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CHEMICAL STUDIES

Surveillance of Batch Samples of DDT 75% Water Dispersible Powders

The program of surveillance of batch samples of DDT powders purchased for the AID program was continued throughout the past fiscal year. During this period some 14,000 samples were received and stored under semi-tropical conditions at Savannah, Georgia. Approximately 2,500 tests were run on the powders under surveillance during the year.

Since the introduction of the 70°C accelerated storage test in Specification 2373-1 the quality of the powders received under this specification has been excellent and few complaints have been received from field missions. During the past year, however, we began to observe failures of some powders after 3 to 6 months in storage. Two companies in particular seemed to be having difficulty with their products. Additional samples from these companies were pulled from storage and tested for suspensibility. In some cases 20 to 30 percent of the batch samples from a given lot of material were found to be defective. The companies were notified and a thorough investigation of the problem was initiated.

It was found that the failures were not due to growth or agglomeration of the particles, but rather to the failure of the wetting agent. The possibility of bacterial decomposition was considered and tests were conducted to determine if the wetting agent had been biodegraded. The results were negative. Further tests indicated that the wetting agent in the faulty powders had not decomposed, but had become associated or bound with the clay or other inert

ingredients of the formulation in such a way that it is not available when needed to suspend the powder. Almost all of the powders tested dramatically improved in suspensibility when soaked for 24 hours in water.

AID and GSA officials and plant managers of the companies involved were notified of the batch sample failures. Complaints have now been received from one country which received some of the faulty material. It has been recommended that additional wetting agent be furnished to countries which have faulty powders on hand with instructions for addition of the wetting agent to those powders which will not suspend. The following wetting agents and concentrations can be used for this purpose: Igepon T-77, Antara Chemical Division of General Aniline & Film Corporation or Duponol WA Dry, E. I. DuPont de Nemours & Company. One percent of wetting agent based on the weight of the dry DDT powder should be added to the faulty powders.

In the meantime, work on an improved specification to prevent recurrence of this problem has been initiated. It is believed that the present powder formulations can be improved by addition of more wetting agent and/or reduction of the amount of clay in the formulation.

Development of New Specifications

A. DDT 75% Water Dispersible Powder.

ICA Specification 2373-1 dated June 1, 1961 was revised and reissued as AID Specification 2373-2 dated July 1, 1964. No major changes were made

in the new specification, however, several improvements in analytical techniques which were developed during the past three years were incorporated in the new specification.

An amendment has also been added requiring closer inspection and high storage stability of batch samples. This device is being used on a temporary basis to correct the wetting agent deficiency discussed above. As soon as an analytical procedure is designed to detect wetting agent deficiency it will be incorporated in the specification.

B. Malathion, 95% Technical.

At the request of AID officials, a specification for malathion, 95% technical was developed. Consideration was given to a new argentimetric method of analysis which was said to be more accurate than the present IR and colorimetric methods. Work at this laboratory indicated that the increase in accuracy did not offset the increased time required to run analyses by the new method. The IR method was therefore recommended for purchases of malathion, technical.

C. Malathion, 50% Water Dispersible Powder.

When we prepared the original specifications for 25 and 50% malathion water dispersible powders, these products were in the experimental stage and manufacturing type specifications were drafted to insure receipt of a product which was known to be effective and stable. We now have considerable experience with malathion formulations and feel that a performance specification

will be satisfactory for the purpose of future purchases. A draft specification for 50% malathion powder was prepared during the year which contained the necessary changes to make it a performance specification. We do not recommend further purchases of malathion 25% water dispersible powders in view of the fact that transportation costs would be greater and they offer no advantage over the 50% products now available.

Collaboration with GSA and Other Laboratories

Responsibility for analysis of samples from DDT powder production for compliance with AID specifications was transferred from commercial laboratories to GSA laboratories during the past fiscal year. The transition took place smoothly and no major problems were encountered. Some minor difficulties arose, but these were resolved by telephone contact between this laboratory and the GSA laboratory. On one occasion a representative from this laboratory visited a GSA laboratory to assist them.

On several occasions one of the manufacturers obtained results which did not agree with the GSA findings. The Savannah laboratory assisted in the investigation of the problem and supported the GSA laboratory. Some suggestions for changes in the wording in the AID specification to improve the clarity were incorporated in the latest revision of the specification as a result of this collaborative work.

At the request of WHO we participated in collaborative tests on methods of analysis for DDVP, malathion and dieldrin. Results of these tests are expected to be of value to AID in the development of specifications in the future.

Development of DDVP Spontaneous Vaporizers

Work on an improved DDVP dispenser was continued during the past fiscal year. The development of a new unit which produces 4 to 5 times the volume of vapor as the old wax and resin formulation is still underway. The new unit is not considered practical in its present state because it absorbs water from the atmosphere too rapidly. Chemical and mechanical means of overcoming this phenomenon are being studied.

BIOLOGICAL INVESTIGATIONS

Residual Fumigant Studies at Savannah, Georgia

A. Culex pipiens quinquefasciatus

Residual fumigants containing dichlorvos were evaluated against Culex pipiens quinquefasciatus breeding in catch basins within Savannah to investigate (a) the comparative effectiveness of five dispenser formulations, (b) the effect of varying the applicate dosage of 20-percent formulation dichlorvos-resin dispensers, and (c) the over-all efficacy of a large-scale treatment of the dichlorvos^{*}-resin formulation.

