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THE NEW SYNTHETIC INSECTICIDES AS DRESSINGS FOR BLOWFLY STRIKE IN SHEEP.

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Hepburn (1943a & b), Mönnig (1943) and Mönnig and Celliers (1944) have presented a considerable amount of evidence on the dominant role played by *Lucilia cuprina* Wied., in the causation of strike in the Union of South Africa. These authors have also shown that, according to the experimental work conducted by them, this species is dependent largely upon the living sheep for its continued existence, particularly during the summer months, due to the competition afforded in carcass material by other species such as *Chrysomyia albiceps* Wied., and *Chrysomyia marginalis* Wied. From this evidence Mönnig (1943) stresses the desirability of an efficient dressing capable of destroying the larvae upon the living sheep in the hope of bringing about a general reduction in the incidence of *L. cuprina.*

Du Toit and Goosen (1949) have pointed out the disappointing results which have followed the extensive use of an efficient blowfly larvicide in South Africa over some years. Similar conclusions have been drawn in Australia where large-scale operations, designed to control blowflies in their larval stages both in carcass material and on living sheep, have been equally ineffective.

The general conclusion from the investigations into the blowfly problem both in Australia and in South Africa points to a greater measure of success being achieved by protecting sheep against attack rather than by attempts at reducing the incidence of the blowflies involved.

In the publication previously cited, du Toit and Goosen (1949) have indicated the degree of protection to blowfly strike afforded by treatment of the fleece with D.D.T. and du Toit *et al.* (1948) have presented some experimental evidence regarding the even better protection afforded by hexachlorocyclohexane (B.H.C.) and Chlordane.

No matter what degree of protection against strike is afforded by the new synthetic insecticides the need for a thoroughly efficient blowfly dressing for fly-struck sheep will always exist. The greater the degree of protection such a dressing can give to such wounds against subsequent restrike the greater its value.

Lennox (1941) has enumerated the properties which he considers the ideal blowfly dressing should possess. He has stressed such characteristics as its stability, resistance to decomposition, its capacity to penetrate the fleece easily without damage to it and its ready removal during the usual industrial processes of woolscouring; larvicidal effect or ability to render larvae innocuous; non-toxicity to sheep with persistent effect to protect against restrike during healing, which should not be interfered with; cheapness; and finally, if in an aqueous system, ease of preparation by the addition of water to the solid components.

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Lennox has not laid undue emphasis upon larvicidal effect which, in the light of our present knowledge, is perhaps not as important as was thought previously.

The present investigation was designed to evaluate the larvicidal properties of a number of the new synthetic insecticides and at the same to ascertain in how far certain formulations containing them conformed to the standard laid down by Lennox.

Material and Methods.

The methods of testing the insecticides consisted of:—

(*a*) *in vitro* tests upon fully mature active third stage larvae of *Lucilia cuprina* bred upon lean beef. The maggots were immersed in various concentrations of the insecticides. Aqueous suspensions of wettable powders were used in order to eliminate the possible effects of solvents which might play a part if used in the form of emulsions. Immersion for 10 seconds was applied in each case; and

(*b*) *in vivo* tests of the insecticides as wettable powder suspensions in water upon third stage larvae in artificially induced strikes upon sheep. In that various formulations of the insecticides were being tested upon these strikes for both larvicidal effect and effect upon the wounds, it was necessary to implant several batches of first stage larvae on successive days in order to ensure well developed strikes upon the sheep. The result was that the larvae varied in size to some extent so that after treatment their behaviour was not strictly comparable to the very uniform larvae used in the *in vitro* tests. Furthermore, the treatment and disturbance of the wool during treatment caused the larvae to disperse rapidly into the wool, which made their collection for subsequent observation very difficult.

All larvae after treatment by the *in vitro* method were placed upon sand in glass jars covered with fine gauze after draining off the excess insecticide suspension. A period sufficient for all formed pupae to hatch and the resultant flies to die was allowed and thereafter the sand was sifted and pupae, pupal casings and dead flies were counted. By subtracting the pupae and casings from the number of larvae originally treated the number of larvae which had been killed by the insecticide was recorded as well as the pupae which had failed to hatch.

A number of random collections of larvae treated by the *in vivo* method were made from the experimental sheep and examined in this way. The observations made upon these larvae revealed that, taking into consideration the lack of uniformity of these specimens, the results were identical to the *in vitro* method at comparable dilutions of the insecticides in aqueous suspensions. For this reason the method was discontinued.

