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THE PROTECTION OF SHEEP AGAINST BLOWFLY STRIKE.
III.—THE EFFECT OF DIFFERENT FORMULATIONS OF GAMMA BENZENE HEXACHLORIDE (B.H.C.)

O. G. H. FIEDLER* and R. DU TOIT, Onderstepoort Laboratory.

A Review of Recent Investigations.

It is the general experience that the efficiency of an insecticide is dependent to a large extent upon the formulation. Thus when made up in the form of a solution, an emulsion, a suspension or a dust each preparation has a field of application in which it is particularly effective. The chemical and physical properties are not, however, the sole index of the efficacy of a compound. A diversity of conditions, both during and after application, may operate to alter the value of a particular preparation entirely.

In the treatment of living sheep it has been shown by du Toit and Fiedler (1953) that the wool grease profoundly influences the residual effect of certain insecticidal compounds applied to the fleece for protection against blowfly strike. Insecticides such as benzene hexachloride which are regarded normally as having a residual effect of a short duration, afford protection to the living sheep for periods considerably longer than compounds like D.D.T., Toxaphene and Chlordane which are characterised by more durable effectiveness in other fields of application.

Recent observations by Addison and Furmidge (1952) have shown that wool grease not only influences the mode of action of the insecticide but, coupled with suint, profoundly affects the activity of the wetting agent in as much as grease and suint, rather than the wool fibre itself, are responsible for the inactivation which occurs in insecticidal dip washes. The wetting agents are held in the grease layer around the wool fibres in quantities considerably in excess of that adsorbed on to the surface. The adsorption exceeds the calculated value by a factor of more than ten, which means that the take-up of wetter by the grease involves more than surface adsorption. The process is to be regarded, therefore, as absorption rather than as adsorption. Furthermore, it has been found that the inactivation of the wetters during dipping or spraying of sheep is only partly due to direct absorption by wool grease and arises in large measure from the insoluble nature of the product of reaction with dissolved suint.

The inactivation rate is particularly high when cationic wetting agents are used (Jolly *et al.*, 1953). This results in an unnecessarily heavy insecticidal deposit on the fleece and a rapid depletion of the dip or spray. According to Addison and Furmidge, it appears probable that an original cationic wetter may be removed completely by precipitation alone. The result is a change in the character of the wetting agent from cationic to anionic, whereas absorption by grease is probably a property of all long-chain compounds irrespective of ionic charge.

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* Senior Research Fellow.

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In addition Laudani (1952) has reported that a selective pick-up of insecticide (D.D.T.) by the wool fibre also occurs when fabricated woollens are washed or soaked in very dilute nonionic insecticidal emulsions. This makes the fabric moth proof for a considerable period of time.

Moncrieff (1950) found that exhaustion of insecticide took place when woollen material was dipped in cationic emulsions under slightly acid conditions. Although the synthetic insecticides have no affinity for the wool fibre owing to the absence of salt-forming groups (Goodal *et al.*, 1946), the position is entirely changed by the addition of a surface-acting agent which, through inactivation, causes a heavy insecticidal deposit on the fibre.

The process is enhanced on the living sheep by wool grease and suint to such an extent that only wetting agents up to a certain level of activity can be used economically.

The stability of a dip or spray and the extent to which insecticide is deposited are largely independent of variations in the grease and suint content of the fleece and depend only upon the chemical nature and concentration of the wetting agent (Addison and Furmidge, 1953). The use of a cationic wetter with any insecticidal formulation, therefore, is undesirable for dipping woolled sheep and such wetters are only of value when a non-returnable spray method is employed (Jolly *et al.*, 1953). For this reason the best wetting agent for insecticidal compounds for use on sheep appears to be one of the anionic type.

The system of reactions in a dipping bath, however, is of an even more complex nature, since it has been the general experience with any type of wetter that exhaustion of the insecticidal component is more pronounced in the case of emulsions than with suspensions (Downing *et al.*, 1952; Jolly *et al.*, 1953). This confirms the findings in South Africa (Bekker, 1948). Furthermore, it has been found (Jolly *et al.*, 1953) that in the control of the sheep tick, *Ixodes ricinus* L., inferior results were obtained with one and the same preparation (B.H.C. at 0·065 per cent gamma) when used as a spray as compared with its use as a dip in the same concentration. Good protection of sheep was afforded, however, by the use of a considerably higher concentration in the spray. An explanation for this observation has not been given.

The varied behaviour of insecticides in different formulations and applied by different methods will have a bearing certainly also on the period of protection against blowfly strike in sheep. The aim of the investigation presented in this paper, therefore, has been to assess the value of different formulations and to find the reason for the divergent results obtained by dipping and spraying.

