Onderstepoort J. vet. Res. 45, 155–163 (1978)

THE EFFICACY OF VARIOUS INSECTICIDES AGAINST THE LARVAE OF *LUCILIA CUPRINA* (WIED.), THE GREEN BLOWFLY OF SHEEP. I. *IN VITRO* TESTS USING A RESISTANT AND SUSCEPTIBLE STRAIN

J. H. VILJOEN, Veterinary Research Institute, Onderstepoort, 0110

ABSTRACT

VILJOEN, J. H., 1978. The efficacy of various insecticides against the larvae of *Lucilia cuprina* (Wied.), the green blowfly of sheep. I. *In vitro* tests using a resistant and susceptible strain. *Onderstepoort Journal of Veterinary Research* 45, 155–163 (1978).

In order to determine the larvicidal action of different insecticides against both susceptible and resistant blowfly strains in the Republic of South Africa, an attempt was made to find a suitable *in vitro* method for mortality counts and potency evaluations. After a series of preliminary trials the Australian method of Roxburgh & Shanahan (1973) was adopted as the most reliable for this purpose. A statistical analysis by computer of the basic data at Onderstepoort established the superiority of the LC50 value in biological assays over the LC99 or the closely related minimum lethal concentration (MLC).

The insecticidal action of different blowfly compounds was evaluated for relative potency against both susceptible and resistant blowfly strains by comparing their LC50 values in their logarithmic form. Comparisons are fully justifiable where the individual regression coefficients of the insecticides under test do not deviate significantly from a common slope initially calculated (Finney, 1971). A diazinon formulation proved the most potent against susceptible blowflies and fenthion ethyl against the resistant strain from Riversdale. Relative insecticidal potencies varied from 0,07–0,62 in the former compound and from 0,35–0,66 in the latter.

Résumé

EFFICACITÉ DE DIVERS INSECTICIDES CONTRE LA LARVE DE LA MOUCHE VERTE DU MOUTON, LUCILIA CUPRINA *(WIED.). I. ESSAIS* IN VITRO *SUR DES SOUCHES RÉSISTANTES ET SUSCEPTIBLES*

Afin de déterminer l'action larvicide de divers insecticides sur des souches susceptibles et des souches résistantes de la mouche verte du mouton en Afrique du Sud, on a recherché une méthode in vitro *qui permet des comptages de mortalité et des évaluations d'efficacité. Après une série d'essais préliminaires la méthode australienne de Roxburgh et Shanahan* (1973) *a été retenue comme la plus fiable à cet égard. Une analyse statistique par ordinateur des données de base à Onderstepoort a montré que, pour les tests biologiques, le taux LC50 est préférable au LC99 ou à la concentration léthale minimum (MLC) qui en est très proche.*

On a procédé à l'évaluation de l'efficacité relative de divers composés contre des souches susceptibles aussi bien que résistantes de la mouche verte du mouton en comparant leurs valeurs de LC50 sous forme logarithmique. Ces comparaisons sont tout-à-fait justifiées lorsque les coefficients de régression individuels des insecticides ne s'écartent pas significativement d'une pente commune calculée à l'origine (*Finney*, 1971). *Une formule à base de diazinone s'est avérée la plus efficace contre des souches susceptibles; contre la souche résistante de Riversdale, ce fut le fenthion-éthyle. Les efficacités insecticides relatives ont varié de* 0,07 *à* 0,62 *pour le premier composé et de* 0,35 *à* 0,66 *pour le second.*

INTRODUCTION

The period of protection afforded in the field by any insecticidal compound against blowflies depends as much on its larvicidal action as on its ability to diffuse into the fleece of treated sheep (Du Toit & Fiedler, 1953a). Fiedler & Du Toit (1956) proved that certain organophosphorus compounds diffuse along wool fibres and in this respect they compare favourably with certain previously tested chlorinated hydrocarbon insecticides and can be accepted as such. The screening of organophosphorus compounds in the laboratory for larvicidal action is, however, an essential preliminary in the selection of effective insecticides for field evaluation in a country where blowflies constitute a problem.

Laboratory methods for determining the effect of insecticides on blowfly larvae have been described by various authors. In the earlier tests artificial media impregnated with insecticides were used and their toxicity judged by observing growth retardation or rate of mortality in the larval stages (Lennox, 1940; Hoskins, Bloxham & Van Ess, 1940). These artificial media act both as nutrients for the larvae and as diluents for the insecticides, but unfortunately their nutritional level may affect the toxicity of the insecticide. In addition, sterilization of these media may accelerate the decomposition of heat-labile compounds.

Received 20 March 1978—Editor

McCulloch (1940) introduced minced beef as a nutrient medium, while Du Toit (1968) also added clean wool to serve as neutral matrix. In both preparations the final concentration of insecticide after introduction to the meat or meat-wool mixture was calculated and the effect on both pupation and first stage larvae determined.

The *in vitro* larvicidal test in which mammalian serum (ovine, bovine or equine) was used both as nutrient medium as well as diluent for the insecticide and in which wool or cotton wool served as the neutral matrix, was described by Hobson (1937), Fiedler & Du Toit (1951) and Du Toit & Fiedler (1953a). This test was subsequently used either in the original or in a modified form by the latter and other workers in various countries (Du Toit & Fiedler, 1953b; Fiedler & Du Toit, 1954; Du Toit & Fiedler, 1954; Fiedler & Du Toit, 1956; Harrison & Johnson, 1961; Greenwood, 1964; Harrison, 1967; Shaw, Page & Blackman, 1968; Shaw & Blackman, 1971; Wood, 1973; Tenquist & Heath, 1975 and Blackman & Baker, 1975). In all these tests the larval mortality counts were time-consuming because the larvae had first to be separated from the neutral matrix in which they had usually become entangled. Results were therefore expressed as either MLC or MAC (=minimum affecting concentration), but rarely as the LC 50.

