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Organophosphorus Insecticides for the Control of Mosquitos in Nigeria

Trials with Fenthion and Malathion Conducted by the WHO Insecticide Testing Unit in 1960-61 *

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The authors describe the entomological, chemical and toxicological findings made by the WHO Insecticide Testing Unit following the spraying of malathion, fenthion and DDT in villages in Western Nigeria.

Chemical observations of the deposits indicate that on the local type of mud malathion is sorbed to a greater extent and to a deeper level than fenthion, and DDT less so. On surfaces of vegetable origin all three appeared to remain to about the same degree.

The greatest effect on both anophelines and culicines as determined by the pyrethrum-spray technique was shown by fenthion on mud, followed by malathion and then fenthion on organic substrates, with malathion and DDT on mud as the least effective. As estimated by window-trap catches, the densities were most affected by fenthion on organic surfaces, least by fenthion on mud, and intermediately by DDT on mud and malathion on organic surfaces.

Anopheline mortality was greatest with fenthion on mud, followed by DDT on mud and malathion on organic surfaces, and least with malathion with mud. With culicines, fenthion was most active, and DDT least so, on both types of surface.

After spraying, the blood cholinesterase levels in the inhabitants of the sprayed villages fell quite markedly with fenthion and less so with malathion. The falls were temporary, being reversed within six weeks, were not confined to any ascertainable population group and were not associated with detectable illness. The authors stress that until more is known of the significance of these changes and of possible effects on health, the introduction of organophosphorus insecticides, especially fenthion, for malaria eradication should be carried out with caution.

In March 1960 the World Health Organization, in collaboration with the Federal Government of Nigeria, established an Insecticide Testing Unit [3] at Lagos, Nigeria, with the objective of evaluating on a village basis new insecticides that might be used as substitutes for DDT, γ-BHC and dieldrin in malaria eradication programmes. The work of the Insecticide Testing Unit from April 1960 to March 1961 was organized in five phases:

(1) Survey of the area adjacent to the laboratories of the Malaria Service (Federal Ministry of Health, Yaba, Lagos) and selection of suitable villages for study.

(2) Observations of mosquito densities, relevant human activities and other features of the selected villages.

(3) Spraying of separate villages with fenthion (Baytex),[4] malathion or DDT.

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[3] The Insecticide Testing Unit consists of two entomologists, a chemist and a laboratory assistant.

[4] Dimethyl 3-methyl-4-methylthiophenyl phosphorothionate.

(4) Observations of mosquito densities and behaviour in sprayed and unsprayed villages, of the residual effect of sprayed surfaces, and of the fate of the insecticides as determined chemically.

(5) Studies of the effects of exposure to the insecticides on the human population of the sprayed villages.

THE AREA OF OPERATIONS

The area chosen was a part of the Western Region of Nigeria, lying to the east of the Lagos-Abeokuta road and readily accessible from the Lagos-Ibadan road. The area is forest and farmland traversed by rivers flowing into Lagos Lagoon, with mangrove swamp and freshwater swamp forest along these providing *Anophele*s densities of fair intensity and duration through the period June-December.

It was not possible to choose villages for spraying and control purposes of exactly equivalent type; each had its own individuality as regards situation, mosquito prevalence, building materials used, and also ethnic origin and economic status of the inhabitants. The results of the spraying had, therefore, to be interpreted in the light of the known differences. The villages may be described briefly as follows.

Oregun

This is the only village west of the Lagos-Ibadan road. Although there is swamp near by, this village is the farthest from the lagoon and seems to have a shorter mosquito season than the others. It is basically a Yoruba village with a Hausa community in addition, the economic basis being general farming, cola-nut production and the operation of sandpits. Houses are of mud, with cement rendering; a few are in concrete. Roofs are mainly of corrugated iron, with some of palm-tiles, a form of thatch made by folding and stitching the leaves of the raphia-palm. This village received the DDT treatment. Spraying was well accepted, but eight out of 12 householders finally rejected the window traps.

Majidun

This is a riverside fishing village, the people being mainly Ilaje. Housing consists mainly of frame houses in which raphia-palm midribs are used for walls and ceilings with corrugated iron or palm-tile roofs, but there are a number of two-storey, concrete houses. Malathion was sprayed here, and the smell of the formulation caused about 32% of householders to refuse it; otherwise they co-operated well throughout the period.

Ajegunle

This is another Yoruba-Ilaje fishing community, but not so prosperous as Majidun. It is flooded badly in the rains and many houses, mostly of wood, palm midribs, and palm-tile thatch construction, are in poor repair. Fenthion, at 1 g/m^2, was sprayed here, the smell—less offensive than that of malathion—causing comment but little resistance.

Ogudu

This is a Yoruba village, with farming, some fishing, and the manufacture of rush mats as the obvious income sources. It is prosperous and probably a good deal of outside income from trade flows in. During the spraying with fenthion at 2 g/m^2, which the chief and his own followers accepted, an opposition faction emerged who rejected it largely on account of the smell. As a result, six unsprayed catching stations and six sprayed ones were operated here, though the over-all coverage was 63%.

Owode

This is a small riverside Yoruba-Ilaje settlement; the houses are mainly of mud with palm-tile roofs. It was selected for a trial of two malathion formulations, to supplement the information from Majidun, which included only one mud house.

Idena

This is another Yoruba-Ilaje fishing settlement, about half the houses being of mud and half of palm midribs. It was used as an unsprayed control; *Anopheles* production was fair until December, when it fell away.

Owutu

This small, roadside Yoruba settlement of thatched mud houses was used as a second unsprayed control. Although distant from Idena, it showed similar mosquito densities.

Ojota

A small Yoruba village, in which blood cholinesterase levels were observed before and after spraying of fenthion; no entomological observations were made.

Ethnological note

Yorubas are the settled inhabitants of the area, most of those in the villages being of the Ijebu subgroup. Ilajes are a related people with their own language, coming from the south of Ondo Province, and specializing in fishing. Hausas are immigrants from the north; many are cola-nut buyers, but the Oregun settlement work in the sand-pits. The people of these villages near Lagos vary from extreme simplicity to a high degree of sophistication; economically, from subsistence cultivators to shrewd and capable business men. In gaining their co-operation, therefore, a flexible approach was needed.

Types of sprayable surface

Mud is not in such wide use in this area as it is further north in Nigeria; however, all three insecticides were tried on this important type of surface. A series of pH determinations on local muds showed levels all on the acid side (5.5-5.8). Besides mud, concrete, plaster and wood were sprayed, and the two building materials derived from the swamp-dwelling raphia-palm. Both palm-tile and palm-midribs are relatively non-absorbent. The midrib, as used for building, is a lath two to three inches (5-7.5 cm) wide, with a concavo-convex section. Walls and ceilings made with this material present a corrugated surface with a sprayable area probably one and a half times that of the wall area. It is not an easy surface to bio-assay.

CHEMICAL INVESTIGATIONS

Formulations

The three insecticides were applied as suspensions of water-dispersible powders containing 75% DDT, 25% and 30% malathion, and 40% fenthion. Suspensibility of the first three complied with the requirements of specifications WHO/SIF/1.R2 and WHO/SIF/10.R1 (World Health Organization, 1961) respectively both before and after three months' tropical storage; the fenthion showed a slightly lower suspensibility (40% against 50% active material in suspension according to the established test procedure), which was also maintained during storage. All four formulations were satisfactory in use.

Storage trials under artificially contrived, stringent conditions, including exposure to air, showed that both malathion formulations after three months had lost up to 3% of their active ingredient by chemical degradation, while fenthion was almost unaffected.

Laboratory methods

The chemical methods used for assessment of the active material present in the above trials and in subsequent investigations on the fate of the deposits were: for malathion, that given in *Specification for Pesticides* (World Health Organization, 1961; pp. 66-69) in specification WHO/SIT/10.R1; for DDT, that given in the same work (pp. 15-16) in specification WHO/SIT/1.R2 (revised Stepanow method); and for fenthion, the analytical method specified by Farbenfabriken Bayer A.G., Leverkusen, Germany.[1]

Chemical sampling methods

The initial deposits were assessed by the analysis of deposits on filter papers (Whatman No. 1, 100 cm^2) hung clear of the wall on nails, and on mud discs of 5 cm diameter and 2 cm thickness attached to the walls. The latter were scraped to a depth of 0.5 mm in batches of four, one hour after spraying, and at intervals thereafter. Owing to interference by children, the discs could not be left *in situ* until tested, but were stored in wooden boxes under shelter.

Parallel with the tests of the deposits remaining on the mud discs, scrapings of mud-wall surfaces were performed at the points used for entomological bio-assay (see below) and at near-by points.

The surfaces of organic origin—wood and palm-leaf midrib walls and palm-tile roofs—were more difficult to sample. Early attempts to assess the insecticide present after chloroform extraction of cut-off portions were abandoned, as the solvent extracted organic substances in quantities sufficient to interfere with the analysis. The earlier results in Fig. 3 are therefore unreliable. The method of washing off the surface deposit with cotton swabs moistened with chloroform-alcohol 1 : 1 mixture was substituted, with satisfactory results.[2]

Initial deposits of insecticides

The analyses of samples done immediately after spraying show, as is usual with field operations, marked irregularities in the dosages achieved. These are summarized in Table 1, from which it will be seen that DDT was under-sprayed, fenthion over-sprayed, and malathion sprayed with reasonable accuracy. These departures from the planned dosages have to be taken into account in the assessment of the biological efficiency of the insecticides.

