/03/97	CLOUDY – SLIGHT WIND	0	[illegible]
09/03/97	SUNNY – CLOUDY PERIODS + WINDY	0	[illegible]
10/03/97	CLOUDY PERIODS	0	[illegible]
11/03/97	CLOUDY	0	[illegible]

metdata.doc xcs

APPENDIX 7

PERSONNEL SHEET

PERSONNEL INVOLVED IN TRIAL OR STUDY

Sponsor: MRC NEEM OIL PROJECT.

Study title: EFFICACY OF NEEM OIL AS AN ANTIFEEDANT TO AID IN BUFFALO FLY CONTROL

Study number: XCSSP BF 25/96

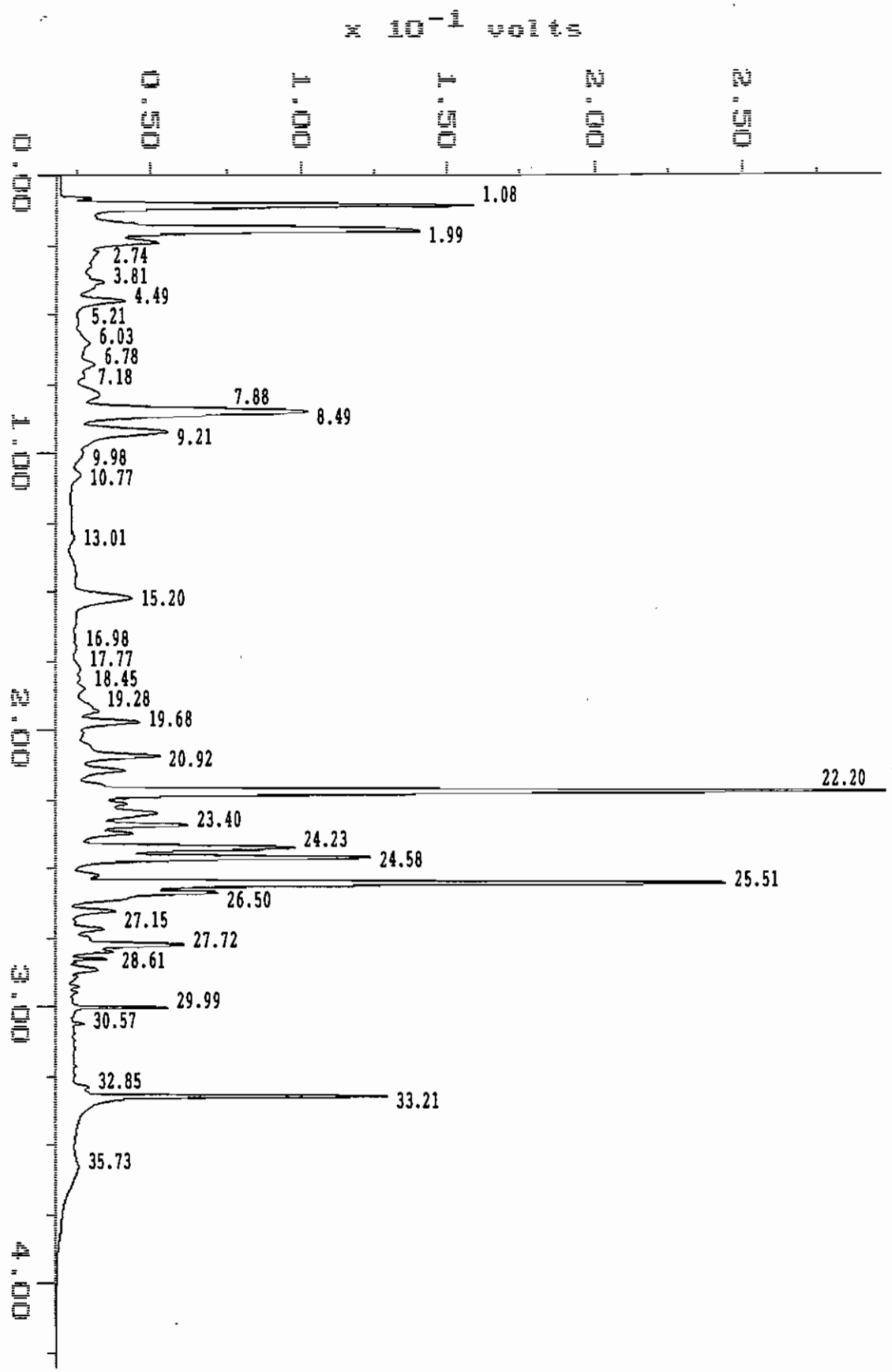
Name:	R. S. TOZER
Company:	FLYCAM PTY LTD
Responsibility:	STUDY SUPERVISION
Signature:	
Name:	S. PENDER
Company:	—
Responsibility:	FLY COUNTS
Signature:	S Pender
Name:	W. BAILEY.
Company:	—
Responsibility:	PROPERTY OWNER
Signature:	W. Bailey
Name:	P. SPRADBERY
Company:	XCS CONSULTING P/L
Responsibility:	STUDY DIRECTOR
Signature:	