It should be stated that the treated larvae both in the *in vitro* and *in vivo* tests were not killed immediately. In some cases a state of irritability with increase in activity occurred immediately after treatment. Thereafter gradually increasing paralysis supervened with death finally occurring only after 24 hours in many cases, or even later.

In Table I the average mortality obtained from a minimum of five replications of each test, in which 25 third stage larvae were immersed in various dilutions of the test insecticide, is reflected.

TABLE 1.

Average percentage mortality of third stage larvae of Lucilia cuprina *at various dosages of insecticides.*

Insecticide.	Concentration applied.									
	6·4%	3·2%	1·6%	0·4 %	0·1%	0·025%	0·0064%	0·0016%	0·0004%	0·0001%
	64,000 Parts per Million.	32,000 Parts per Million.	16,000 Parts per Million.	4,000 Parts per Million.	1,000 Parts per Million.	250 Parts per Million.	64 Parts per Million.	16 Parts per Million.	4 Parts per Million.	1 Part per Million.
D.D.T.	36	24	10	—	—	—	—	—	—	—
DFDT (Gix) (Farbenwerke Höchst. Germany)	64	48	30·5	12	—	—	—	—	—	—
D.D.D. (Rothane)	10	3	—	—	—	—	—	—	—	—
Methoxychlor	10	3	—	—	—	—	—	—	—	—
B.H.C. (Gamma Isomer)	—	—	—	100	100	98·5	85·5	28	10·7	1
Toxaphene	—	91·2	64	25	3	—	—	—	—	—
Chlordane	—	100	89	50	21	5	—	—	—	—
Aldrin	—	—	100	100	91	61	23	—	—	—
Dieldrin	—	—	100	100	75	42	15	—	—	—
Thiophos (Parathion)	—	—	—	100	100	86	57	7	—	—
EPN-300 (Dupont)	—	—	100	88	70	45	17	—	—	—
No. 4049 (Amer. Cyanamid Co.	—	—	94	30	17	8	—	—	—	—
Controls*	AVERAGE HATCHING = 96·5%.									

* A control group of larvae was immersed in tap water before and after each series of immersions in insecticide and treated similarly to the larvae used in the insecticide immersion tests.

In addition to the insecticides enumerated in Table 1, which were used in wettable powder form with the exception of DFDT (Gix), which was available in the form of an emulsifiable concentrate only, two proprietary preparations of Toxaphene and Chlordane in emulsion form were tested. At dilutions similar to those in the wettable powder form both these preparations proved to be inferior in larvicidal properties. The Chlordane preparation did not yield 100 per cent. mortality even at a concentration of 5 per cent. of the active ingredient.

In figure 1 the mortality of third stage larvae of *L. cuprina* at the various dilution rates of the test insecticides is given in graphical form for the sake of comparison.

It will be noted from figure 1 that the mortality percentages fairly closely approximate straight lines between 10 and 90 per cent. They deviate considerably however at either end of this range. From this graph it is possible to plot the mean 50 per cent. mortality (LD 50) for the various insecticides as follows:—

LD50 for *L. cuprina,* third stage larvae.

Insecticide.	*Dilution in parts per million.*
B.H.C. (gamma isomer)	25
Thiophos (Parathion)	50
Aldrin	170
E.P.N.-300	335
Dieldrin	335
Chlordane	4,000
No. 4049	6,000
Toxaphene	10,000
D.F.D.T.	130,000
D.D.T.	130,000

The effect of the components of various blowfly dressings upon the skin of sheep.

In order to determine the effects of the various constituents of certain blowfly dressings upon the skin of sheep with the object of finding a solvent into which to incorporate B.H.C., the most promising of the larvicides tested, a series of patch tests was conducted upon sheep.

The method adopted was to press one end of an open glass cylinder firmly against the skin of the side of a recently shorn sheep placed upon the ground in a fully extended recumbent position and to pour just sufficient of the material under test into the cylinder so as to saturate the area without wastage when the cylinder was removed. The skin over the lateral aspects of the thorax and abdomen allowed of seven to eight such patch tests, one side only being used.

Observations upon the effects of the test materials on the skin were made at intervals of 3, 6, 12, 24 and 36 days and the results are given in Table 2.

FIGURE 1.