Busvine & Barnes (1947) exposed various insects to insecticides by confining them for different periods to films whose surfaces consisted of a neutral matrix of

filter-paper impregnated with a volatile solvent with an insecticide. This method was modified by Busvine & Nash (1953) who used a mixture of volatile solvent and a mineral oil to avoid irregular size and deposition of crystals and thereby improved the relation between concentration and mortality. Both Roxburgh & Shanahan (1973) as well as Arnold & Whitten (1975) used the surface exposure technique to test insecticides against blowfly larvae whereby these larvae were exposed for a 24 h period to either insecticide-impregnated chromatography paper or rolled plugs of cellular cellulose fabric. Ovine serum was used as nutrient during the exposure period. By adding lanolin to their volatile solvent Arnold & Whitten (1975) avoided the disadvantage mentioned previously by Busvine & Nash (1953).

In the Republic of South Africa arsenic and coal tar distillates were originally used as blowfly insecticides and Mönnig (1943) concentrated on the last mentioned group to develop a suitable blowfly dressing.

The chlorinated hydrocarbon insecticides, dichlorodiphenyl-trichloro-ethane (DDT) and hexachlorocyclo-hexane (BHC) became available during the mid 1940's. Du Toit (1946) and Du Toit & Goosen (1949) demonstrated the value of DDT, and Du Toit, Goosen & De Kock (1948) showed that BHC afforded even better protection. Some 8 years after their introduction, BHC and especially DDT were largely replaced by the cyclodienes, Aldrin and Dieldrin, because these insecticides gave excellent protection (Fiedler & Du Toit, 1951; Du Toit & Fiedler, 1953a).

However, a change in the response of *Lucilia cuprina* to Dieldrin was recognised for the first time in Australia during late 1957 (Shanahan, 1958). Subsequently, Guneidy & Busvine (1964) reported that this change in response had developed in South Africa in this blowfly, which had undergone genetic changes similar to those of the Australian strain of the species.

Since there is a group relationship between Dieldrin and BHC, the latter was also rendered less effective for the control of the South African blowfly.

The value of organophosphorus insecticides was recognised even before the appearance of the Dieldrin/BHC tolerant blowfly in the Republic. Parathion and EPN-300 proved to be highly effective but were excluded because they are highly toxic to mammals (Du Toit & Fiedler, 1953a). Further tests by Fiedler & Du Toit (1956) and Du Toit (1968) on other group-related organophosphorus insecticides were highly satisfactory and proved them to be superior to Dieldrin Aldrin and BHC.

Organophosphorus tolerance in the sheep blowfly was recognised late in 1965 in Australia (Shanahan & Hart, 1966) and in 1968 in the Republic (Howell, 1970). In the latter case the insecticidal effect of 26 organophosphates and carbamates on blowfly-larvae from Riversdale was compared with that of the reference strain from Onderstepoort, and factors of tolerance or resistance varying from 2–100× were found.

Recent work by Blackman & Baker (1975) confirmed the presence of OP-tolerant or resistant blowflies in the Republic. The change in response varied from slight to moderate with diazinon and even excessive with dichlofenthion and chlorfenvinphos. All calculations were, however, based on the concept of minimum lethal concentrations.

In the present survey the main object was to determine the potency of several different organophosphorus compounds and a pyrethroid against both OP susceptible and resistant strains of blowflies in the Republic. After a statistical analysis of the basic data, the LC 50 values were (i) compared with the associated LC 99 values, (ii) used to illustrate the presence or absence of a significant difference between the LC 50 values of all insecticides when used against the 2 different blowfly strains, and (iii) utilized to determine factors of tolerance or resistance.

Eventually the improved estimations of the log LC 50 values were used to determine the relative potency of insecticides against both blowfly strains.

Materials and Methods

Two strains of *L. cuprina* were used, namely

1. *The R-strain.* This is an OP-resistant strain obtained from 2 adjacent farms in the Riversdale district where resistant blowflies were found originally during 1968. Larvae were collected from infested sheep and reared at Onderstepoort to establish the R-colony.

2. *The S-strain.* This is an OP-susceptible reference strain kept at Onderstepoort.

The 12 different insecticides used in these *in vitro* tests, 8 of which are currently registered as blowfly insecticides in the Republic, are listed in Table 1. Their larvicidal effect was determined by using a slightly modified version of the method of Roxburgh & Shanahan (1973) and following the principles outlined by Busvine & Barnes (1947).

TABLE 1 Insecticides used in larvicidal tests

Active Ingredient/s	Trade Name	Formulation		Company
		%		
Fenthion-ethyl	Lujet	50	EC	Bayer S.A.
Triazophos	Hostathion*	42,8	EC	Hoechst
Chlorfenvinphos (1)	Supona 30	30	EC	Shell Chemical Co.
Chlorfenvinphos (2)	Steladone 30	30	EC	Ciba Geigy
Diazinon (1)	Topclip Blue Shield	20	EC	Ciba Geigy
Diazinon (2)	Dazzel	30	EC	Agricura
Dichlofenthion	Bromfos	25,6	EC	Milborrow & Co.
Chlorfenvinphos and Fenchlorphos	Golden Fleece	72+48	EC	Coopers S.A.
Bromophos-ethyl	Nexa-Jet	50	EC	Hoechst
Quinthiophos	Bacdip NF2	50	EC	Bayer S.A.
Carbophenothion	Trithion*	60	EC	Robert Young & Co., Glasgow
Pyrethroid	RU 24 366*	2,5	EC	Procida

* Not registered as blowfly insecticide in the Republic of S.A.
EC=emulsifiable concentrate

All tests were done from September 1975–June 1976, first on the resistant R-strain and then on the susceptible S-strain. The procedure was as follows:

(1) Eggs were collected on raw sheep's liver during an oviposition period of 2 hours. These eggs were transferred to the bottom of a funnel lined with moist Whatman No. 3MM chromatography paper and covered with a small piece of liver. To prevent possible desiccation of the chromatography paper (especially overnight), the stem of the funnel was plugged with absorbent cotton wool and then immersed in a beaker of water (Fig. 1).

