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THE PROTECTION OF SHEEP AGAINST BLOWFLY STRIKE.
I.—AN EVALUATION OF CERTAIN ORGANIC INSECTICIDES.

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A. INTRODUCTION.

With the development of the new synthetic insecticides a method now exists for the long term and effective protection of sheep against blowfly strike. The value of a number of these compounds in this field has been demonstrated in the Union of South Africa (Du Toit *et al.*, 1948, 1949) as well as in many other countries. Waterhouse and Scott (1950) in Australia and Stones (1951) in England, found that some of these insecticides, such as D.D.T. and B.H.C., exert a twofold action upon the dipterous parasites. They act both as insecticides and repellents for the gravid females as well as possessing larvicidal properties against the hatching parasites.

Previous experiments have shown that the repellent properties of such insecticides as D.D.T. are not of great practical significance. It was clearly demonstrated in the course of many tests that the larvicidal action of all of these new insecticidal compounds persisted long after their repellent properties against the adult flies had disappeared. In view of the transitory nature of the repellent properties of an insecticidal compound it is held that its long term larvicidal potency is the only reliable criterion, therefore, by which such a compound can be judged as a protecting agent against blowfly strike in sheep. Furthermore, the residual effect and stability of the insecticide in the wool will determine its value for practical use.

A number of the synthetic insecticides readily available to the public have been tested in order to determine the compound most suitable for long-term protection of sheep. Throughout the course of these experiments special emphasis has been laid upon the property of larvicidal action and the behaviour of the insecticides in the wool of the living sheep. With the single exception of Fluoro-D.D.T., which was available in the form of an emulsifiable concentrate only, (Gix), all the compounds tested were used in the form of wettable or dispersable powders in order to exclude the possible insecticidal action of any organic solvent.

B. EFFECT OF CONTACT OF SHORT DURATION ON FIRST INSTAR LARVAE.

In the first series of experiments freshly hatched larvae of *Lucilia cuprina*, Wied., were placed in contact with the insecticides for short periods with the object of establishing their mode of action and in order to compare the susceptibility of first instar with fully grown larvae which had been tested in previous trials (Du Toit and Fiedler, 1952).

1. *Method.*

Freshly-emerged first instar larvae were washed from pieces of beef, on which they were cultured, into luke-warm water in a conical measuring cylinder. The

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larvae sink to the bottom of the cylinder leaving the egg shells floating on the surface and can be lifted readily by means of a pipette fitted with a rubber suction teat. By means of the pipette approximately 100-200 larvae were transferred to a disk of moistened filter paper 1 inch in diameter placed on the surface of a sintered glass filter, porosity 2×2. The filter was connected to a suction pump which drew off all superfluous fluid through the filter paper and horizontal sintered glass rapidly. Six drops of the insecticidal suspension under test were then distributed over the larvae by means of a pipette. As soon as all surplus fluid had been removed, in about 5 seconds the filter paper containing the larvae was lifted by means of forceps, and inverted on to a piece of lean beef in a glass jar so as to bring the larvae into close contact with the nutritional medium. The jars containing the meat were covered with fine gauze and incubated at a temperature of $\pm 24°$ C. ($\pm 75°$ F.). The maggots were examined at intervals of 24, 48 and 72 hours.

The young larvae left the filter paper immediately and commenced feeding upon the meat. By the method described the maggots remained in contact with the insecticide for only a very short period, usually less than two minutes. Thereafter adherent particles of insecticide were removed mechanically by contact between the larvae and the juicy surface of the meat.

Each series of tests was repeated seven times and the average mortality noted.

2. *Results.*

The 12 insecticides tested at various serial dilutions displayed significant differences in larvicidal properties as shown in Figure 1.

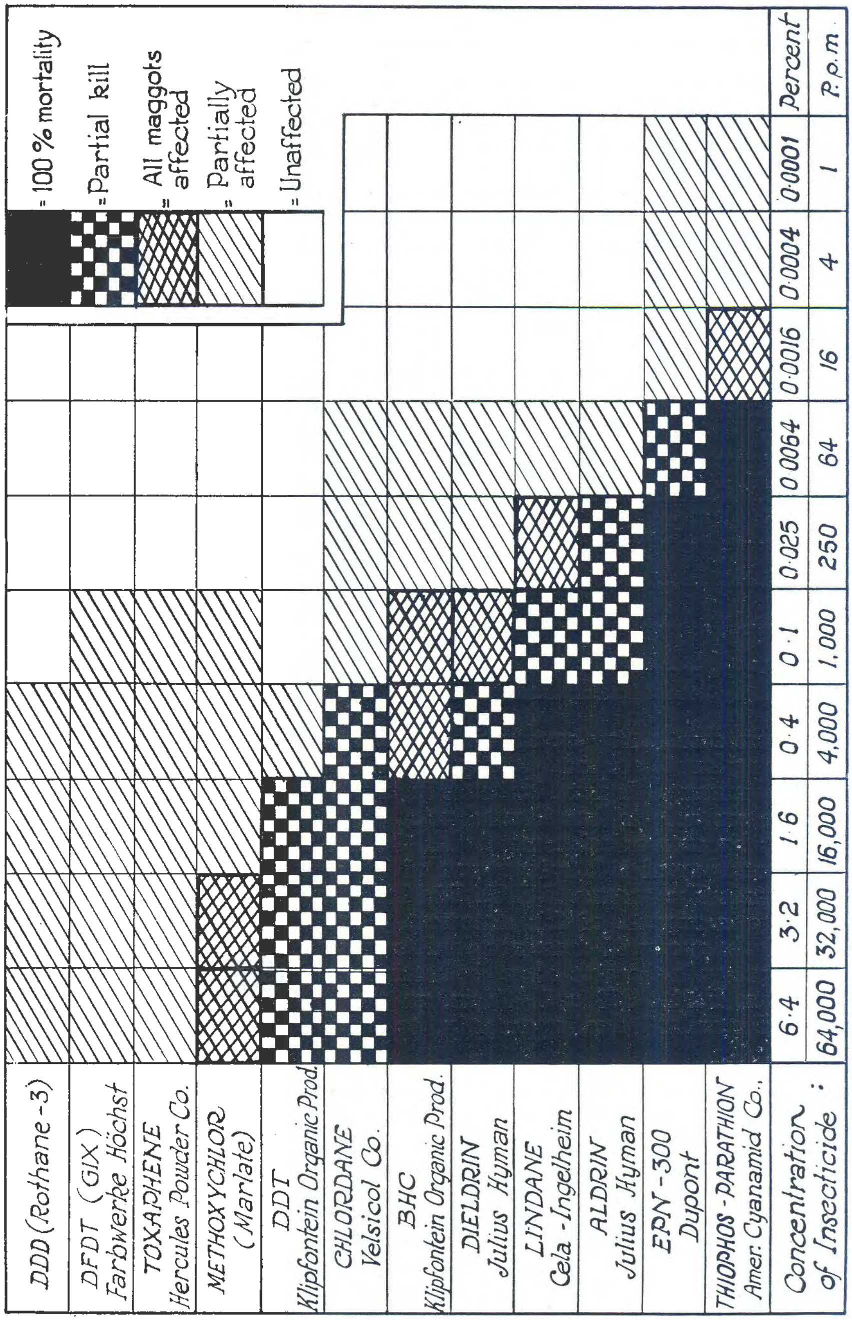
Thiophos-parathion, E.P.N. 300, Aldrin and Lindane, Dieldrin and B.H.C. were the only insecticides which destroyed all the exposed larvae, their relative effectiveness, as determined by the minimum lethal concentration, being in that descending order. Thiophos was fully larvicidal in as high a dilution as 64 p.p.m. whereas Dieldrin and Gamma B.H.C. required 16,000 p.p.m. to produce the same effect. In a dilution of 16 p.p.m. Thiophos retarded the development of all the larvae and even affected some larvae at 1 p.p.m., whereas 1,000 and 64 p.p.m. respectively of Gamma B.H.C. were required to produce the same results. Of the other insecticides only Chlordane and D.D.T. were capable of destroying a percentage of the larvae in any of the concentrations tested, e.g. from 4,000 to 64,000 p.p.m. Toxaphene, DFDT and DDD (T.D.E.) were found to be capable of doing no more than retard the rate of development of larvae at concentrations of from about 1,000 p.p.m. upwards. Below that concentration they appeared to be completely inactive.