Selection of the test areas was based on portions of the city which normally sustain heavy Culex production. In the large-scale treatment, the additional factor of minimizing the hazards normally encountered in a control operation of this type was considered (i.e., danger to personnel working under congested traffic conditions, etc.). The two areas selected extended over 260 square blocks of the city of which 212 of these blocks (containing 1,263 basins) were used for large-scale treatment. Thirty catch basins were utilized for each of the other studies.

Based on previous studies, a 20-gram dichlorvos dosage, characteristic of a 10-inch length of the resin strip formulation, was selected for use in the large-scale treatment. Formulations evaluated in the comparative dispenser type studies were as follows:

* Dichlorvos is now the accepted common name for DDVP.

- (1) Twenty percent dichlorvos in polyvinyl chloride resin plastic strip; $3/16''$ x $2-1/2''$ x $10''$ strips containing 20 grams of technical dichlorvos by weight of dispenser unit.
- (2) Twenty percent dichlorvos in polyvinyl chloride resin plastic strand, $1/8''$ diameter; applied at 20 grams of technical dichlorvos by weight of the strand.
- (3) Twenty percent dichlorvos in polyvinyl chloride resin pellets; applied at 20 grams of technical dichlorvos by weight of pellets.
- (4) Twenty-five percent dichlorvos in wax and dibutyl phthalate; a cylindrical unit containing approximately 50 grams technical dichlorvos per unit.
- (5) Seventy percent dichlorvos in liquid; contained in a permeable plastic dispenser; unit weight of technical dichlorvos approximately 30 grams.

In the dosage studies, 20 percent dichlorvos resin-plastic dispensers containing 8, 12, 16, 20, and 24 grams of technical dichlorvos were tested. These dispenser units measured $3/16''$ by $2-1/2''$ by 4", 6", 8", 10", and 12", respectively.

All dispensers of the strip type were hung within the basin by means of a length of number 12 coated copper wire fastened to the front support rod

of the basin top. The pellet formulation, not suitable for mounting within the basin, was seeded at the bottom of the basin. Dispensers of the strand type were mounted at the top of the basin. Both the wax and liquid dispenser units required either a protective casing or special bracket to insure a lasting installation of proper function.

The average physical characteristics of the catch basins under study adhered closely to those described in previous work reported in June 1963.

Temperatures within the test basins remained favorable to dichlorvos vaporization through the entire 18-week test period. The maximum temperatures varied from 62°F to 106°F while minimums were recorded at 62°F to 78°F. Ambient air temperatures at the hour of testing ranged from 60°F to 106°F. Water temperatures of 69°F to 82°F were recorded, a range well within tolerance levels for the breeding of C. p. quinquefasciatus.

With only slight variation, the evaluation of the effectiveness of treatment was based on the techniques described in the AID Annual Report for the year ending June 30, 1963.

Mosquito larvae were reduced markedly after treatment in all studies, but breeding reappeared at a low level from 2 to 4 weeks after dispenser installation. Immatures continued to appear in small numbers through the remainder of the test period. Although low in magnitude the average number of larvae per dip in the basins routinely inspected reflected the same 3- to 4-week cyclic breeding fluctuations as in the control basins. The normal

late seasonal increases in breeding intensity of C. p. quinquefasciatus appeared in September.

Data (Table 1) for the weeks of effective adult control in the large-scale treatment showed satisfactory mortalities in the regularly tested basins for 14.3 weeks; the range was from 10 to 18 weeks. The five basins randomly selected from the treatment at large for testing each week yielded acceptable kills averaging 14.2 weeks and ranging from 13 to 15 weeks.

Table 1

Evaluation of Large-scale treatment with dichlorvos for control of Culex pipiens quinquefasciatus in catch basins.

Type of Testing	Basins Tested Weekly	Individual Catch Basins Tested	Average Weeks Effective Adult Control
Routine	15	15	14.3
Random	5	90	14.2

Considerable variation occurred in the length of residual action with each formulation used in the comparative dispenser type study (Table 2). The resin plastic strip formulation gave satisfactory kills from 13 to 16 weeks, averaging 14.7, while the resin plastic strand, wax and liquid formulations yielded 6 to 15, 6 to 14, and 8 to 17 weeks with averages of 10.5, 10.5, and 11.6 weeks, respectively. The resin pellet formulation seeded within the water volume of the basin gave no adult kill.

Table 2

Evaluation of five types of dichlorvos dispensers against
Culex pipiens quinquefasciatus.

Dispenser Type	Technical Dichlorvos (g.) per Dispenser Unit	Average Weeks* Effective Adult Control
Resin Plastic Strip	20	14.7
Resin Plastic Strand	20	10.5
Resin Plastic Pellets	20	0.0
Montan Wax	50	10.5
Liquid Drip	30	11.6

* Five basins for each formulation.

In the comparative dosage tests, dispenser units containing 8 and 12 grams of technical dichlorvos sustained effective control for 3.7 and 5.8 weeks within ranges of 2-6 and 4-8 weeks, respectively (Table 3). Dispensers with dosages of 16, 20, and 24 grams technical dichlorvos each averaged 14.7 in ranges of 11-18, 13-15, and 10-18 weeks, respectively.

Table 3

Effectiveness of various dosages of dichlorvos-resin against Culex pipiens quinquefasciatus.

Grams Technical Dichlorvos per Dispenser Unit	Individual Basins Tested	Number of Individual Tests	Average Weeks Effective Adult Control
8	5	180	3.7
12	5	180	5.8
16	5	90	14.7
20	5	90	14.7
24	5	90	14.7

The resin strip formulation used for the large-scale treatment demonstrated certain advantages over the other types of dispensers in that it reduced (a) dispenser losses due to water and debris abrasion, (b) installation time and materials, and (c) manpower and equipment needed for maintenance of satisfactory control levels against C. p. quinquefasciatus.