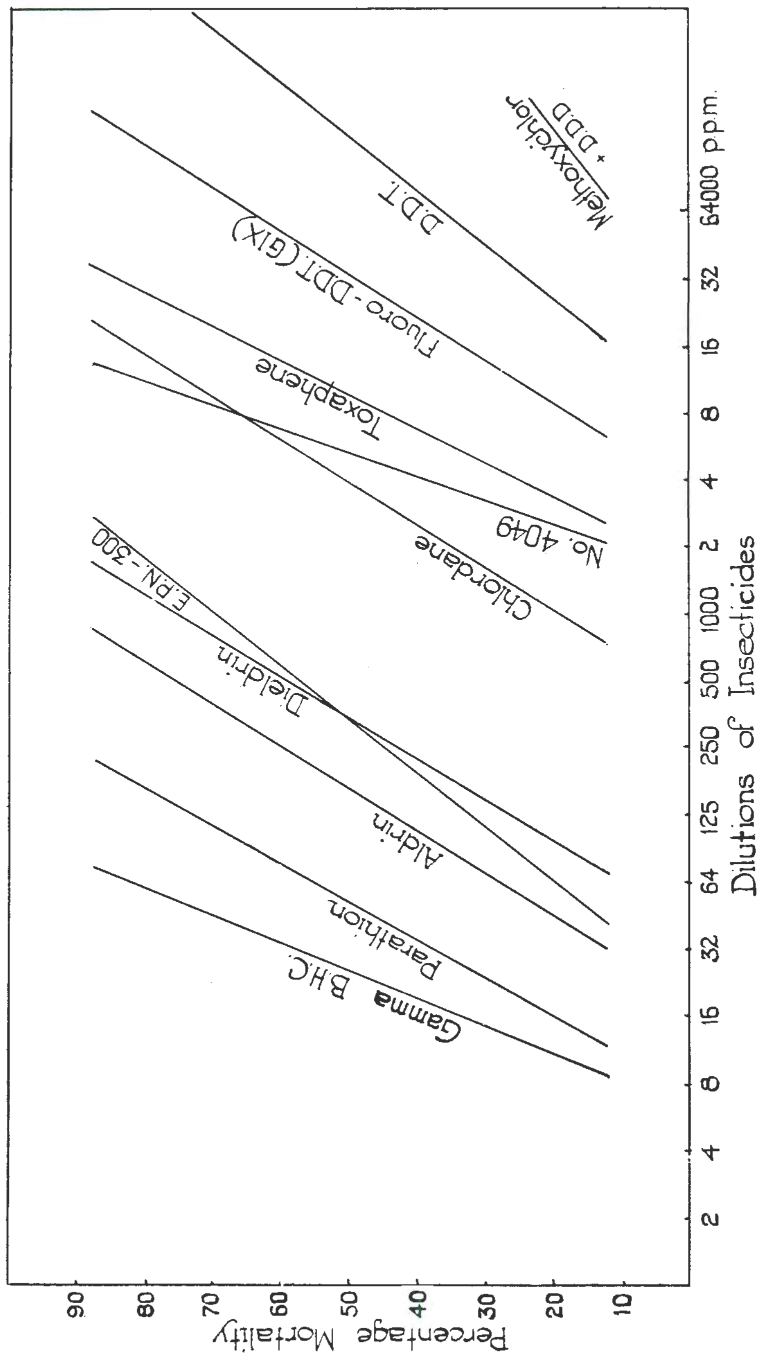
D.D.T.
Methoxychlor
+ D.D.D.
Fluoro - DDT (GIX)
Toxaphene
No. 4049
Chlordane
E.P.N.-300
Dieldrin
Aldrin
Parathion
Gamma B.H.C.
Percentage Mortality
90
80
70
60
50
40
30
20
10
Dilutions of Insecticides
2
4
8
16
32
64
125
250
500
1000
2
4
8
16
32
64000 p.p.m.

TABLE 2.

Patch Tests upon Sheep. (Average of Effects upon Two Sheep per Test.)