B. Dipping Experiments.

1. *Method.*

Benzene hexachloride (B.H.C. = Hexachlorocyclohexane) was chosen as the test insecticide as it possesses good larvicidal qualities combined with a marked propensity for diffusing along the wool fibre from the treated zone into the ever expanding untreated new growth of wool below. This compound is a very suitable protecting agent, therefore, against blowfly strike (du Toit and Fiedler, 1952, 1953). As the analysis of the entire complex of B.H.C. isomers presents rather involved chemical procedures which detract from accuracy in the case of polluted washes, Lindane, the pure gamma isomer of B.H.C. was employed in the tests as it is assessed easily by simple chemical means. It has been proved experimentally that the larvicidal action and residual effect of Lindane in wool are entirely comparable with that obtained with the whole complex of isomers at the appropriate concentration. The results obtained with Lindane can be compared directly, therefore, with those obtained with technical B.H.C.

The following Lindane formulations were used for the experiment:—

(1) Lindane emulsifiable concentrate at 10 per cent gamma, containing an anionic emulsifier.

(2) Lindane wettable powder at 10 per cent gamma, containing an anionic wetter.

(3) Lindane dust at 10 per cent gamma in talc.

The emulsion and the suspension were applied by dipping. This method of administration ensures an even distribution of the insecticide in the fleece and, furthermore, facilitates assessment of the quantity of fluid absorbed as well as of the insecticide. Use was made of a small vat and, in making the dilutions with water, a gamma concentration of 0·1 per cent (1,000 p.p.m.—parts per million) was the aim. The volume of the dipping fluid was limited in both cases to 120 gallons (546 litres) in order that a rapid depletion of the insecticide would result from treatment of a limited number of sheep. The dust was applied by means of a small hand apparatus, special care being taken to ensure that the dust was evenly distributed over the entire body and well worked into the fleece down to skin level. An amount of Lindane in dust form was applied equal to that deposited by dipping in the wettable powder suspension in order that the subsequent duration of protection against blowfly strike could be compared.

Three groups each of twelve merino sheep with a wool length of between $1\frac{1}{2}$ and 2 inches were used as test animals. The quality of the wool, as well as its grease content, were determined for each sheep prior to treatment. Wool grease analyses of the two dipped groups were repeated after dipping in order to note any possible influence due to the actual dipping process. The grease content of the wool of all the sheep was determined for a third time a month later.

Each of the three groups of twelve sheep were subdivided into two groups of six animals each, in order that one half of each group of animals treated with a particular formulation might be immersed for 5 to 7 minutes in plain water four weeks after the insecticidal treatment. The object was to determine whether portion of the insecticide deposited in the fleece would be removed possibly with some of the wool grease.

The following procedure was adopted for the dipping and for the sampling of the wash together with the drippings from the sheep:—

> Samples of the dip wash, which was kept well agitated throughout, were taken prior to the commencement of dipping and then after 3, 6, 9 and 12 sheep respectively had been dipped. The drippings were collected from sheep Nos. 1, 3, 6, 9 and 12 of each dipped group. The weight of each animal was taken before it went into the vat, where it remained singly for two minutes. Thereupon, it was placed on a metal tray until no more fluid dripped from it and weighed again.

2. *Depletion of the dip washes* (c/f Tables 1 and 2).

From the figures obtained for fluid removal per sheep, and the chemical analyses of the washes after every third sheep dipped, it was possible to determine the actual amount of insecticide removed per animal.

TABLE 1.

Fluid removal and gamma depletion rate by four groups of three merino sheep each from a dipping vat containing 120 gallons (546 litres) of a 970 p.p.m. Lindane emulsion equalling a gamma total of 530 gm.

COLUMN.	I.		II.	III.			IV.			V.	VI.	VII.
Groups of Sheep.	Amount of Dip Wash removed.		Gamma Strength of Vat after each Group in p.p.m.	Actual. Gamma Removal per—			Expected* Gamma Removal per—			Relation between Actual and Expected Gamma Depletion (see Text).	Dipwash removed equivalent to a Gamma Suspension of (see Text).	Relation between Gamma concentration of Wash removed and fluid in Tank.
					Animal.			Animal.				
	Gallon.	Litre.		Group in gm.	In gm.	In %.	Group in gm.	In gm.	In %.			
1.	6·0	27·3	825	102	34·0	6·4	26·5	8·8	1·7	3·4	0·37%	4·1
2.	5·4	24·6	665	100	33·3	7·7	23·9	8·0	1·5	4·2	0·41%	5·5
3.	6·8	30·9	525	85	28·3	8·6	30·0	10·0	1·9	2·8	0·28%	4·7
4.	6·7	30·5	375	81	27·0	11·1	29·6	9·9	1·9	2·7	0·27%	6·0
TOTAL	24·9	113·3	—	368	—	—	110·6	—	—	—	—	—
AVERAGE PER SHEEP	±2	±9·5	—	—	30·6	—	—	9·2	—	3·3	0·33%	5·1

* Calculated on the basis that only the volume of emulsion indicated in Column I was absorbed by each fleece.

TABLE 2.

Fluid removal and gamma depletion rate by four groups of three merino sheep each from a dipping vat containing 120 gallons (546 litres) of a 940 p.p.m. Lindane suspension equalling a gamma total of 514 gm.