As several concurrently run laboratory experiments have indicated that the activity pattern of newly emerged adults and their greater susceptibility to dichlorvos vapor (versus the 3-day-old adults used in evaluating these tests), in practice, may well extend the 14-week control period demonstrated by the techniques used in this study.

Evaluation of the five types of dichlorvos formulations and/or dispensers supported the conclusions drawn from the large-scale treatment

study. Although the resin plastic strip formulation, with an average of 14.7 weeks effective kill, appears the most promising for catch basin mosquito control, the remaining strand, wax, liquid and pellet formulations show promise of other specialized uses (i.e., larvicidal, etc.).

Differences in the length of residual action observed in the study of various dosages indicate that for field use the 16-gram dosage (8" length) of the resin plastic strip offers as much promise as the higher dosages with this formulation and design.

B. Comparative Susceptibility of Four Species of Mosquitoes to Dichlorvos Vapor.

Exposure of the Cleveland strain of Anopheles quadrimaculatus, Trinidad strain of Aedes aegypti, Savannah strain of Culex pipiens quinquefasciatus, and Anopheles albimanus to dichlorvos vapor (Table 4) indicate that with 1-, 2-, and 4-hour exposure A. albimanus is the most susceptible, closely followed by C. p. quinquefasciatus. A. quadrimaculatus and A. aegypti were the most difficult to kill and the mortalities for the two species were quite similar. With a 12-hour exposure, the same pattern was followed but the differences were not as great.

Table 4

Results obtained with exposure of four different species of mosquitoes to dichlorvos vapor.

Dispenser Age Wks.	<u>Anopheles quadrimaculatus</u>				<u>Aedes aegypti</u>				<u>Anopheles albimanus</u>				<u>Culex pipiens quinquefasciatus</u>			
	Mortality at indicated exposure period in hours															
	1	2	4	12	1	2	4	12	1	2	4	12	1	2	4	12
1	100	100	100	-	100	100	100	-	100	100	100	-	100	100	100	-
2	43	100	100	-	95	100	100	-	100	100	100	-	82	100	100	-
3	100	100	100	-	93	100	100	-	100	100	100	-	100	100	100	-
4	12	35	77	-	11	42	60	-	76	98	100	-	61	92	96	-
5	58	97	100	-	54	90	98	-	100	100	100	-	94	100	100	-
6	8	33	96	-	1	8	61	-	70	96	100	-	20	82	90	-
7	2	9	75	-	1	20	70	-	29	90	100	-	18	75	100	-
8	-	62	95	100	-	33	97	100	-	86	99	100	-	58	100	100
9	-	4	45	100	-	4	34	100	-	79	100	100	-	30	93	100
10	-	1	7	100	-	2	36	98	-	23	88	100	-	3	66	100
11	-	-	-	89	-	-	-	87	-	21	75	100	-	-	3	100
12	-	-	-	92	-	-	-	70	-	-	-	100	-	-	-	88
13	-	-	-	25	-	-	-	36	-	-	-	81	-	-	-	73
14	-	-	-	1	-	-	-	5	-	-	-	37	-	-	-	11

Field Experiment in Haiti with Dichlorvos Residual Fumigant.

The area selected in 1962 as a test site to determine the effectiveness of dichlorvos as a vapor in controlling Anopheles albimanus was treated for the sixth and final time in April 1964. The last biological evaluation is now in progress. The number of houses treated has ranged from 4,618 during the first treatment to 5,760 during the fifth treatment. In the original treatment an attempt was made to treat at the rate of six dispensers per house but the average was actually five per house. The second and third treatments were similar to the first but during the fourth treatment all houses were remeasured and treated and the average number of dispensers per house for the fourth, fifth, and sixth treatments was 7.2, 6.5, and 7.0, respectively.

Each treatment was evaluated by placing caged A. albimanus at the 2- and 6-foot levels and exposing for 12 hours during the night. As many as 50 houses were tested weekly during the first two treatments but this number was reduced for the remainder of the treatments and tests made on alternate weeks on a particular group of houses. The results for the first four treatments were similar. The week in which 25 percent or more of the houses had average mortalities of 70 percent or less for treatments 1 through 4 were 13, 11, 13, and 11, respectively. Twenty-five percent of the houses had average mortalities less than 90 percent for treatments one through four on weeks 11, 11, 11, and 10, respectively. The fifth treatment gave better results and none of the houses had mortalities of

less than 70 percent until week 16. Seventeen percent of the test houses had less than 90 percent mortality on week 11 but this did not increase any for the next 5 weeks.

Parasitological data to date indicate that interruption of malaria transmission has not been obtained with either the dichlorvos treatment or the DDT house spraying even though the incidence rates in both areas have been reduced. Preliminary data on the activity pattern indicate that the major biting of humans occurs out-of-doors. Of the number that enter homes and bite, many escape to exterior without contacting the walls. One additional parasite survey is now underway.

Evaluation of Compounds as Residual Agents for the Control of Adult Mosquitoes.

The effect of surface and other factors on the potential of new organophosphorus and carbamate insecticides was evaluated in tests conducted out-of-doors. The test panels were placed under a shed which protected them from rain but exposed them to variations in temperature, relative humidity and air movement. The test surfaces included clay, whitewashed clay, thatch, galvanized metal, plywood, whitewashed plywood, bamboo, cement plaster and brick. The compounds tested were those that had shown promise in laboratory tests with plywood panels.