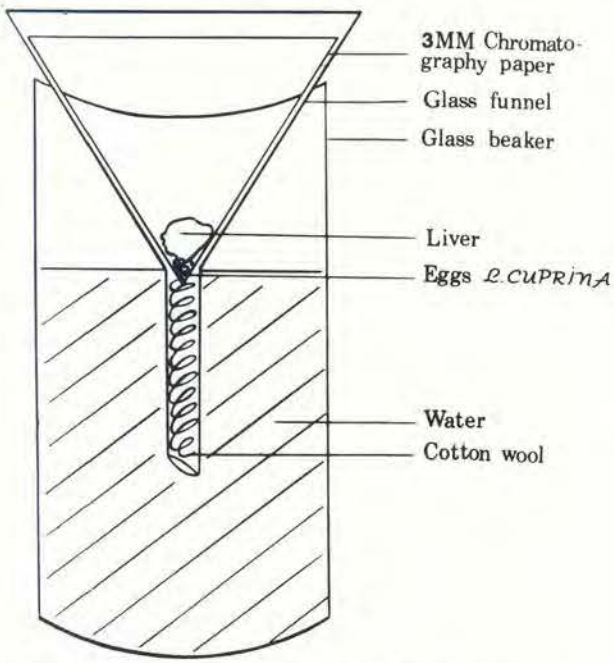

FIG. 1 Apparatus for hatching of *Lucilia cuprina* eggs

(2) Eggs were allowed to hatch during a 12–18 h period and the 1st instar larvae collected from the upper surface of the liver or the sides of the funnel after migration.

(3) Immediately before each test insecticides were diluted in chemically pure acetone to give a range of concentrations of approximately equal logarithmic intervals.

(4) A volume of 1,5 mℓ* of the insecticidal solution in acetone was then applied to 90×40 mm strips of Whatman No. 3MM chromatography paper on a drying board at room temperature. Three strips were prepared for each concentration and 3 with acetone only for the controls.

(5) All the strips were dried for 2 h at room temperature before they were rolled and inserted in flat-bottomed glass tubes of 50×16 mm.

(6) Sterile bovine serum (1,5 mℓ) was then applied to each roll of paper including that of the controls. For even application the glass tubes were held in a horizontal position and slowly rotated.

(7) At least 30 first instar larvae were transferred to each tube and the tubes plugged with open cell plastic bungs. The tubes were left 300 mm beneath a 40 W fluorescent light in a humidity room at 70% R.H. and 28 °C.

* Chromatography paper of the above specified thickness and surface absorbed 1,5 mℓ of the insecticidal solution in acetone during preliminary immersion tests

(8) Mortality was recorded after a period of 24 h in the humidity room. The foam plugs were removed and the tubes as well as their unrolled paper strips thoroughly rinsed with warm water into square perspex counting chambers. Larvae were recorded as dead or alive, according to the method of Lennox (1940).

(9) The percentage mortality for each concentration of both insecticides and the untreated controls was analysed by computer in an existing probit analysis programme written by Dr H. van Ark of the Plant Protection Research Institute and the results used in further calculations.

(10) When the calculated Chi squared (χ^2) value indicated heterogeneity the tests were repeated until more reliable results were obtained.

Statistical Techniques

The results obtained by computer in the existing probit analyses programme were used in the following calculations.

1. The width of the 95% fiducial bands in the probit regression lines of at least 2 insecticidal tests at different levels of response (Finney, 1971).

2. The significance of the difference between LC 50 values of each insecticide after tests with the 2 different blowfly strains (R and S) (Paterson, 1939).

3. Significant differences between LC 50 values and resistant factors. Adaptation of Paterson (1939).

4. The relative potencies of the different insecticides (relative to the best in each series of tests) against both the R and S strains of *L. cuprina.*

The sequence used in potency calculations was identical with that of Finney (1946, 1971), viz:

A. Variance analysis of heterogeneity and parallelism
B. Estimation of a common slope
C. Improved estimation of all probit regression equations
D. Determination of the different Log LC 50 (M) values for the different insecticides and the calculation of relative potencies.

Significance was established by using either the Table of t or χ^2 at P=0,05 and the appropriate number of degrees freedom (Fisher & Yates, 1943).

Results

The results of the original data analysed in the probit analysis programme are given in Tables 2A and 2B. Of the 24 insecticidal tests (12 per blowfly strain) only 5 were repeated a second time, because the data were not homogeneous enough for further calculations.

After the second repetition, another 3 tests were still heterogeneous but their χ^2 values were considered to be within reasonable limits and the heterogeneity factor $h=\chi^2/n-2$ was employed to increase all variances in the existing computer programme. In these tables standard errors at 2 different levels of response as well as their corresponding 95% fiducial limits can be compared.

Fig. 2A and 2B demonstrate the 95% fiducial bandwidth at different levels of response.

In Table 3A and 3B the difference between LC 50 values for different insecticides when used against the 2 different blowfly strains was used to calculate the significance of differences as well as the magnitude of the different resistant factors to denote significance.

TABLE 2A Results of probit analysis by computer (S-strain)

Insecticide	LC 50 p.p.m.	SE LC 50 p.p.m.	95% Fiducial Limits of LC 50 p.p.m.		LC 99 p.p.m.	SE LC 99 p.p.m.	95% Fiducial Limits of LC 99 p.p.m.		χ^2
			Lower	Upper			Lower	Upper	
Diazinon (1)	0,09	0,02	0,05	0,12	2,29	0,89	1,07	4,93	1,719
Triazophos	0,14	0,02	0,11	0,19	3,72	1,21	1,97	7,04	2,400
Fenthion-ethyl	0,17	0,03	0,12	0,23	2,84	1,13	1,30	6,22	4,489
Chlorfenvinphos (2)	0,21	0,3	0,16	0,28	4,69	1,94	2,08	10,58	9,540
Dichlofenthion	0,23	0,03	0,18	0,29	2,97	0,86	1,69	5,23	6,341
Chlorfenvinphos (1)	0,24	0,06	0,11	0,51	4,77	2,85	—	—	14,116*
Diazinon (2)	0,38	0,05	0,30	0,49	3,66	1,10	2,02	6,61	6,184
Chlorfenvinphos + Fenchlorphos	0,39	0,06	0,29	0,52	7,07	2,90	3,17	15,75	7,369
Bromophos-ethyl	0,45	0,05	0,35	0,57	4,95	1,38	2,86	8,57	1,720
Quinthiophos	0,58	0,07	0,45	0,73	10,43	3,05	5,87	18,52	1,230
Carbophenothion	0,94	0,12	0,74	1,21	14,50	4,86	7,52	27,98	7,681
Pyrethroid RU 24366	1,28	0,15	1,01	1,61	13,00	4,49	6,60	25,62	6,502