Deterioration of deposits on mud surfaces

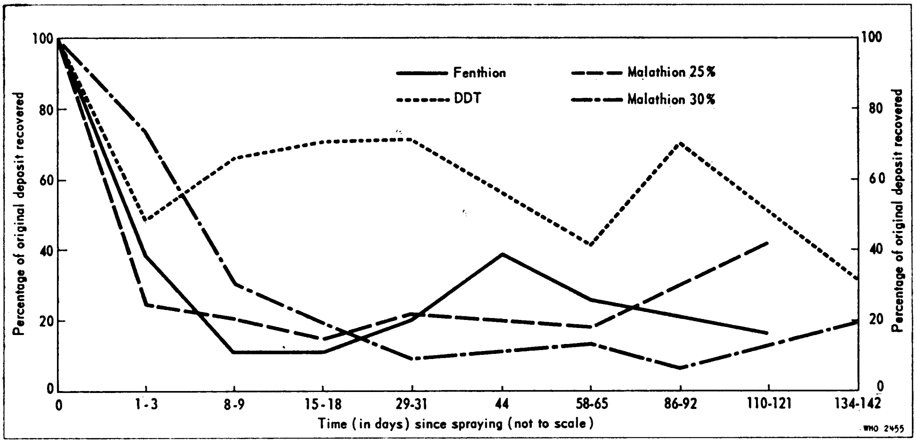
After the initial observations, at day 0, chemical analyses of both wall scrapings and mud discs were

[1] Dr Heusser—personal communication, 17 March 1959.

[2] F. Barlow—personal communication.

FIG. 1

INSECTICIDE RECOVERED FROM SCRAPINGS 0.5 mm IN DEPTH ON MUD WALLS [a]



[a] Expressed as the percentage of the original deposit recovered from 5 sampling points at each time interval.

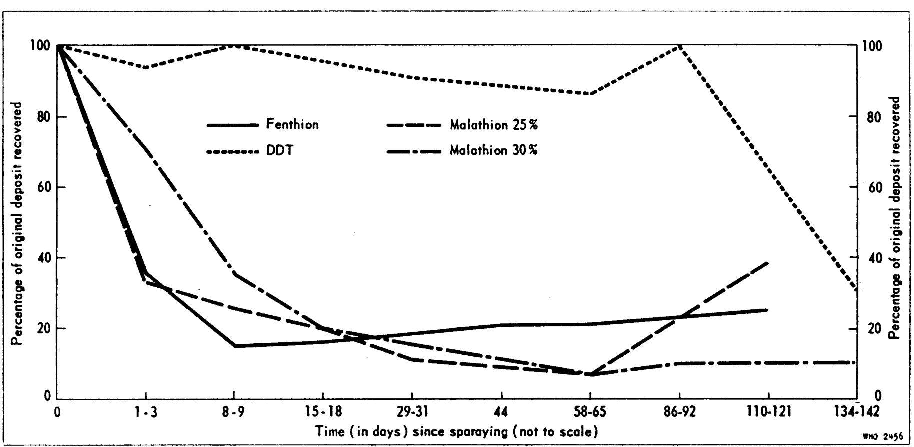
carried out at days 1 to 3 and at lengthening intervals thereafter. The results of scrapings to a depth of 0.5 mm, in terms of percentage of the original deposit present after a given time, are summarized in Fig. 1 and 2. It will be noted that the results from the wall scrapings are in substantial agreement with those from the mud discs.

Another series of chemical estimations was done to ascertain the amount of insecticide present at a greater depth than 0.5 mm; in the case of the walls the layer below the 0.5-mm scraping was removed to a further depth of 2-3 mm; in the case of the blocks, the whole block 2 cm thick (less the 0.5 mm scraping) was extracted and analysed. The results of these tests are summarized in Table 2, the figures for the two malathion formulations being placed together in order of time elapsed.

The observations in Fig. 1 indicate a drop in the proportion of the original deposit present in the superficial layer to one-third or less by the end of the first week in the case of the two organophosphorus insecticides, after which there was a fluctuation

FIG. 2

INSECTICIDE RECOVERED FROM SCRAPINGS 0.5 mm IN DEPTH ON MUD DISCS [a]



[a] Expressed as the percentage of the original deposit recovered from batches of 4 discs at each time interval.

TABLE 1

THE INITIAL DEPOSITS OF INSECTICIDES [a]

Formulation	Deposit intended (g/m²)	Average deposit achieved (g/m²)	Minimum deposit observed (g/m²)	Maximum deposit observed (g/m²)
Fenthion 40 %	2	2.92	2.18	3.36
Fenthion 40 %	1	1.57	0.66	2.89
Malathion 25 %	2	2.07	1.72	2.45
Malathion 30 %	2	2.05	1.82	2.27
DDT 75 %	2	1.42	0.82	2.29

[a] As determined from analysis of test papers and scrapings of mud blocks.

[b] Mean of 5 observations.

possibly due to migration of the insecticides between the surface and the deeper layer of the mud. DDT remained in a greater proportion at the surface, to the extent of two-thirds or three-quarters of the original deposit for 16 weeks or more, after which a decrease was registered.

In comparing Fig. 1 and 2, the proportion of the organophosphorus insecticides in the surface layer was less with the 2-cm-thick mud discs than with the mud walls, but otherwise showed a similar pattern with time. DDT, however, remained almost entirely in the surface layer of the discs for about 12 weeks, after which it penetrated farther.

The observations in Table 2 show a difference between fenthion and malathion in that a greater proportion of the remaining fenthion is at the surface, which is in agreement with the observations in Fig. 1 and 2 at the same period. The two DDT observations indicate a migration into the depth of the mud, but offer no clue as to whether this is a continuous process or an intermittent one with reverse movements also occurring.

The movements of the insecticides which are dimly outlined by these chemical investigations could not be shown to correspond to any observed changes in the insecticidal activity of the deposits as determined by the biological assays.

Deterioration of deposits on organic surfaces

Both chemically and biologically, the behaviour of the insecticides on the three main surfaces of vegetable origin appeared similar; palm-leaf tiles, palm-leaf midrib, and wood are therefore not separated in Fig. 3. Similarly, the results for the two malathion formulations, and for the two fenthion

TABLE 2

DISTRIBUTION OF INSECTICIDES BETWEEN SURFACE (0.5 mm) AND DEEPER LAYERS IN MUD WALLS AND BLOCKS [a]

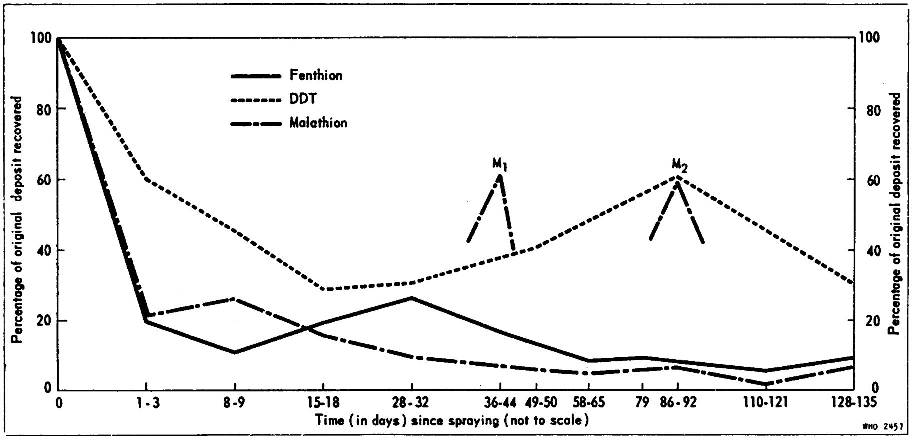
Insecticide	Day	Percentage of original deposit in 0.5-mm layer	Percentage of original deposit in deeper layers	Percentage of remaining deposit in 0.5-mm layer
		Mud Walls		
Fenthion	44	38	46	45
,,	65	26	38	41
Malathion 25 %	31	22	55	29
,, 30 %	62	13	36	27
,, 25 %	64	18	48	27
,, 30 %	86	7	33	18
,, 25 %	110	42	53	44
,, 30 %	142	19	37	34
DDT	92	70	27	72
,,	134	32	63	34
		Mud Blocks (2 cm thick)		
Fenthion	44	21	55	28
,,	65	21	51	29
,,	121	16	32	33
Malathion 25 %	9	26	14	65
,, 25 %	31	11	98	11
,, 30 %	62	8	55	13
,, 25 %	64	7	49	12
,, 30 %	86	10	44	19

[a] Means of 4 or 5 observations at each time interval. Certain of the assays may have been made at points where the insecticide was over-sprayed and may give misleadingly high values, including total values above 100 % of the original deposit.

dosage rates are consolidated. It will be seen that all three insecticides were found to leave the surface rapidly, DDT less so than the organophosphorus insecticides, and with some tendency to a partial return. The two points M_1 and M_2 represent high values obtained for malathion; M_1 at 43 days is a mean of 10 high values provided by a wood surface; M_2 at 86 days is a mean of four values of which two were exceptionally high, one on a palm-midrib surface and one on wood. Both M_1 and M_2 were observed with the 30% formulation.

