# THE ACTIVITY OF DL-ALLETHROLONE D-TRANS CHRYSANTHEMATE AND OTHER PYRETHROIDS IN MOSQUITO COILS

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The burning of "mosquito coils" occupies an unusual place in the various methods of preventing mosquito attack. Under calm conditions (MacIver, 1963), a coil will give 6 or 8 hours protection from biting, a result difficult to achieve by other methods. Coils are most often used in tropical areas where mosquitoes are always present, but some are sold in temperate countries for protection from "midges and gnats" during the evenings.

MacIver (1963, 1964a, b) described the production of coils and studied the burning process and biological activity. He found that pyrethrins had about 2.2 times the knockdown activity of allethrin against Aedes aegypti L.

This paper describes further studies on coils containing natural pyrethrins or various synthetic pyrethroids.

The NRDC group of compounds have been described by Elliott *et al.*, (1967) whilst Martel *et al.*, (1967a, b) have given methods leading to the large scale production of *dl* allethrolone *d-trans* chrysanthemate.

### MATERIALS

Blank coils based on pyrethrum marc were made by Kenya Apiaries. Toxicant was added to these by pipetting the appropriate volume of solution in acetone as evenly as possible onto both sides of the coil which was then air-dried. The active materials used are listed below.

The abbreviation *d-trans* C of *dl* A is used in conformity with French practice. The unofficial names Bio-allethrin and Biolethrin also have been applied to this compound.

#### **METHODS**

Knockdown activity was examined using either a Peet-Grady chamber (6.13 m<sup>8</sup>) or an aerosol test room (34 m<sup>8</sup>) as the test chamber. Batches of about twenty-five 3-4 day-old male and female A. aegypti were confined in glass cylinders, 10 cm in diameter and 11.5 cm long, fitted with open mesh gauze ends. Four of these cylinders held horizontally were placed

# Pyrethrins

Allethrin

d-trans C of dl-A

NRDC 104 NRDC 105

NRDC 106 NRDC 107 d-trans-105

Neopynamin (tetramethrin)

d-trans-Neopynamin Piperonyl butoxide From 25% pyrethrins (P.M.B.K. 1954 method) Oleo-resin grade extract.

3-allyl-2-methyl-4-oxocyclopent-2-enyl-dl-cis-trans chrysanthemate.

3-allyl-2-methyl-4-oxocyclopent-2-enyl-d-trans chrysanthemate (dl allethrolone d-trans chrysanthemate).

5-Benzyl-3-furylmethyl *dl-cis-trans* chrysanthemate.

5-Benzyl-2-methyl-3-furylmethyl dl-cis-trans chrysanthemate.

5-Benzyl-3-furylmethyl d-trans-pyrethrate.

5-Benzyl-3-furylmethyl *d-trans* chrysanthemate. 5-Benzyl-2-methyl-3-furylmethyl *d-trans* chrysanthemate.

3,4,5,6-Tetrahydrophthalimidomethyl *dl-cistrans* chrysanthemate.

3,4,5,6 Tetrahydrophthalimidomethyl *d-trans* chrysanthemate. Commercial material, technical grade: 6-(propyl-piperonyl)-butyl carbityl ether.

50 cm above the floor on stands at the corners of a square marked about the center of the room and either 75 or 150 cm from the center depending on which chamber was used. The coil was placed on its metal stand in the center of the square and lit. Counts of the numbers of mosquitoes knocked down were then made at 2- or 3-minute intervals for up to 15 minutes exposure in the Peet-Grady chamber and up to 30 minutes in the aerosol room. The knockdown figures recorded were corrected for the effect of the smoke from blank coils with no added toxicants using Abbott's correction (Abbott, 1925). The figures from the four cylinders in each test were combined and regarded as a single unit when calculating results.

Where biting activity was considered, batches of 10 unfed female mosquitoes, caged in the cylinders already described, were exposed to the smoke from a newly lit coil for the stated time. The coil was then extinguished. The cylinder was placed on the observer's forearm for 5 or 10 minutes. The number which had fed was found by crushing the mosquitoes between two filter papers and observing if blood was present.