COLUMN.	I.		II.	III.			IV.			V.	VI.	VII.
Groups of Sheep.	Amount of Dip Wash removed.		Gamma Strength of Vat after each Group in p.p.m.	Actual Gamma Removal per—			Expected Gamma Removal per—			Relation between Actual and Expected Gamma Depletion (see Text).	Dipwash removed equivalent to a Gamma Suspension of (see Text).	Relation between Gamma concentration of Wash removed and fluid in Tank.
	Gallon.	Litre.		Group in gm.	Animal.		Group in gm.	Animal.				
					In gm.	In %.		In gm.	In %.			
1	5·6	25·5	860	67	22·3	4·3	24·0	8·0	1·6	2·8	0·26%	2·9
2	6·9	31·4	840	37*	12·3	2·8	29·5	9·7	1·9	1·3	0·12%	1·4
3	6.1	27·8	780	50	16·7	4·1	26·2	8·7	1·7	1·9	0·18%	2·2
4	6·5	29·6	720	49	16·3	4·5	27·8	9·3	1·8	1·8	0·17%	2·3
TOTAL	25·1	114·3	—	203	—	—	107·5	—	—	—	—	—
AVERAGE PER SHEEP	±2·0	±9·5	—	—	16·9	—	—	8·9	—	2·0	0·18%	2·2

* The wool of two sheep in this group was squeezed in error on the draining pan, resulting in a lower insecticidal deposit.

(a) Emulsion dip.

The results of the dipping experiment with the emulsion are given in Table I. The amount of dipping fluid removed from the tank, which averaged 2 gallons or 9·5 litres per head, is shown in column I of the table. The gamma concentration of the bath, which was 0·097 per cent (970 p.p.m.) prior to commencement of dipping, dropped to 375 p.p.m. after the twelve sheep had gone through (column II). The actual quantity of insecticide in gamma taken out with the fluid for each group of three animals as well as for the single sheep is shown in column III. 368 gm. or 70 per cent Lindane of the 530 gm. originally included in the wash were picked up by the twelve sheep. The removal of Lindane per sheep, which averaged 30·6 gm., decreased from 34·0 to 27·0 gm. during the course of dipping (column III), whereas the gamma exhaustion rate of the bath per animal increased from 6·4 to 11·1 per cent of the quantity in the vat. In order to assess the amount of gamma selectively removed, the actual Lindane take-out had to be compared with the so-called expected removal, which is calculated on the basis that only the quantity of insecticide originally dispersed in the amount of fluid absorbed in the fleece, is carried out of the dip wash. The expected removal is dependent, therefore, only on the insecticidal strength of the dip and the volume of fluid retained by the fleece. This varied in the test between 8·0 and 10·0 gm. per animal with a mean of 9·2 gm. (column IV), which is equivalent to an exhaustion rate of between 1·5 and 1·9 per cent. The quotient between actual and expected Lindane removal represents the relation between the two and expresses the level of preferential or selective removal of insecticide (column V). On an average 3·3 times the quantity of Lindane that could be expected theoretically was taken out per animal from the emulsion dip. The fluid carried out by each sheep, therefore, represents a wash of insecticidal strength considerably in excess of that of the bath (column VI). This may be illustrated by the following example:—

> The quantity of active ingredient (34·0 gm. Lindane) removed by each of the three sheep of the first group with six gallons (27·3 litres) of fluid equalled in reality a wash containing 0·37 per cent Lindane, and the actual removal for all twelve animals was equivalent to an emulsion of 0·33 per cent Lindane. If the gamma concentration of the wash removed is compared with the insecticidal strength present in the tank at the moment of dipping (column VII) it will be seen that the insecticidal concentration of the wash that impregnated the fleece, is on the average, 5·1 times higher than the actual gamma concentration in the tank as determined by chemical analysis.

(b) Suspension dip.

Table 2 represents the dipping results with the suspension. The gamma strength initially was 0·094 per cent (940 p.p.m.). Each sheep removed about the same quantity of fluid from the vat as in the case of the emulsion, namely 2 gallons or 9·5 litres (column I). The dip wash showed a gamma content of 720 p.p.m. after the twelve sheep had gone through (column II). In the case of the suspension 230 gm. Lindane of the 514 gm. originally placed into the tank was removed during the course of dipping, or approximately 45 per cent. The actual Lindane take-out per sheep decreased from 22·3 for the first group to 16·3 gm. for the last three animals, with an average of 16·9 gm., which is considerably less than that removed in the case of the emulsion (column III). The percentage exhaustion rate on the other hand showed only an insignificant rise from 4·3 to 4·5. The expected gamma removal, which is calculated in accordance with the volume of wash taken out and its initial strength, must also be expected to be slightly lower in this case than with the emulsion, as the initial strength was lower. The expected Lindane depletion averaged 8·9 gm. per animal with a range of variation from 8·0 to 9·7 gm. (column IV). The actual mean removal of insecticide, therefore, was twice as high as could be extected theoretically (column V). This indicates that the dip carried out per sheep actually represented a wash with a Lindane concentration of between 0·12 and 0·25 per cent, an average of 0·18 per cent (column VI). Thus the fleeces of the sheep were saturated during dipping by a wash, the average insecticidal concentration of which was equivalent to a wash 2·2 times higher than the corresponding fluid in the vat.