FIG. 3

INSECTICIDE RECOVERED FROM WASHINGS FROM SURFACES OF ORGANIC ORIGIN [a]



[a] Expressed as the percentage of the original deposit recovered from 3-10 sampling points at each time interval.

ENTOMOLOGICAL INVESTIGATIONS

Methods

Three series of entomological observations were made in the selected villages:

(*a*) pyrethrum spray-catches were made;

(*b*) window traps were operated in 12 catching stations per village for a period of six to eight weeks before spraying and about 16 weeks thereafter; and

(*c*) bio-assays with *Anopheles gambiae* females were made on the various types of sprayed surface for up to 26 weeks after spraying.

The pyrethrum spray-catches were made weekly, starting at 7 a.m. in the two unsprayed and five sprayed villages. Pyrethrum at 0.25% was used and collections were made from a standard area of floor per catching-station which might be located in one large or two small rooms. The window traps were operated in the same catching-stations, being set up as exit traps at 7 p.m. and removed before the spray-catches were made. The type of trap used was of Terylene netting on a 12-inch cubical wooden frame, the entrance side being drawn into a truncated cone with a one-inch (2.5 cm) diameter hole at its apex, which was one inch from the opposite face of the trap. They were clipped against 12-inch (30 cm) square openings in sheets of hardboard individually fitted to each window. All windows are normally kept shut at night, so that the traps represented the most convenient, but by no means the only, exit for mosquitos leaving the houses. The traps were removed to the laboratory and the catch sorted into *Anopheles* and culicines, dead and alive, and the survivors were placed in paper cups supplied with sugar-water for 24 hours, at which time mortality was again observed.

The bio-assays were carried out with young (three to six days old) female *A. gambiae* of the Lagos susceptible strain, blood-fed on the day previous to exposure. The WHO standard bio-assay kit was used, batches of 10-20 females being placed in each plastic cone for exposure periods of 30-240 minutes, and removed to paper cups under the same conditions as the window-trap survivors. Surfaces to be bio-assayed were in some cases very irregular and, besides the use of plastic sponge strips ¼ inch (6 mm) in thickness round the base of the cones, additional packing in the shape of cotton wads was used, especially on the palm-midrib surfaces. This provided an extra area of available untreated surface for the mosquitos to rest on, but, in fact, the great majority were seen to spend almost the whole exposure period on the test surface. For the shorter bio-assay periods, five points were used, three fixed and two at random, two batches being exposed at each. No significant difference in mortality between successive batches could be detected. For the longer periods, three fixed and seven random points were bio-assayed once. Thus each weekly observation represented the mean mortality of 100 or more females exposed on each combination of surface and insecticide. The fixed points were scraped or washed for chemical analysis after a period of use and replaced by newly chosen ones.

The results of the methods described are considered in two main categories; data on densities and population dynamics based on mosquitos, both *Anopheles* and culicines, remaining to rest in the houses (spray-catches) and leaving (window traps); and data on the lethal effects of exposure to treated surfaces, both facultatively (window trap mortalities) and under forced contact (bio-assay mortalities).

Mosquito densities and rainfall. Fig. 4 gives the relation between the trend of rainfall at the nearest meteorological station and *Anopheles* densities. The year 1960 was rather unusual in that the dry interval between the two main wet periods commenced in July and continued through August, instead of being more or less confined to the latter month. Therefore mosquito densities, which loosely follow the trend of rainfall, were falling in September when the villages were sprayed. The impact of insecticides was, as a consequence, less apparently impressive than it would have been on a steady or rising density, and the main comparison of the effects of the treatments is to be found in the extent to which the recovery of the population was delayed. This applies to both *Anopheles* and culicine populations, although the latter, being partly composed of *Mansonioides* species, are less dependent on rainfall than *A. gambiae*, which formed the overwhelming majority of the *Anopheles* populations.

Mosquito densities : anophelines

Fig. 5 and 6 show the observations from spray-catches and window-trap catches in the unsprayed villages. For the sake of convenience, these will be referred to as RD (resting density) and ED (exit density) in the following discussion. It is at once noticeable that the two sets of data are by no means entirely in agreement. At Idena, both show a small peak of density in mid-September and a decline at the end of the month, but ED shows a high level through October-November which is

FIG. 4
RAINFALL AND ANOPHELINE DENSITY

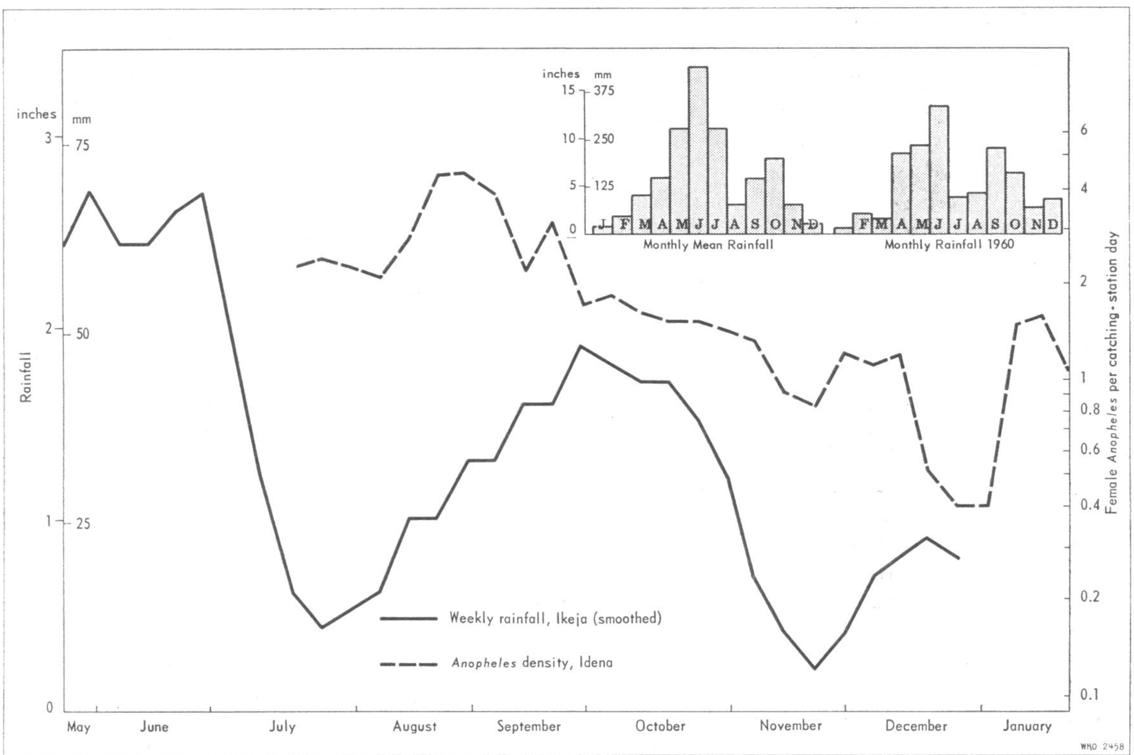


FIG. 5
ANOPHELINE RESTING DENSITIES IN UNSPRAYED VILLAGES

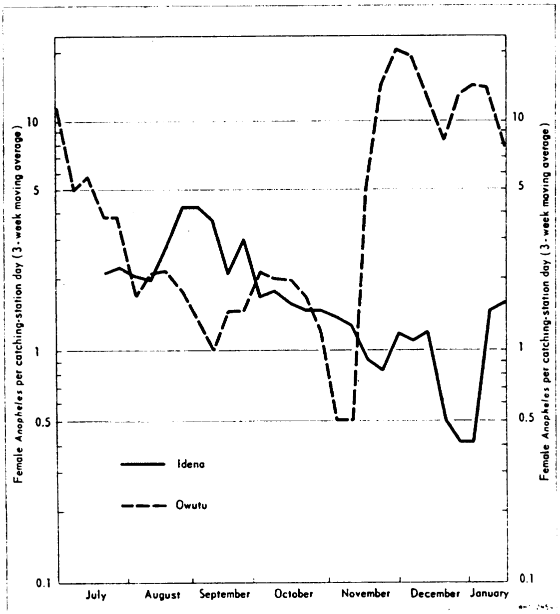
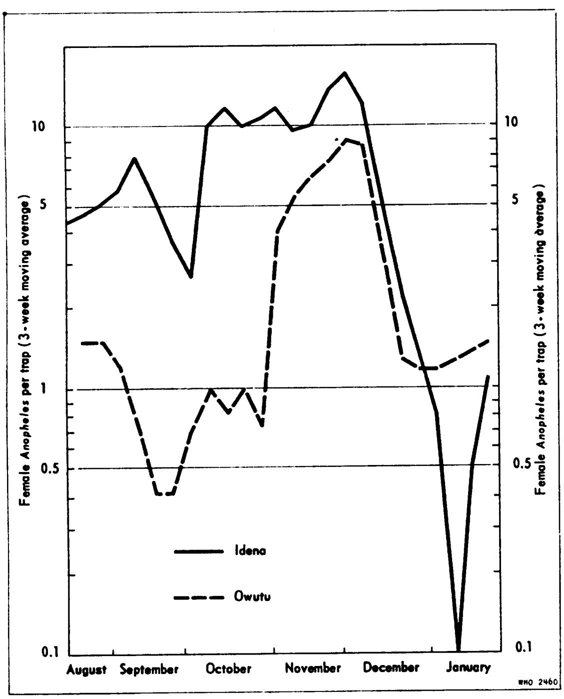