All observations were made at 27° C. and 50 percent R.H.

#### EXPERIMENTAL WORK

- I. Tests Under Different Conditions. The effects of ventilation and of confining the mosquitoes were examined in the Peet-Grady chamber. A commercial coil, containing 0.224 percent pyrethrins on analysis (PBK method) was burned in the chamber at a mean rate of 2.18 g. per hour. Mosquitoes were exposed under 3 set of conditions:
  - i. caged in cylinders with gauze ends; Peet-Grady chamber not ventilated (standard conditions).
  - ii. free-flying within the Peet-Grady chamber; chamber not ventilated.iii. caged as in (i); chamber venti-

lated at rate of 1.04 air changes per minute.

The different rates of knockdown are shown in Figure 1. Free-flying mosquitoes were knocked down less rapidly than caged ones in the unventilated chamber. This may be due to their tendency to rest on the walls and partly escape contact with the smoke. When the chamber was ventilated, the 50 percent knockdown time increased from 11.6 to 21 minutes. This condition of test is roughly the equivalent of a room in the tropics with the windows open and with little wind blowing.

2. The Effect of Piperonyl Butoxide. Two coils were prepared by pipetting solutions in acetone onto blank coils to give 0.2 percent pyrethrins and 0.2 percent pyrethrins plus 1.0 percent piperonyl butoxide. The percentage knockdown figures recorded at the end of 20-minute exposure of A. aegypti in the Peet-Grady chamber are shown in Table 1.

TABLE I.

	, -	n after 20 minutes sposure
Test Number	o.2% Pyre- thrins	0.2/1.0% Pyre- thrins/pip. but.
I	49	2
2	77	23 18
3	58	18
4	43	27
5	68	10

Piperonyl butoxide evidently slowed knockdown. A t-test on data, normalised by the sine-squared transformation, showed P<0.01. Another comparison showed a KD 50 time of 9.76 minutes from a coil containing 0.3 percent pyrethrins and 12.63 minutes from a coil containing 0.3 percent pyrethrins and 1.5 percent piperonyl butoxide. The unsynergised pyrethrins thus were 1.29 ± 0.056 times as fast in action as pyrethrins plus piperonyl butoxide. MacIver (1964a) also found that

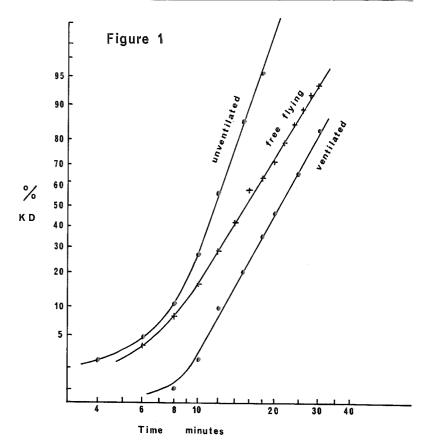


Fig. 1.—The knockdown of A. aegypti exposed to smoke when free-flying in the unventilated Peet-Grady chamber or cages with or without ventilation of the chamber.

piperonyl butoxide slowed knockdown.

3. Comparison of the Activity of Different Pyrethroids. Solutions were pipetted onto blank coils to give 0.5 percent w/w of pyrethroid in the finished coil. These were tested against *A. aegypti* in the Peet-Grady chamber. The mean corrected 50 percent knockdown times from four replicates are given in Table 2.

Pyrethrins, allethrin, NRDC 104 and NRDC 107 showed similar activity, whilst *d-trans* C of *dl-A* was significantly more active.

Table 2.—Mean 50% knockdown times of A. aegypti exposed to smoke from coils containing 0.5% of pyrethroids.

Compound	50% KD Time (Mins)		
d-trans C of dl-A	5.5		
Pyrethrins	8.8		
NRDC 104	8.9		
Allethrin	8.9		
NRDC 107	9.6		
d-trans Neopynamin	11.7		
d-trans NRDC 105	14.5		
Neopynamin	13.8		
NRDC 105	19.3		
NRDC 106	24.0		

Using a range of concentrations, *d-trans* C of *dl-A* was then compared with pyrethrins and allethrin; the two insecticides commonly used in mosquito coils (Table 3).

was opened so that close comparison with the normal test technique is not possible. *d-trans* C of *dl-A* again gave the fastest 50 percent knockdown at 3.0 minutes, pyrethrins taking 6.6 minutes and alle-

Table 3.—Mean 50% knockdown times (minutes) of A. aegypti after lighting the coil in the Peet-Grady chamber, using indicated concentrations of material.