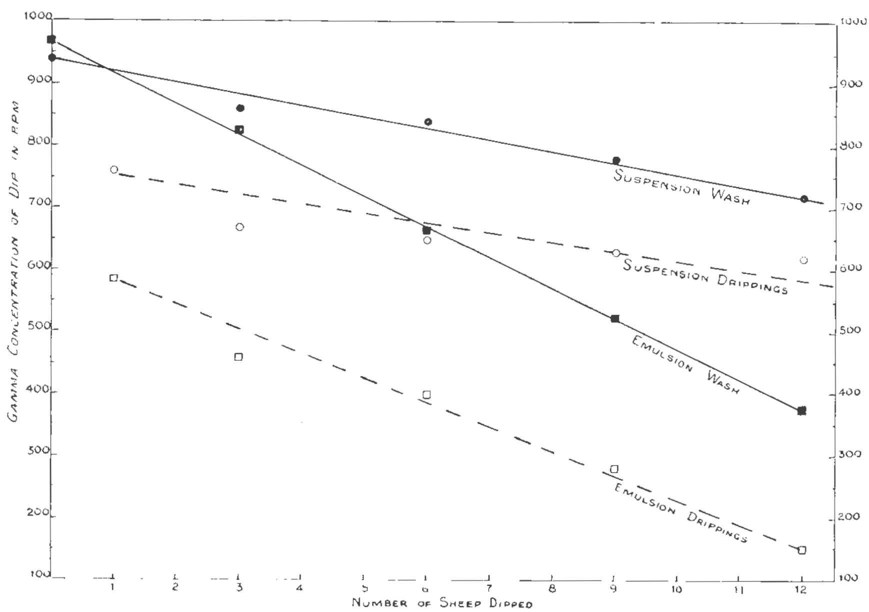

FIG. 1.—*Depletion rate of a Lindane emulsion and suspension wash of* 120 *gal.* (546 *litre*) *and the insecticidal concentration of the drippings from sheep.*

3. *Discussion.*

The dipping experiments described offer a great deal of evidence in support of past findings regarding the selective removal of insecticide during dipping which occurs particularly in respect of emulsion dips. This is indicated in Figure 1. If the graphs for the two dip washes were to be extended to zero it will be noted that in the case of the emulsion dip the bath would be compltely exhausted after immersion of 19 sheep whereas exhaustion would be reached only after 51 sheep had been dipped in the suspension.

A completely satisfactory explanation for this phenomenon has not yet been found. It would appear, however, that the combination of emulsifying agent and solvent in the case of the emulsion displays greater activity than is the case with the suspension where the emulsifying agent is bound to clay particles. The exhaustion of dip washes appears not to be a mere sifting action brought about by physical properties of the fleece but rather a true adsorption in the case of washed and fabricated wool as shown by Laudani (1952) and a combination of absorption and adsorption in the case of the living sheep where the wool grease and suint play a part as well, as demonstrated by Addison and Furmidge (1952). This may be shown by comparing the insecticidal content of the drippings from sheep with that of the corresponding dipping fluid (Table 3 and Figure 1).

TABLE 3.

The decrease in Lindane concentration in p.p.m. of the tank wash and of the drippings from sheep with an emulsion and with a suspension.

	Emulsion.		Suspension.	
	Dip Wash.	Drippings.	Dip Wash.	Drippings.
Initial Concentration	970	—	940	—
Sheep No. 1	—	585	—	760
After 3 Sheep	825	—	860	—
Sheep No. 3	—	460	—	670
After 6 Sheep	665	—	840	—
Sheep No. 6	—	400	—	650
After 9 Sheep	525	—	780	—
Sheep No. 9	—	280	—	630
After 12 Sheep	375	—	720	—
Sheep No. 12	—	150	—	620

The exhaustion level during the dipping process was markedly higher with the emulsion than with the suspension. It was also found, however, (Tables 1 and 2, column III) that the percentage exhaustion rate increased during dipping. This was particularly noticeable in the case of the emulsion, where it increased from 6·4 to 11·1 per cent per animal. On the other hand, this increase was not so significant with the suspension. It can be stated thus that at least with the emulsion dip, the percentage exhaustion rate rises as the insecticide available in the bath decreases.

Laudani (1952) found that the insecticidal deposit in woollen cloth increased proportionately to the amount of insecticide available. When the total amount of insecticide was kept constant but the concentration varied by altering the volume of fluid, the deposit in the cloth remained constant. This indicates that there exists a correlation between the absolute quantity of insecticide in the container and the resulting deposit on the wool. It can be deduced from his results, however, that this correlation does not follow a fixed pattern as relatively more insecticide was removed from small containers than from large containers with equal concentration and a decrease of available insecticide in a given volume was followed by an increase in the percentage exhaustion rate. The results obtained by Laudani in his *in vitro* experiments with emulsions, have been confirmed in the dipping tests with living animals.