FIG. 6
ANOPHELINE WINDOW-TRAP CATCHES IN UNSPRAYED VILLAGES



not shown by RD. A decline at the end of the year shows in both, detectable first in the RD. It is possible that RD and ED represent different segments of the *Anopheles* population as regards the age of the individuals comprising them. At Owutu, the two figures agree more closely: a low point in September, a peak at the end of the month, a decline in October to a low point, and a rise to a second peak at the end of November. A possible explanation of some, but not all, of the sequences is that the RD population is, on the whole, composed of younger mosquitos than the ED population. If this were so, increases and decreases in *Anopheles* production would show first in the RD, later in the ED, catches. This occurs in about two-thirds of the population changes shown in Fig. 5 and 6.

Fig. 7 and 8, showing the corresponding figures for the malathion-treated villages, provide an interesting contrast in that, while RD figures are of the same order in both, the mud village of Owode shows ED figures about 50 times higher relatively than the wood and palm-midrib village of Majidun. The probable reason is the many alternative exits available in the loosely built Majidun houses. At Majidun, spraying was followed by a decline in RD for 10 weeks and in ED for four weeks. At Owode, RD was depressed for five weeks only and ED hardly at all, indicating that malathion was effective on surfaces of organic origin but ineffective on the local mud.

Fig. 9 and 10 show that fenthion depressed RD at Ajegunle for seven weeks and ED for five weeks on organic surfaces: in Ogudu, on mud walls, RD was depressed in sprayed catching-stations for at least 16 weeks while unsprayed stations maintained a high level. ED was depressed for two weeks only in sprayed stations, although, as will be seen later, the catch was almost entirely dead in the trap for some weeks.

At Oregun, the *Anopheles* densities in Fig. 11 and 12 show recovery in five weeks for RD and in four weeks for ED.

Mosquito densities: culicines

In Fig. 13 and 14, for the unsprayed villages, culicine RD and ED do not entirely correspond, though, as with the *Anopheles*, there is a tendency for rises and falls in RD to be followed at an interval of two or three weeks by rises and falls in ED.

The malathion-treated villages showed depressions in RD of about 10 and five weeks for Majidun (organic surface) and Owode (mud) respectively;

