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THE PROTECTION OF SHEEP AGAINST BLOWFLY STRIKE.

IV. AN EVALUATION OF CERTAIN ORGANIC PHOSPHORUS COMPOUNDS.

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A. INTRODUCTION.

Several organic phosphorus compounds have been developed lately which show excellent insecticidal properties especially against house flies resistant to most of the chlorinated hydrocarbons (D.D.T., B.H.C., etc.) as well as against certain groups of noxious insects and mites that cannot be controlled successfully by the latter compounds. As many of the newer phosphorus insecticides possess only a low grade toxicity to warm-blooded animals, in any case, not higher than the well established hydrocarbons, their application in the field of animal husbandry seems to be indicated.

Since the authors had shown (Du Toit and Fiedler, 1953) that two of the earlier organic phosphates, Parathion and EPN 300, were exceptionally good larvicides capable of producing complete mortality of first instar maggots of sheep blowflies (*Lucilia cuprina* Wied. and *Chrysomyia chloropyga Wied.*) at a concentration of 0·25 p.p.m. of the active ingredient in the nutritional medium, an investigation was undertaken to evaluate all available phosphorus compounds with the view to their possible application as protecting agents of sheep against blowfly strike. The aim was to determine those compounds most suitable for long-term protection of sheep.

In addition to the larvicidal properties, it was necessary to investigate the behaviour of the insecticides in the wool of living sheep. It has been proved that in the case of the chlorinated hydrocarbons, apart from other factors, the protection of sheep is dependent largely on the capability of the compound to diffuse along the wool fibres from the treated zone into the ever increasing new growth of wool underneath. In order to assess this very important property, the compounds were examined by the biological assay method of Fiedler and Du Toit (1951). For this purpose it is essential to eliminate factors such as excretions in the crutch region, which would influence the residual effect of an insecticide adversely, and the compounds were applied, therefore, to the clean, uncontaminated wool over the rump.

It must be stressed that with compounds applied to such uncontaminated wool the period of protection achieved under what might be termed semi-laboratory conditions is only relative and should in no way be compared with the results of field trials. On the other hand the bio-assay method represents a quick and accurate means of selecting the most suitable compounds out of a large number of insecticides and these may then be subjected to field tests in order to determine the durabilities of protection under natural conditions.

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B. Experiments.

The following phosphorus compounds have been tested in the present series of trials:—

1. Parathion
2. EPN 300
3. Malathion

(all three from the American Cyanamid Co.).

4. Diazinon (Geigy).
5. Oko 4487 (Bayer, Leverkusen), a dialkyl coumarin thiophosphate.
6. Oko 4490 (Bayer, Leverkusen), an aliphatic dialkylester of phosphoric acid.

The insecticides were applied as wettable powders except for Oko 4490, which was used as a solution as it is readily soluble in water.

1. *Larvicidal Properties.*

According to the method described by Fiedler and Du Toit (1951) first instar larvae of *Lucilia cuprina* were subjected to serial dilutions of the insecticides through the range 1000 to 0·064 p.p.m. of the active principles to determine the minimum concentrations of the different compounds in the nutritional medium (horse serum) necessary to kill or affect the maggots over given periods of time. The results which represent the means of four repetitions are shown in Table I.

Table I.

The Minimum Concentrations of Insecticide in p.p.m. in the Nutritional Medium Required to Kill or Affect Newly Hatched L. cuprina *Larvae in Time Specified.*

Insecticide.		Time (hours).					
		1	3	6	24	48	72
Parathion.....	K	—	16	1	0·25	0·15	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
Malathion.....	K	—	—	—	64	64	64
	A	—	250	64	4	4	4
Diazinon.....	K	—	1,000	64	1	0·25	0·25
	A	4	4	1	0·064	0·064	0·064
Oko 4487.....	K	250	64	16	1	0·25	0·25
	A	4	1	1	0·25	0·25	0·25
Oko 4490.....	K	250	16	16	1	1	1
	A	16	4	4	0·25	0·25	0·25

(K=100% Mortality; A=Affected or showing partial kill.)