APPENDIX 8

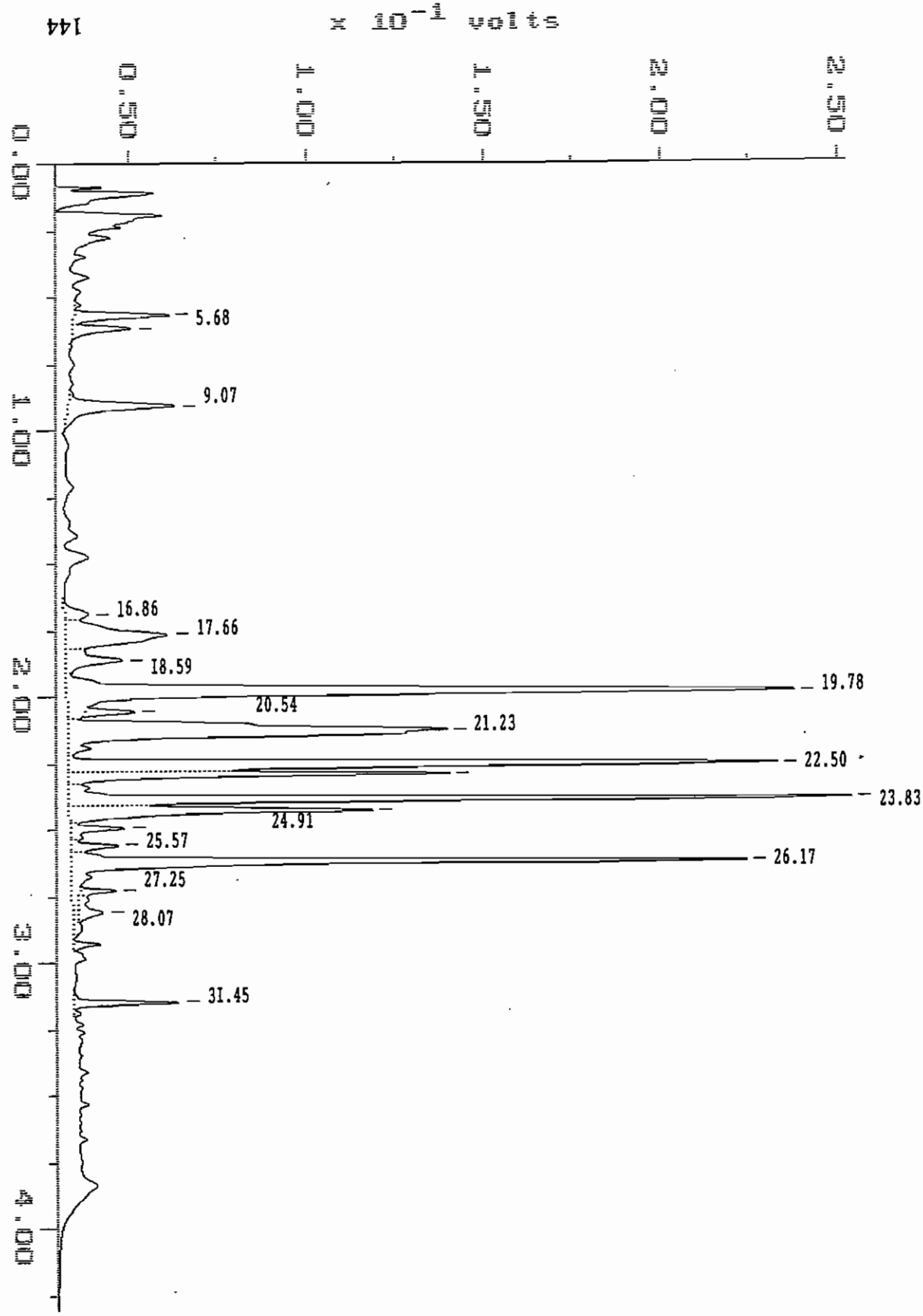
GC PROFILES OF NEEM OIL

(Neem Extracts Pty Ltd, Lismore, NSW)

Sample: 35% No filters Channel: 214 Filename: nofilter
Acquired: 04-OCT-95 11:32 Method: C:\NEEM\NEEM Operator: Dav
Comments: 16% Azadirachtin Powder