These findings are of the greatest significance in the whole procedure of sheep dipping in emulsion dips and indicate factors which do not appear to have been considered previously. It seems obvious that if the concentration of a dip wash is given merely as a percentage neither the exhaustion rate nor the amount of insecticide deposited upon the fleece can be estimated, or predicted. To correlate depletion rate with insecticidal deposit the total amount of insecticide available must be considered in terms of the tank size or volume of dip wash, as varying depletion rates of dip washes will occur in dipping vats of different sizes containing different volumes of wash of the same percentage concentration.

In addition it has been proved experimentally by Laudani that the quantity of wool immersed in an insecticidal wash has no influence on the amount of insecticide deposited but definitely affects the exhaustion rate of the bath. This means that an insecticidal deposit of equal intensity per unit of wool will be achieved with either long or short fleeces. More rapid exhaustion results, however, with long woolled sheep.

The amount of insecticide deposited on the sheep of each of the two groups in the experiment described above differed as the result of using two different formulations of the same active ingredient. Selective removal of the insecticide resulted in the deposit being higher with either formulation than could have been expected theoretically. The portion of the dipping fluid retained in the fleece of each animal actually constituted a wash several times higher in insecticidal strength than the corresponding fluid in the bath, whereas the drippings from these sheep showed a considerably lower concentration (Table 3). These findings are of extreme significance and must be borne in mind when the biological efficiency of a dip is compared with that of a spray. For this reason the percentage concentration of insecticide in a non-returnable spray must always be substantially higher than in a dip bath in order to ensure equal deposition and comparable control on woolled sheep. The insecticidal concentration should be about five times higher with emulsions and about $2\frac{1}{2}$ times higher with suspensions to produce equal results if the same type of wash is applied as a spray (Tables 1 and 2, column VII). These findings are indirectly confirmed by Jolly *et al.* (1953) who state that the biological effect, i.e. the period of protection against ticks, was not as good with a spray as with a dip using fluids of the same insecticidal concentration.

The type of formulation used did not materially influence the amount of insecticide deposited in the fleece of the first or last sheep of the respective groups. In the case of the emulsion the last sheep absorbed 79 per cent of the insecticide picked up by the first sheep; in the case of the suspension 73 per cent. The maintenance of a high deposit rate for the entire group treated with the suspension could be anticipated as neither the total amount of available insecticide nor the percentage concentration decreased to any very great extent during the dipping process. With the emulsion dip, however, depletion was considerable and the percentage concentration dropped rapidly, but the rise in the percentage exhaustion rate compensated for the loss of total insecticide available with the result that the amount absorbed remained constant.

4. *Conclusions.*

The following conclusions, which have a bearing on present day conceptions regarding the application of insecticides to woolled sheep, may be drawn from the results of the dipping tests:—

(1) The selective removal of insecticides from a dipping bath, which results in a considerably higher insecticidal deposit in the fleece in the case of emulsions than in the case of suspensions, is dependent upon the type and the amount of wetting agent used.

(2) In the case of emulsion dips the deposition of insecticide in the fleece is approximately maintained during the process of dipping until the available insecticide is depleted completely. This is due to the fact that as the total amount of available insecticide decreases the percentage exhaustion rate rises.

(3) In the case of suspension dips deposition of insecticide in the fleece is maintained at a constant level for a considerable period, but not until the insecticide is exhausted completely. As the total amount of insecticide decreases there is a slight but not proportional increase in exhaustion rate with the result that the amount deposited on the wool is bound to decrease progressively until it reaches a low level of little biological value, long before complete depletion of the tank has occurred.

(4) Both emulsions and suspensions must be used at a higher percentage concentration of insecticide when used as sprays than as dips to attain comparable deposition in the wool and so afford equivalent protection. The reason is to be found in the greater selective removal of active ingredient in dips due to the wetters.

In addition the following significant inferences, may be drawn from the *in vitro* tests of Laudani (1952):—

(5) As the total amount of insecticide available alone is responsible for the degree of deposition, the larger the dipping tank, the more insecticide is picked up per animal.

(6) The quantity of wool immersed in a dip wash influences the rate of exhaustion only and does not influence the deposition of concentration of insecticide on the wool. Long and short woolled sheep, therefore, leave the bath with equal relative insecticidal concentrations in the fleece, yet the depletion rate is greater with long wool as more actual insecticide is withdrawn.

The latter two conclusions are reported with reservations and must be subjected to further investigation. To the fact that the length of the wool has no influence on the concentration of insecticidal deposit, must be added the observation that the efficiency of a deposit on living sheep is largely dependent on the ability of the active ingredient to diffuse from the treated zone of the fleece into the constantly expanding new growth of wool nearer the skin. (du Toit and Fiedler, 1953). It has been shown (idem 1954) that a period of protection of longer duration is achieved when an insecticide with a high diffusion power is applied to sheep with long wool than to those with short wool. This means that the absolute amount of insecticide absorbed in the fleece, which is in direct proportion to the length of the fibres, and not the concentration in the fluid determines the duration of biological efficiency.