Composition of Material Tested (Percentages).	Observations upon Skin.				
	3 Days.	6 Days.	12 Days.	24 Days.	36 Days.
T.O.V.S.*........................	+++ 00000 D	00000	00000 C	00000	00000 L
Blowfly Spray†....................	+++ 0000 D	0000	0000 C	00000	00000 L
Alcohol 75 + T.O.V.S. 25..........	++ 000	000	000 C	0000	0000 L
Alcohol 80 + T.O.V.S. 20..........	+ 000 D	000	000 C	000	000 L
Alcohol 85 + T.O.V.S. 15..........	00	00	00 C	00	00 L
Alcohol 90 + T.O.V.S. 10..........	0	0	0	—	—
Alcohol 95 + T.O.V.S. 5..........	—	—	—	—	—
Coal Tar Oil (minus Cresol and Tar Acids) = CTO..................	+++ 0000 D	0000	00000 C	00000	00000 L
Alcohol 75 + C.T.O. 25............	++ 0000 D	0000	0000 C	00000	00000 L
Alcohol 80 + C.T.O................	+ 00 D	00	000 C	00	00 L
Alcohol 85 + C.T.O. 15............	0	0	0	—	—
Alcohol 90 + C.T.O. 10............	—	—	—	—	—
Alcohol 95 + C.T.O. 5............	—	—	—	—	—
Alcohol 97·5 + Cresol 2·5.........	0	0	0	—	—
Alcohol 98·5 + Cresol 1·5.........	—	—	—	—	—
Alcohol 99·0 + Cresol 1·0.........	—	—	—	—	—
Alcohol 99·33 + Cresol 0·67.......	—	—	—	—	—
Alcohol 99·67 + Cresol 0·33.......	—	—	—	—	—
Alcohol 96........................	—	—	—	—	—
Benzol (pure)......................	B 000 D	000	0000 C	0000	0000
Alcohol 65 + Benzol 35............	B 000 D	000	0000 C	0000	0000 L
Alcohol 80 + Benzol 20............	0 D	000	000 C	000	000 L
Alcohol 90 + Benzol 10............	—	—	—	—	—
Alcohol 95 + Benzol 5............	—	—	—	—	—
Acetone (pure)....................	—	—	—	—	—

Crust Formation.

00000 = Thick hard crust.
0000 = Hard crust.
000 = Crust.
00 = Slight crust.
0 = Slight hardening of skin.
— = No visible effect.

Wool Staining.

+++ = Dark brown.
++ = Middle brown.
+ = Light brown.
B = Wool showing bleached appearance.

D = Skin dark in colour.
C = Crust cracking.
L = Superficial layers of skin lifting.

* T.O.V.S. = Tar Distillate containing 7% cresol.

† Blowfly Spray = Standard spray produced at Onderstepoort.

Formula: Cresol 2·5%;
Coal tar derivatives 32·25%;
Inorganic acid 0·25%;
Alcohol (96%) 65%.

It will be noted that the neutral tar oil advocated by Mönnig (1943) showed distinct crust formation of the healthy skin with subsequent lifting of the superficial layers together with the wool covering the area. This is in accordance with reports received from extensive use of this dressing in the field over a period of several years.

Of the materials tested none was entirely satisfactory with the exception of alcohol alone. This was considered to be too costly to make its use feasible.

In view of the excellent larvicidal properties possessed by several of the insecticides reported upon earlier when used in wettable powder form, it was concluded that no material advantage could be anticipated from the inclusion of a solvent. Some increase in the rate of penetration of the fleece would result but this alone did not appear to justify the added expense involved in its use.

Effects of gamma B.H.C. in wettable powder form and as emulsions upon blowfly infested wounds.

The investigation at this stage had indicated the efficient larvicidal properties of certain of the new synthetic insecticides and the irritant effect of the more common solvents upon the skin of sheep. It appeared advisable, therefore, to study more closely the actual healing of blowfly infested wounds.

For this purpose two proprietary emulsions containing B.H.C. were selected. Their effect upon blowfly infested wounds on sheep was compared with a proprietary wettable powder containing this insecticide. Of the two emulsions chosen one contained a saponified coal tar distillate as a base and the other toluol as solvent with saponified pine resin and casein as emulsificant. The former was in the form of a miscible oil and the latter as a mayonnaise type emulsion concentrate. The wettable powder contained a small quantity of a proprietary wetting agent milled into the mixture of B.H.C. and clay. By way of comparison these three preparations were compared with the blowfly spray mixture advocated by Mönnig as regards effect upon maggots and subsequent healing of the wounds.