	0.15%	0.20%	0.25%	0.30%	0.40%	0.50%
Pyrethrins		11.4	10.3	10.0		8.3
Allethrin			12.3	9.4	9.5	8.9
d-trans C of dl-A	9.3	9 • 4	8.55	5.9		5.5

From these figures estimates were made of the concentrations of the three compounds required to give 50 percent knockdown in different times under the particular conditions of test (Table 4). It appears

Table 4.—Approximate concentration of pyrethrins, allethrin and *d-trans* C of *dl-A* to give 50% knockdown in various times in the Peet-Grady chamber.

KD 50 Time	Alle- thrin	Pyre- thrins	<i>d-trans</i> C of <i>dl-</i> A
11.0 mins.	0.30%	0.23%	0.12%
10.0 mins.	0.37%	0.29%	0.16%
9.0 mins.	0.49%	0.38%	0.20%
8.0 mins.		0.60%	0.24%

that the effect of any given concentration of pyrethrins can be duplicated by about six-tenths of that concentration of *d-trans* C of *dl-A*. Commercial coils appear to contain between 0.20 percent and 0.35 percent pyrethrins on analysis.

4. Tests Using an Already Smoke-Filled Chamber. A comparison of coils containing 0.3 percent of pyrethrins or allethrin or *d-trans* C of *dl-A* was made under test conditions intended to simulate those encountered by a mosquito entering a smoke-filled room. The coil was burned for 10 minutes in the closed Peet-Grady chamber and the four cylinders containing mosquitoes then carried in quickly. This entailed some loss of smoke as the door

thrin 6.7 minutes (3 replicates). *d-trans* C of *dl*-A gave 100 percent mortality and the other two compounds gave 98 percent.

The more rapid knockdown found in this test compared with that found when starting the exposure of the mosquitoes with a newly lit coil is only to be expected.

5. Comparison of the Activity of DIFFERENT PYRETHROIDS IN THE AEROSOL The activity of pyrethrins, allethrin and d-trans C of dl-A was examined using a 34 m<sup>3</sup> aerosol test room. This was considered to give a more realistic test than the Peet-Grady chamber which is smaller than any normal room. Comparisons were made at different concentrations and using coils made from two different batches of blank coils. Some difficulty was found in keeping coils from one of the two batches burning well and these were sprayed with potassium nitrate solution and redried before treatment. caused some slowing of knockdown presumably resulting from the higher temperature of burning which would cause more degradation of the pyrethroid. The comparisons are set out in Table 5.

6. The Effect of Smokes on Biting Activity. A preliminary experiment in the Peet-Grady chamber showed that 28 out of 50 unfed females would take blood in 5 minutes when not exposed to smoke whilst only 7 out of 50 fed after exposure to the smoke from 5 minutes burning of

TABLE 5.-Mean 50% knockdown times of A. aegypti exposed in the aerosol room.

Insecticide	Pyrethroid Concentration	Coil Base	50% KD Time (mins)
Pyrethrins	0.5%	untreated	18.7
Allethrin		untreated	18.6
d-trans C of dl-A	0.5% 0.5%	untreated	13.8
Pyrethrins	0.3%	untreated	13.8
Allethrin	0.3%	untreated	14.5
d-trans C of dl-A	0.3%	untreated	14.5 8.8
Pyrethrins	0.3%	KNO <sub>3</sub> -sprayed	17.7
Allethrin	0.3%	KNO <sub>3</sub> -sprayed	19.8
d-trans C of dl-A	0.3%	KNO <sub>a</sub> -sprayed	11.7

a coil containing 0.224 percent pyrethrins. Longer periods of exposure resulted in some knockdown. In a second experiment the mosquitoes were exposed to smoke during 5 minutes burning of the coil and then allowed 10 minutes with the cylinder held on the observer's forearm. The results of this experiment are shown in Table 6.