Comment.—The two proprietary formulations of B.H.C. included in these tests yielded different results. The wettable powder produced by Klipfontein Organic Products, normally used as a dipwash for the destruction of ticks on livestock, contains the gamma isomer in the form of technical B.H.C. bound to clay particles of fairly large dimensions characterized by fair suspensibility. The Cela-Ingelheim product consists of pure Lindane held in suspension by kaolin particles to which a special wetting agent is added to give a suspension with physical properties usually associated only with emulsions. It would appear, therefore, that the greater larvicidal activity of the latter product is due to the finer particle size and superior suspensibility.

The complete lack of larvicidal action with contact of short duration of some of the insecticides, notably toxaphene, merits attention.

FIGURE 1.

Mortality Rate of First Instar Larvae of Lucilia cuprina *after Short Contact with the Test Insecticide.*



3. *Comparison with the effect on fully grown larvae.*

It will be noticed that fairly high concentrations of the insecticides as suspensions are necessary to kill all maggots on contact of short duration. The amount of insecticide necessary is in most cases greater for young larvae than for fully grown maggots. Parathion, E.P.N. 300 and Aldrin are exceptions, however, as can be seen from Table 1.

TABLE 1.

Toxicity of Some Organic Insecticides to Newly Hatched and Fully Grown Blowfly Larvae (L. cuprina). *Percentage Concentrations causing Complete mortality on Contact of Short Duration.*

Material.	Newly hatched Larvae.	Fully grown Larvae.
Parathion	0·0064	0·1
E.P.N. 300	0·025	1·6
gamma B.H.C.	0·4	0·1
Aldrin	0·4	0·4
Dieldrin	1·6	0·4
Chlordane	above 6·4	3·2
Toxaphene	above 6·4	6·4

These results may appear somewhat incongruous as it is commonly accepted that young larvae are more susceptible to insecticidal compounds than those which are fully mature. It must be borne in mind, however, that young, actively-feeding maggots are able to free themselves more readily from the suspended insecticide particles by mechanical contact with the flesh on which they are feeding. Thus the penetration of a lethal amount of insecticide through the cuticle is avoided. Compounds like gamma B.H.C., Dieldrin, Chlordane and Toxaphene afford good examples in support of this statement. Concentrations of these insecticides considerably in excess of those required to produce mortality in fully matured larvae are necessary in the case of newly hatched maggots with contact of short duration. Parathion, E.P.N. 300 and Aldrin, on the other hand, conform to the generally accepted standard due, apparently, to their capacity of penetrating the cuticle rapidly.

The conclusion must be drawn, therefore, that in order to achieve effective protection at the lower concentrations it is essential that newly hatched maggots must remain in continuous contact with the insecticide.

C. EFFECT OF CONTINUOUS CONTACT.

The serous fluid exuding from the superficial wounds caused by larvae of the primary blowflies on the skin of sheep constitutes the principal food of these maggots (Fiedler, 1951).

In order to determine the minimum concentration of insecticides capable of destroying all first instar larvae when kept in contact with it, a series of *in vitro* tests was conducted in which the insecticides were added to the nutritional medium.

1. *Method.*

First stage larvae were placed in glass tubes measuring 3 inches by 1 inch each containing a constant sized pledget of cotton wool soaked in 5 c.c. of normal serum into which was suspended the insecticides in wettable powder form, over the range 1,000 to 0·004 parts per million. Insecticides in wettable powder form were chosen again to eliminate the possible effect of solvents. Approximately 100 freshly hatched maggots were placed on the soaked cotton wool in each tube which was plugged with cotton wool and held in an incubator at a constant temperature of $\pm 27^\circ$ C. ($\pm 80 \cdot 5^\circ$ F.). Observations were made at 1, 3, 6, 24, 48 and 72 hours. It was not necessary to extend the observations beyond this limit as the maggots become more resistant to insecticidal action with increasing age and, if not killed or injured within 72 hours, are no longer affected.