All panels (approximately 1' x 4') were treated with suspension formulations in vertical position under the shed using a 1-gallon

compression sprayer equipped with an 8002 nozzle. Plywood sheets held on both sides of the panel being treated protected the adjacent panels from contamination during the spraying operation. The following toxicants and dosages were tested:

<u>Compound</u>	<u>Percent Active Ingredient</u>	<u>g./sq. m.</u>
1. dicalthion	50	0.25, 0.5, 1
2. EPN	25	0.5, 1
3. OMS-122	25	0.5, 1, 2
4. Guthion	25	0.25, 0.5, 1
5. HRS-1422	75	1, 2
6. U-12927	75	0.25, 0.5, 1
7. malathion	25	1
8. DDT	75	0.5, 1
9. DDT	90	0.5, 0.75, 1
10. Sevin	50, 80, 85	1, 2

Evaluation of the treatments was by exposure of dieldrin-resistant Anopheles quadrimaculatus females to the deposits for 1 hour beneath a plastic cone. Three replicates were made on each surface at each exposure with 10 females in each cone. A different location was employed in each test at biweekly intervals. After exposure, the specimens were held at 80°F and 70 percent relative humidity for 24 hours, at which time the mortality counts were made.

The number of weeks which the indicated compounds gave 90 percent or higher female mortality is given in Table 5.

The data again show that insecticidal deposits were least effective on clay and whitewashed clay while those on plywood and thatch gave the greatest longevity.

EPN at 0.5 and 1.0 g./sq. m. was the only experimental insecticide that was effective on all test surfaces. Deposits at the lower dosage were slightly less effective on only two surfaces, whitewashed clay and metal.

Dicaphon at 1.0 g./sq. m. gave 8 or more weeks of effective mortalities on all surfaces except cement plaster and metal. Lower dosages of this compound were relatively ineffective on several surfaces.

HRS-1422 at 2.0 g./sq. m. gave 6 or more weeks of effective mortalities on all surfaces except clay. The period of effectiveness of deposits at 1.0 g./sq. m. dropped to 2 and 6 weeks, respectively, on whitewashed clay and metal.

In tests involving comparison of DDT formulations, no difference was found between the 75 and 90 percent water-wettable powders when deposits of 1.0 g./sq. m. were applied. However, deposits calculated to give 0.5 g./sq. m. from the 75 percent wettable powder gave results superior to similar deposits from the 90 percent material on bamboo, plywood and clay.

Table 5

Number of weeks that various insecticides on indicated surfaces gave at least 90 percent mortality of dieldrin-resistant A. quadrimaculatus females exposed for 1 hour.

Toxicant	g./sq.m.	Brick	Cement Plaster	Bamboo	WW Clay	WW Plywood	Plywood	Metal	Thatch	Clay
dicapthon	0.25	10*	0	0	0	10*	10*	2	10*	0
	0.5	8*	2	4	6*	2	8*	2	14+	2
	1.0	10	4	14+	8*	8	14+	4	14+	10*
EPN	0.5	14+	14+*	14+	12*	14+	14+	12	14+	14+*
	1.0	14+	14+	14+	14+	14+	14+	14+	14+	14+
OMS-122	0.5	0	0	0	0	0	0	0	0	0
	1.0	3	3	0	0	0	11*	7*	15*	0
	2.0	0	7	3	0	7*	9*	7*	17+	0
Guthion	0.25	3	0	0	0	0	0	0	17+*	0
	0.5	0	0	17+*	0	17+*	3	5	17+	0
	1.0	inc.	0	17+*	3	17+	0	3	17+	0
HRS-1422	1.0	inc.	12	12	2	12	12	6	16+	0
	2.0	6	16+	16+	6	16+	16+	16+*	16+	0
U-12927	0.25	0	0	3	0	0	13	5	17+	0
	0.5	7	11*	11	0	7	17+*	5	17+	0
	1.0	15	17+*	17+	5	17+	17+	13*	17+	0
malathion	1.0	0	0	5	0	inc.	13*	5	15	0
DDT (75)	0.5	17+*	17+	17+	17+*	17+	17+*	9	17+	17+*
	1.0	17+*	17+*	17+	17+	17+	17+*	17+*	17+	17+
DDT (90)	0.5	15*	15	0	17+*	17+	5	17+	17+	3
	0.75	17+	17+	17+*	17+	17+	17+	15*	17+	17+
	1.0	17+	17+	17+	17+	17+	17+*	17+	17+	17+

(Table continued on next page)

Table 5 - continued

Toxicant	g./sq.m.	Brick	Cement Plaster	Bamboo	WW Clay	WW Plywood	Plywood	Metal	Thatch	Clay
Sevin(50)	1.0	-	-	-	9	-	17+	-	-	inc.
	2.0	-	-	-	17+	-	17+	-	-	13*
Sevin(80)	1.0	-	-	-	17+	-	17+	-	-	15*
	2.0	-	-	-	17+	-	17+	-	-	15
Sevin(85)	1.0	-	-	-	13	-	15	-	-	13*
	2.0	-	-	-	17+	-	17+	-	-	13

Plus sign indicates weather terminated tests.

* - mortality fell below 90 percent during test period.

inc. - inconclusive results due to fluctuations in mortality.

In tests involving water-wettable formulations of Sevin, the deposits from the 80 percent wettable powder were found to be superior to the 50 and 85 percent materials on the basis of longevity of effectiveness of deposits at 1.0 g./sq. m.