Table 6.—Numbers of mosquitoes feeding after exposure to smoke in the Peet-Grady chamber.

Treatment	No. Exposed	No Feeding	% Feeding
None	20	17	85
Blank coils	44	27	61
0.5% pyrethrins	50	1	2.
0.5% d-trans C of dl-A	50	1	2

Smoke from the blank coil caused a small reduction in the biting rate, whilst pyrethrins and *d-trans* C of *dl-A* almost completely prevented biting. However *d-trans* C of *dl-A* gave knockdown as well so the experiments were transferred to the aerosol room and a lower concentration of pyrethroid used in the coils to reduce the knockdown. The coils were burned for 5 minutes and the mosquitoes again given 10 minutes to feed. The results are given in Table 7.

This level of knockdown precluded any observations on the direct effect on feeding. The work was therefore transferred to a room of 57 m. A fan was kept running during each experiment to ensure

Table 7.—Number of mosquitoes, percent knockdown and percent feeding after exposure to smoke in the aerosol room

		% Knocked Down			
Treatment	Num- ber	5	After 15 Mins.	% Fed	
Blank coil	50	0	0	82	
0.3% pyrethrins 0.3% d-trans	50	O	46	24	
C of dl-A	50	0	66	22	

distribution of the smoke. The results are given in Table 8.

At the lower concentration of pyrethroids there was little knockdown but a marked reduction in the percentage feeding compared to blank coils.

Table 8.—Numbers of mosquitoes, percent knockdown and percent feeding after exposure to smoke in a lurge room of 57 m<sup>2</sup>.

		d Down		
Treatment	Num- ber	After 5 Mins.	After 15 Mins.	% Fed
None	88	0	0	69
Blank coil	55	0	2	87
0.3% pyrethrins	50	0	46	24
0.3% d-trans	-		•	•
C of dl-A	50	0	70	22
0.25% pyrethrins	64	6	13	52
0.25% d-trans			.,	
C of dl-A	82	2	6	46

7. Reasons for the Greater Activity of Smoke from Coils Containing d-trans C of dl-A. The experiments described in Sections 3 to 5 show that the smoke from coils containing d-trans C of dl-A is more effective than that from coils containing pyrethrins at the same concentration. This may be because d-trans C of dl-A has a greater knockdown activity than pyrethrins against A. aegypti or because a greater proportion of the d-trans C of dl-A in the coil survives the burning to appear in the smoke.

The first possibility was examined by exposing mosquitoes to residual films of d-trans C of dl-A or pyrethrins. Deposits of 87 mg/m2 of pyrethroid on paper rectangles were prepared using silicone fluid as a carrier solvent. The mosquitoes were then exposed to the paper in Busvine-Nash cylinders (W.H.O., 1958) and the knockdown observed. Under these conditions d-trans C of dl-A gave 50 percent knockdown of Aedes in 6.8 minutes and pyrethrins in 10.8 minutes. Other experiments showed that the killing powers of the two compounds were similar; the LC 50 values being 85 mg/m<sup>2</sup> and 90 mg/m<sup>2</sup> respectively.

The second possibility was most conveniently examined by chemical methods. Blank mosquito coils were impregnated to a nominal 0.5 percent with either pyrethrins or d-trans C of dl-A. Pieces of coil were then burned in an apparatus similar to that described by Webley, (1968) and the smoke and other deposits, with the exception of ash, were collected in acetone and analysed. The combined smoke absorption-guard tube contained silica gel 200-300 mesh, and air flowed through the apparatus at about 350 ml/min, giving a coil burning rate of 15-20 mg/min. This is a slower burning rate than found with free standing coils, but the comparison is made between coils burned under the same conditions in this experiment. In order to form the basis for a comparison, acetone extracts of appropriate pieces of unburnt coil were also analysed.