C. Biological Tests.

The duration of the insecticidal properties of various formulations of compounds with a high diffusion potential in growing wool has still to be assessed accurately. Contradictory opinions on the point have appeared in the literature. Downing *et al.* (1952) report that an emulsion is markedly inferior to a watery suspension in conferring long term protection, yet on the same page (791) at the end of the next paragraph, the statement is made that the protection conferred is more efficient and of longer duration where an emulsion is used.

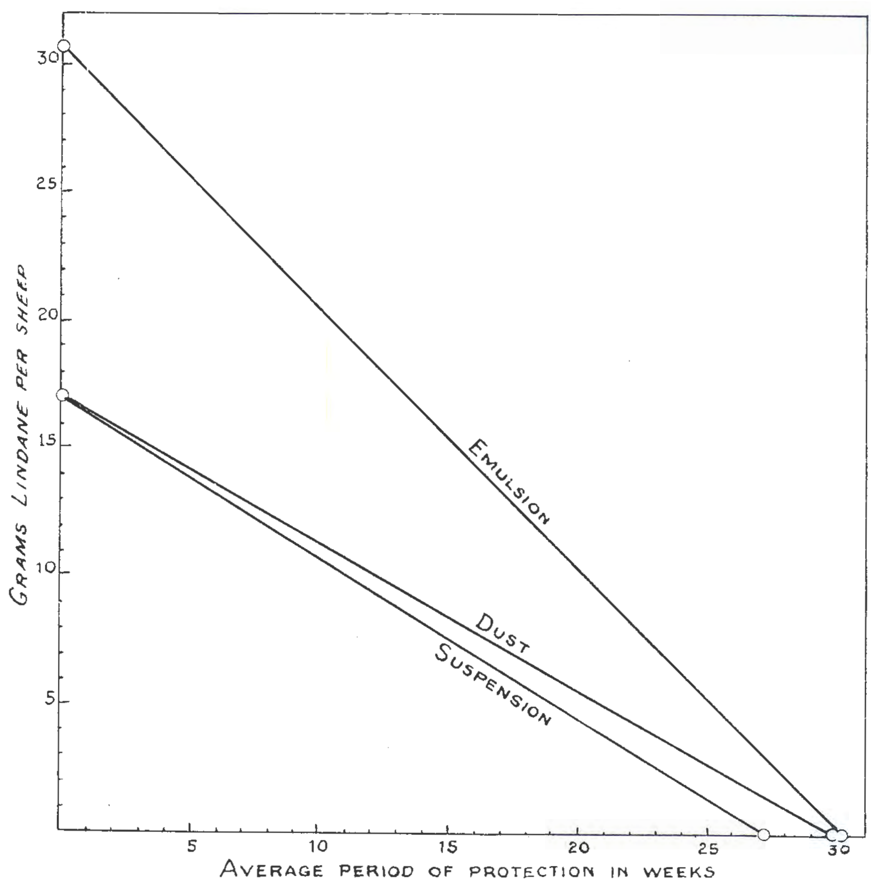

Fig. 2.—*Relation between period of protection against strike and insecticidal deposit on the sheep as conferred by different formulations of Lindane.*

The following biological tests were conducted with a view to throwing some light on this aspect. At the same time the most suitable and economical formulation for long term protection of woolled sheep against blowfly strike was investigated, and attention was paid to several additional factors likely to influence the period of protection, e.g. grease content of the fleece, and quality of the wool.

1. *Period of protection.*

The average quantity of Lindane deposited on each sheep in the three different groups was determined as follows:—

(1) Emulsion group ... ... 30·6 gm.
(2) Suspension group ... ... 16·9 gm.
(3) Dust group ... ... 17·0 gm.

The insecticidal deposit on the sheep in the groups treated with wettable powder and dust was the same, whereas the animals dipped in emulsion absorbed nearly double that amount.

The period of protection against strike was determined by means of the previously described bio-assay method (Fiedler and du Toit, 1951), where wool samples of individual sheep were periodically subjected to *in vitro* tests with first instar maggots of *Lucilia cuprina* Wied. The results were then verified by artificially induced strikes on the animals according to the method described by McLeod (1937).

The period for protection for each sheep, as assessed by these two methods, is given in Table 4. It will be recalled that six of the twelve sheep in each group were immersed in water four weeks after the insecticidal treatment.

TABLE 4.

Period of protection against blowfly strike conferred by different formulations of Lindane.

Group.	Emulsion.		Suspension.		Dust.	
Treatment after Dip.	None.	Immersion Water.	None.	Immersion Water.	None.	Immersion Water.
Period of Protection in Weeks for the Individual Sheep.	27	27	24	26	29	26
	27	27	26	27	29	27
	30	27	26	28	31	27
	30	29	27	30	33	27
	31	30	27	30	34	29
	36	31	27	32	36	31
	30·2	28·5	26·2	28·6	32·0	27·7
	29·4		27·4		29·8	

Results.