Four replications of the tests gave the results enumerated in Table II.

2. *Results.*

The eleven compounds tested showed marked differences in their effects. According to the minimum concentration required to achieve a complete kill they can be divided into 5 groups:—

(1) 0·25 p.p.m.—Parathion, E.P.N. 300.

(2) 1 p.p.m.—Dieldrin.

(3) 4 p.p.m.—gamma B.H.C., Aldrin, Methoxychlor, D.D.T.

(4) 16 p.p.m.—D.F.D.T., Chlordane.

(5) 64 p.p.m.—Toxaphene, D.D.D.(TDE).

In general there is no obvious correlation between insecticidal potency and the speed of action of the various compounds with the exception of Parathion and E.P.N. 300. The time elapsed between the commencement of the test and the death of all the maggots at the lowest effective dilution differs with each insecticide (Figure 4). So far as their speed of action is concerned the compounds may be grouped as follows:—

(1) 12-24 hours—Parathion, E.P.N. 300, D.F.D.T.

(2) 24-48 hours—gamma B.H.C., Toxaphene, D.D.D.(TDE).

(3) 48-72 hours—Dieldrin, Aldrin, Methoxychlor, D.D.T. and Chlordane.

According to their larvicidal properties D.D.T. and gamma B.H.C., which are most generally used for long-term protection of sheep, fall into the same category. Both are capable of killing first stage larvae at a concentration of 4 p.p.m. However, the results of many field and laboratory trials (Du Toit *et al.* 1948) have shown that gamma B.H.C. consistently gives more durable protection than D.D.T. used at 5 or 10 times the concentration. Under experimental conditions D.D.T. at 5 per cent. affords protection for approximately 3 months whereas B.H.C. at 0·5 per cent. gamma still protects after 6 months provided that at the time of application the wool had a length of 1 to $1\frac{1}{2}$ inches. The difference in behaviour of the two insecticides in both field trials and *in vitro* tests suggested at the time that some factor in the wool of sheep played an important part by either assisting the action of B.H.C. or retarding that of D.D.T.

D. Behaviour of insecticides in the fleece.

A number of experiments was conducted to determine the fate of insecticides applied to the wool of living sheep with the ultimate aim of finding compounds capable of providing the highest degree of protection. Only insecticides harmless to warm-blooded animals at the concentrations applied were considered. The two highly toxic organic phosphates (Parathion and E.P.N. 300) were excluded as their general use on livestock cannot be recommended. Fluoro D.D.T.(D.F.D.T.) could not be included as the available supply was limited.

In the following experiments eight compounds, which had proved their value in many fields of application, were tested, namely, (1) D.D.T., (2) Methoxychlor, (3) D.D.T.(TDE), (4) B.H.C., (5) Toxaphene, (6) Chlordane, (7) Aldrin, and (8) Dieldrin.

TABLE II.

The minimum concentration of insecticide in parts per million of the nutritional medium to kill or affect newly hatched L. cuprina *larvae in time specified. (K = Complete mortality; A = Partial kill or all adversely affected.)*

Insecticide.		Time (Hours).					
		1	3	6	24	48	72
DDT	K	—	—	1,000	16	16	4
	A	1,000	250	64	4	4	—
DDD (TDE)	K	—	—	—	250	64	64
	A	—	1,000	250	16	16	16
Methoxychlor	K	—	1,000	250	16	16	16
	A	250	64	16	4	4	—
DFDT	K	—	1,000	64	16	16	16
	A	250	64	16	4	4	4
Toxaphene	K	—	—	1,000	250	64	64
	A	—	—	64	16	4	4
Chlordane	K	—	—	—	250	64	16
	A	—	—	1,000	64	16	4
Aldrin	K	—	1,000	1,000	16	16	4
	A	—	250	64	4	4	1
Dieldrin	K	—	1,000	250	16	4	1
	A	—	64	16	0·25	0·25	0·25
Lindane and γ-BHC	K	—	250	64	16	4	4
	A	16	4	4	1	1	1
Parathion	K	—	16	1	0·25	0·25	0·25
	A	4	0·25	0·064	0·064	0·064	0·064
EPN-300	K	—	16	1	0·25	0·25	0·25
	A	4	1	0·25	0·064	0·064	0·064

1. *Protection tests on sheep.*

Eight groups of from 4 to 6 merino sheep were treated by thoroughly saturating the wool to the skin over an area on the rump about 12 inches in diameter. Suspensions of the insecticides containing 0·5 per cent. of the active ingredient were used in each case. The fibre length of the wool varied from $1\frac{1}{4}$ to $1\frac{3}{4}$ inches. A ninth group was left untreated to serve as a control.

Starting 9 weeks after saturation, first instar larvae were implanted weekly on to the treated areas by the method described by McLeod (1937). Death of the larvae on the third day after implantation served as the index of protection. In each case the weekly implantations of larvae were carried out on one of the control sheep as well, to serve as a means of judging the viability of the larvae used. Throughout the experiment the maggots implanted on the untreated sheep succeeded in completing their larval development.

The results are reflected in Figure 2.

At the concentration used the compounds of the D.D.T. group [D.D.T., D.D.D. (TDE) and Methoxychlor] afforded protection against strike for the shortest period of time, the average being 9 weeks. Next in sequence was Toxaphene with 13 weeks followed by Chlordane with 19 weeks. Gamma B.H.C., Dieldrin and Aldrin, on the other hand, protected sheep for extremely long periods, averaging 33, 37 and 39 weeks respectively.

Combinations of equal parts of gamma B.H.C. with Aldrin or Dieldrin, using 0·5 per cent. of either compound to bring the total concentration of insecticide to 1 per cent., did not increase the duration of protection. The mixture of B.H.C. and Dieldrin gave the same degree of protection as Dieldrin at 0·5 per cent. alone, namely 37 weeks, whereas B.H.C. combined with Aldrin protected for a shorter period than either of the constituents alone, namely 31 weeks. This seemed to indicate some antagonistic action between these two compounds.