All solutions representing smoke from coils, extractives from unburnt coils and standard insecticides were subjected to the following chromatographic clean-up. Solvent acetone was carefully removed and the residue was dissolved in sufficient 50:50 diethylether:petroleum ether (b.p. 60-80° C.) so that 0.5 ml solution contained the residue from, or equivalent to, 1.0 g of mosquito coil. This solution was transferred to the top of a column of Woelm basic alumina, Grade W200 deactivated with water to activity III, and eluted with the same solvent; for solutions equivalent to one gram of mosquito coil a column 6 cm high, diameter 0.5 cm was formed from 1.5 g of alumina and the same mixed solvent, 25 ml of eluate being collected.

Solvent was removed from the eluate from a clean-up column and the residue dissolved in petroleum ether so that 1 ml solution contained the equivalent of the extractive or smoke from 2 g of mosquito coil. Two ml were then gas chromatographed through a 46 cm long glass column 2.4 cm in diameter packed with 1 percent OV17 stationary phase on Chromosorb G 60-80 mesh, AW DCMS, nitrogen carrier gas flow 27 ml/min. column and flame ionisation detector temperature 186° C. and injection port temperature 236° C. Area measurements were made using a disc integrator. The conditions used for the gas chromatography were inadequate to obtain separation of pyrethrins from jasmoline; as the latter are present in small amounts, their presence has been neglected. The results obtained are shown in Tables 9 and 10.

These results show, in terms of weight, that the efficiency of transfer of unchanged

TABLE 9.—Percent recovery of insecticide in mosquito coil smoke.

d-trans C of dl-A	63%
pyrethrin I	34%
cinerin I	47%
pyrethrin II	18%
cinerin II	45%

Table 10.—Ratios of various components of natural pyrethrins in smoke, mosquito coil and pale extract.

	Smoke	Coil	Extract
pyrethrin I/cinerin I pyrethrin II/cinerin II	3 I	4 3	4.5

insecticide into smoke, when coils were burnt under the conditions described, was *d-trans* C of *dl-A*>cinerin I=cinerin II>pyrethrin I>pyrethrin II. They also agree with the results obtained by Webley (*loc. cit.*) that in general there was a better recovery of pyrethrin I than of pyrethrin II.

## DISCUSSION

Formerly, coils were made from a mixture of ground pyrethrum flowers, a filler such as coconut shell flour and a binding agent which traditionally was 'Tabu' powder. This is the ground leaves and bark of the tree Machilis thunbergii and contains a polysaccharide which forms a gum when wetted. Nowadays synthetic binding agents are more often used and these form a smaller proportion of the total weight than does Tabu powder. Furthermore the pyrethrins content of ground flowers is greater than it was. This makes it necessary to incorporate an additional quantity of filler so that the pyrethrins content is maintained at the desired level. Pyrethrum marc may be used for this purpose.

The active material may, on the other hand, be added to a coil consisting entirely of filler with the necessary binding agent. Up to the present, pyrethrum extract or allethrin has been used. The work reported here suggests that NRDC 107 or NRDC 104 could perhaps be used. However, *d-trans* C of *dl-A* is markedly superior to all the other compounds tested and would seem to offer the possibility of producing more effective coils having the same content of active material. Alternatively it might be used at a lower content to make an equally effective coil.

Knockdown activity is not the only way that smoke from a coil can affect mosquitoes. Figure 2 outlines the sequence of activities which a mosquito must follow in the feeding cycle. The arrows show the points in this sequence at which different control agents may act. Residual insecticides can contact only resting mosquitoes, whereas space sprays and smoke from coils are primarily aimed at the flying in-Space repellents are essentially agents which prevent the mosquito oriienting itself to fly towards the host. No evidence is presented here for the possibility that smoke exerts such an action. However it is generally accepted that pyrethrins in small doses cause disoriented movement, and observation of caged Aedes during biting experiments suggests that many smoke-treated mosquitoes never alight on the observer's arm. Pyrethrins can certainly act as contact repellents when spread on the skin, but this effect would not occur with smoke.