The lowest concentration capable of destroying the larvae in a given period of time (72 hours) can be used as an index of the relative efficiency of the compound. Four insecticides, Parathion, EPN 300, Diazinon and Oko 4487, produced complete mortality down to 0·25 p.p.m. of the active ingredient. They surpass, therefore, in lethal effect all the chlorinated hydrocarbons evaluated in previous tests. Oko 4490 gave a complete kill at a concentration of 1 p.p.m., which places it into one group with Dieldrin, whereas Malathion failed to achieve a 100 per cent kill within 72 hours at a concentration below 64 p.p.m. The quickest initial kill was achieved by the two Oko-compounds (250 p.p.m. within 1 hour), followed by Parathion and EPN 300 (1000 p.p.m. in about 1 hour), Diazinon (1000 p.p.m. within 3 hours) and Malathion (1000 p.p.m. in over 6 hours). The rate of larvicidal action of the six phosphorus compounds in comparison with two well established protecting agents (Dieldrin and B.H.C.) is given in Fig. 1.

It can be concluded from this test that the larvicidal properties of the compounds tested, with the exception of Malathion, is very good. Oko 4490, however, is not very stable in solution and tends to disintegrate even under refrigeration. Thus after storage for one week complete mortality was only achieved with 4 p.p.m., and the concentration for an equivalent result after eight weeks was 250 p.p.m.

A peculiar phenomenon was observed in this connection which has never been seen with the chlorinated hydrocarbon insecticides and was apparent in the case of all the organic phosphates. It was particularly pronounced with Oko 4490. At all concentrations which did not produce a complete kill of the newly hatched maggots, the surviving specimens showed rapid growth and were considerably larger than the larvae in the control tubes after an incubation period of 72 hours. It would appear that the phosphate insecticides at sub-lethal concentrations tend to stimulate the growth of blowfly larvae and this is particularly obvious with the less stable compounds where by decomposition more phosphorus is present in a less complex form.

2. *Behaviour of Insecticides in the Fleece.*

To assess the value of the phosphorus compounds as larvicides in the wool of living sheep and at the same time to determine their diffusing power along the fibres, areas about 12 inches in diameter on the rump of six groups of six Merino sheep, each with wool of from 1 to 1½ inches in length, were saturated with a suspension containing 0·5 per cent of the active principle. The results of the bio-assay method, which were checked by means of artificial strikes, are presented in Table 2.

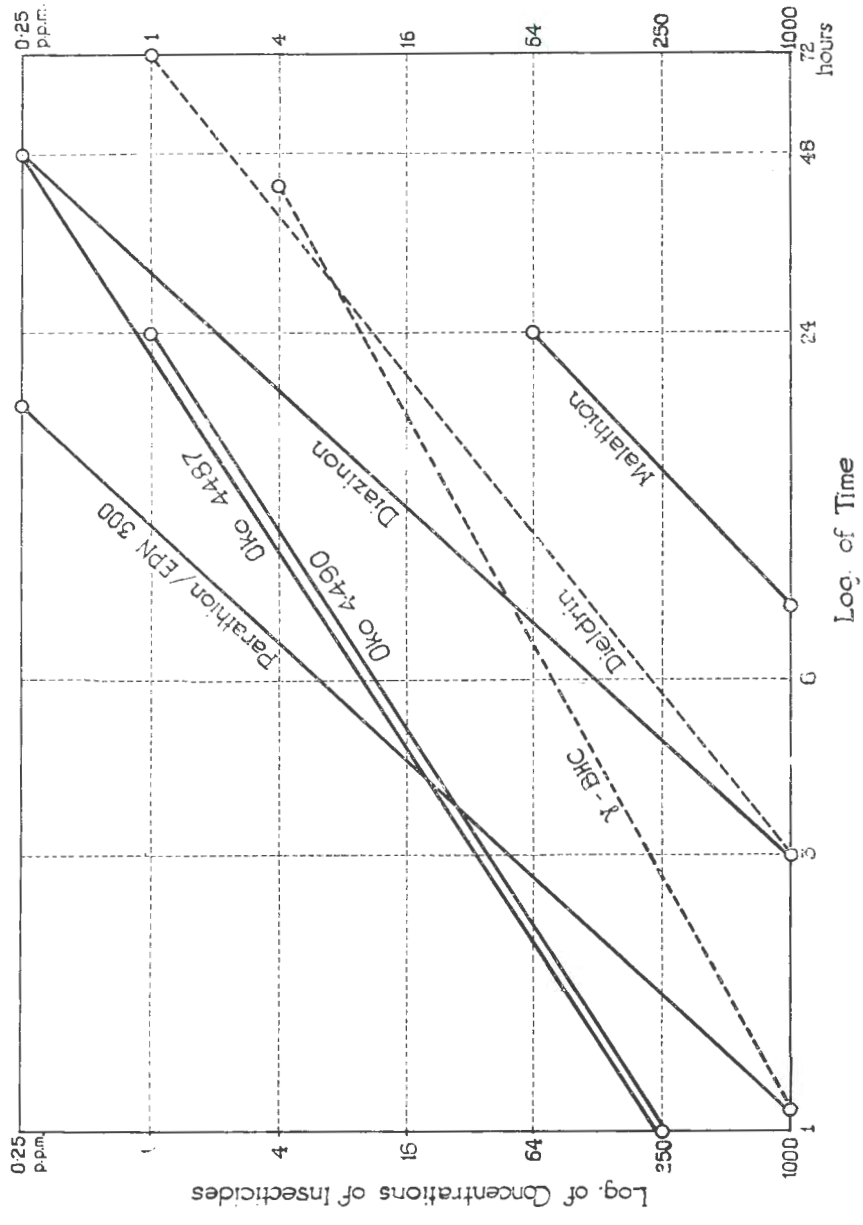
TABLE II.