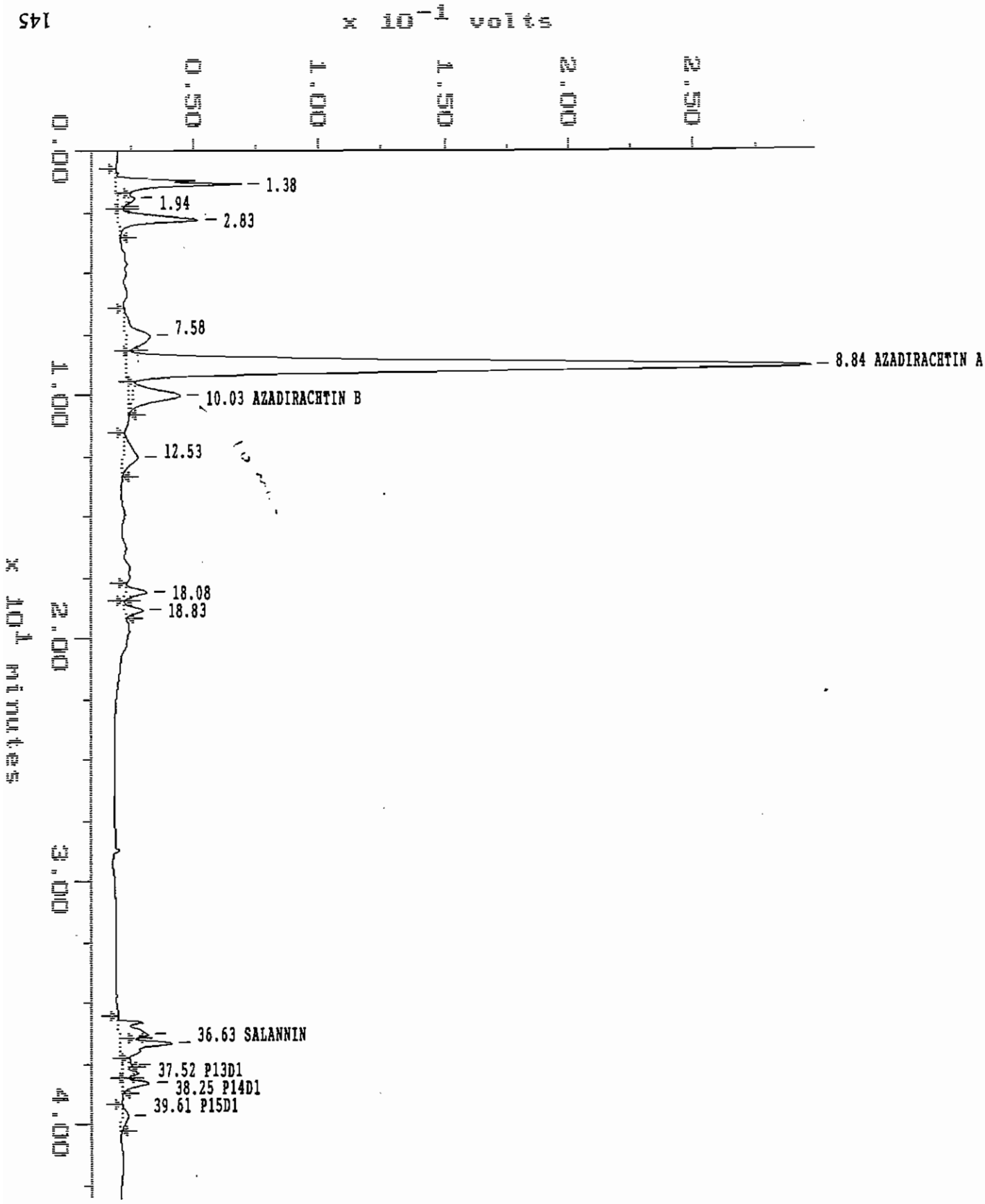


Sample: Ian Bally Oil Channel: Ian Bally oil Filename: ayroil_1
Acquired: 12-SEP-95 10:58 Method: C:\NEEM\OILAYR95 Operator: Dav