Heterogeneity value (χ^2)=11,07 at P=0,05 and DF=5

* Heterogeneity established and heterogeneity factor $h=\frac{\chi^2}{n-2}$ used for calculations of variances. 95% Fiducial limits of LC 99 not calculated for chlorfenvinphos (1) because chi-squared value indicated excessive heterogeneity

TABLE 2B Results of probit analysis by computer (R-strain)

Insecticides	LC 50 p.p.m.	SE LC 50 p.p.m.	95% Fiducial Limits of LC 50 p.p.m.		LC 99 p.p.m.	SE LC 99 p.p.m.	95% Fiducial Limits of LC 99 p.p.m.		χ^2
			Lower	Upper			Lower	Upper	
Fenthion-ethyl	3,09	0,26	2,60	3,66	13,52	2,40	9,53	19,17	4,275
Triazophos	4,65	0,38	3,95	5,47	20,51	3,17	15,09	17,64	0,924
Chlorfenvinphos (2)	4,85	0,38	4,14	5,67	18,66	2,70	14,02	24,74	1,795
Diazinon (1)	5,24	0,78	2,92	8,66	17,58	5,40	—	—	14,142*
Dichlofenthion	5,26	0,46	4,46	6,14	22,91	4,21	15,97	32,87	6,280
Chlorfenvinphos + Fenchlorphos	5,41	0,50	4,57	6,40	20,22	3,33	14,62	27,99	2,787
Diazinon (2)	5,53	0,46	4,70	6,51	19,39	2,93	14,42	26,08	0,308
Chlorfenvinphos (1)	5,93	0,45	5,02	6,82	20,84	2,92	15,82	27,45	9,090
Bromophos-ethyl	6,19	0,51	5,26	7,30	24,12	4,23	17,00	33,84	1,727
Quinthiophos	6,61	0,92	3,69	9,84	29,47	9,25	—	—	10,399*
Carbophenothion	8,116	0,56	6,86	9,46	33,46	6,30	23,13	48,44	4,770
Pyrethroid RU 24366	8,854	0,71	7,55	10,38	36,83	7,19	25,09	54,00	6,225

Heterogeneity value (χ^2)=9,49 at P=0,05 and DF=4

* Heterogeneity established and heterogeneity factor $h=\frac{\chi^2}{n-1}$ used for calculation of variances. 95% Fiducial limits of LC 99 not calculated for diazinon (1) and quinthiophos because chi-squared values indicated excessive heterogeneity

TABLE 3A Significance of differences in LC 50 values of insecticides tested against R and S blowfly strains

Insecticide	LC 50 p.p.m.		SE LC 50 p.p.m.		*t Value calculated
	R	S	R	S	
Fenthion-ethyl	3,09	0,17	0,26	0,03	11,15
Triazophos	4,65	0,14	0,38	0,02	11,86
Chlorfenvinphos (2)	4,85	0,21	0,38	0,03	12,21
Diazinon (1)	5,24	0,09	0,78	0,02	6,60
Dichlofenthion	5,26	0,23	0,46	0,03	10,94
Chlorfenvinphos + Fenchlorphos	5,41	0,39	0,50	0,06	9,96
Diazinon (2)	5,53	0,38	0,46	0,05	11,19
Chlorfenvinphos (1)	5,93	0,24	0,45	0,06	12,53
Bromophos-ethyl	6,19	0,45	0,51	0,05	12,10
Quinthiophos	6,61	0,58	0,92	0,07	6,54
Carbophenothion	8,12	0,94	0,56	0,12	12,54
Pyrethroid RU 24366	8,85	1,29	0,71	0,15	10,44

$$* \; t = \frac{LC\ 50\ (R) - LC\ 50\ (S)}{\sqrt{SE\ LC\ 50\ (R)^2 + SE\ LC\ 50\ (S)^2}}$$

with t=2,262 at P=0,05 and DF=9 all calculated t values denote significant differences between R and S-strains

TABLE 3B Significant differences between LC 50 values and resistant factors

Insecticides	Difference between LC 50 values p.p.m.		Factors of resistance (RF)	
	Calculated	Significant *	Calculated **	Significant ***
Fenthion-ethyl	2,92	>0,59	18,7	>4,6
Triazophos	4,51	>0,86	32,3	>6,9
Chlorfenvinphos (2)	4,64	>0,86	23,2	>5,1
Diazinon (1)	5,15	>1,76	60,4	>—
Dichlofenthion	5,03	>1,04	22,7	>5,5
Chlorfenvinphos + Fenchlorphos	5,02	>2,14	13,9	>3,9
Diazinon (2)	5,15	>1,04	14,6	>3,7
Chlorfenvinphos (1)	5,69	>1,02	25,1	>—
Bromophos-ethyl	5,74	>1,59	13,9	>2,3
Quinthiophos	6,03	>2,09	11,5	>—
Carbofenothion	7,18	>1,30	8,7	>3,9
Pyrethroid RU 24366	7,56	>1,64	6,9	>2,3
Mean significant RF value (Org. Phosphates)****				>4,5

* Significant difference = SE_D (SE of difference) $\times$ t (t=2,26 at P=0,05 and DF=9)

** Calculated RF values = $\frac{LC\ 50\ (R)}{LC\ 50\ (S)}$

*** Significant RF-values = $\frac{LC\ 50\ (S) + Significant\ difference}{LC\ 50\ (S)}$

**** Mean significant RF value for organophosphorus insecticides=4,5 (excluding those derived from significant heterogeneous data)