The average period of protection for the groups of twelve sheep was 29·4 weeks in the case of the emulsion, 27·4 weeks for the suspension, and 29·8 weeks for the dust. Emulsion and dust, therefore, conferred protection for the same length of time, whereas the effect of the suspension was slightly shorter. This finding, however, changes to the disadvantage of the emulsion when the time of protection is viewed in the light of the amount of insecticide deposited (Figure 2). The emulsion which produced nearly twice as much deposition as the other formulations (30·6 gm. against 17 gm.) lost its larvicidal efficacy in about the same period as the dust and suspension. The bio-assay tests revealed that the diffusion rate of Lindane was relatively lower with the higher deposit from the emulsion than with the other formulations. This points to the fact that a certain masking effect of the active ingredient had taken place resulting in a marked loss of larvicidal activity. The part which the grease content of the wool plays in this respect will be discussed below.

2. *Influence of an additional immersion in water.*

To determine whether the entire amount of insecticide deposited in the fleece was held in the wool grease or whether a certain portion of the Lindane remained in the wool in the original form, six sheep of each group were immersed in plain water for a period of about six minutes a month after treatment, as previously stated. This would allow any insecticide, not taken up by the grease, to disperse in the water.

Result.

The results (Table 4) demonstrate in all three cases some influence by the water on the length of the protecting period.

The water immersion treatment shortened the period for about three weeks for the emulsion and for over three weeks in the case of the dust, thus indicating especially in the case of the latter formulation, that a considerable quantity of the insecticide had remained as dust in the fleece and could again be separated easily from the wool. The animals treated with suspension, on the other hand, contrasted quite markedly with the other two groups, as the period of protection was extended by about two weeks due to the water treatment. Whether the additional treatment with water had effected a further penetration of the Lindane wettable powder into the new growth wool, remains a matter for speculation.

3. *Influence of the quality of the wool.*

The wool of all the merino sheep in this experiment was graded prior to the insecticidal treatment. The animals were then placed in the three groups, care being taken that equal numbers of sheep with similar wool were subjected to treatment with the three insecticidal formulations. Three classes of wool, namely strong, medium and fine with quality numbers between 60's and 100's and an average fineness of the fibres between 21·74 and 15·94 μ were available for the experiment.

Result.

An analysis of the results showed no correlation between the duration of protection against strike and the fineness of the wool fibre. This indicates that the quality of the wool, within the limits of the range investigated, has no bearing on the efficiency of the insecticide.

4. *Influence of the wool grease.*

The grease content of the wool was determined for each sheep prior to treatment. This was repeated after dipping, and again a month later after one half of each group had been immersed in water. No change in grease content was apparent after any of the treatments.

The data connected with the protection conferred upon the different groups and sub-groups were analysed after the biological tests had been completed with the view to determining any influence of the grease content upon the duration of protection. For this purpose half of the sheep of each sub-group (3 animals) showing the longest protection was compared with the half showing the shortest protection. The findings are shown in Table 5.

TABLE 5.

Influence of the grease content of the wool on the period of protection conferred by a Lindane emulsion, suspension and dust.

Treatments.		Long Protection.		Short Protection.	
Insecticide Formulation.	One month later.	In Weeks.	Per Cent Grease.	In Weeks.	Per Cent Grease.
Emulsion............	Nil..................	36 31 30	11·8 13·6 12·2	27 27 30	14·2 14·4 18·6
	Water Treatment.....	31 30 29	12·8 11·9 13·2	27 27 27	22·1 23·0 13·3
	AVERAGE.........	31·1	12·6	27·5	17·6
Suspension...........	Nil..................	27 27 27	19·6 11·5 12·2	24 26 26	14·0 13·6 19·1
	Water Treatment.....	32 30 30	16·3 12·3 10·3	26 27 28	15·8 10·6 12·7
	AVERAGE.........	28·8	13·7	26·2	14·3
Dust................	Nil..................	36 34 33	9·4 14·4 20·3	29 29 31	13·7 18·9 14·4
	Water Treatment.....	31 29 27	12·3 16·6 15·4	26 27 27	16·4 18·2 17·7
	AVERAGE.........	32·2	14·7	28·2	16·6

Result.

No significant influence on the duration of protection attributable to the percentage of wool grease is demonstrable for the groups treated with either suspension or dust. The protection conferred by the emulsion, however, is influenced decidedly by the grease. The animals in the two emulsion sub-groups showing long protection, possessed a grease content between 11·8 and 13·6 per cent with an average of 12·6 per cent, whereas the grease content of animals showing short protection ranged between 13·3 and 23·0 per cent, averaging 17·6 per cent. The difference of 5·0 per cent in the wool grease content, therefore, corresponds to a difference in protection of 3·6 weeks, or over 11 per cent of the long period of protection. The antagonistic influence of a high grease content on the duration of protection afforded by an emulsion may explain at least in part the reduced activity of emulsions in general as compared with suspensions or dusts in affording protection against blowfly strike.

5. *Discussion.*

The duration of the larvicidal efficiency of Lindane when used in the form of an emulsion, a suspension or as a dust for long term protection of sheep varied considerably.