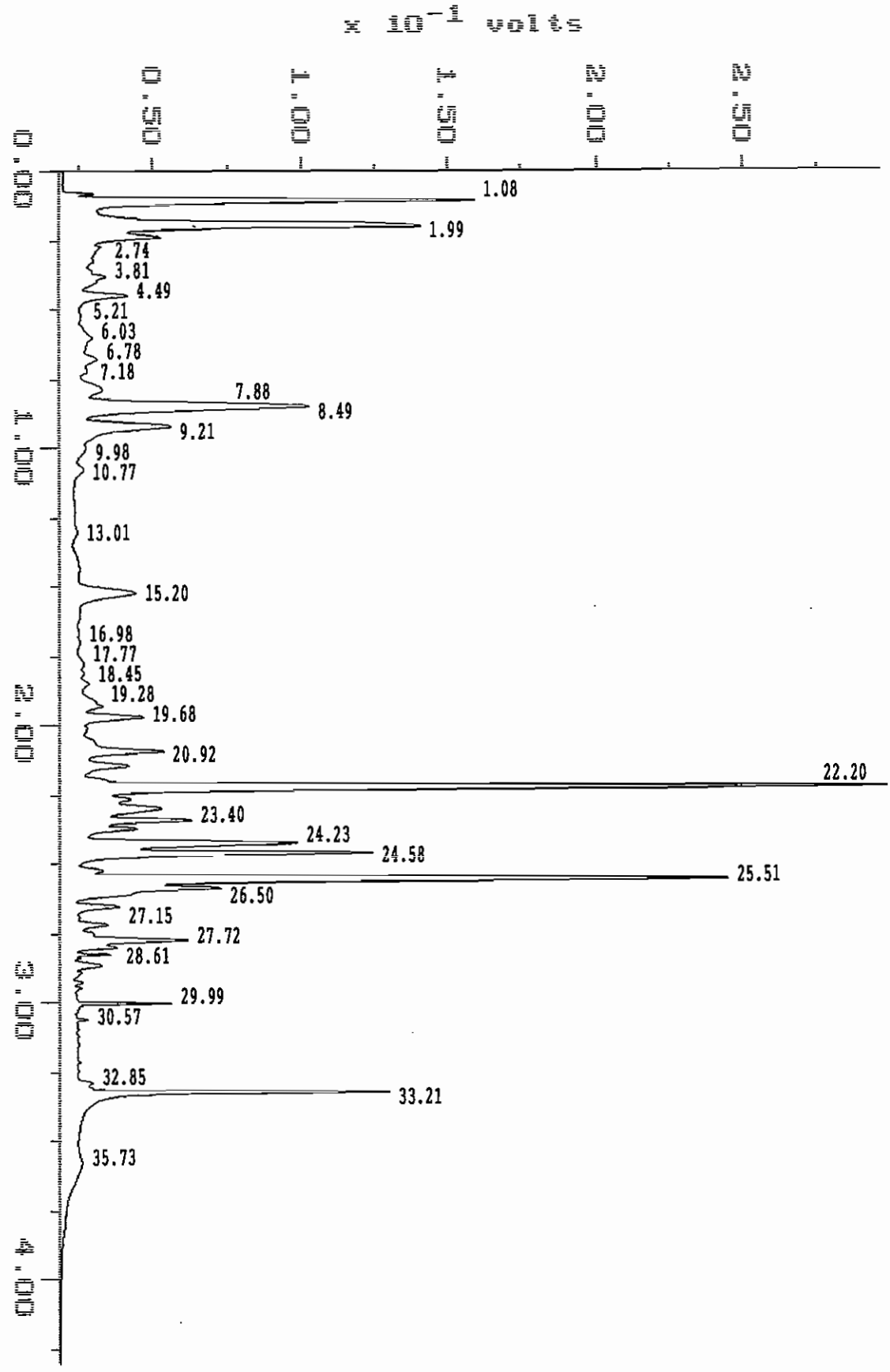


710

Sample: Fraction #9 Channel: 214 Filename: Flsh#2_3
Acquired: 05-OCT-95 12:25 Method: C:\NEEM\TRIALS\FLSHCOL2 Operator: Dav



Sample: 35% No filters Channel: 214 Filename: nofilter
Acquired: 04-OCT-95 11:32 Method: C:\NEEM\NEEM Operator: Dav
Comments: 16% Azadirachtin Powder