FIG. 7

ANOPHELINE RESTING DENSITIES IN MALATHION-SPRAYED VILLAGES

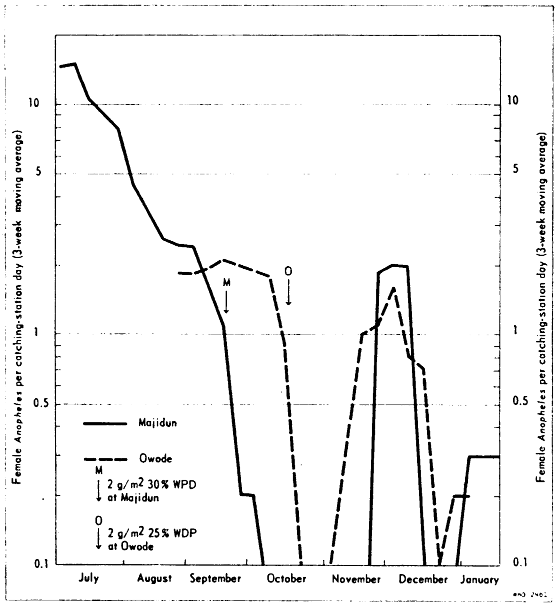


FIG. 9

ANOPHELINE RESTING DENSITIES IN FENTHION-SPRAYED VILLAGES

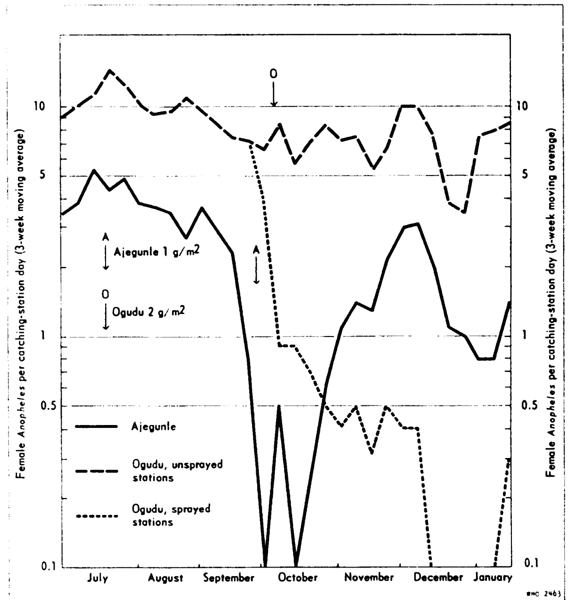


FIG. 8

ANOPHELINE WINDOW-TRAP CATCHES IN MALATHION-SPRAYED VILLAGES

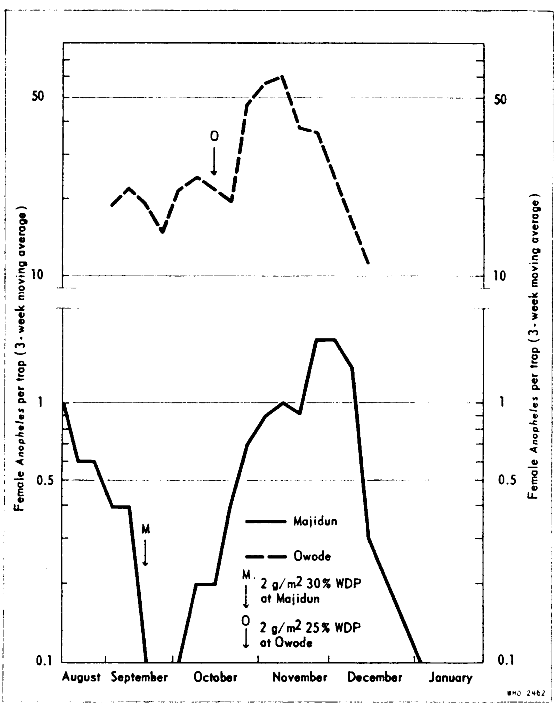


FIG. 10

ANOPHELINE WINDOW-TRAP CATCHES IN FENTHION-SPRAYED VILLAGES

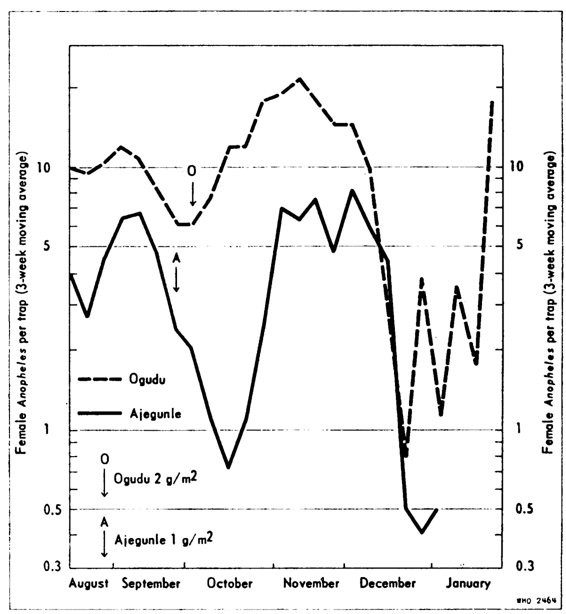


FIG. 11
ANOPHELINE AND CULICINE RESTING DENSITIES IN DDT-SPRAYED VILLAGE

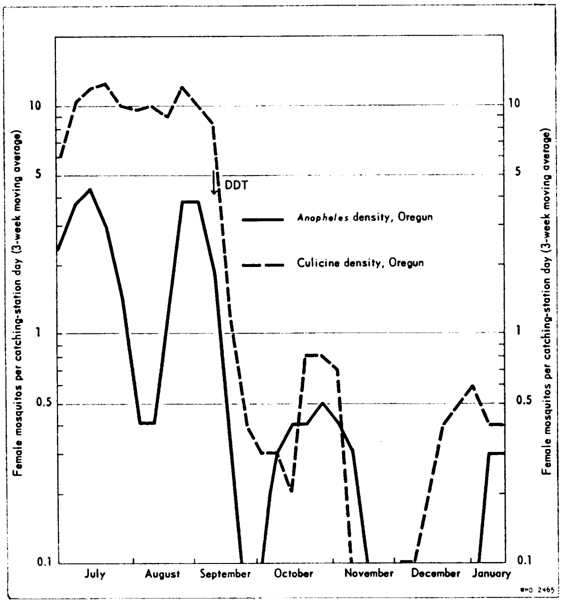


FIG. 13
CULICINE RESTING DENSITIES IN UNSPRAYED VILLAGES

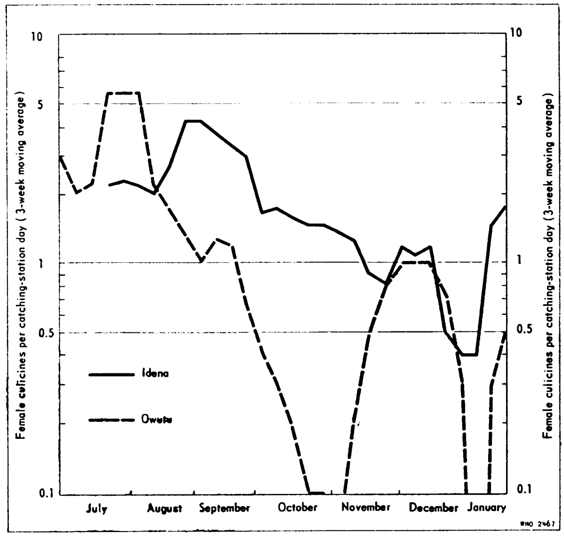


FIG. 12
ANOPHELINE AND CULICINE WINDOW-TRAP CATCHES IN DDT-SPRAYED VILLAGE

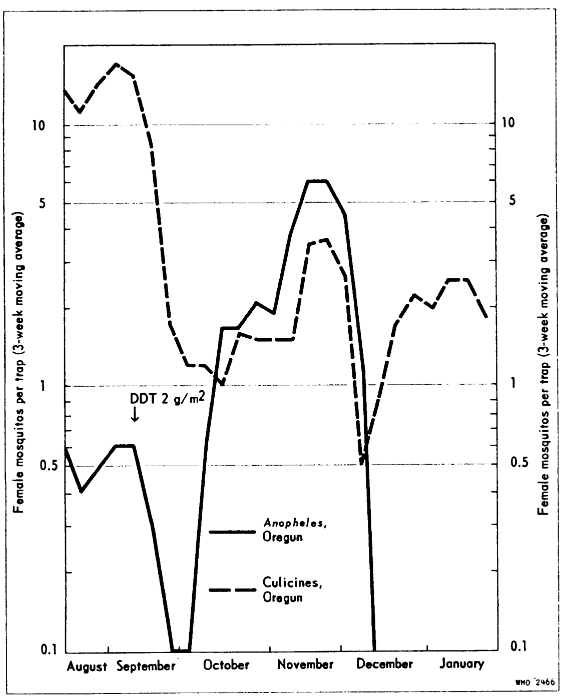
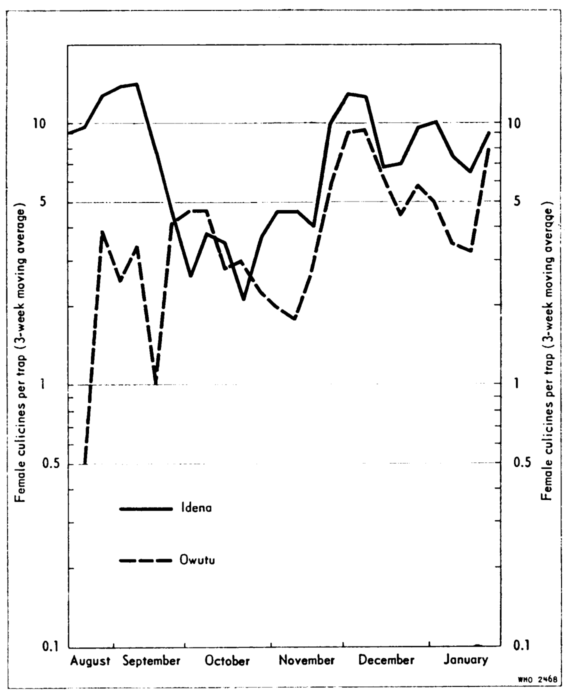


FIG. 14
CULICINE WINDOW-TRAP CATCHES IN UNSPRAYED VILLAGES



ED values showed much shorter periods of depression (Fig. 15 and 16).

For the fenthion-sprayed villages, Fig. 17 and 18 show 10 weeks' depression of RD at Ajegunle, and 13 weeks' in the Ogudu sprayed stations. At Ajegunle ED was depressed for six weeks; at Ogudu for four.

Finally, returning to Fig. 11 and 12, culicine RD and ED figures show about six weeks of depression at Oregun after DDT spraying.

Table 3 summarizes these observations and indicates that the most striking effects were those shown by 2 g/m² of malathion on organic surfaces at Majidun, and by 2 g/m² of fenthion on mud at Ogudu.

Mortality in window-trap catches : anophelines

Window traps had not been operated before on a large scale in Nigeria and various technical difficulties showed themselves. Most were solved, but two—the unavoidable use of insecticide-contaminated vehicles and the fact that catches from unsprayed villages were done on a separate day and therefore are not an exact control for those from the sprayed villages—remained to the end. In work of this kind it seems desirable to place a few traps each night in an unsprayed village to act as daily controls for those from the sprayed village. Under the circumstances, to minimize the effects of accidents to the catches from the unsprayed villages, mortalities have been

TABLE 3

DEPRESSION OF FIGURES [a] FOR ANOPHELINE AND CULICINE SPRAY-CATCH FIGURES (RD) AND WINDOW-TRAP CATCHES (ED)

Village	Insecticide and dosage	Type of surface	Period of depression (weeks)			
			Anophelines		Culicines	
			RD [b]	ED [c]	RD [b]	ED [c]
Majidun	Malathion 2 g/m²	Organic	10	4	10	3
Owode	Malathion 2 g/m²	Mud	5	2	5	3
Ajegunle	Fenthion 1 g/m²	Organic	7	5	10	6
Ogudu	Fenthion 2 g/m²	Mud	16	0	13	4
Oregun	DDT 2 g/m²	Mud	5	4	6	6

[a] As judged by comparison of Fig. 5-18.
[b] Density of resting mosquitos; pyrethrum-spray catches.
[c] Density of mosquitos leaving houses; window-trap catches.