2. *Laboratory tests with treated wool.*

In order to confirm the results obtained by the implantatiion method above and, at the same time to ascertain the larvicidal properties of the wool staple at various intervals after treatment, samples of wool from the treated area were removed weekly for laboratory tests from those sheep upon which larval implantations were made at the same time.

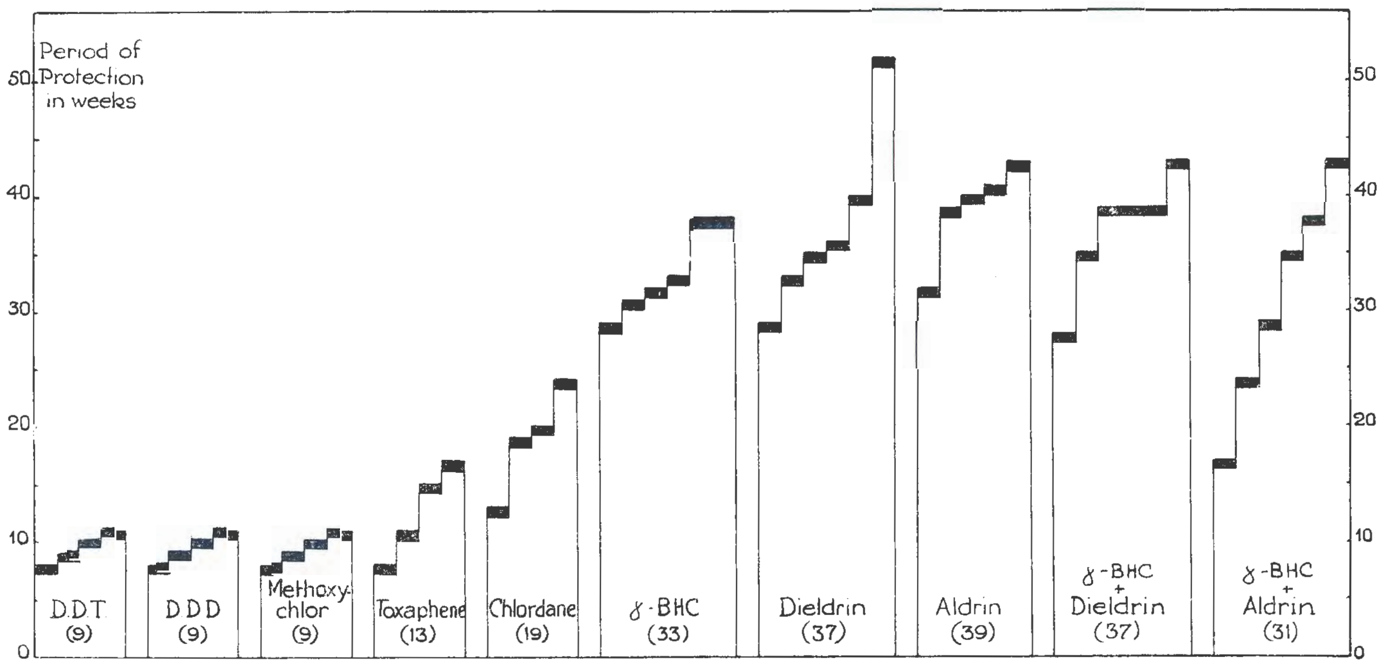
Under South African conditions wool grows at the fairly constant rate of $\frac{1}{4}$ inch per month. At the time of commencement of testing, therefore, 9 weeks after the application of the insecticides, a new growth of at least $\frac{1}{2}$ inch of wool could be expected below the treated portion of the staple.

(*a*) *Method.*

The wool samples, consisting of 2 to 3 grams, were clipped from the skin by means of a pair of curved scissors, care being taken to sever the fibres as close to the skin as possible. In the laboratory the samples were divided into two or more portions, depending on the time after the original treatment and the length of the staple as shown in Figure 3. The routine procedure was adopted in every case of measuring the half inches between cuts from skin level so as to ensure that no portion of the wool zone originally treated would be included in any of the samples consisting of new growth only. Thus, the wool sample shown in Figure 3 taken 18 weeks after treatment was divided into 3 segments, the two $\frac{1}{2}$

FIGURE 2.

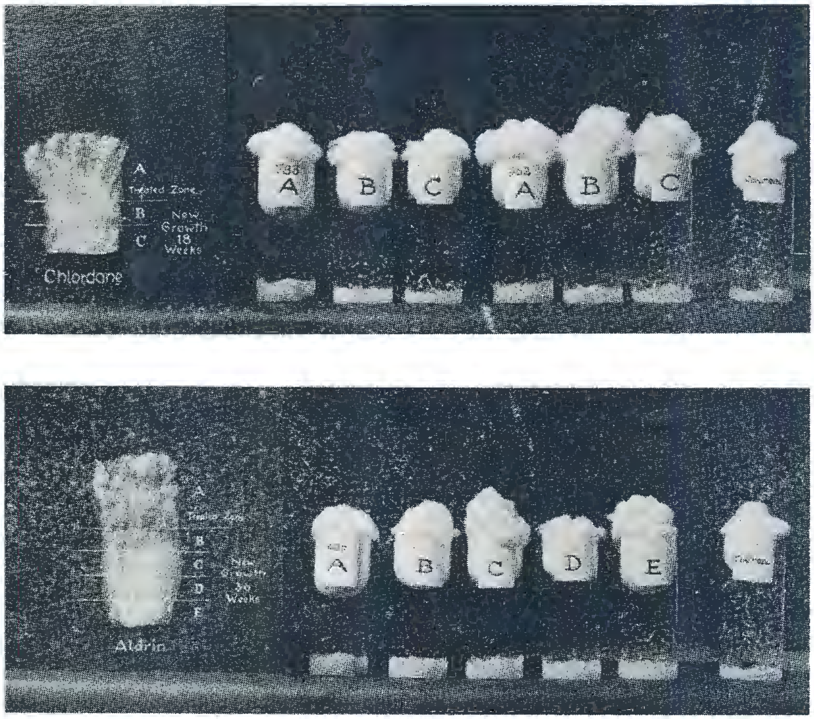
Period of protection against artificial strikes with different insecticides applied as suspensions containing 0·5 per cent. of active ingredient to merino sheep with wool between $1\frac{1}{4}$ and $1\frac{3}{4}$ inches long. Columns refer to individual sheep in each group and figures in brackets indicate the average duration of protection in weeks.



inch segments nearest the skin representing the new growth while the third portion consisted of the wool zone treated originally together with a small portion of the wool which had grown subsequently. This ensured that any insecticide present in the samples of newly grown wool could only have been derived from that with which the original wool zone had been saturated.