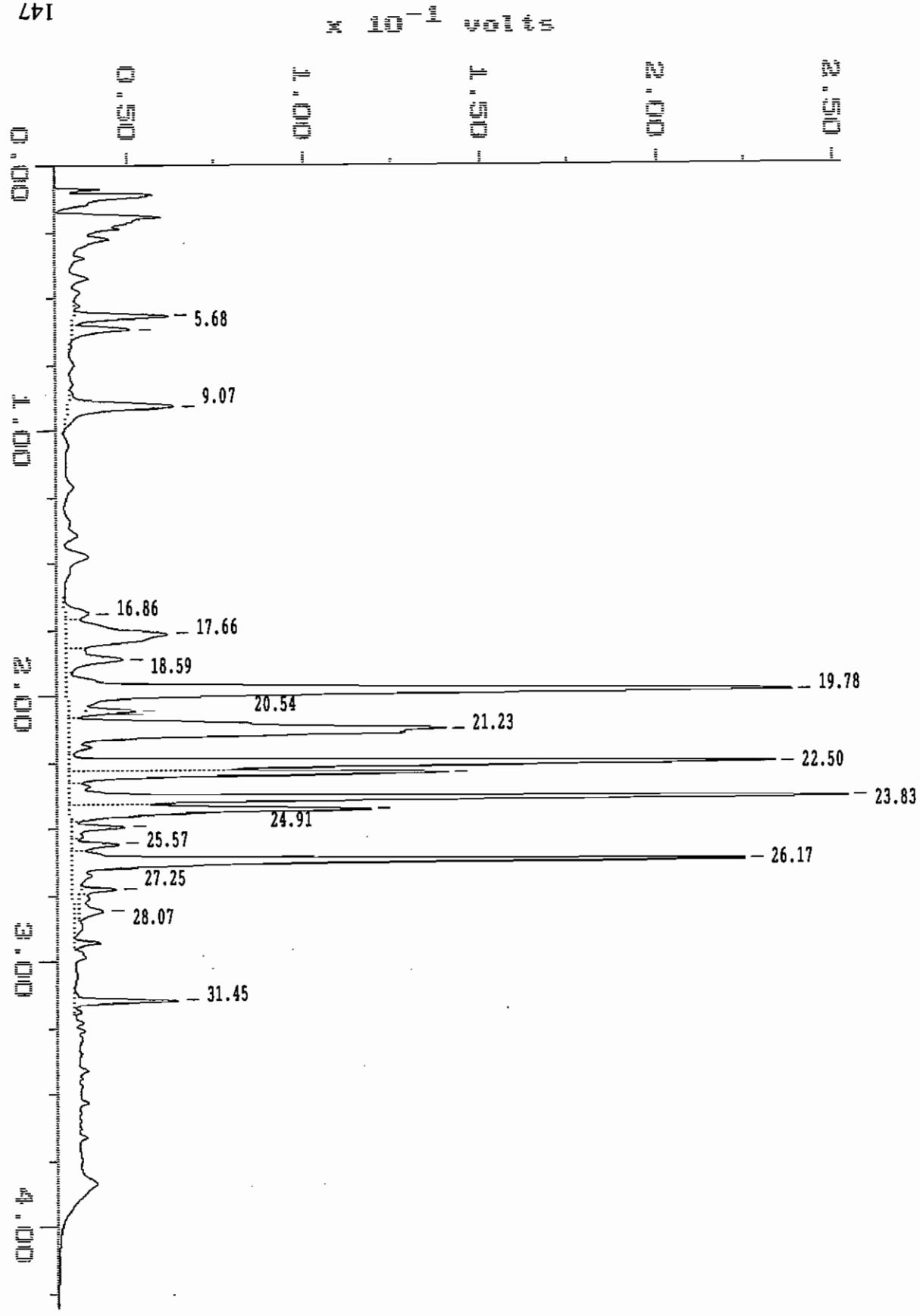
XEROX

Sample: Ian Bally Oil
Acquired: 12-SEP-95 10:58

Channel: Ian Bally oil
Method: C:\NEEM\OILAYR95

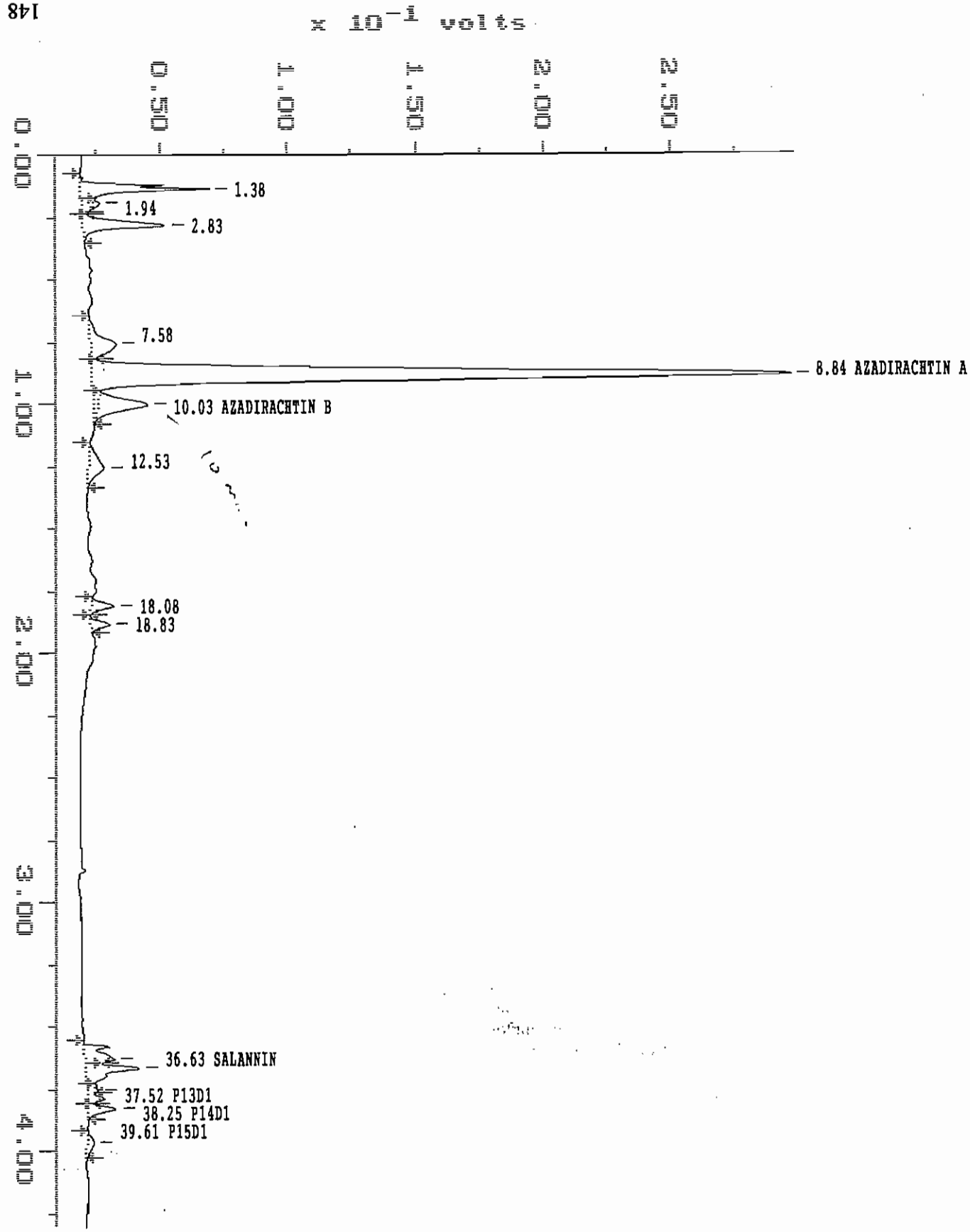
Filename: ayroil_1
Operator: Dav

147



710

Sample: Fraction #9 Channel: 214 Filename: Flsh#2_3
Acquired: 05-OCT-95 12:25 Method: C:\NEEM\TRIALS\FLSHCOL2 Operator: Dav



APPENDIX 9

NRA TRIAL PERMIT

National Registration Authority
FOR AGRICULTURAL & VETERINARY CHEMICALS

First Floor, Industry House, National Circuit, Barton ACT
PO Box 240, Queen Victoria Terrace ACT 2600
Tel: (06) 272 5158 Fax: (06) 272 4753

PERMIT

TO ALLOW THE CONDUCT OF SMALL SCALE TRIALS

Permit Number - TPM0001A

General

This permit, issued under the Agvet Codes, allows any person listed in ***1. Person(s)*** and of those jurisdictions listed in ***2. State(s)/Area(s)*** to have the products listed in ***3. Product(s)/Active(s)*** in their possesion or custody and use these product(s) for the purposes of conducting research in small scale trials as outlined in ***4. Small Scale Trials.*** If this permit were not issued possession or custody of these products (if unregistered or unapproved) and their use in the manner outlined below would constitute an offence under the Agvet Codes.

The persons listed in ***1. Person(s)*** must comply with all conditions listed in ***CONDITIONS OF PERMIT*** to be effectively covered by this permit

This permit is effective from 15 MARCH 1995 until suspended or cancelled.

DETAILS OF PERMIT

1. Persons

All persons who are trained or experienced in the handling and use of agvet chemicals and who handle and use agvet chemicals as part of their normal duties in their employment for the research facility, the company or organisation for which they are conducting a trial;

2. State(s)/Area(s)

All states and territories.