FIG. 15

CULICINE RESTING DENSITIES IN MALATHION-SPRAYED VILLAGES

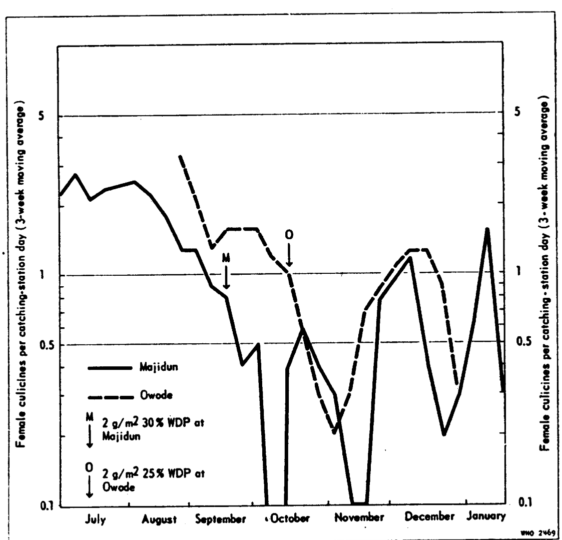


FIG. 16

CULICINE WINDOW-TRAP CATCHES IN MALATHION-SPRAYED VILLAGES

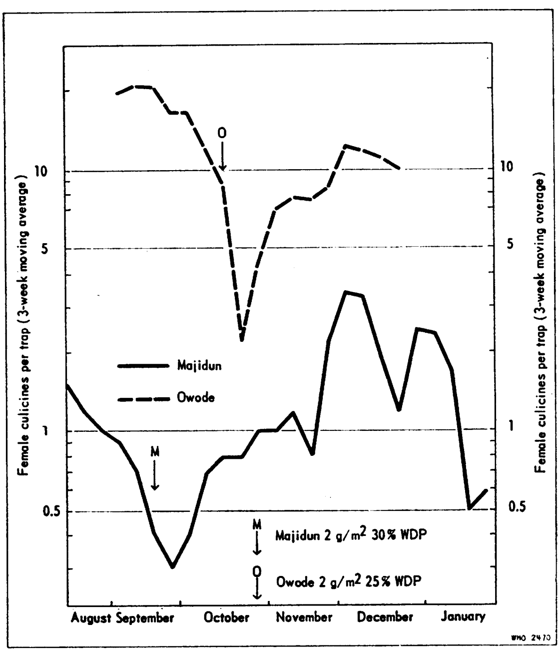


FIG. 17. CULICINE RESTING DENSITIES IN FENTHION-SPRAYED VILLAGES

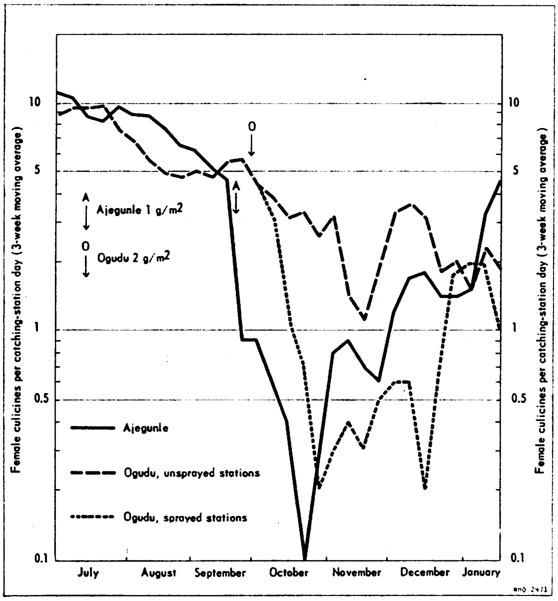
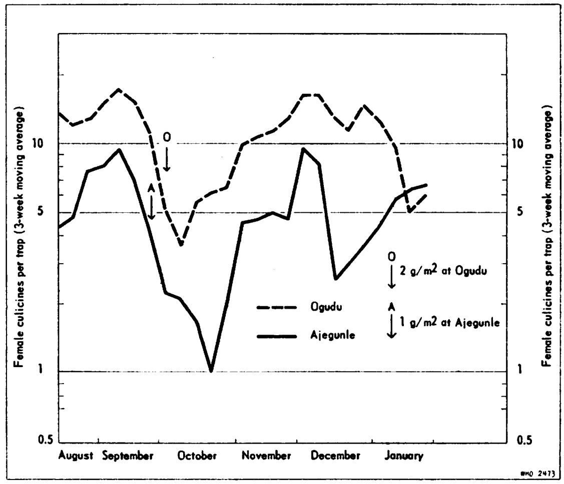


FIG. 18. CULICINE WINDOW-TRAP CATCHES IN SPRAYED VILLAGES



summarized in Table 4 as means over four-week periods before and after spraying. From these, it is clear that mortality increased in catches from unsprayed villages and stations as the experiments

TABLE 4. ANOPHELINE WINDOW-TRAP MORTALITIES

Village	Insecticide and dosage	Type of surface	Time at which mortality count was made	Mean percentage mortality over 4-week periods					
Idena	Nil (unsprayed control)	Organic	Immediate	1	4	12	1	7	17
			In 24 hours	1	6	26	38	72	44
Owutu	Nil (unsprayed control)	Mud	Immediate	0	0	0	5	15	17
			In 24 hours	0	30	44	63	68	67
Ogudu	Nil (unsprayed stations)	Mud	Immediate	3	7	33	28	15	9
			In 24 hours	12	18	80	81	79	61
Majidun	Malathion 2 g/m²	Organic	Immediate	5	50 [a]	86	50	—	—
			In 24 hours	8	75	97	98	—	—
Owode	Malathion 2 g/m²	Mud	Immediate	0	12	73 [a]	37	27	—
			In 24 hours	1	21	98	83	80	—
Ajegunle	Fenthion 1 g/m²	Organic	Immediate	5	8	97 [a]	67	42	8
			In 24 hours	12	13	100	98	73	30
Ogudu	Fenthion 2 g/m²	Mud	Immediate	3	7	88 [a]	68	71	60
			In 24 hours	12	18	98	95	91	87
Oregun	DDT	Mud	Immediate	4	100 [a]	88	22	11	—
			In 24 hours	33	100	100	85	67	—

[a] Time of insecticidal application.

went on, and that this is more marked in the 24-hour mortalities than in the immediate mortalities. Contamination of vehicles and personnel with insecticide seems the most probable reason for this, particularly as over-all, immediate mortality in unsprayed villages was 6% and from the unsprayed stations at Ogudu only 16%. Comparison of immediate mortalities at once singles out Ogudu (fenthion on mud) as giving the longest-lasting immediate kill of *Anopheles* entering the houses.

Average trap-kills over the first three four-week periods are in the order:

Ogudu (fenthion on mud)	76 %
Oregun (DDT on mud)	70 %
Ajegunle (fenthion on organic surface)	69 %
Majidun (malathion on organic surface)	62 %
Owode (malathion on mud)	46 %

By the fourth period, the figures for Oregun (DDT) and Ajegunle (fenthion)—11% and 8%—indicate complete loss of immediate activity, as does that for Owode (malathion on mud)—27%—in the third. Twenty-four-hour mortalities averaged 13% in unsprayed villages before any insecticide was used, 53% after insecticide was used, and 75% in catches from the unsprayed stations in Ogudu village. Under these circumstances it seems inadvisable to place much reliance on the order of magnitude of mortality in the first three four-week post-spraying periods which was:

Oregun and Ogudu (DDT and fenthion on mud)	95 %
Ajegunle and Majidun (fenthion and malathion on organic surfaces)	90 %
Owode (malathion on mud)	87 %

However, the drop to 30% mortality at Ajegunle in the fourth four-week period seems to indicate that the lower dosage of fenthion had by then lost its effectiveness.

Mortality in window-trap catches: culicines

The immediate mortality for unsprayed villages, and for the others before the commencement of spraying (Table 5), averaged 8%, the maximum

TABLE 5. CULICINE WINDOW-TRAP MORTALITIES

Village	Insecticide and dosage	Type of surface	Time at which mortality count was made	Mean percentage mortality over 4-week periods					
Idena	Nil (unsprayed control)	Organic	Immediate	2	9	12	5	4	5
			In 24 hours	2	39	20	25	46	50
Owutu	Nil (unsprayed control)	Mud	Immediate	7	9	6	25	7	1
			In 24 hours	22	22	54	34	51	82
Ogudu	Nil (unsprayed stations)	Mud	Immediate	4	16	55	28	15	24
			In 24 hours	18	19	69	55	54	61
Majidun	Malathion 2 g/m²	Organic	Immediate	8	58 [a]	45	29	37	46
			In 24 hours	27	100	84	70	57	87
Owode	Malathion 2 g/m²	Mud	Immediate	2	9	34 [a]	11	12	—
			In 24 hours	4	27	69	66	51	—
Ajegunle	Fenthion 1 g/m²	Organic	Immediate	4	90 [a]	65	34	4	4
			In 24 hours	9	100	93	71	47	69
Ogudu	Fenthion 2 g/m²	Mud	Immediate	4	16	64 [a]	72	61	55
			In 24 hours	18	19	91	95	91	78
Oregun	DDT 2 gm/²	Mud	Immediate	3	17 [a]	5	18	40	8
			In 24 hours	6	36	12	74	77	49

[a] Time of insecticidal application.

figure being 25%. The average for the Ogudu unsprayed stations after the spraying of the rest of the village was 30%; this suggests quite strongly that the mosquitos were obtaining a lethal dose elsewhere in the village before being caught in the unsprayed houses. Since the Owutu catches, brought in along with those from Majidun, were little different from Idena, it seems probable that these immediate mortality differences were not due entirely to contamination.

DDT at Oregun showed low mortalities, which from the outset reflect the natural tolerance of most culicines to this toxicant. The order of effectiveness of the treatments based, as with the *Anopheles*, on the mean mortality for the first three four-week periods after treatment was:

Ogudu (fenthion on mud)	66 %
Ajegunle (fenthion on organic surface)	63 %
Majidun (malathion on organic surface)	44 %
Owode (malathion on mud)	19 %
Oregun (DDT on mud)	13 %

Against the culicine mosquitos, therefore, fenthion appeared more effective than malathion on both types of surface, while malathion was little more effective than DDT on the mud surface at Owode.

Twenty-four-hour mortalities were so high in the unsprayed villages, averaging 37% with a maximum of 82%, that comparison of the figures from the sprayed villages becomes unreliable. The order of effectiveness based on means for the first three periods after spraying remained as before:

Ogudu (fenthion on mud)	92 %
Ajegunle (fenthion on organic surface)	88 %
Majidun (malathion on organic surface)	85 %
Owode (malathion on mud)	62 %
Oregun (DDT on mud)	41 %

Bio-assay results

Bio-assay tests were conducted at weekly intervals on the principal types of surface, and occasionally on others such as matting used for screens. The initial exposure period was 30 minutes, and when the 24-hour mortality levels fell below 70% the period was extended to 60, 120, and in some cases 240 minutes. Where a mortality less than 70% was recorded once for a given exposure period and high mortalities continued at the next longer period the shorter interval was, in a few cases, reinstated. In all trials control batches were exposed simultaneously on unsprayed surfaces. Control mortalities were throughout very low, and the introduction of a correction factor was unnecessary. All values quoted in Table 6 are crude mortalities with a corresponding control mortality below 10%, and in most cases below 5%.

The results shown in Table 6 underline the ineffectiveness of malathion on the specific mud substrate present, and its very fair performance on every other type of surface. Fenthion falls second to malathion on every surface except mud, on which it was the most effective of the three insecticides. DDT performed well on wood and concrete and reasonably well on mud at the longer exposures. Under the conditions of forced contact used in the bio-assay test DDT is, however, favoured over the other two, since its irritant or locomotor-stimulant effect does not operate to shorten the contact period.