Panels were also made of goat wool tent material received from Iran and placed under the shed described above. Suspension formulations of DDT (1.0 and 2.0 g./sq. m.), dieldrin (0.5 g./sq. m.), Sevin (1.0 and 2.0 g./sq. m.), and malathion (1.0 and 2.0 g./sq. m.) were applied. Susceptible A. quadrimaculatus were exposed to the deposits for 1 hour under plastic cones at biweekly intervals and mortality counts were made 24 hours later. Sevin (1.0 and 2.0 g.), malathion (2.0 g.) and DDT (2.0 g.) gave effective kills for the entire 16-week test period. Kills of 90 percent or more were obtained through week 12 with malathion (1.0 g.) and week 8 with dieldrin. Results with DDT at 1.0 g./sq. m. were inconclusive due to fluctuating mortalities.

A comparison of thatch panels made from a native sedge and from sugar cane from Haiti showed no significant differences in effect of surface on residual activity of insecticides. Dieldrin-resistant A. quadrimaculatus were exposed to panels of each material treated with 1.0 g./sq. m. of Sevin, malathion, Bayer 39007, and Bayer 37344.

Additional plywood, whitewashed plywood, and cement plaster panels were placed under the shed, treated with experimental compounds, and tested against DDT-resistant Aedes aegypti and DDT-dieldrin resistant Culex pipiens quinquefasciatus. Similar panels were tested against dieldrin-resistant A. quadrimaculatus and the results are included in Table 6.

Table 6

Number of weeks that various insecticides on indicated surfaces gave at least 90 percent female mortality against three species of mosquitoes.

Toxicant	g./sq. m.	<u>A. quadrimaculatus</u>			<u>C. p. quinquefasciatus</u>			<u>A. aegypti</u>		
		Plywood	WW Plywood	Cement Plaster	Plywood	WW Plywood	Cement Plaster	Plywood	WW Plywood	Cement Plaster
dicapthon	0.25	10*	10*	0	1	1	1	0	0	0
	0.5	8*	2	2	1	1	1	0	0	0
	1.0	14+	8	4	9*	5	1	0	0	0
EPN	0.5	14+	14+	14+*	17+	15+	5	inc.	inc.	0
	1.0	14+	14+	14+	15+	15+	11	17+*	9	0
OMS-122	0.5	0	0	0	0	1	1	0	9*	0
	1.0	11*	0	3	1	1	1	13*	15	0
	2.0	9*	7*	7	1	1	1	17+*	15*	7
HRS-1422	1.0	12	12	inc.	15	13	3	inc.	1	1
	2.0	16+	16+	6	17	19+	3	inc.	15	0
U-12927	0.5	17+*	7	11*	11*	3	3	3	0	0
	1.0	17+	17+	17+*	17	inc.	3	inc.	inc.	0
malathion	1.0	13*	inc.	0	13	7	0	13	7	0
Sevin(50)	1.0	-	-	-	11	15+	15+*	1	0	0
	2.0	-	-	-	11	17+	17+*	0	0	0

Plus sign indicates weather terminated tests.

* - mortality fell below 90 percent during test period.

inc. - inconclusive results due to fluctuations in mortality.

Sevin and dicapthon were ineffective against A. aegypti while OMS-122 showed little residual activity against C. p. quinquefasciatus.

Effectiveness of Deposits of Sevin, DDT, Bayer 39007, and Bayer 37344 against Anopheles albimanus in Haiti.

Studies were made in 1962 and 1963 in the rural section of Source Matelas to determine the residual activity of three carbamate compounds and DDT when applied to the interior surfaces of occupied houses. The houses are usually 8-10 feet wide and 12-18 feet long with mud walls and thatched roofs. Doors and shutters are wooden. The walls are made by plastering native clay over a frame of wood and whitewashing when dry. Most houses have from 3 to 6 doors and a similar number of windows.

All formulations were from wettable powders and treatment made by spray brigades from the Service National d'Eradication de la Malaria using cylindrical hand-compression sprayers.

To evaluate the treatments, the plastic cone method in which adult female mosquitoes are confined on the treated surfaces for 1 hour was used. Ten females were placed in each cone, and single cones placed on each of three surfaces (wood, thatch, whitewashed mud) in five to seven houses at weekly or biweekly intervals. After exposure the mosquitoes were removed to clean cages, a sugar-water pad placed on the cage and held for 24-hour mortality determinations. An average mortality of 70 percent on a surface residue was considered satisfactory.

To obtain a broad coverage of the surfaces in each house and to minimize the effect of possible variations in the amount of toxicant present thereon, each successive test was made on a different site. All test specimens were 3- to 4-day-old females obtained from a laboratory colony of A. albimanus.

Table 7 gives average mortalities obtained on the whitewashed mud, thatch, and wood surfaces treated with Sevin and Bayer 39007. Sevin at 1 g./sq. m. gave 100 percent mortality at week 3 but had dropped to 78 percent by week 5 due to the low kill on mud. The kills on mud were unsatisfactory after week 3. On thatch, kills were above 70 percent for the 15 weeks tested with the exception of week 11 when it was only 61 percent. Results on wood were satisfactory except for weeks 11 and 14. Sevin at 2 g./sq. m. was highly satisfactory for the duration of the test. Bayer compound 39007 at 1 g./sq. m. gave satisfactory average kills for 3 weeks and at 2 g./sq. m. for 4 weeks. Kills above 70 percent were obtained on wood for 13 weeks with Bayer 39007 at 2 g./sq. m. Mortalities were the poorest on whitewashed mud. As the A. albimanus used were slightly resistant to DDT, the results obtained against DDT were considered invalid for comparison with those of the experimental compounds.