Interference with feeding is shown by the figures given in Table 8. Smith and Obudho (1967) showed a very marked inhibition of feeding of Anopheles gambiae (Giles) entering experimental huts in which a pyrethrins coil was burning. They also found that a greater percentage of mosquitoes left huts in which a coil was burning; a clear expellent effect. Smith and Chadwick (1964) caught fewer mosquitoes in a hut treated with a pyrethrum emulsion but could not determine with the type of hut used whether the mosquitoes were repelled from entering or were expelled after entry. All these effects can be regarded as the result of interference with the normal sensory mechanism of the insect.

#### **SUMMARY**

Experiments examining the knockdown activity against *A. aegypti* L. of mosquito coils containing natural pyrethrins or various synthetic pyrethroids are described. The *d-trans* chrysanthemate of *dl* allethrolone 3-allyl-2-methyl-4-oxocy-

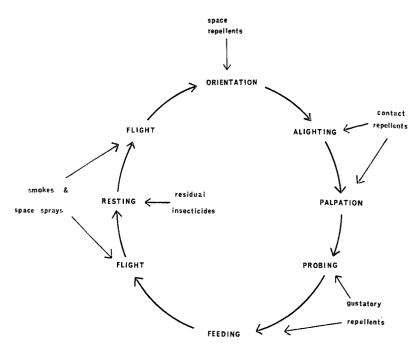


Fig. 2.—The sequence of activities in the feeding cycle of a mosquito with possible points of attack.

clopent-2-enyl-d-trans chrysanthemate) was the most effective knockdown agent giving equal percentage knockdown in about 60 percent of the time taken when pyrethrins were used at the same concentration. Piperonyl butoxide at 1:5 ratio slowed the knockdown by pyrethrins. The d-trans chrysanthemate of dl allethrolone and pyrethrins both caused a reduction in the number of mosquitoes feeding after exposure to smoke. The greater activity of smoke from a coil containing the d-trans chrysanthemate of dl allethrolone appeared to be due both to its greater knockdown activity compared with pyrethrins and to a greater proportion surviving the burning of the coil.

ACKNOWLEDGMENTS. I am indebted to Mr. R. H. McLellan of Kenya Apiaries for making the blank coils and to Mr. H. Stephenson for chemical analysis of the

smoke. *dl*-allethrolone d-trans chrysanthemate was supplied by Messrs. Roussel-Uclaf and the other synthetic pyrethroids were made by Mr. G. S. Poll.

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# EVALUATION OF FIVE PERCENT METHOXYCHLOR GRANULES AS A PRESEASON LARVICIDE

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Introduction. The Aedes group of mosquitoes constitutes a major control problem not only for the upland region of New York State but also the other northeastern States. A non-persistent larvicide, having the capability of slow release would be of great value in combating these early spring Aedes. With these criteria in mind, a 5 percent methoxychlor granular formulation supplied by E. I. du-Pont De Nemours and Co., Wilmington, Delaware, was chosen for an evaluation study in Dutchess County, New York. A literature survey completed at this time revealed no previous field trials with this formulation as a preseason larvicide.

METHODS AND MATERIALS. In February, 1969, nine water sites ranging from one-tenth acre to one acre were chosen for the study. These areas were consistently heavy producers of *Aedes* and were thoroughly mapped as to area and location in Dutchess County. All sites were closed

landlocked ecosystems which prevented insecticide runoff during the spring thaw.

Field evaluations were conducted during the month of March, applying the 5 percent methoxychlor granules at the rate of 1.0 lb. actual/acre. The first preseason application was conducted March 11, 1969, at four woodland pool areas. A back pack unit registered under the trade name Mitey Mite (Buffalo Turbine Agricultural Equipment Co., Inc.), calibrated at 1.2 lbs./min. dosage rate, was used to apply the granules. Further evaluations were conducted March 24, 1969 at three additional woodland pools, one woodland swamp, and one meadow pool. A truck mounted Model CS Buffalo Turbine (Buffalo Turbine Agricultural Equipment Co., Inc.) calibrated at 5.0 lbs./15 secs was used to apply the granules. Pertinent data collected during both evaluations are found in tables 1 and 2. A recheck every 10 days of the nine sites was initiated April 1, 1969 and continued to June 15, 1969. Observations of nontarget organisms were also recorded.

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