An interesting phenomenon at Ogudu was that bio-assays on unsprayed mud and concrete surfaces at shoulder level gave appreciable mortalities, but at seven feet (2 m) up on the walls gave nil mortalities. The transfer of the insecticide, apparently, on people's clothes, is relevant to the toxicology studies reported below.

CORRELATION OF CHEMICAL AND ENTOMOLOGICAL DATA

Population studies and chemical data

Since the two sets of population data do not entirely agree *inter se*, the correlation of chemical data with them must be difficult. The most striking point with the chemical results was the rise in the amount of DDT and fenthion (and to some extent malathion) present at the surface. This occurred at day 92 for DDT, day 44 for fenthion, and day 102 for malathion (during November). The *Anopheles* density at Oregun (DDT) by spray-catch did decline at this point, after a small rise, but nothing definite happened at Ogudu (fenthion). Culicine density by spray-catch at Oregun showed a similar drop, but again not at Ogudu. Neither anopheline nor culicine populations, as measured by the window traps, showed any change at this period, nor did either immediate or 24-hour mortalities.

Chemical data and bio-assay results

On mud, there seems to be no possible correlation with DDT between the results of chemical assays and the immediately preceding bio-assay. With malathion, there was a steady decline in bio-assay mortality and the amount of insecticide present. In the fenthion village, there was an incomplete correspondence between biological and chemical

TABLE 6

BIO-ASSAY RESULTS SHOWING DURATION OF INSECTICIDAL ACTION ON DIFFERENT SURFACES

Surface	Insecticide and dosage	Duration (in days) of mortality over 70 % at three exposure periods			Results of latest tests		
		30 min.	60 min.	120 min.	Exposure period (min.)	Days after treatment	Mortality (%)
Mud	Malathion 2 g/m²	7	14	35	240	63	67
	Fenthion 2 g/m²	98	—	—	60	119	19
	DDT 2 g/m²	28+	119	140+	120	140	92
Palm-tile	Malathion 2 g/m²	112	—	—	60	119	33
	Fenthion 1 g/m²	91	105	—	60 120 [a]	262 133	32 14
	Fenthion 2 g/m²	77	98	105	120	119	47
	DDT 2 g/m²	28	154+	—	60 60 [a]	154 175	88 35
Wood	Malathion 2 g/m²	119+	—	—	30 30 [a]	112 119	90 99
	Fenthion 1 g/m²	98	126+	—	60	126	94
	DDT 2 g/m²	161	273+	—	60	273	100
Palm midrib	Malathion 2 g/m²	126	168+	—	60	196	60
	Fenthion 1 g/m²	175+	189+	—	30 60 [a]	175 235	100 55
Concrete and plaster	Malathion 2 g/m²	112	—	—	30	119	63
	Fenthion 2 g/m²	98+	—	—	30	98	100
	DDT 2 g/m²	—	112+	—	60	112	96
Matting [b]	Fenthion 1 g/m²	91	—	—	30	91	35

[a] Different bio-assay point.

[b] A palm-fibre product used for internal partitions at Ajegunle.

results; both showed increases in the eighth and twelfth weeks.

The results on other surfaces showed virtually nothing of interest at all. In particular, pairs of results on the same surface at the same time were more often than not contradictory.

The most interesting observation was, perhaps, the very low surface residues of the organophosphorus insecticides on " non-absorbent " surfaces which yet produced a high kill. It seems that perhaps these surfaces are, in fact, slightly absorbent, and may hold below the surface a reserve of insecticide which has a powerful effect, perhaps fumigant.

TOXICOLOGICAL STUDIES

In view of the known toxic effects of some organophosphorus insecticides on man and warm-blooded animals, investigations on the effects of the use of malathion and fenthion on both the spraymen and the inhabitants of the treated villages were carried out; these consisted of the medical examination of any persons showing signs of illness in the immediate post-spraying period and of periodic estimations of the level of cholinesterase activity in the blood of the spraymen and a representative sample of the villagers.

Chemical methods

The method used was that of Edson (1957); blood from a normal individual was taken as a standard in each set of determinations, and reduction of cholinesterase activity determined in a series of eight steps each of 12.5%: in practice, it was found possible to subdivide these to include intermediate readings 6.25% apart.

In a healthy population not exposed to organophosphorus insecticides, fluctuations may be expected in the findings for each individual at different times, amounting to 10%-15% (Callaway et al., 1951). Such variation would be expected to be scattered equally above or below the cholinesterase level at the original determination.

Observations on spraymen

Four out of six operators employed on spraying fenthion for six hours a day for five days at 1.5 g/m² and 2 g/m² showed no fall in cholinesterase activity on the final day. The other two showed falls to 37.5% and 12.5% of normal, the latter being the foreman, who did not use the protective clothing of rubber hat, gloves and overalls worn by the others. The previous spraying of malathion at 2 g/m² had not caused significant lowering of cholinesterase levels in this group.

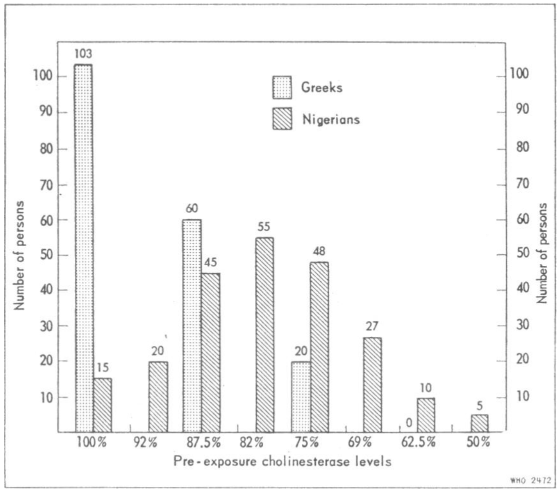
Observations on villagers without contact with organophosphorus insecticides

In Fig. 19 the cholinesterase levels determined for 225 Nigerians in 1960 are compared with those for 183 Greeks surveyed by the same team using the same method in 1959. From this, it is clear that the Nigerian norm for cholinesterase activity lies at the 82% level, while that for the Greeks lies close to the 100% level. Nigerians showing 100% activity are a minority of 7%.

Without contact with organophosphorus insecticides, the cholinesterase activity of individuals may be seen to fluctuate quite widely; in most cases, but not in all, the individuals showing rises in level between successive observations are balanced by those showing falls. In Table 7, the first untreated village showed fairly balanced rises and falls; the second also, over only a week, showed small rises in about half the population; the third, over a five-month interval, showed the same tendency.

FIG. 19

BLOOD CHOLINESTERASE LEVELS FOR 183 GREEKS AND FOR 225 NIGERIANS



Observations on villagers after contact with organophosphorus insecticides

The fourth pair of villages, sprayed with malathion, showed a preponderance of falls in cholinesterase level a week after spraying, but these can be set off against about half their number of rises, while a larger number still showed no change.

The next group of villages, sprayed with fenthion, showed a very different picture. Altogether 122 out of 160 people (76%) examined showed falls, two-thirds of these in the lowest categories, but balanced by only one rise. There seems no reason to doubt the relationship between exposure to fenthion and depression of cholinesterase activity as cause and effect. Ajegunle, sprayed with fenthion at an actual dosage of 1.5 g/m² on non-absorbent surfaces, and Ogudu, sprayed with double this on mud, showed greater depressions than Ojota, which was sprayed in November for toxicological observations only, and received the intended dose of 2 g/m² on mud. This suggests that exposure is less in the population of mud villages, where the insecticide is quickly sorbed and probably becomes less readily available.

TABLE 7

EFFECT ON LEVEL OF CHOLINESTERASE ACTIVITY IN INHABITANTS OF SPRAYED VILLAGES

Village	Insecticide and dosage	Interval between surveys	Falls in cholinesterase level			No change	Rises in cholinesterase level			Total number examined
			37.5 % and over	25 %	12.5 %		12.5 %	25 %	37.5 % and over	
Idena	Nil	1 week	0	0	4	26	6	0	1	37
Oregun	DDT	1 week	0	0	1	12	7	3	1	24
Ojota	Nil	5 months	0	1	1	12	13	1	0	28
Total not sprayed with organophosphates		—	0	1	6	50	26	4	2	89
Majidun and Owode	Malathion 2 g/m²	1 week	3	5	20	37	13	0	1	79
Ajegunle	Fenthion 1 g/m²	1 week	5	9	35	15	0	0	0	64
Ogudu	Fenthion 2 g/m²	1 week	9	7	16	13	0	1	0	46
Ojota	Fenthion 2 g/m²	1 week	0	9	32	9	0	0	0	50
Total sprayed with fenthion		—	14	25	83	37	0	1	0	160

Table 8 shows the changes observed in the rather small group who were examined three times. At Idena (unsprayed), the small majority showing rises between the first and second examination was balanced by falls between the second and third; most showed no change. At Ojota, where there was no spraying between the first and second observation, about half the population showed slight falls, and this tendency was exaggerated after the fenthion treatment. At Owode, the group examined three times did not show the slight depression immediately after spraying seen in the Fig. 7 sample, but showed a general rise in the following period.