The insecticidal deposit in the case of the suspension group was similar to that of the dusted group but approximately only half of that of the emulsion group.

Lindane in dust form conferred the longest period of protection relative to the amount of the deposit and was closely followed by the suspension. The duration of protection afforded by the emulsion was not proportional to the amount of insecticide actually deposited in the fleeces, however, and did not exceed that of the other formulations deposited in a smaller amount.

The quality of wool normally produced by merino sheep in South Africa appeared to have no effect on the protecting properties of either of the three formulations.

The wool grease content of the fleece did appear to play a part. Protection of shorter duration resulted from the use of the emulsion in fleeces with a high grease content, whereas this detrimental effect was not observed with either the dust or the suspension. It may be concluded from these findings that wool grease in general exerts a detrimental effect on the larvicidal efficiency of an emulsion, whereas this does not apply to the other formulations. The decrease in efficiency is proportionate to the percentage grease content of the fleece.

The emulsion type of insecticide must be regarded as both the least effective formulation for long term protection of woolled sheep against blowfly strike as well as the most inferior protective dressing for strike wounds.

D. Conclusions.

From the results of the experiments conducted, the following conclusions may be drawn which are of importance in the practical application of insecticides to sheep for protection against blowfly strike:—

(1) Insecticides with high diffusion potential such as gamma B.H.C., Aldrin and Dieldrin, should be used as wettable powders or dusts and not in the form of emulsions to achieve long lasting protection at low cost.

(2) Wettable powders designed for use on sheep when applied as non-returnable sprays, should be used at a concentration $2\frac{1}{2}$ times higher than in the dipping tank. In emulsion form with an anionic wetter, the concentration should be 5 times the strength used for dipping.

E. Summary.

1. Three groups each of twelve merino sheep were treated separately with Lindane in emulsion, suspension and dust form to determine the influence of the formulation on the duration of protection against blowfly strike.

2. The emulsion and suspension contained a wetting agent of anionic type. The emulsion, containing 970 p.p.m. gamma B.H.C. and the suspension with 940 p.p.m. gamma B.H.C. were applied by immersion in a small vat of 120 gallons (546 litres) capacity. Twelve sheep were used for each formulation. The removal of dip wash per animal and the depletion rate of the insecticide were determined.

3. The rate of depletion of insecticide from the dipping bath was higher per sheep treated in the emulsion than in the suspension. This is supported by the finding that the average deposition of Lindane per sheep was 30·6 grams for the emulsion, but only 16·9 grams after immersion in the suspension.

4. The percentage rate of exhaustion of insecticide continued to rise progressively as the absolute quantity available in the tank decreased. This rise in percentage depletion was far more marked in the case of the emulsion that the suspension.

5. There was no significant difference between the amount of insecticide deposited in the fleece of the first and last of *twelve* sheep treated in either the emulsion or the suspension formulation. This may be ascribed to the finding that in the case of the emulsion the progressively higher percentage depletion accounted for the higher rate of deposition in the wool, in other words the higher rate of selective removal. In the case of the suspension the lower absolute depletion rate together with the appreciably lower percentage removal were the factors involved in maintaining the even deposition of insecticide with the progress of dipping. Had more sheep been dipped the end result would probably be different.

6. Continued immersion of sheep in an emulsion dip will result in a sudden and rapid complete depletion of insecticide below the level of biological effectiveness. Using a suspension dip it will take longer to reach this point.

7. Using a non-returnable spray as the means of applying an insecticide a suspension spray must be mixed at 2½ times the concentration of a dip and an emulsion of anionic type at about 5 times the concentration of a dip to obtain the same concentration of insecticide on treated sheep.

8. Attention is directed to Laudani's observation that the amount of insecticide absorbed by wool is dependent not only on the concentration of the active ingredient in the dip wash but also on the absolute amount present. Full consideration must therefore be paid to the capacity of a dip or bath.

9. The third group of animals was treated with dust at a rate of 17 gm. of the active ingredient per head, i.e. the amount absorbed by each sheep dipped in the suspension, in order to facilitate comparison of larvicidal efficacy of a dust and a suspension. The bio-assay tests with first instar maggots of *Lucilia cuprina* revealed an average protection of relatively shorter duration, 29·4 weeks with the considerably heavier deposit (30·6 gm.) in the case of emulsion, than the 27·4 weeks with the suspension (16·9 gm.) and 29·8 weeks for the dust (17 gm.). The dust, therefore, gave the best protection of the three formulations per unit of insecticide absorbed by the fleece.

10. The grease content of the fleece was shown to exert a marked influence on the degree of efficacy of the insecticide deposited from an emulsion. This does not apply, however, in the case of the dust or the suspension. The loss of larvicidal activity is proportionate to the level of wool grease which is not affected by dipping or spraying.

11. The quality of the wool has no effect on the protecting properties of any of the formulations.

12. The use of B.H.C. or any compound possessing good powers of diffusion in the wool is recommended for the long term protection of woolled sheep against blowfly strike. Such compounds may be used in the form of dusts or wettable powders but the present investigation suggests that their use as emulsions is contraindicated.

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