3. Products/Actives

Any active constituent or chemical product, not including:

- genetically manipulated organisms (GMOs); or
- veterinary biologicals used outside the confines of a research facility; or
- any active constituent or chemical product where the trial is conducted in a state where that active constituent or chemical product is proscribed by legislation;

4. Small Scale Trials

Small scale trials include:

(i) screening tests, laboratory assessment and other research conducted within the confines of a research facility. (A research facility includes research station, research laboratory, research glasshouse, veterinary surgery or hospital, university or similar institution); or

(ii) trials conducted to generate data relating to efficacy, residues, crop or animal safety or other scientific information on small plots outside the confines of a research facility where the size of the trial, under the control of a person, does not exceed the following:

a. a total of 1 hectare (100m x 100m) in any one state, or a total of 5 hectares in all states, in the case of a major crop such as a cereal crop; or
b. a total of 225 sq. metres (15m x 15m) in any one state, or a total of 2 hectare in all states, in the case of a crop other than a major crop; or
c. 50 fruit trees or vines in any one state; or
d. a total of 100 cattle, pigs, or deer; 1000 sheep or goats; or 2000 poultry; or 100 other non-food species.

CONDITIONS OF PERMIT

1. Disposal of any produce from plants and animals treated during the trials cannot be done in a manner that can result in direct or indirect consumption of this produce by humans.

2. All trials involving animals must comply with conditions laid down in animal welfare legislation or guidelines which are applicable in the state where trials are conducted;

3. Detailed records must be maintained listing
   a. the date the trials are conducted;
   b. for trials conducted within the confines of a research facility, the name and address of the research facility; for trials conducted outside the confines of a research facility, the state and specific location of that state in which trials are conducted;
   c. the trial details, including crops or animals treated, the pest controlled or reason for treating, the rates and frequency of application;
   d. the active constituents or chemical products used plus the total amounts used;
   e. the method of disposal of produce from treated plants or animals; and
   f. the names of the persons conducting or controlling the trials.

4. These detailed records of each trial must be maintained for a period not less than 2 years from the date of commencing each trial and be made available to the NRA upon request by the National Manager.

Authorised by

(GN HOOPER) 15/3/95
Executive Manager, Registration.

NOTE

The NRA, in considering an application for a permit, must evaluate the permit against criteria set out in the Agvet Codes. The NRA issues a permit if it is satisfied that by issuing the permit the use would:

- not be an undue hazard to users of the products;
- not be likely to have an unintended direct effect or indirect effect (residues) that is harmful to humans;
- not be likely to have an unintended effect that is harmful to animals, plants or to the environment;
- not unduly prejudice trade;
- be effective for the intended purpose.

In making a decision, whether to issue a permit, the NRA must often balance the need for the permit against known and uncertain scientific and other factors.

Persons using actives or products under a permit issued by the NRA must make their own judgement as to the suitability and effectiveness of the chemicals for that use, and do so at their own risk.

APPENDIX 12

PROBIT ANALYSES OF AZA PER ANIMAL / CONTROL DATA FOR 1, 2, AND 3 DAYS AFTER TREATMENT

Trans dose	dose	Obs Number used	Est Number respond	Contrib Number respond	diff	to chi2	
0.005-2.301		100	93	74.5	-18.5	17.96	
0.010-2.000		100	67	80.5	13.5	11.70	
0.050-1.301		100	82	90.8	8.8	9.23	
0.750-0.125		100	99	98.3			
0.750-0.125		100	99	98.3			
1.500 0.176		100	99	99.0	3.7	4.63	Pooled
1.500 0.176		100	100	99.0			
3.000 0.477		100	100	99.4			
3.000 0.477		100	100	99.4			

Snw = 182.0 Snwx = -260.3831 Snwy = 1136.077
1/Snw = 0.005493756 Mean x = -0.5051 Mean y = 6.9677
Snwx2 = 116.48873 Snwxy = 112.3125 Snwy2 = 116.4887

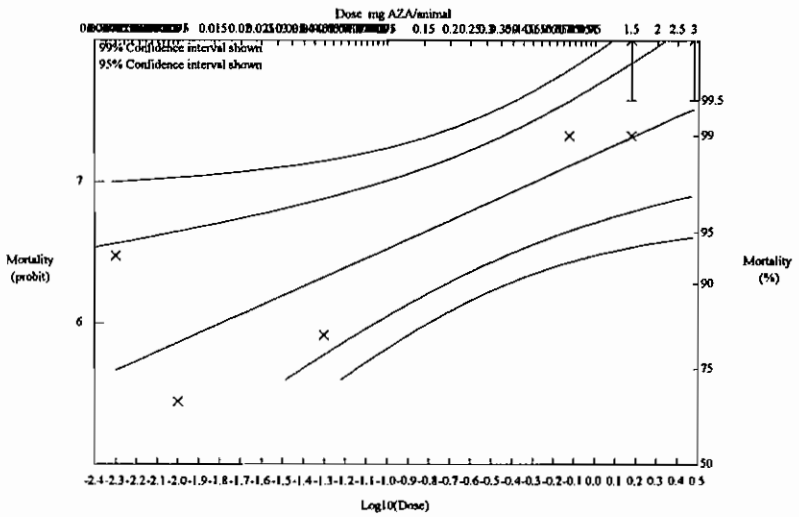
Chi Squared based on pooling = 43.52 with 2 degrees of freedom P = 0.
Chi Squared based on all data = 41.50 with 7 degrees of freedom P = 0.