At Ogudu, the people whose cholinesterase levels fell in the week after spraying showed complete recovery in the next five weeks.

TABLE 8

RECORD OF CHANGES IN CHOLINESTERASE LEVELS IN THOSE TESTED ON THREE OCCASIONS

Village	Insecticide and dosage	Interval between surveys, and time (in days) after treatment	Falls in cholinesterase level			No change	Rises in cholinesterase level			Total number examined
			37.5 % and over	25 %	12.5 %		12.5 %	25 %	37.5 % and over	
Idena	Nil	Aug.-Oct.	0	0	6	17	8	0	1	32
	Nil	Oct.-Dec.	0	2	7	20	3	0	0	
Ojota	Nil	July-Dec.	0	1	8	9	1	1	0	20
	Fenthion 2 g/m²	Dec.-Jan. (Day 20)	1	5	8	5	1	0	0	
Owode	Malathion 2 g/m²	October [a] (Day 7)	0	0	4	15	2	1	1	19
	Malathion 2 g/m²	Oct.-Nov. (Day 42)	0	0	2	10	5	2	0	
Ogudu	Fenthion 2 g/m²	October [a] (Day 7)	9	6	10	6	0	0	0	31
	Fenthion 2 g/m²	Oct.-Nov. (Day 42)	0	0	0	8	10	7	6	

[a] Both surveys carried out in October.

TABLE 9

CHANGES IN CHOLINESTERASE LEVELS IN CHILDREN UNDER 11 YEARS OF AGE [a]

Village	Insecticide and dosage	Falls in cholinesterase levels			No change	Rises in cholinesterase levels			Total number examined
		37.5 % and over	25 %	12.5 %		12.5 %	25 %	37.5 % and over	
Idena and Oregun	Nil	0	1	5	27	9	1	1	44
Majidun and Owode	Malathion 2 g/m²	3	2	9	27	3	2	0	46
Ajegunle and Ogudu	Fenthion 2 g/m²	11	9	10	16	0	0	0	46

[a] Data from Table 7.

Distribution of the effect of organophosphorus insecticides in the population

Accepting, from the above evidence, that spraying with fenthion lowers the levels of cholinesterase activity in the inhabitants of sprayed premises, it is next necessary to decide if these effects are general over the population or are concentrated in a particular group. The first group to be considered is the children, who leave the village less than adults, and, in their play, may have more opportunity of contact with the insecticide. Table 9 separates three groups of children under 11 years, and it can be seen that the proportion of children showing a moderate or severe fall in cholinesterase activity is greater than the corresponding proportion of adults.

At Ajegunle a few, and at Ogudu over 30%, of the houses were unsprayed. An investigation of the domiciles of as many members of the sample showing falls in cholinesterase level as could be contacted showed that a fair proportion resided in unsprayed houses. A close check of these showed that there was little more depression in the inhabitants of sprayed than in those of unsprayed houses, and that no correlation appeared to exist between age or sex and the degree of cholinesterase depression in the people living in unsprayed houses.

Cholinesterase levels and health

In the immediate post-spraying period in each village a search for sick people, especially children, was made, and these were seen by a medical officer, as were a sample of those children showing the most marked depression of cholinesterase activity. In no case was any sign of illness attributable to the insecticide detected.

DISCUSSION AND CONCLUSIONS

The results of chemical and entomological assessment together show that both the organophosphorus insecticides may be expected to show results equal or superior to those of DDT when used in houses constructed of wood, palm-thatch and similar materials of vegetable origin. Fenthion, moreover, at high dosages, can be expected to produce good results on absorbent mud surfaces. These results are in general agreement with those of Schoof et al. (1961) working in El Salvador, and those of Smith & Hocking (1962) in East Africa, except as regards malathion.

The apparent failure of malathion on mud was observed at the all-mud village of Owode and at Majidun, where the single mud house in the village was used for bio-assay. The pH values for mud from the two villages were 5.8 and 5.5 respectively, the extremes of the range observed locally. In the East African work quoted above, malathion performed better on non-sorptive than on sorptive mud. Limewashing, with associated high pH value, improved its performance on sorptive mud, but had the reverse effect on non-sorptive mud. So far, the type of mud classified as non-sorptive has not been found in Nigeria.[1] So far as the evidence available at present can be relied upon, there is a possibility that active or sorptive muds, on which malathion appears to undergo a process of deep sorption and become unavailable to mosquitos, are predominant in Nigeria and possibly elsewhere. If the El Salvador results were obtained on a non-sorptive, inactive type of mud, and if this type is widespread, the prospects of successful use of malathion in malaria

[1] Unpublished data of Langbridge & Elliott.

eradication are relatively bright; if the Nigerian type is predominant, however, they will be correspondingly limited.

The irritant effects of DDT are illustrated by the contrast between prolonged high kills under bioassay conditions of forced contact, and short duration of effect shown under facultative contact conditions by the window-trap catches. The high mortality due to fenthion under both these sets of conditions suggests that little irritation or repellency is provoked by this substance. Malathion appears to be intermediate in this respect, but with repellency, though present, relatively unimportant.

If the effects on the mosquito were the sole criterion, it would be possible at this point to recommend, for purposes of malaria eradication, the use of either of these insecticides in areas where the dwellings were predominantly of organic material, and of fenthion alone where a certain type of mud is the predominant building material.

But the effects on the human population call for a note of caution. Where, as in Nigeria, the local norm for cholinesterase activity is, by European standards, low, either of these insecticides, but especially fenthion, may produce a significant decrease in its level. This process appears to be both temporary and reversible, as the level recovers by the sixth week. Moreover, even when severe, it has not been shown to be associated with any detectable loss of health and well-being. However, the temporary depression of a physiological index due to the absorption of an insecticide, even in the absence of any detectable toxic symptoms, seems to call for further investigation. It will be necessary to repeat the observations on other residents of houses sprayed with fenthion. Although the evidence seems quite clear that the insecticide was responsible for the falls in cholinesterase observed, the fact that people living in unsprayed houses also showed effects appears very anomalous. This experience in Nigeria does emphasize the need to obtain good base-line data on the cholinesterase levels of people in different communities *before* organophosphorus insecticides are introduced. Nutrition, disease and possibly racial and genetic factors may play a part in determining the " normal " cholinesterase levels and may also lead to fluctuations that might be attributed to other causes such as exposure to insecticides.

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RÉSUMÉ

Les essais sur le terrain effectués dans des villages de Nigéria avaient un triple but: comparer l'efficacité, sur anophélinés et culicinés, de divers insecticides pulvérisés dans des habitations faites de matériaux indigènes, de déterminer par voie chimique la durée de l'effet rémanent, de contrôler si les insecticides affectaient physiologiquement les habitants des cases traitées.

L'étude chimique des dépôts indique que sur le pisé local le malathion est sorbé en plus grande quantité et à une plus grande profondeur que le fenthion et que le DDT l'est moins. Sur les surfaces d'origine végétale, ces trois insecticides semblent persister au même degré.

Les observations entomologiques faites par la technique d'aspersion au pyrèthre ont montré que, par ordre d'efficacité décroissante sur la densité de moustiques, les insecticides peuvent être classés comme suit: fenthion sur pisé; malathion, puis fenthion sur surfaces organiques et enfin malathion et DDT sur pisé. Cette comparaison est valable aussi bien pour les anophèles que pour les culicinés, mais il faut tenir compte de la dose élevée (presque 3 g/m^2) de fenthion nécessaire sur pisé. Les observations parallèles faites par la technique des pièges de fenêtre ont donné, par ordre d'efficacité décroissante sur la densité de moustiques, la classification suivante:

fenthion sur surfaces organiques, DDT sur pisé et malathion sur surfaces organiques, fenthion sur pisé.

La mortalité des anophèles à l'intérieur des pièges de fenêtre a diminué en fonction des insecticides dans l'ordre suivant: fenthion sur pisé, DDT sur pisé et malathion sur surfaces organiques, malathion sur pisé. Sur les culicinés, le fenthion a été le plus efficace des insecticides sur les deux types de surface et le DDT le moins actif.

Les essais biologiques ont confirmé les mortalités relevées dans les pièges de fenêtre, sauf dans les conditions de contact forcé où le DDT a été le plus efficace. Le malathion sur pisé a été ici encore le moins actif.

Avant les aspersions, le taux d'activité cholinestérasique du sang chez les Nigériens n'était en moyenne que 82% du taux normal européen. Après les aspersions il a diminué d'une manière appréciable lorsqu'on avait utilisé du fenthion et moins lorsqu'on avait utilisé du malathion. Les chutes du taux ont été momentanées, avec retour à la normale, en moins de 6 semaines; elles n'ont pas paru affecter un groupe particulier de la population, et n'ont été associées à aucun état pathologique appréciable. En attendant d'avoir des renseignements supplémentaires concernant la signification de ces modifications et leurs effets possibles sur la population, il convient de ne recourir qu'avec précaution aux organophosphorés, en particulier au fenthion, dans les opérations d'éradication du paludisme.

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