Table 8 gives data on the 1963 treatments with Sevin, Bayer compound 37344, and DDT. The three formulations of Sevin at 2 g./sq. m. gave average mortalities of 90 to 100 percent for 21 weeks. Mortalities were usually slightly lower on whitewashed mud than on wood or thatch but were 79 percent or above through week 21 with the three formulations. No kills below 84 percent occurred on wood or thatch until week 24.

Table 7

Average percent mortality of female A. albimanus after 60-minute exposure to deposits of Sevin and Bayer 39007 on whitewashed mud, thatch, and wood.

Residue Age, Weeks	Sevin 50W		Bayer 39007	
	1 g./sq. m.	2 g./sq. m.	1 g./sq. m.	2 g./sq. m.
2-3 ^{1/}	100	100	90	99
4-5	78	99	55	72
6-7	-	100	45	58
8	-	94	-	59
9	66	94	-	-
10	71	97	-	55
11	47	95	-	59
12	72	83	-	38
13	63	96	-	69
14	50	95	-	37
15	89	94	-	72

^{1/} Test at 1 and 2 g./sq. m. made on alternate weeks.

Table 8

Average percent mortality of female A. albimanus after 60-minute exposure to deposits of Sevin, Bayer 37344 and DDT on whitewashed mud, thatch and wood.

Residue Age, Weeks	Sevin, 2 g./m. ²			Bayer 37344		DDT	
	50W	80W	85W	1 g./m. ²	2 g./m. ²	1 g./m. ²	2 g./m. ²
5	100	100	-	67	92(wk 6)	95	100
7	100	100	96	97	99	-	100
9	100	100	95	96(wk 10)	100(wk 10)	99*(wk 10)	96(wk 10)
11	100	99*	100*	-	-	100*	-
13	98	100	95	99	99*	95	100
14	99	98	99	100	100*	91	99
15	95	100	-	65	98	-	-
16	97	99	100	91	91	98	97
17	-	97	90	84	40	22	99
18	90	97	95	91	95	71	95
19	96	99	96	91	78	70	100
20	100	100	100	87	82	95	100
21	99	100	97*	80	63	78	92
22	93	79	80	52	60	48	77
23	80	76	95	71	49	33	73
24	74	55	69	63	75	40	28
25	53	87	73	18	69	38	45
26	87	51	50	48	39	-	40

* Control mortality greater than 20 percent.

Bayer compound 37344 at 1 g./sq. m. gave an average mortality of 67 percent at the initial test at week 5. However, after this time the average mortalities ranged from 80 to 100 percent through week 21 with the exception of week 15. Residues on whitewashed mud gave unsatisfactory results at weeks 5, 15, 17, and 20. The treatment at 2 g./sq. m. gave similar average results to those obtained with 1 g./sq. m., on whitewashed mud unsatisfactory kills after week 18. Both dosages gave kills above 70 percent on thatch for 24 weeks and on wood for 21 weeks except for week 17 with the 2 g./sq. m. dosage.

Mortalities were satisfactory with DDT at 1 g./sq. m. for 21 weeks except at week 17. Mortalities on whitewashed mud usually were slightly lower than those on wood and thatch but were satisfactory through week 16. At 2 g./sq. m. DDT gave average mortalities of 77 to 100 percent for 22 weeks. Results on whitewashed mud were equal to those on thatch and wood through week 21.

Effects of Mutagenic Compounds upon *Culex pipiens quinquefasciatus*.

A. Using citrated whole beef blood containing 10 percent sucrose (technique described in 1963 report) the basic information relative to laboratory behavior of *Culex* has been completed.

The first egg rafts are normally produced after the females have been offered the sucrose-blood for 4 days. The average number of eggs per raft is about 110 and of these, 95 percent or more hatch. The duration of each instar of larval development is the same as that of the original colony fed

on a rabbit and the size, vigor and life span of the adults has not been altered. The normal adult life span on sucrose-blood is 60 days for females and 14 days for males. Males do not live as well on sucrose-blood alone; therefore the males in all chemosterilant tests are fed honey-water for 4 days prior to being placed with the females.

The eggs produced normally hatch within 24 hours after oviposition (occasionally 48 hours).

The caging of 25 normal males with 25 normal females in 1-gallon ice cream cartons results in approximately 90 percent successful mating and individually caged females oviposit an average of three times during their life span. Those egg rafts produced after the females are 30 days of age are very small (10-20) but almost all are viable.

B. Four-day-old adult males have been exposed to residues of tepa, metepa and apholate using the technique described in the 1963 report.

1. Four-day-old males were exposed for 4 hours to 12-hour-old residues of 500 mg. of tepa/sq. ft. on plywood. These males were then placed with virgin females (25/25) in gallon cartons. Eggs were collected daily, counted and hatched. From the data in the table below, little or no sterility was produced using this technique.

Table 9

Egg hatch from females mated with males exposed to tepa residues 12 hours old.

Test	Number Eggs	Number Larvae	Percent Hatch
A	1792	1553	87
B	1204	1089	90
C	1513	1076	71
D	803	618	77
Total	5312	4336	Mean 82
Control	1660	1528	92

2. Identical tests using 500 mg. of apholate/sq. ft. 1 week old were made. Of 3,887 eggs produced by females mated with treated males, 3,821 or 98 percent hatched.

3. Tests using 500 mg. of metepa/sq. ft. 14 hours old and 7 days old (1963) resulted in male sterility. The data in the table below indicate that almost all of the males exposed for 4 hours to residues 14 hours old were affected.

Table 10

Egg hatch from females mated with males exposed to metepa residues 14 hours old.