In order to simulate as closely as possible blowfly struck sheep under conditions of natural strike in the field four or five implantations of first stage larvae were made upon the experimental sheep at intervals of three or four days. At intervals varying between eight and eleven days after the first implantations of first stage larvae the strikes were found to be well advanced with fairly extensive superficial erosions of the skin. Most of the maggots of the first few implantations had by this time left the wounds but numerous maggots in various stages of development from later implantations were present throughout the struck areas. They showed a tendency to congregate in pockets in the fleece as is generally the case with natural strikes.

The test was not quite comparable to conditions which would obtain under field conditions where, during a wave of blowfly activity, the sheep would probably show repeated natural strikes by both primary and secondary blowflies. Under the conditions of the experiment natural strike was almost absent, only one sheep being struck during its duration. The result was that upon completion of larval development the maggots left the wounds which healed normally thereafter. For purposes of the experiment, however, where the object was a study of the rate of healing after treatment with the test remedy, the conditions were considered satisfactory.

Three groups of ten sheep each were selected, nine animals in each group being treated and one left untreated to serve as a control. The nine sheep of each group were subdivided into three groups of three each and these subgroups were each treated with the test dressings at concentrations of 0·01 per cent., 0·1 per cent and 0·5 per cent gamma isomer respectively. A separate group of two sheep only were treated with the Onderstepoort blowfly spray (Mönnig) after infestation with two lots of first stage larvae.

The results are best reflected in general terms as averages of the effects on the sheep in each group.

1. The three groups of three sheep each treated with the dressings at a concentration of 0·01 per cent gamma B.H.C. showed the presence of living and apparently unaffected third stage maggots two days after treatment. The group treated with the wettable powder showed the least effect of all so far as living larvae were concerned, all three sheep remaining infected. In the case of the coal tar distillate emulsion one sheep showed no maggots after two days and in the toluol emulsion group two sheep were free from maggots on the second day.

Three days after treatment first stage maggots were again implanted upon all three groups to test whether the concentration of B.H.C. was sufficient to protect against restrike. Three days later all the sheep showed infestation.

In view of the reinfestation which took place three days after treatment the 0·01 per cent gamma B.H.C. concentration was regarded as inadequate and this experiment was closed.

2. The second group was treated with the three formations of B.H.C. at a concentration of 0·5 per cent of the gamma isomer.

Two days after treatment a few dead maggots only were still present in pockets in the fleece from which they appeared to have been unable to escape before being overcome. The pine resin emulsion group showed the greatest number of dead maggots in the wool. At this stage the wettable powder group showed the most advanced healing of the superficial skin erosions caused by the blowfly larvae, both the other groups showing some scab formation with suppuration.

On the third day after treatment the wounds on the sheep treated with the wettable powder were covered by dry thin scabs and the wool was dry whereas both other groups showed a considerable amount of moisture in the wool with suppuration of the wounds. In the case of the pine resin emulsion group the struck area exuded an unpleasant odour. At this stage first stage larvae were implanted upon all the sheep to test the degree of protection afforded. Three days later no development of larvae had occurred upon the treated sheep whereas the larvae had developed normally upon the control (untreated) sheep.

Healing of the wounds on the wettable powder treated group compared with those on the untreated control sheep took place at approximately the same rate once the maggots on the latter sheep had left the wound. In the two emulsion groups there was a distinct retardation of healing although six days after treatment all wounds were healed. Moisture was still present in the case of the pine-resin emulsion group.

3. In order to reduce the quantity of inert or adjuvant ingredients which might have a retarding effect upon the healing process, the third group was treated with the remedies diluted to give a concentration of 0·1 per cent. gamma B.H.C.

On the second day after treatment the wettable powder group again showed the greatest progress so far as healing was concerned, the wool being practically dry. The coal tar distillate group showed the presence of more moisture than the pine-resin emulsion group and some suppuration was still present in both these groups. Dead maggots were present in all three groups in limited numbers only, the majority having left the fleece. Examination on the third day revealed the progress of healing to be in favour of the wettable powder group with the pine-resin group second and the coal tar distillate group third.

First stage maggots implanted on the 5th day after treatment failed to develop except in the control group where development was normal.

As has been stated a fourth group of two sheep infested with two implantations of first stage larvae was treated with Onderstepoort blowfly spray (Mönnig) and compared with the above three groups.