FIGURE 3.

Examples of actual tests on two insecticides (Chlordane and Aldrin). Wool from treated sheep showing zones of varying degrees of protection (left). The decreasing insecticidal concentrations in wool samples from the various zones A, B, etc., as evidenced by larval development compared with control tubes showing normal growth after incubation for 24 hours at 27° C. = 80·5° F. (right).

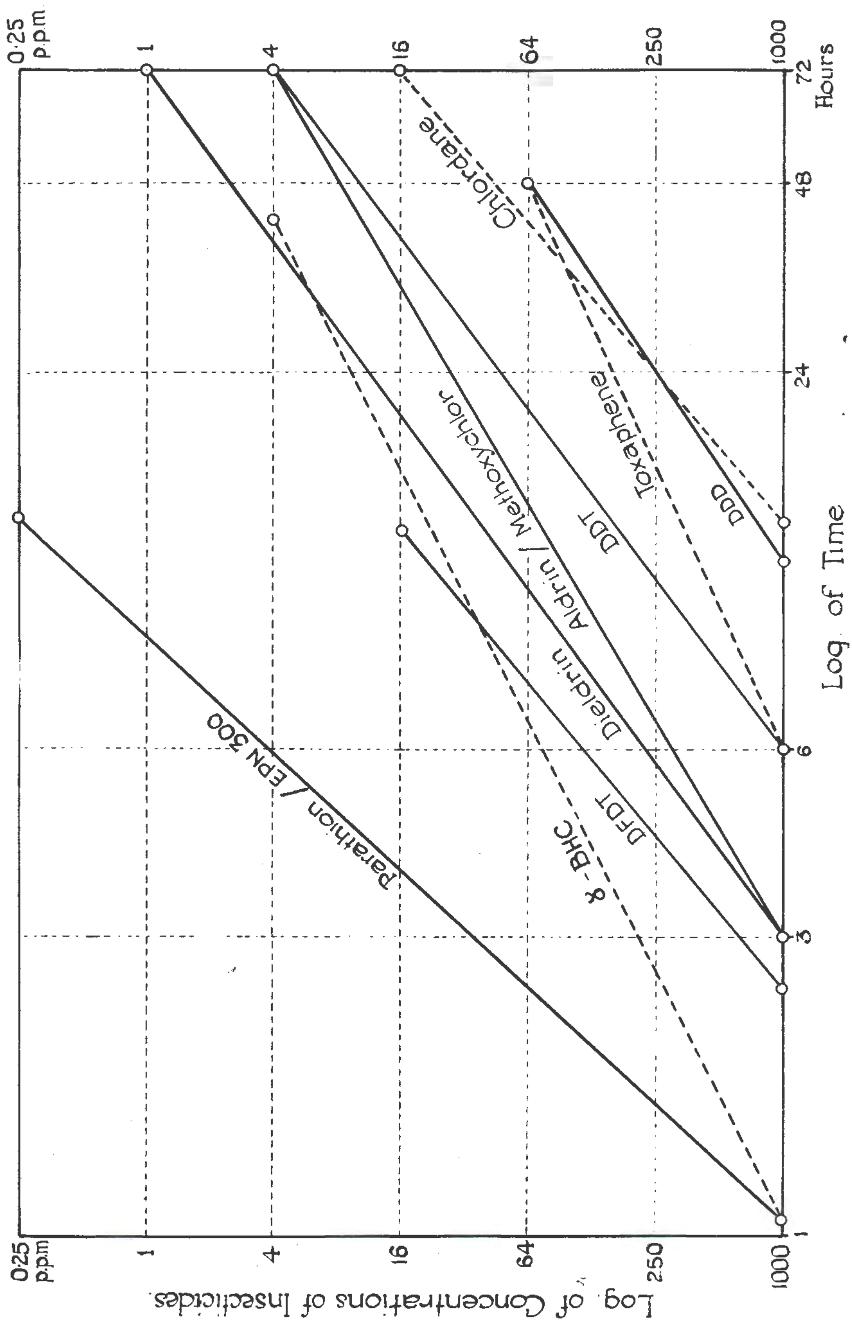


Each wool segment so divided from the staple was placed in a glass specimen tube (3 inches by 1 inch) and saturated with serum. Into each tube were then pipetted 50 to 100 first instar larvae of *L. cuprina* and the tubes incubated at 27° C. for subsequent observation.

This method represented the reverse of that used previously as the insecticide was now present in the wool instead of being suspended in the nutritional medium. The duration of survival of the larvae served as an index of the concentration of the insecticide present in the different segments of the wool samples. A comparison of the mortality rates with those given in Table II provides an accurate means of estimating the concentration of insecticide in the particular portion of the fleece.

FIGURE 4.

Concentrations of insecticides in the nutritional medium and time required to achieve complete kill of newly hatched Lucilia cuprina *larvae.*



(*b*) *Results.*

The *in-vitro* tests indicated clearly that sheep were no longer protected against strikes when the concentration of insecticide in the proximal portion of the new growth wool fell below the level capable of killing first stage larvae as indicated in Figure 4. In the case of D.D.T. and gamma B.H.C. this is four parts per million. As soon as the larvae survive and develop normally in those tubes containing the $\frac{1}{2}$ inch of new growth wool nearest the skin, strikes will develop simultaneously on the particular sheep when larvae are implanted on it.