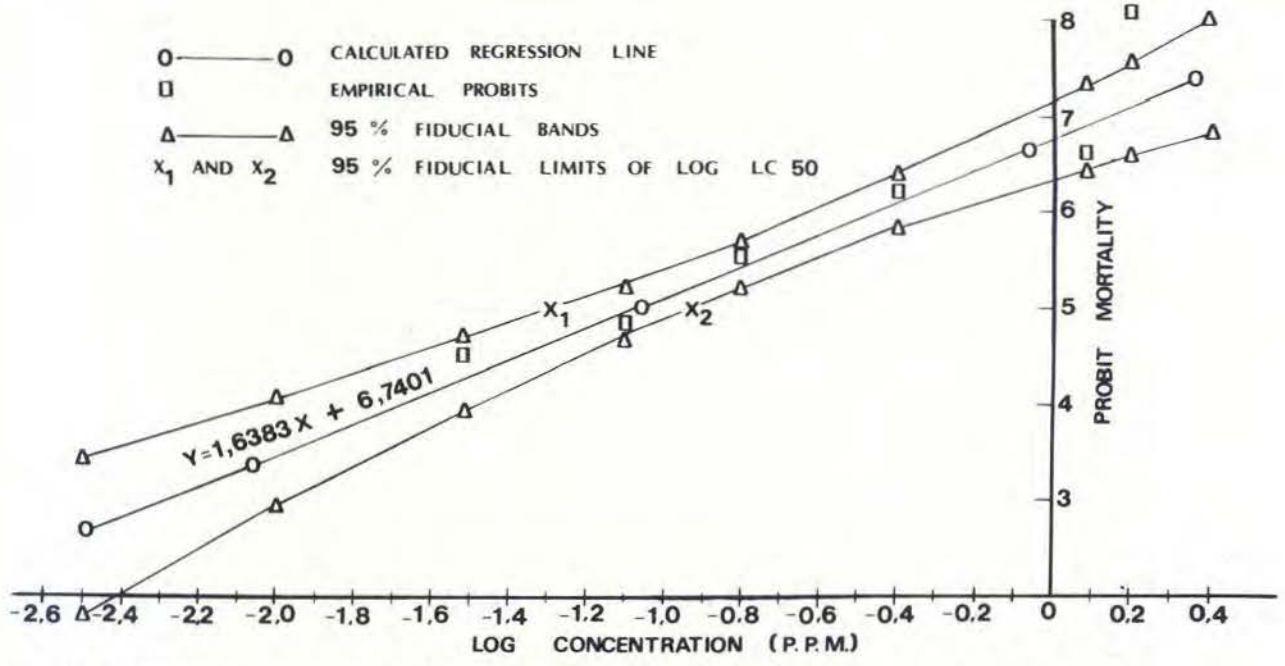

FIG. 2A Probit regression line and 95% fiducial bands for Diazinon (I) versus the S-strain of *Lucilia cuprina*

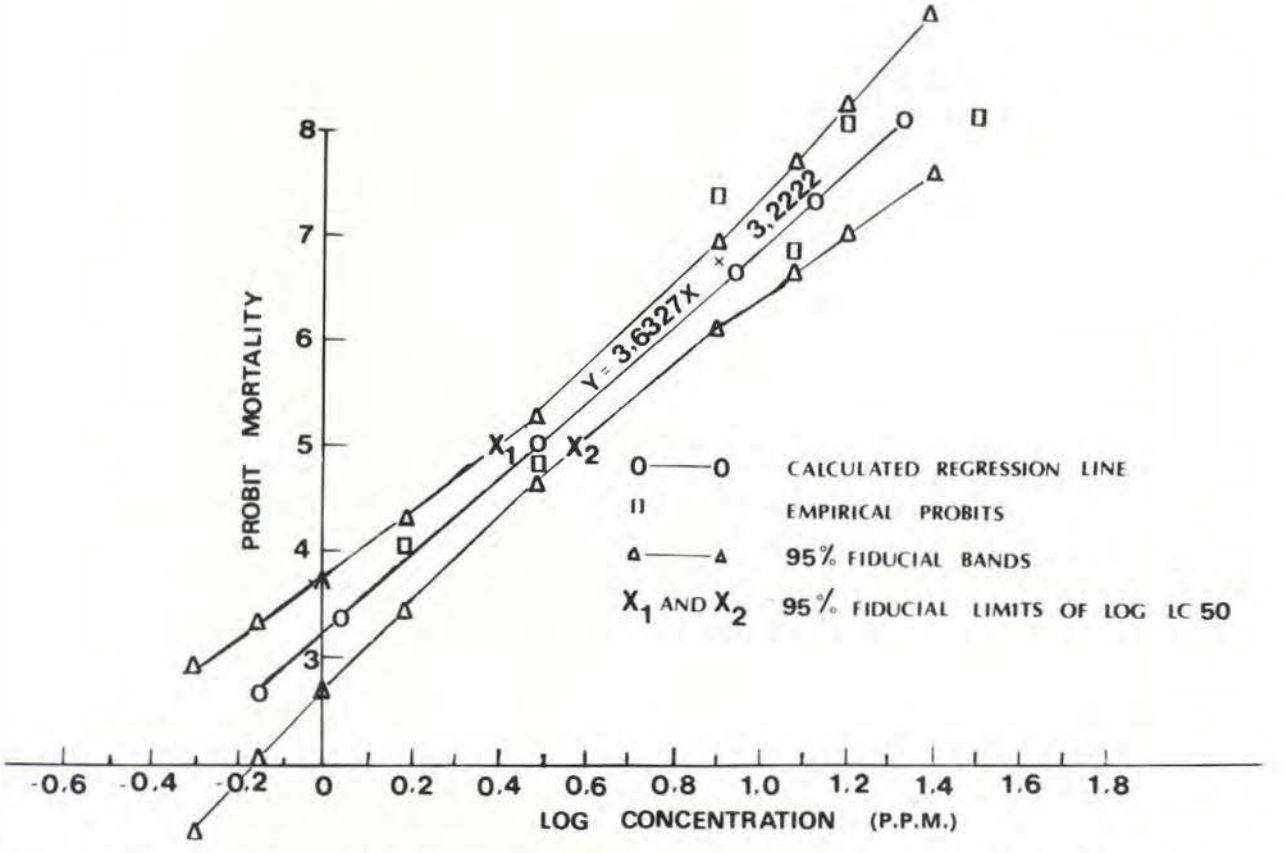

FIG. 2B Probit regression line and 95% fiducial bands for Fenthion ethyl versus the R-strain of *Lucilia cuprina*

In Tables 4A and 4B variancy analyses of the heterogeneity and parallelism of regressions were undertaken for the 2 different experiments and the sum of the squares of both tested for significance as χ^2 values. Both proved to be non-significant at the test level of P=0,05 and the appropriate number of D.F.