Test	Number Eggs	Number Larvae	Percent Hatch
A	2197	270	12
B	1391	62	4
C	1893	114	6
D	1676	58	3
Total	7157	504	Mean 7

Initially, it was thought that a male, after being treated with metepa, would either be totally sterile or completely normal. Thus any given egg raft produced by a female mated to a treated male would consist of all or no viable eggs. This is not the case. By placing each raft in a separate labeled container and keeping a daily record of the hatch, (and if larval development is being investigated, a record of the complete life cycle of all individuals from each raft), it was found that 18/68 rafts (26 percent) were completely sterile (zero hatch); 41/68 (60 percent) had 5 percent hatch or less; 64/68 (94 percent) had 25 percent hatch or less and only one egg raft had more than 30 percent hatch (37 percent). Females when mated to 4-day-old males exposed to the metepa residues 11 days old produced 6,245 eggs from which 5,226 larvae (84 percent) hatched.

From these data concerning residues of tepa, metepa and apholate, it appears that residues of these compounds on plywood are not satisfactory in the sterilization of Culex pipiens quinquefasciatus. Since insecticidal residues on plywood normally are superior in effectiveness to these on surfaces encountered in the field, the possible use of the three agents in practical sterilization and control of Culex pipiens quinquefasciatus appears unlikely.

C. As these compounds tend to polymerize rapidly upon exposure to air, the application of residues to plywood surfaces results in unknown and varying concentrations of chemosterilant actually available for tarsal absorption by the mosquito, to provide a method of direct application of the chemosterilants to the body surfaces of the mosquitoes to permit valid comparisons between the compounds under test. A modified Hoskins-Caldwell spray chamber was used. Two ml. of an 85:15 mixture of 95 percent ethanol:glycerol solution containing the chemosterilant was sprayed as a fine mist into an upper chamber. A small exhaust fan was used to gently move the mist from the upper chamber through the screened ends of a 1-pint carton containing 25 adult males. As the mist passes through the carton, particles impinge upon the body of the adults. The treated specimens are removed from the exposure carton and placed in 1-gallon cartons for mating.

1. Concentrations of 0.5, 1.0 and 2.0 percent apholate were used. These concentrations did not result in any abnormal mortality and the

behavior of the specimens appeared normal. All concentrations resulted in mean percent hatch below 20 percent when treated males were placed with virgin females. The data (Table 11) show that concentrations of 2 percent or higher are necessary for obtaining satisfactory sterility.

Analysis of hatch from individual egg rafts (Table 12) shows that while concentrations of 0.5 and 1.0 percent did result in hatch, none of the rafts had normal hatch and the majority of the rafts had a low percentage hatch. The 2 percent concentration resulted in almost all egg rafts having less than 5 percent hatch and of these 91 percent were completely sterile and had no hatch.

Table 11
Apholate mist.

Test	Percent Hatch Using		
	Mist Concentrations of:		
	0.5%	1.0%	2.0%
A	19%	32%	3.0%
B	13%	8%	0.5%
C	11%	5%	0.6%
D	33%	10%	3.5%
Mean	19%	14%	1.9%
Control	94%	94%	92%

Table 12

Apholate mist. Percent of egg
rafts with hatch of five ranges.

Percent Conc.	Percent Hatch Groups				
	0-5	6-25	26-50	51-75	76-100
0.5	33	0	67	0	0
0.5	50	29	7	14	0
0.5	50	33	17	0	0
0.5	14	24	43	19	0
1.0	27	20	13	40	0
1.0	83	0	17	0	0
1.0	63	37	0	0	0
1.0	73	14	0	13	0
2.0	80	20	0	0	0
2.0	98	2	0	0	0
2.0	97	3	0	0	0
2.0	90	8	0	2	0
Control	0	0	0	6	94

2. Similar tests using tepa mists of 0.5, 1.0, 2.0, 3.0, 4.0 and 6.0 percent concentrations were made. The data in Table 13 summarize the results of these tests. While 6 percent tepa mist was necessary to obtain satisfactory sterilization, 4 percent tepa mist caused 83 percent of the rafts to have hatch of less than 5 percent.

Table 13

Tepa mist.

Percent Conc.	Number Eggs	Number Larvae	Percent Hatch
0.5	9085	1485	16.0
1.0	8852	835	9.0
2.0	8078	949	11.7
3.0	8886	1114	12.5
4.0	7835	319	4.1
6.0	2327	2	0.08
Control	9709	8757	90.1

3. Tests using 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 6.0 percent metepa mists showed (Table 14) that while all concentrations markedly reduced the percent of viable eggs obtained, a concentration of 8 percent was necessary in order to obtain a satisfactory degree of sterility. Probably more significant are the data in Table 15, particularly in the group of egg rafts having zero to 5 percent hatch. Note that as the concentration of material used is increased, the percent of rafts having little or no hatch increases from none to almost all. Actually in the tests run using 8% material, only 3 rafts had percentage hatch above 6 percent. While this test was not set up to identify which female produced a given raft, analysis of data showed that two of the

Table 14
Metepa mist.

Percent Conc.	Number Eggs	Number Larvae	Percent Hatch
0.5	1711	1086	63
1.0	2742	890	32
1.5	2534	817	32
2.0	2977	754	25
3.0	4002	639	16
4.0	5211	433	8
8.0	2941	33	1
Control	6047	5570	92

Table 15
Metepa mist. Percent of egg rafts having hatch of five ranges.