Equation is b = 0.6677 Y = 7.1964 + 0.6677x

Variance of b V(b) = 0.035 Therefor b = 0.668± 0.188
Heterogeneity factor = 5.9280
Heterogeneity factor exceeds 1, Variances corrected
!! Warning !! this is only valid if there is no systematic variation

Response level	lower CI 99%	lower CI 95%	EDx	upperCI 95%	upperCI 99%	
ED .00001	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .0001	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .001	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .01	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .1	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .5	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED 1	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED 5	0.000	0.000	0.000	0.000	0.001	Extrapolated
ED 10	0.000	0.000	0.000	0.001	0.002	Extrapolated
ED 25	0.000	0.000	0.000	0.002	0.005	Extrapolated
ED 50	0.000	0.000	0.001	0.009	0.018	Extrapolated
ED 75	0.000	0.000	0.005	0.039	0.072	
ED 90	0.000	0.000	0.043	0.193	0.381	
ED 95	0.000	0.010	0.149	0.721	4.650	
ED 99	0.161	0.359	1.568	88.340	inf	
ED 99.5	0.412	0.759	3.706	912.694	inf	Extrapolated
ED 99.9	1.507	2.766	21.81[illegible]	4704.488	inf	Extrapolated
ED 99.99	5.437	11.470	190[illegible]	2862.476	inf	Extrapolated
ED 99.999	15.283	37.541	253.373	inf	inf	Extrapolated
ED 99.9999	37.503	106.656	754.873	inf	inf	Extrapolated
ED 99.99999	84.046	274.371	431.331	inf	inf	Extrapolated

CI: Confidence limits ED: Effective dose (=Lethal dose LD)



Trans dose	dose	Obs Number used	Est Number respond	Contrib Number respond	diff	to chi2	
0.005-2.301		100	33	42.3	9.3	3.53	
0.010-2.000		100	59	51.3	-7.7	2.39	
0.050-1.301		100	75	71.1	-3.9	0.73	
0.750-0.125		100	96	92.5	-3.5	1.74	
0.750-0.125		100	92	92.5	0.5	0.04	
1.500	0.176	100	91	95.2	4.2	3.95	
1.500	0.176	100	93	95.2			
3.000	0.477	100	97	97.1	-0.4	0.02	Pooled
3.000	0.477	100	99	97.1			

Snw = 315.8 Snwx = -330.3416 Snwy = 1915.343
1/Snw = 0.003166888 Mean x = -0.5051 Mean y = 6.1448
Snwx2 = 210.97377 Snwxy = 260.9392 Snwy2 = 210.9738

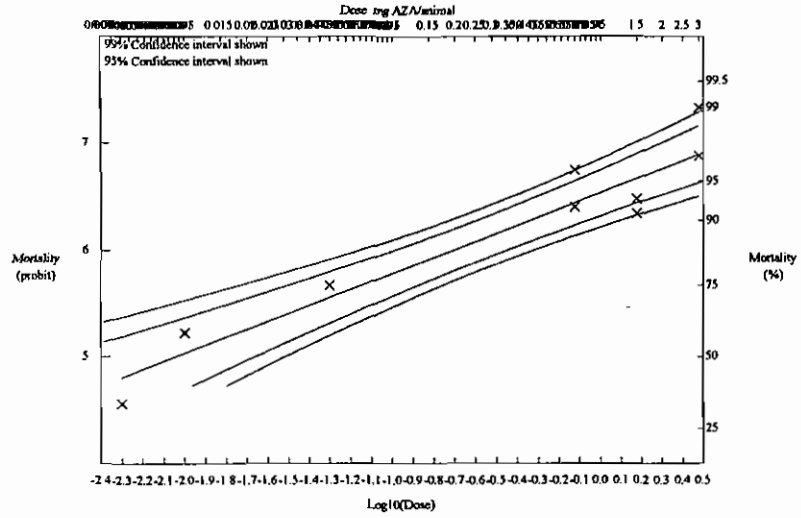
Chi Squared based on pooling = 12.40 with 5 degrees of freedom P = 0.
Chi Squared based on all data = 14.75 with 7 degrees of freedom P = 0.

Equation is b = 0.7520 Y = 6.5357 + 0.752x

Variance of b V(b) = 0.006 Therefor b = 0.752± 0.078
Heterogeneity factor = 2.1072
Heterogeneity factor exceeds 1, Variances corrected
!! Warning !! this is only valid if there is no systematic variation

Response level	lower CI 99%	lower CI 95%	EDx	upperCI 95%	upperCI 99%	
ED .00001	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .0001	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .001	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .01	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .1	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .5	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED 1	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED 5	0.000	0.000	0.000	0.000	0.001	Extrapolated
ED 10	0.000	0.000	0.000	0.001	0.002	Extrapolated
ED 25	0.000	0.000	0.001	0.004	0.006	Extrapolated
ED 50	0.001	0.002	0.009	0.022	0.031	
ED 75	0.019	0.032	0.072	0.134	0.176	
ED 90	0.188	0.254	0.460	0.874	1.264	
ED 95	0.567	0.746	1.398	3.176	5.431	
ED 99	3.311	4.642	11.272	43.280	113.859	Extrapolated
ED 99.5	6.046	8.815	24.195	115.780	362.156	Extrapolated
ED 99.9	20.310	32.420	116.852	897.883	4054.328	Extrapolated
ED 99.99	86.819	156.307	800.852	11187.00	79876.691	Extrapolated
ED 99.999	302.590	607.312	5258.50	190790.	1995744.664	Extrapolated
ED 99.9999	919.880	2038.550	000.	0723604	27544.363	Extrapolated
ED 99.99992	530.488	144.732	406.	3287	21551.198	Extrapolated