As illustrated in Tables 5A and 5B, the potency of the different insecticides (relative to the most potent for each blowfly strain) was calculated from the improved estimations of the regression equations by using the value of a common slope (b).

TABLE 4A Variance analysis of heterogeneity and parallelism of regressions (S-strain) and different insecticides

Variance	Degrees of freedom	Sum of squares
Common slope.....	1	$\left(\frac{(\Sigma Sxy)^2}{\Sigma Sxx}\right)$ =884,0
Deviation from parallelism	11	12,41*
Separate slopes.....	12	$\left(\Sigma\frac{(Sxy)^2}{Sxx}\right)$ =896,41
Heterogeneity.....	60	($\Sigma\chi^2$) = 69,29*
Total.....	72	(Σ Syy) =965,70

TABLE 4B Variance analysis of heterogeneity and parallelism of regressions (R-strain) and different insecticides

Variance	Degrees of freedom	Sum of squares
Common slope.....	1	$\left(\frac{(\Sigma Sxy)^2}{\Sigma Sxx}\right)$ =839,57
Deviation from parallelism	11	4,12*
Separate slopes.....	12	$\left(\Sigma\frac{(Sxy)^2}{Sxx}\right)$ =843,69
Heterogeneity.....	48	($\Sigma\chi^2$) = 53,72*
Total.....	60	(Σ Syy) =897,41

* Non-significant at P=0,05 and appropriate DF (χ^2-test)

TABLE 5A Potency of different insecticides against the S-strain relative to Diazinon (1)

Insecticides	Regression equations		M**	Potency relative to diazinon (1)
	Original (computer)	Improved estimations*		
Diazinon (1).....	y=6,740 1+1,638 3 x	y=6,949 0+**1,906 5** x	−1,022 3	—
Triazophos.....	y=6,388 7+1,649 6 x	y=6,548 2+**1,906 5** x	−0,812 1	0,62
Fenthion-ethyl.....	y=6,475 2+1,886 8 x	y=6,488 8+**1,906 5** x	−0,780 0	0,57
Chlorfenvinphos (2).....	y=6,172 5+1,725 2 x	y=6,290 7+**1,906 5** x	−0,677 0	0,45
Dichlofenthion.....	y=6,335 7+2,102 1 x	y=6,205 8+**1,906 5** x	−0,632 4	0,41
Chlorfenvinphos (1).....	y=6,118 0+1,785 2 x	y=6,195 8+**1,906 5** x	−0,627 2	0,40
Diazinon (2).....	y=5,996 1+2,367 5 x	y=5,804 6+**1,906 5** x	−0,422 0	0,25
Chlorfenvinphos + Fenchlorphos....	y=5,756 8+1,851 9 x	y=5,781 6+**1,906 5** x	−0,410 0	0,24
Bromophos-ethyl.....	y=5,782 3+2,229 5 x	y=5,647 9+**1,906 5** x	−0,339 8	0,21
Quinthiophos.....	y=5,444 1+1,852 0 x	y=5,453 1+**1,906 5** x	−0,237 6	0,16
Carbophenothion.....	y=5,055 2+1,958 6 x	y=5,050 9+**1,906 5** x	−0,026 7	0,10
Pyrethroid RU 24366.....	y=4,753 9+2,312 2 x	y=4,758 7+**1,906 5** x	0,103 6	0,07

* Improved estimation of regression equations $(y)=\bar{y}+b\ (x-\bar{x})$, where $b=\frac{\Sigma Sxy}{\Sigma Sxx}$

** Log LC 50 $(M)=\bar{x}+\frac{5-\bar{y}}{b}$

Common slope $(b)=\frac{\Sigma Sxy}{\Sigma Sxx}=$**1,906 5**

TABLE 5B Potency of different insecticides against the R-strain relative to Fenthion-ethyl

Insecticides	Regression equations		M**	Potency relative to fenthion-ethyl
	Original (computer)	Improved estimations*		
Fenthion-ethyl.....	y=3,222 2+3,632 7 x	y=3,083 3+**3,882 4** x	0,493 7	—
Triazophos.....	y=2,587 8+3,614 3 x	y=2,383 0+**3,882 4** x	0,674 1	0,66
Chlorfenvinphos (2).....	y=2,273 2+3,978 2 x	y=2,346 4+**3,882 4** x	0,683 5	0,65
Diazinon (1).....	y=1,815 1+4,430 1 x	y=2,222 8+**3,882 4** x	0,715 3	0,60
Dichlofenthion.....	y=2,372 1+3,643 8 x	y=2,189 3+**3,882 4** x	0,724 0	0,59
Chlorfenvinphos + Fenchlorphos....	y=2,019 2+4,066 8 x	y=2,161 9+**3,882 4** x	0,731 0	0,58
Diazinon (2).....	y=1,825 5+4,274 6 x	y=2,147 9+**3,882 4** x	0,734 6	0,57
Chlorfenvinphos (1).....	y=1,702 0+4,267 1 x	y=2,037 9+**3,882 4** x	0,763 0	0,54
Bromophos-ethyl.....	y=1,874 3+3,946 8 x	y=1,927 1+**3,882 4** x	0,791 5	0,50
Quinthiophos.....	y=2,055 0+3,589 9 x	y=1,793 6+**3,882 4** x	0,825 9	0,47
Carbophenothion.....	y=1,557 3+3,786 2 x	y=1,465 6+**3,882 4** x	0,910 4	0,38
Pyrethroid RU 24366.....	y=1,434 9+3,764 0 x	y=1,315 0+**3,882 4** x	0,949 2	0,35

* Improved estimations of regression equations $(y)=\bar{y}+b\ (x-\bar{x})$ where $b=\frac{\Sigma Sxy}{\Sigma Sxx}$

** Log LC 50 $(M)=\bar{x}+\frac{5-\bar{y}}{b}$

Common slope $(b)=\frac{\Sigma Sxy}{\Sigma Sxx}=$**3,882 4**

Discussion

In vitro evaluations were done with larvae rather than with adults of the 2 blowfly strains because blowfly control in the veld depends primarily on the prevention of larval myiasis on the living sheep. Larvae are also more resistant to insecticidal action than adult blowflies (Harrison, 1967; Roxburgh & Shanahan, 1973 and Arnold & Whitten, 1975), and their levels of OP susceptibility do not correlate (Arnold & Whitten, 1975). The use of adult flies in these tests could therefore only lead to unreliable results and the insecticidal requirements as to the concentration of blowfly compounds either under- or overestimated.