Period of Protection in Weeks Conferred by Different Insecticides to Individual Sheep.

Oko 4490.	Malathion.	Oko 4487.	EPN 300.	Parathion.	Diazinon.
> 9	13	18	29	46	40
< 9	17	20	38	46	48
> 9	18	22	43	47	53
> 9	18	23	43	47	54
> 9	18	27	43	53	55
> 9	19	29	46	54	56
Average ..... > 9	16·2	23·2	40·3	48·8	51·0

FIGURE 1.

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



Three of the compounds, Diazinon, Parathion and EPN 300, possess outstanding powers of diffusion in the fleece and, therefore, afford protection of very long duration. This protection averaged 51 weeks for Diazinon and was closely

followed by Parathion with about 49 weeks, whereas EPN 300 protected for 40 weeks. The results for the remaining compounds were comparatively inferior. Oko 4487 gave a protection of 23 weeks and Malathion 16 weeks. The water soluble Oko 4490 had already broken down in the wool 9 weeks after application when the tests of the treated sheep were due to commence.

C. Discussion and Conclusions.

An evaluation of the phosphorus insecticides by means of the bio-assay method has revealed that certain of these compounds combine very good larvicidal action with outstanding diffusing power in the fleece of living sheep. They compare favourably in this respect with the insecticides previously tested (Du Toit and Fiedler, 1953), the best of which was Aldrin with an average period of protection of 39 weeks. Dieldrin gave a protection of 37 weeks, and gamma B.H.C. 33 weeks. In addition to the properties mentioned, however, on which the duration of protection depends such compounds must comply with other demands necessitated by the environmental factors encountered on the bodies of sheep. To start with such protecting agents must not stain or damage the wool fibres and secondly they should be stable in a slightly alkaline medium in order to produce the maximum residual effect in all situations such as even in the soiled wool of the crutch region. From this point of view Parathion and EPN 300 must be excluded for practical purposes as they discolour the treated wool imparting to it a strong yellow-green tint. Furthermore, their general use cannot be recommended on account of their toxicity to warm-blooded animals. The only phosphorus insecticide which seems to comply with all the requirements of a protecting agent for sheep against blowfly strike is Diazinon. Its larvicidal action as well as its diffusing power in the fleece are superior to insecticides like Dieldrin and B.H.C. which have proved to be very efficient protecting agents in practice. In the light of the properties studied Diazinon may be expected to produce a longer period of protection than B.H.C., which is not stable in an alkaline medium; and it may be found even to surpass Dieldrin in this field of application.

Field experiments to demonstrate the actual period of protection of Diazinon in comparison with Dieldrin and B.H.C. are well under way in some of the worst blowfly areas of the Karoo and will be reported on in due course.

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