FIGURE 5.

Example of a protection diagram showing: (1) *The concentrations of insecticides in the treated wool (A) and in the different zones of the new growth wool (B-E) tested by the biological method.* (2) *The outcome of artificial strikes applied monthly to the same sheep from* 13 *to* 41 *weeks.*

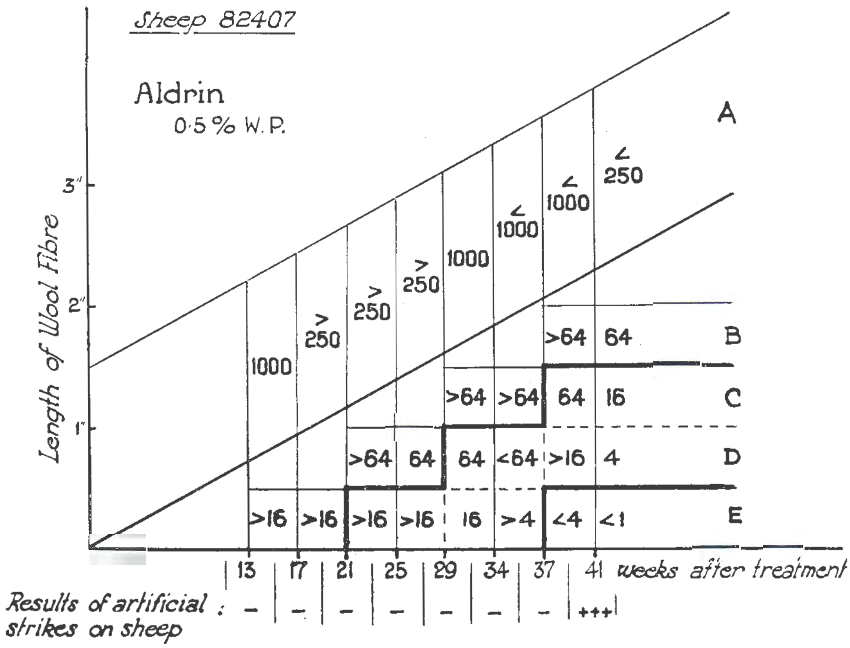


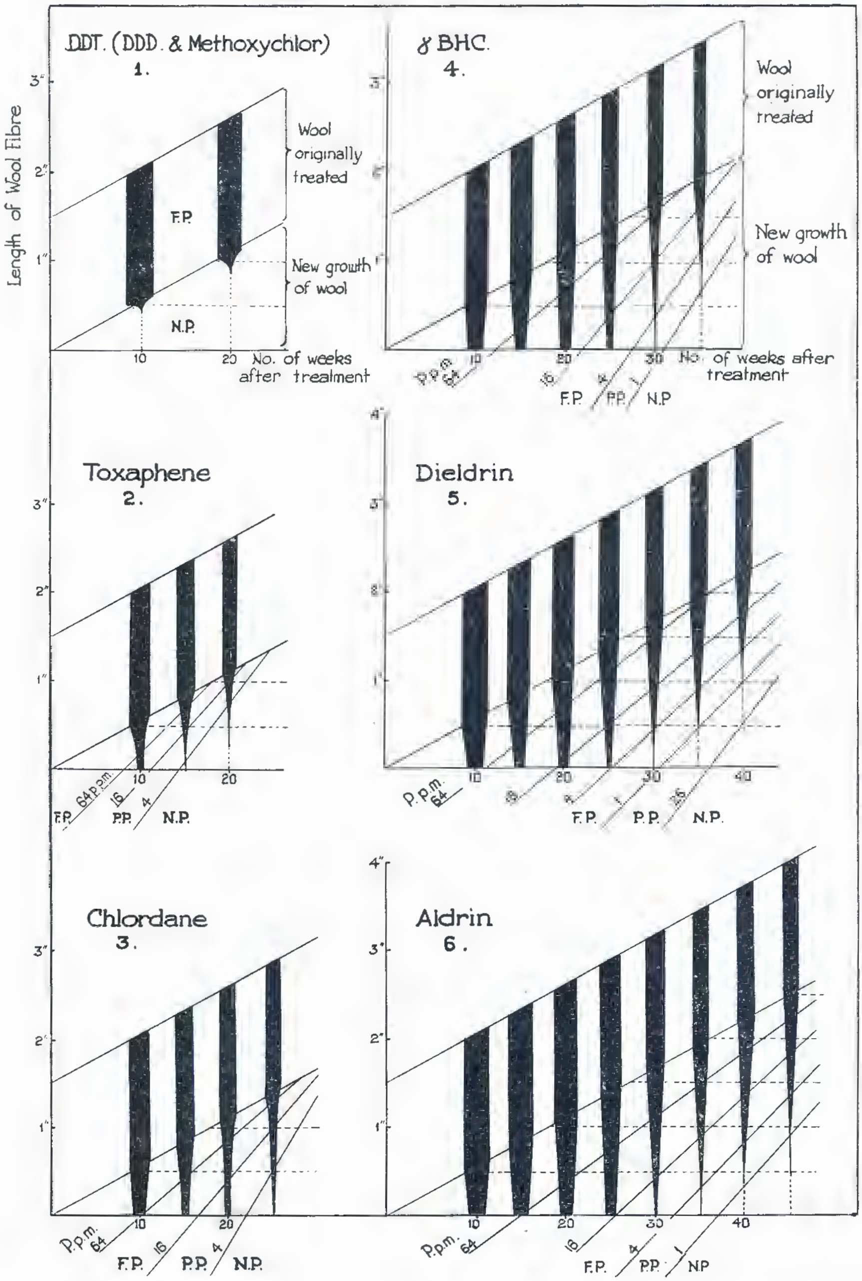
Figure 5 shows in diagrammatic form the varying concentrations of Aldrin in the different zones of the fleece between 13 and 41 weeks after the original treatment of the wool. The result of artificial strikes which were applied simultaneously on the particular sheep from which each wool sample was taken are indicated below.