All maggots were killed instantly and remained in the fleece. On the second day after treatment the wounds together with the healthy skin surrounding them, which had come into contact with the remedy, showed a hard dry crust which had formed and dark discoloration of the skin. On the 7th day after treatment the superficial layers of the skin together with the wool covering the whole of the treated area showed a tendency to lift. Subsequently the wool over the wounds and treated area surrounding them lifted completely leaving a bare patch over which, however, the wool again grew normally.

Discussion.

From the data presented in the investigation it will be noted that, of the various insecticides tested, the following may be looked upon as efficient larvicides capable of producing 100 per cent. mortality of third stage larvae of *Lucilia cuprina* at dilutions which make their use practical and economically possible;

1. Parathion (Thiophos)—at 0·1 per cent.
2. B.H.C. (gamma isomer)—above 0·1 per cent.
3. Aldrin—at 0·3 per cent.
4. Dieldrin—at 0·4 per cent.
5. E.P.N. 300—at 1·6 per cent.
6. Chlordane (Velsicol 1068)—at 3·5 per cent.

D.D.T. and its related compounds, on the other hand, have yielded very poor results as also has toxaphene in wettable powder form. These compounds must be looked upon as unsuitable for purposes of a dressing for blowfly struck sheep e.g.

7. Toxaphene—at 3·2 per cent. only 90 per cent. kill

(At higher concentrations the suspension became too thick and creamy to make its use possible).

8. Fluoro D.D.T. (D.F.D.T. Gix)—at 6·4 per cent. only 65 per cent. kill.
9. D.D.T.—at 6·4 per cent. only 35 per cent. kill.
10. Methoxychlor (Marlate)
11. D.D.D. (T.D.E. or Rothane 3)

} at 6·4 per-cent. no appreciable effect.

Of the insecticides tested, Parathion, although extremely potent, cannot be recommended for use as a dressing on account of its extreme toxicity for warm blooded animals and man. B.H.C. is undoubtedly the insecticide of choice from the point of view of effectiveness and economy. The gamma isomer of B.H.C. in its pure form as Lindane, although extremely lethal to larvae, is expensive and its use would not be justified unless the unpleasant odour of the technical product demanded it; an unlikely contingency.

The other insecticides tested e.g. Aldrin, Dieldrin E.P.N.-300 and Chlordane, although effective at concentrations which make their use possible on economic grounds, must give way to B.H.C. in South Africa where the latter product is produced and is readily available in bulk.

The patch tests together with the tests of certain proprietary emulsions of B.H.C. indicate that no material advantage is to be gained by the addition of solvents which, although possibly enhancing the penetrating properties of the dressing, in no way promote healing and may actually retard it.

CONCLUSIONS.

The evidence presented in this investigation points to the gamma isomer of B.H.C. as most closely conforming to the desiderata for a blowfly dressing enumerated by Lennox. For reasons of economy B.H.C., is recommended for use in its technical form combined with clay as a wettable powder readily dispersible in water. In this form B.H.C. at a concentration of 0·5 per cent. of the gamma isomer yields 100 per cent. mortality of mature third stage larvae. The insecticidal effect is slow thus permitting the majority of the larvae to leave the wound before they are destroyed and healing is in no way interfered with. There appears to be no advantage in the incorporation of a solvent for B.H.C. and such solvents may prove irritating even to the healthy skin surrounding the wound and lead to fairly extensive excoriation with the loss of a considerable amount of wool. The protection against restrike afforded by B.H.C. is at least ample to permit of complete healing of all wounds caused by blowfly larvae.

SUMMARY.

1. The more recent literature on the blowfly problem in South Africa is reviewed and the general conclusions regarding control measures discussed.

2. The larvicidal properties of a number of the new synthetic insecticides for mature third stage maggots of *Lucilia cuprina*, Wied., are investigated for purposes of their use in blowfly dressings.

3. The methods of assessment of larvicidal properties by *in vitro* and *in vivo* tests are stated and tabulated for the different insecticides tested.

4. Patch tests upon sheep for the more common organic solvents of the insecticides are described and the results enumerated.

5. Tests of B.H.C. in wettable powder form and as emulsions are conducted upon blowfly strikes artificially produced upon sheep and the results compared.

6. Recommendations for the use of B.H.C. in wettable powder form at a concentration of 0·5 per cent of the gamma isomer are made.

7. The advantage possessed by B.H.C. of killing third stage maggots slowly thus permitting them to leave the wound before death is pointed out.

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