First instar larvae were used because it is essentially this developmental stage that must be controlled in any blowfly attack and because of a difference in susceptibility between the first and later stages (Du Toit & Fiedler, 1953a).

All insecticidal tests were done by using a slight modification of the method of Roxburgh & Shanahan (1973). Despite the merits of standard films of oil solutions of insecticides on surfaces to be exposed (Busvine & Barnes, 1953), only chemically pure acetone was used for all insecticide solutions because changes in concentration gave fairly sensitive changes in recorded mortality of exposed larvae. Chromatography paper No. 3MM was used as neutral matrix because this facilitates counting and ensures more reliable data of deaths for computer analysis.

Bovine serum was used instead of ovine throughout these experiments since it gave better results in the preliminary trials and was more readily available. The use of specially prepared funnels for the hatching of blowfly eggs prevented desiccation and enabled the larvae to be concentrated.

The statistical calculations for heterogeneity (χ^2 test) showed that only 5 of the original 24 tests were significantly heterogeneous. This high level of reliability proves that the *in vitro* test as employed in these experiments was sufficiently dependable for further consideration.

The superiority of LC 50 over LC 99 values in all bioassays where results were statistically analysed is obvious from the tabulated values of their standard errors and 95% fiducial limits as well as from the width of the 95% fiducial bands as illustrated in Fig. 2A and 2B. This level of response should be preferred in screening tests for candidate insecticides as well as for the calculation of factors of resistance. A minimum lethal concentration (MLC) is even less reliable than a LC 99 value and both are difficult to estimate accurately.

By using the more reliable LC 50 values it was proved statistically that the Riversdale strain of blowflies possesses a marked degree of resistance to the majority of organophosphorus insecticides. Resistance factors vary from 8,7 in carbofenothion (which is not registered for blowfly control in S.A.) to a factor of 60,4 in one of the diazinon formulations. Despite its high factor of resistance the latter formulation is still widely used in the Riversdale area because of the excellent period of protection afforded.

Resistance factors only become significant in the organophosphorus insecticide group where these values exceed the mean value of 4,5 (excluding those derived from significant heterogeneous data). This is in accordance with the statement of Shanahan & Hart (1966) where a factor under 5 was termed a tolerance and not a resistance.

The difference between the 2 chlorfenvinphos formulations in terms of their factors of resistance is not marked, whereas that between the 2 diazinon formulations is exceptionally large and not easy to explain.

The insecticidal action of different blowfly compounds can only be compared on the basis of relative potencies. A common slope must therefore be estimated and the individual slopes of the different insecticides must not depart significantly from this estimation. Another approach, especially where slopes differ considerably (i.e., more than they do in the present experiments) would be to treat relative potency as a function of response level as postulated by Confield (1964).

In the present example the sums of squares of both heterogeneity and deviation from parallelism were tested in a variance analysis for significance as χ^2 values (Tables 4A and 4B). Both proved to be non-significant at P=0,05, and DF=>30 and 11, respectively, for each blowfly strain tested. Potency determinations could therefore be undertaken by using the common regression coefficient (b) to calculate the improved estimations of the different regression equations in each of the R and S series of tests.

In a direct comparison of insecticidal potency as determined in the laboratory it can therefore be stated that for the susceptible blowfly strain of *L. cuprina* (S-strain) and relative to the most potent insecticide tested, namely diazinon (1), potencies varied from 0,07 (pyrethroid RU 24366)—0,62 (triazophos). For the resistant Riversdale strain (R-strain) and relative to the most potent insecticide, namely, fenthion ethyl, potencies varied from 0,35 for the pyrethroid RU 24366—0,66 for triazophos.

At present candidate insecticides for blowfly control are screened at Onderstepoort at an LC 50 value of 10 p.p.m. or at the less reliable LC 99 value of 40 p.p.m. These 2 values represent the poorest performance of any organophosphorus insecticide tested against resistant blowflies from the Riversdale area.

Acknowledgements

I am indebted to Mr G. van der Westhuizen for his expert technical assistance; to Mrs A. L. Viljoen for drawing the graphs; to Mr A. M. du Bruyn for the photography; to Dr C. J. Howell, Head of the Department of Entomology, for valuable suggestions and to the companies concerned for supplying the insecticides for these tests.

References

ARNOLD, J. T. A. & WHITTEN, M. J., 1975. Measurement of resistance in *Lucilia cuprina* larvae and absence of correlation between organophosphorus resistance levels in larvae and adults. *Entomology, Experimental and Applied*, 18, 180–186.

BLACKMAN, G. G. & BAKER, J. A. F., 1975. Resistance of the sheep blowfly *Lucilia cuprina* to insecticides in the Republic of South Africa. *Journal of the South African Veterinary Association* 46, 337–339.

BUSVINE, J. R. & BARNES, S., 1947. Observations on mortality among insects exposed to dry insecticidal films. *Bulletin of Entomological Research*, 38, 81–90.

BUSVINE, J. R. & NASH, R., 1953. The potency and persistence of some new synthetic insecticies. *Bulletin of Entomological Research* 44, 371–376.

CORNFIELD, J., 1964. Comparative bioassay and the role of parallelism. *Journal of Pharmacology* 144, 143–149.

DU TOIT, R. M., 1946. D.D.T. for the protection of sheep against blowfly. *Farming in South Africa* 21, 542–544.