This *in-vitro* method proved to be sufficiently sensitive to indicate even partial protection. Where this only results, the larval development is retarded in comparison with the development which occurs when the medium is entirely free of insecticide. So long as the $\frac{1}{2}$ inch wool nearest the skin contains sufficient insecticide to influence the normal growth of newly hatched maggots detrimentally, no strike will develop on the sheep. The concentrations of different compounds that are still able to impede normal development are reflected in Table II.

Furthermore, the biological assay method has disclosed distinct differences in the behaviour of different insecticides in the fleece apart from those just described. (See Figure 6.)

FIGURE 6.

The diffusing power of insecticides in the wool of sheep applied as suspensions (wettable powders) containing 0·5 per cent. active ingredient, showing actual concentrations in parts per million (p.p.m.) in the new growth wool.



F.P. = Full protection against artificial strike. P.P. = Partial protection. N.P. = No protection.

(i) *D.D.T. and Related Compounds.*

Upon implantation of first instar larvae, normal diffuse strikes developed on sheep treated nine weeks previously with 0·5 per cent. of D.D.T., Methoxychlor or D.D.D.(TDE).

Wool samples which were submitted to the *in-vitro* test revealed that, even after 20 weeks, sufficient insecticide to produce a complete kill was always present in the distal or treated zone of the fleece. Whereas a concentration of insecticide of between 1,000 and 250 p.p.m. could still be determined for D.D.T. and D.D.D. (TDE) in the case of Methoxychlor the decrease in concentration in the treated zone was more rapid and amounted to about 16 p.p.m. after 10 weeks and sometimes reached its lowest lethal level of 4 p.p.m. 20 weeks after application (Figure 6/1).

The new growth wool on the other hand contained insufficient insecticide 10 and 20 weeks after treatment to either kill or even affect maggots brought into contact with it in a nutritional medium. The insecticidal titre, therefore, was below 4 p.p.m. in the case of D.D.T. and Methoxychlor, and below 16 p.p.m. in the case of D.D.D. (TDE). The new growth of wool beneath the portion treated with any one of these three compounds is unable to afford any protection against blowfly strike.

(ii) *Toxaphene.*

On an average artificial strikes developed normally on sheep 14 weeks after application of the insecticide at a strength of 0·5 per cent., thus indicating that at comparable concentration toxaphene affords a slightly better protection than insecticides of the D.D.T. group.

In-vitro tests also demonstrated results differing from those with D.D.T. and its relatives (Figure 6/2).

The amount of Toxaphene decreased steadily in the treated zone to a level of about 64 p.p.m. after 20 weeks, which is still within the lethal range of this compound. Furthermore, the biological *in-vitro* method revealed the presence of about 64 p.p.m. of toxaphene in the new growth wool up to 9-10 weeks after application and this is still sufficient to kill all newly hatched larvae. After 18-20 weeks, however, the proximal $\frac{1}{2}$ inch of wool next to the skin contains less than 4 p.p.m. which allows development of larvae. At the same time the distal $\frac{1}{2}$ inch of the new wool still possesses a content of about 16 p.p.m., which is sufficient to interfere with the normal development of maggots. The fact that the proximal $\frac{1}{2}$ inch of wool 18-20 weeks after treatment is free of insecticide permits of the development of strikes and protection is lost.

(iii) *Chlordane.*

As is the case with Toxaphene a considerable amount of Chlordane could be detected in the new growth wool proximal to that originally treated (Figure 6/3). The concentration of Chlordane in the new wool remained consistently higher than was the case with Toxaphene and the larvicidal titre of the proximal $\frac{1}{2}$ inch averaged 64 p.p.m. after 10 weeks, about 16 p.p.m. after 15 weeks and 4 p.p.m. or less after 20 weeks. As Chlordane is capable of killing young maggots at a concentration of 16 p.p.m., the whole length of the wool fibre possesses larvicidal properties up to 15 weeks followed by partial protection which diminishes rapidly thereafter. At 20 weeks after application the concentration dropped to 4 p.p.m. or less and at this stage normal strikes are liable to occur as could be demonstrated by implantations of larvae on the sheep.

(iv) *Gamma B.H.C.*

Whilst the active ingredient of B.H.C. applied to the fleece decreases in the treated zone and may reach a level of as low as 16 p.p.m. or less at 30 weeks after application, the concentration at skin level is still entirely larvicidal at this stage. The larvicidal titre of the proximal ½ inch of new growth wool averages 64 p.p.m at 10 weeks, 16 p.p.m. at 20 weeks, 4 p.p.m. at 25 weeks and 1 p.p.m. at 30 weeks (Figure 6/4). As the larvicidal threshold of gamma B.H.C. is 4 p.p.m., with retardation of normal larval development at 1 p.p.m., protection against strikes under the conditions of these tests is assured up to 33 weeks as could be shown simultaneously on sheep by means of the larval implantation method.

(v) *Dieldrin.*

It was significant that the larvicidal concentration of this compound remained at a high level for a very considerable time. Up to 1,000 p.p.m. could still be found in the treated zone of wool after 40 weeks (cf. Figure 5, Dieldrin). The distribution of insecticide in the untreated new wool was approximately similar to that of gamma B.H.C. The protection afforded by Dieldrin, however, was of considerably longer duration and as this compound is able to kill young maggots at 1 p.p.m. the whole fibre length of wool remains toxic up to 30 weeks and longer, after the original application. The concentration in the most proximal ½ inch of wool dropped below 0·25 p.p.m. after 37 weeks as a rule after which normal larval development could again proceed (Figure 6/5).

(vi) *Aldrin.*

Of all the insecticides tested Aldrin proved to be the insecticide of which the highest concentration persisted in the untreated new growth wool. The larvicidal titre of the proximal ½ inch decreased very slowly and the concentrations shown were 64 p.p.m. at 15 weeks, 16 p.p.m. at 25 weeks, 4 p.p.m. at 32 weeks and 1 p.p.m. at about 37 weeks. As 1 p.p.m. still kills a certain percentage of the young larvae or at least affects their normal development, diffuse strikes would only be expected to occur on an average after 40 weeks (Figure 6/6).