Percent Conc.	Percent Hatch Group:				
	0-5	6-25	26-50	51-75	76-100
0.5	0	11	22	28	39
1.0	7	34	31	27	0
1.5	18	28	31	20	3
2.0	24	37	22	17	0
3.0	46	32	14	7	1
4.0	70	18	6	5	0
8.0	94	6	0	0	0
Control	1	0	0	2	97

rafts came from one test carton and, based upon our knowledge of oviposition, probably were produced by one female.

4. In Table 16 the effectiveness of the three compounds are compared. In all of these tests, the amount of material atomized, the number of specimens per replicate, the period of exposure and the velocity of mist particles was constant; only the concentration of active ingredient was varied.

These data show apholate to be the most effective of the 3 compounds for the sterilization of males when mist trucks are used. However each agent was capable of decreasing viable eggs to minimum levels.

Table 16

Comparison of effectiveness of apholate, tepa and metepa mists for sterilization of male Culex pipiens quinquefasciatus.

Percent Conc.	Percent Hatch		
	Apholate	Tepa	Metepa
0.5	19.0	16.0	63
1.0	14.0	9.0	32
1.5	-	-	32
2.0	1.9	11.7	25
3.0	-	12.5	16
4.0	-	4.1	8
6.0	-	0.08	-
8.0	-	-	1
Control	94.0	90.1	92

EQUIPMENT AND PACKAGE INVESTIGATIONS

Sprayer Specifications

The compression sprayer specifications were further revised, as required by new developments. The latest AID specification number is 7705-4 dated July 1, 1964.

Nozzle Tip Erosion Studies

A group of 3002 HSS nozzle tips were studied for compliance with the current AID specification. A group of 3002 and 3004 ceramic nozzle tips were tested for AID specification compliance and were observed to respond very favorably to erosion and pattern studies.

Sprayer Sub-assembly Studies

A new design sprayer cut-off valve was tested and passed all of the required performance tests. Two items in the valve design are now under study before final recommendations are made.

Field Equipment Studies

A group of hand pressure sprayers were returned from Saigon for laboratory evaluation of objections in design, and methods of correcting them. These items of correction were then passed on to the manufacturer.

Disc Flow Regulator Field Trial.

An intensive field test of the new material for disc flow regulator construction known as Viton-A has been completed. Prior laboratory studies

and initial evaluation of the field test data have shown that the Viton-A has much better fatigue characteristics than the material originally used in the flow regulator and we believe its use will permit full recommendation of the regulator for operational use. As soon as all data from the field test is fully analyzed a detailed report will be issued.

Insecticide Shipping Containers

Additional tests were conducted on various styles of fiber drums and corrugated boxes during the year for the purpose of revising drum and box specifications and to determine if possible if one type of container was better suited or provided greater protection for the shipment of DDT powders.

A. Description of Containers

Fiber drums were furnished by the Continental Can Co. having the following nomenclatures and description:

1. Lever locking band style MV2305-4-Y19 having a capacity of 23 gallons, cubed as 4.5 cu. ft. and holding 100 lbs. net.
2. Five way flip clip closure lugs SV2305-4x having a capacity of 23 gallons, cubed as 4.3 cu. ft. and holding 100 lbs. net.
3. Five way turnlocks SV2305-4x having a capacity of 23 gallons, cubed as 4.3 cu. ft. and holding 100 lbs. net.
4. All fiber style AV2305-4x: Apak with a slip cover having a

capacity of 23 gallons, cubed as 4.14 cu. ft. and holding 100 lbs. net. A fiber drum furnished by Grief Bros. having the following description::

1. Ro-con round corner drum with a slip cover having a capacity of 17.69 gallons, cubed as $15 \times 15 \times 20 = 2.6$ cu. ft. and holding 75 lbs. net.

Corrugated boxes were furnished by the Connelly Container Co. and the Container Corp. of America with descriptions respectively as follows:

1. An RSC box made of V3c board with an inner liner and pads of triple wall corrugated board having a capacity of 17.75 gallons, cubed as $13 \times 13 \times 28 = 2.74$ cu. ft. and holding 75 lbs. net.

2. An RSC box made of V3c board with an inner liner and pads of high strength double wall corrugated board having a capacity of 17.75 gallons, cubed as $14 \times 16 \times 21 = 2.72$ cu. ft. and holding 75 lbs. net.

B. Tests Conducted

All containers were subjected to the following series of tests:

1. Static load test, top to bottom, dry and wet, container empty.
2. Static load test, side to side where applicable, dry and wet, container empty.
3. Corner drop test, dry and wet, container loaded.
4. Horizontal drop test, dry and wet, container loaded.

Other tests were conducted as the need arose or to investigate some particular weak point or container defect. In addition, dynamic loading tests were conducted and observed at the Continental Can Co. laboratories in Chicago, Ill. and at the Fernandina, Fla. plant of the Container Corp. of America.

C. Results

It was determined that the five way flip closure and the five way turnlock style drums were not suitable for shipping DDT powder, however the remaining drums and the corrugated boxes appeared to provide reasonable protection. With the exception of the Ro-con drum the corrugated boxes offered additional savings in space and possibly freight charges in addition to being easier to handle. Further savings would be possible by utilizing a box with a Hi-strength double wall liner, however, since cost figures were not available the exact amount could not be determined. The fiber drums lost much of their strength when wet, however this could be overcome by the application of a coating to the exterior of the drum to make them water resistant.

D. Summary

Based on the results of test conducted revised specifications for fiber drums and corrugated boxes for the shipping of 75% water wettable DDT powders were written and submitted to AID for their consideration. Tests are being continued on a wax impregnated corrugated box which could result in a further reduction to box cube and possibly a reduction in box cost also.