DU TOIT, R., 1968. An improved method of evaluating insecticides for the protection of sheep against blowfly strike. *Journal of the South African Veterinary Medical Association* 39, 77–79.

DU TOIT, R. & FIEDLER, O. G. H., 1953a. The protection of sheep against blowfly strike. I. An evaluation of certain organic insecticides. *Onderstepoort Journal of Veterinary Research* 26, 65–81.

DU TOIT, R. & FIEDLER, O. G. H., 1953b. Report on the value of Isodrin and Endrin as potential protecting agents against blowfly strike in sheep. *Onderstepoort Journal of Veterinary Research* 26, 221–224.

DU TOIT, R. & FIEDLER, O. G. H., 1954. The protection of sheep against blowfly strike. II. The influence of the length of wool at the time of treatment on the duration of protection. *Onderstepoort Journal of Veterinary Research* 26, 405–408.

DU TOIT, R. & GOOSEN, P. J., 1949. D.D.T. for the protection of sheep against blowfly strike. *Onderstepoort Journal of Veterinary Science and Animal Industry* 22, 285–290.

DU TOIT, R., GOOSEN, P. J. & DE KOCK, J. M., 1948. Blowfly strike in sheep: Application of the new insecticides. *Farming in South Africa* 23, 380–382.

FIEDLER, O. G. H. & DU TOIT, R., 1951. A new biological method for evaluating the efficiency of insecticides for the protection of sheep against blowfly strike. *Nature, London*. 168, 608–609.

FIEDLER, O. G. H. & DU TOIT, R., 1954. The protection of sheep against blowfly strike. III. The effect of different formulations of gamma benzene hexachloride (B.H.C.). *Onderstepoort Journal of Veterinary Research* 26, 409–425.

FIEDLER, O. G. H. & DU TOIT, R., 1956. The protection of sheep against blowfly strike. IV. An evaluation of certain organic phosphorus compounds. *Onderstepoort Journal of Veterinary Research*, 27, 77–81.

FINNEY, D. J., 1946. The analysis of a factorial series of insecticide tests. *Annals of Applied Biology*, 33, 160–163.

FINNEY, D. J., 1971. Probit analysis. 3rd ed. Cambridge University Press. 333 pp.

FISHER, R. A. & YATES, F., 1943. Statistical tables for biological, agricultural and medical research. 2nd ed. Edinburgh: Oliver & Boyd.

GREENWOOD, E. S., 1964. Fly strike control in sheep—an evaluation of the efficiency and persistence of available dip preparations. *New Zealand Journal of Agricultural Research* 7, 375–381.

GUNEIDY, A. M. & BUSVINE, J. R., 1964. Genetic studies of dieldrin resistance in *Musca domestica* L. and *Lucilia cuprina* (Wied.). *Bulletin of Entomological Research* 55, 499–507.

HARRISON, I. R., 1967. The development of organophosphorus insecticide resistance in the Australian sheep blowfly, *Lucilia cuprina* (Wied.). *Veterinary Record* 30, 205–206.

HARRISON, I. R. & JOHNSON, C. A., 1961. The depletion of insecticides on sheep fleece. *Annals of Applied Biology* 49, 588–600.

HOBSON, R. P., 1937. Sheep blowfly investigations. VI. Toxicity of stomach poisons to sheep maggots. *Annals of Applied Biology* 24, 808–814.

HOSKINS, W. M., BLOXHAM, H. P. & VAN ESS, M. W., 1940. The insecticidal effects of organic compounds. I. Toxicity of sulfur and nitrogen compounds to fleshfly larvae. *Journal of Economic Entomology* 33, 875–881.

HOWELL, C. J., 1970. *Entomologiese berigte. II. Brommerweerstand.* Resistance of blowflies to insecticides. *Journal of the South African Veterinary Medical Association* 41, 73–74.

LENNOX, F. G., 1940. Studies on the physiology and toxicology of blowflies. 2. The action of stomach larvicides on *Lucilia cuprina*. *Pamphlet of the Council for Scientific and Industrial Research, Australia* 101, 9–49.

McCULLOCH, R. N., 1940. Observations on the toxicity of some insecticides as stomach poisons to blowfly maggots. *The Journal of the Australian Institute of Agricultural Science* 6, 105–108.

MÖNNIG, H. O., 1943. Sheep blowfly research. VI. The treatment of myiasis. *Onderstepoort Journal of Veterinary Science and Animal Industry* 18, 73–84.

PATERSON, D. D., 1939. Statistical techniques in Agricultural research. 1st ed. McGraw-Hill Book Company Inc. New York and London, 263 pp.

ROXBURGH, N. A. & SHANAHAN, G. J., 1973. A method for the detection and measurement of insecticide resistance in larvae of *Lucilia cuprina* (Wied.) (Dipt., Calliphoridae). *Bulletin of Entomological Research* 63, 99–102.

SHANAHAN, G. J., 1958. Resistance to dieldrin in *Lucilia cuprina* Wied., the Australian sheep blowfly. *Nature, London* 181, 860–861.

SHANAHAN, G. J. & HART, R. J., 1966. Changes in response of *Lucilia cuprina* Wied. to organophosphorus insecticides in Australia. *Nature, London* 212, 1466–1467.

SHAW, R. D. & BLACKMAN, G. G., 1971. A method of measuring susceptibility to insecticides of larvae of the sheep blowfly, *Lucilia* spp. *Australian Veterinary Journal* 47, 268–271.

SHAW, R. D., PAGE, K. W. & BLACKMAN, G. G., 1968. Dieldrin resistance in *Lucilia sericata* (Meig.) from Co. Dublin, Eire. *Veterinary Record* 81, 755–757.

TENQUIST, J. D. & HEATH, A. C. G., 1975. Sheep dipping compounds for blowfly control: a note. *New Zealand Journal of Experimental Agriculture* 3, 191.

WOOD, J. C., 1973. 1.1-Bis (P-ethoxyphenyl)-2-nitropropane, a new insecticide for the control of flies on domestic animals. *Proceedings of the 7th British Insecticide and Fungicide Conference* (1973), 273–279.