E. Discussion and Conclusions.

The insecticides under test, which were applied to the sheep at the uniform concentration of 0·5 per cent. of the active ingredients, may be divided into three groups according to the duration of protection afforded, provided the length of wool at the time of application is between 1¼ and 1¾ inches—

(1) up to 10 weeks—D.D.T., Methoxychlor., D.D.D.(TDE);

(2) between 10 and 20 weeks—Toxaphene, Chlordane;

(3) over 30 weeks—Gamma B.H.C., Dieldrin, Aldrin.

The *in-vitro* biological assay method has shown differences in the behaviour of the various insecticides in the fleece of living sheep. Compounds of the above group 1 remain confined to the wool zone originally treated and show no tendency to diffuse into the new growth of wool. The result is that, as soon as an insecticide-free growth of wool of about ½ inch in length has formed in about 2 months after treatment, strikes can develop.

Compounds belonging to group 2 on the other hand, and, to an even greater extent, those of group 3 demonstrate an entirely different behaviour in the wool. These substances possess the ability of diffusing along the wool fibres into the new growth of wool, namely the zone which is constantly expanding at the rate of about $\frac{1}{4}$ inch per month. The diffusion power of the different compounds varies considerably. The value of this property may be expressed either in terms of the time necessary for the concentration near the growing end of the wool fibre to drop to 4 p.p.m. from the original 5,000 p.p.m., applied to the fleece, or by measuring the actual growth of new wool at this particular stage (Table III). The concentration of 4 p.p.m., has been selected as it represents the lowest common dilution still effective for these insecticides.

TABLE III.

Diffusion power of certain insecticides along the wool fibres in terms of time and length of new wool growth based upon the concentration of insecticide having reached 4 p.p.m. at skin level, which is still effective in preventing blowfly strike.

Material.	Time in Weeks.	Length of new Wool in Inches.
Toxaphene	14	0·8
Chlordane	22	1·2
Dieldrin	25	1·4
gamma B.H.C.	27	1·6
Aldrin	32	1·8

The greater the time required for the insecticidal concentrations to reach the level of 4 p.p.m., the greater is the length of newly grown wool beneath the treated zone. It follows, therefore, that the compound maintaining the highest concentration in the wool at skin level for the longest period would possess the greatest power of diffusion. Of the five compounds under discussion Aldrin occupies first place in this respect followed by gamma B.H.C., Dieldrin, Chlordane and finally Toxaphene.

The diffusion power alone does not determine the duration of protection, however. Dieldrin, for instance, diffuses more slowly than gamma B.H.C. yet affords longer protection against strikes for the reason that its toxic effect on larvae is greater and it produces a complete kill at 1 p.p.m.

The actual period of protection afforded by any larvicidal compound is dependent upon the combined effect of two important properties, namely the diffusion power in wool and the larvicidal value. The greater these two factors are the longer is the resulting period of protection.

Although a number of the synthetic insecticides included in the present investigation have been used for the protection of sheep against blowfly strike no clear exposition of their mode of action seems to have appeared. D.D.T. and B.H.C. most commonly used in field trials, appear to have been equally favoured but in the light of the evidence presented the latter compound is vastly superior to the former.

It would appear advisable, therefore, to re-examine the new synthetic insecticides as a whole in terms of their behaviour in wool when the specific problem of the long term protection of sheep against blowfly strike is considered. Economy and efficiency will be determined by factors such as larvicidal action and rate of diffusion in the actively growing wool fibre at skin level.

As far as our present knowledge goes gamma B.H.C., Dieldrin and Aldrin are the larvicides of choice. Obviously Chlordane may be included but the D.D.T. group of compounds and Toxaphene, which show little tendency to diffuse along the growing wool fibres, are inferior by comparison.

In the present investigation an attempt has been made to evaluate and compare certain of the new synthetic insecticides particularly with a view towards protecting sheep against crutch strike which is by far the commonest form of myiasis in South Africa. Within limits it may be stated that the higher the concentration of active ingredient used the longer the protection. It must be borne in mind, however, that although sheep are able to tolerate high concentrations of insecticides when applied to limited areas of the body such as the crutch region, these concentrations may not be tolerated, or even be economically possible, when applied over the whole body. This would be necessary when body strike is prevalent and must be protected against. Suitable concentrations of insecticides for application by the method of total immersion for the prevention of body strike, in countries where this form of calliphorine myiasis plays a dominant role, have still to be determined.

Evidence of the role played by the length of wool at the time of application to localised areas of skin indicates that this factor is of importance. This phase of the investigation, however, has not been thoroughly examined and will form the subject of future study.

F. Summary.

1. Higher concentrations of the new organic insecticides are necessary to achieve a complete kill of first instar larvae of *Lucilia cuprina* Wied., when contact with the insecticides is short than is necessary for mature larvae. The former are able to free themselves rapidly from the toxicant in the nutritional medium owing to their feeding habits.

2. The larvicidal action of eleven synthetic insecticides has been studied and the minimum lethal dose determined on young maggots within a nutritional medium.

3. A new biological assay method for determining the larvicidal value of wool treated with various insecticides has been developed.

4. Insecticides of equal toxicity to first stage blowfly larvae afford different lengths of protection against strike even when their active ingredients are of the same concentration.

5. The importance of the larvicidal properties of insecticides in protecting sheep over long periods is stressed as of very much greater importance than the more transitory repellent properties to adult blowflies possessed by such insecticides.

6. The biological assay method has revealed that a number of insecticides such as gamma B.H.C., Dieldrin, Aldrin and to a lesser extent Chlordane and Toxaphene possess properties of diffusion which permit of their penetration from previously treated wool on sheep into the constantly expanding zone of growing wool. Compounds of the D.D.T. group do not diffuse along the wool fibres.

7. The duration of protection of sheep afforded by any insecticide is dependent upon the larvicidal value and diffusion power of the particular insecticide. For this reason gamma B.H.C., Dieldrin and Aldrin have been found to be the compounds most suitable for this particular purpose.

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