CI: Confidence limits ED: Effective dose (=Lethal dose LD)



Trans dose	dose	Obs Number used	Est Number respond	Contrib Number respond	diff	to chi2
0.005-2.301		100	13	7.8	-5.2	3.69
0.010-2.000		100	0	13.3	13.3	15.39
0.050-1.301		100	43	34.4	-8.6	3.27
0.750-0.125		100	75	78.6	3.6	0.76
0.750-0.125		100	93	78.6	-14.4	12.36
1.500 0.176		100	75	86.4	11.4	10.99
1.500 0.176		100	91	86.4	-4.6	1.82
3.000 0.477		100	84	92.0	8.0	8.58
3.000 0.477		100	97	92.0	-5.0	3.43

Snw = 372.3 Snwx = -196.3939 Snwy = 2004.029
1/Snw = 0.002686234 Mean x = -0.5051 Mean y = 5.3877
Snwx2 = 403.83772 Snwxy = 338.6399 Snwy2 = 403.8377

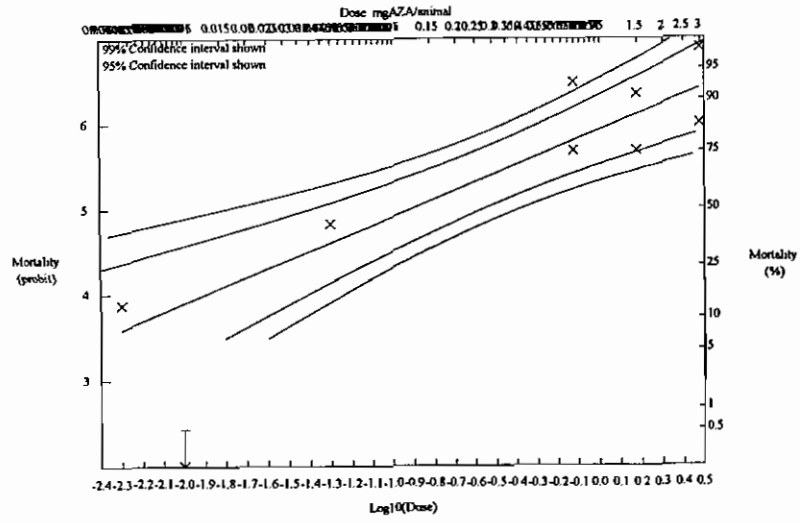
Chi Squared based on pooling = 60.29 with 7 degrees of freedom P = 0.
Chi Squared based on all data = 60.26 with 7 degrees of freedom P = 0.

Equation is b = 1.0146 Y = 5.9185 + 1.0146x

Variance of b V(b) = 0.026 Therefor b = 1.015± 0.161
Heterogeneity factor = 8.6079
Heterogeneity factor exceeds 1, Variances corrected
!! Warning !! this is only valid if there is no systematic variation

Response level	lower CI 99%	lower CI 95%	EDx	upperCI 95%	upperCI 99%	
ED .00001	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .0001	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .001	0.000	0.000	0.000	0.000	0.000	Extrapolated
ED .01	0.000	0.000	0.000	0.000	0.001	Extrapolated
ED .1	0.000	0.000	0.000	0.001	0.002	Extrapolated
ED .5	0.000	0.000	0.000	0.003	0.005	Extrapolated
ED 1	0.000	0.000	0.001	0.004	0.007	Extrapolated
ED 5	0.000	0.000	0.003	0.013	0.021	Extrapolated
ED 10	0.000	0.001	0.007	0.024	0.037	
ED 25	0.001	0.005	0.027	0.072	0.104	
ED 50	0.016	0.041	0.124	0.282	0.421	
ED 75	0.163	0.253	0.574	1.590	3.479	
ED 90	0.639	0.918	2.280	10.827	47.401	
ED 95	1.244	1.822	5.202	37.151	263.489	Extrapolated
ED 99	3.863	6.138	24.433	402.546	7381.741	Extrapolated
ED 99.5	5.736	9.460	43.037	974.622	25481.820	Extrapolated
ED 99.9	12.743	22.822	138.271	6100.186	333367.530	Extrapolated
ED 99.99	33.173	66.135	575.825	60113.707	7071547.966	Extrapolated
ED 99.999	75.373	165.481	986.821	139812.282	22401.964	Extrapolated
ED 99.9999	156.340	374.805	019.221	975386.228	3993.820	Extrapolated
ED 99.99999	303.426	789.016	556.122	31431.141	inf	Extrapolated

CI: Confidence limits ED: Effective dose (=Lethal dose LD)



PLATES

Plates 1-3

Self Applicator in field use

Plates 4-5
Self Applicator
in field use

Plate 6. Experimental field station at "Ikawana", Peak Crossing

Plate 7. Control property with untreated cattle

Plate 8. Solar powered electric fence system at "Ikawana"

Plate 9. Presentation of molasses in drum feeder at "Ikawana"

Plate 10. Experimental (treated) cattle at "Ikawana"

Plate 11. Cattle race and weighing platform at "Ikawana"

Plate 12. Counting buffalo flies on cattle at "Ikawana"

Plate 13. Fly count written down on pro-forma

Plate 14. Untreated cattle at control property