PRELIMINARY STUDIES OF MOSQUITO RESISTANCE TO INSECTICIDES IN THE PHILIPPINES[1]

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INTRODUCTION. To the author's knowledge, no studies have been made in the Philippines to establish the susceptibility levels of various populations of mosquitoes to insecticides. The present studies were initiated to ascertain these levels in order to detect the emergence of resistant strains with increased insecticidal usage. A second purpose of this study was to determine if resistance had developed in certain mosquito populations.

Insecticides have been used in areas adjacent to the base, but virtually no data were available. The malaria eradication program in this area is in the surveillance stage. An active spraying program with residual treatments of 5 percent DDT was instituted in 1952 and ended about June 1960. Mosquito control in the area has been virtually non-existent. Based on personal observations, essentially no insecticides were used agriculturally in conjunction with the rice and sugarcane crops in the areas adjacent to Clark Air Base. Although most of the American housing developments are adjacent to flooded rice fields, control measures have been limited to occasional fogging by Clark Air Base personnel. On the base proper, which consists of about 12,000 acres, the mosquito fauna has not been subjected to the selection pressures common in many areas of the United States. Many parts of the base are relatively inaccessible and, for this reason alone are seldom, if ever, treated.

The insecticides DDT, lindane, dieldrin, and malathion have been the only insecticides used since 1959 for mosquito control. Data for periods prior to 1959 were not available. Table 1 gives the approximate amounts of insecticides used in larviciding operations since 1959. Unlike current practice in many areas of the United States, chlorinated hydrocarbon insecticides have been widely used for larviciding. The diluent used was either diesel oil or kerosene. DDT was almost solely used in 1961 but has not been used so far in 1965. Dieldrin was first used in 1964, but appears to have replaced the other insecticides in 1965. There was considerable use of insecticides on the base for control of insect pests of ornamental trees and bushes. DDT, dieldrin, malathion, and some diazinon were used. However, after talking to base personnel, it was evident that there was no set plan for insecticide employment. Rather, there was a random pattern dictated by the supply situation. Fogging operations have been very spasmodic and infrequent. The

TABLE 1.—Insecticide usage on Clark Air Base for mosquito larviciding from 1959 to May 1965 (gallons of finished spray).

Year	Insecticides				Totals
	DDT	Dieldrin	Lindane	Malathion	
1959	7,920	0	0	0	7,920
1960	5,496	0	276[a]	0	5,772
1961	1,716	0	0	2,669	4,385
1962	0	0	2,920	2,472	5,392
1963	5,550	0	3,225	1,380	10,155
1964	0	3,255	1,946	1,660	6,861
1965	0	2,790	0	0	2,790
Totals	20,682	6,045	8,367	8,181	43,275

[a] Available data gave 552 gal. of DDT and lindane used. For the purposes of this table, this was equally divided to approximate usage per insecticide.

[1] The opinions expressed herein are those of the author only and do not necessarily reflect those of the United States Air Force. The present address of the author is the Department of Entomology, Colorado State University, Fort Collins, Colorado.

housing areas on base may be fogged once a month if at all. Rice fields on the base proper were cultivated by Negritos who have not been observed to use insecticides.

METHOD. All mosquitoes used were collected from areas on or adjacent to Clark Air Base. The base is located about 60 miles north of Manila on the Island of Luzon in the proximity of Angeles City. Mosquitoes studied were *Aedes* (*Stegomyia*) *aegypti* (Linnaeus), *Aedes* (*Stegomyia*) *albopictus* (Skuse), *Aedes* (*Aedimorphus*) *vexans* ssp. *nocturnus* (Theobald), *Culex* (*Culex*) *pipiens* ssp. *quinquefasciatus* Say, and *Culex* (*Culex*) *fuscocephalus* Theobald. The above nomenclature is that of Stone, Knight, and Starcke (1959). These species are hereafter referred to without their subspecific names. All species except *A. vexans* were found breeding in artificial containers. *C. fuscocephalus* was not commonly found in this habitat.

All tests were conducted by using the standard WHO Test Kits obtained from WHO-Geneva. The methods used for the determination of resistance in larvae and adults were those published by WHO (Anonymous, 1963a and b). The larval kits contained standard, ethanol serial dilutions of the insecticides DDT, BHC, dieldrin and malathion. All larval tests were conducted with a 24-hour exposure period in 1-pint mason jars using fourth instar larvae. All tests were replicated three to five times, dependent upon the availability of larvae. The insecticide solutions were kept tightly sealed, except for the brief period involving pipetting, to prevent concentration of the solution by evaporation. The adult kits consisted of papers commercially impregnated with insecticide—Risella oil solution; plastic, cylindrical, exposure and holding tubes with screen tops; and associated transferring devices. The impregnated papers were kept tightly sealed in plastic boxes to prevent evaporation of the oil. Test papers were used only for a period of one week before they were replaced. The screening in the tops of the tubes was replaced with 36-mesh plastic screening to preclude adult escape. The adult tests were conducted, using blooded or gravid females exposed for 60, 120, or 240 minutes at varying concentrations, followed by a 24-hour holding period. All tests were run in duplicate.

Colonies were started and maintained for the following species and strains: *A. aegypti* from Clark Air Base and *C. pipiens* from Clark Air Base (strains 1 and 2 from Negrito Village and 3 from the Dau Area) and from Balibago, Angeles City (Figure 1). Attempts to colonize *A. albopictus* and *C. fuscocephalus* were unsuccessful. The established colonies were successfully maintained by offering the females albino mice for blood meals and the males Karo corn syrup (about 5 percent aqueous solution) in cotton pads. Oviposition took place, for *Aedes,* in evaporation dishes lined with paper toweling and for *Culex,* on open water in small beakers. The larvae were fed ground, high-protein, mouse chow and brewer's yeast. The larvae were reared in 10″ by 15″ pans containing distilled water to a depth of about 1½″. The formation of surface scum was prevented by bubbling air under the water using an aquarium aerator.

The colonies were continually replenished by field collections of both larvae and adults, thus the colonies, except one, were not static. *C. pipiens* strain 1 was static from January 1965. Strain 2 was a continuation of strain 1, the two being separated by a period when all the collecting stations were sprayed with either dieldrin or lindane.

Without temperature controls, it was not possible to insure that the temperature did not exceed 30°C. For 105 larval tests, the average control mortality was 3.2 percent. Thus, the low control mortalities indicated that this lack of temperature control was not deleterious to the larvae. The water temperature was generally cooler than the surrounding air. The average control mortality for 36 adult tests was 5.5 percent. Weather data were maintained for each test date. The monthly

averages are listed in Table 2 to illustrate temperature and humidity variations over the period of testing.

RESULTS AND DISCUSSION

The larval tests for *A. albopictus* were conducted in September and October 1963. Sufficient numbers were not found thereafter to conduct adult tests. The remaining tests were conducted from September 1964 to June 1965. Since many of the test series were completed in a short time span (a month or two, or less), the response of the mosquitoes to seasonal variation was not determined.

Table 3 lists the estimated larval LD_{50} and LD_{90} values obtained by plotting the average mortality data on log-dosage-probit

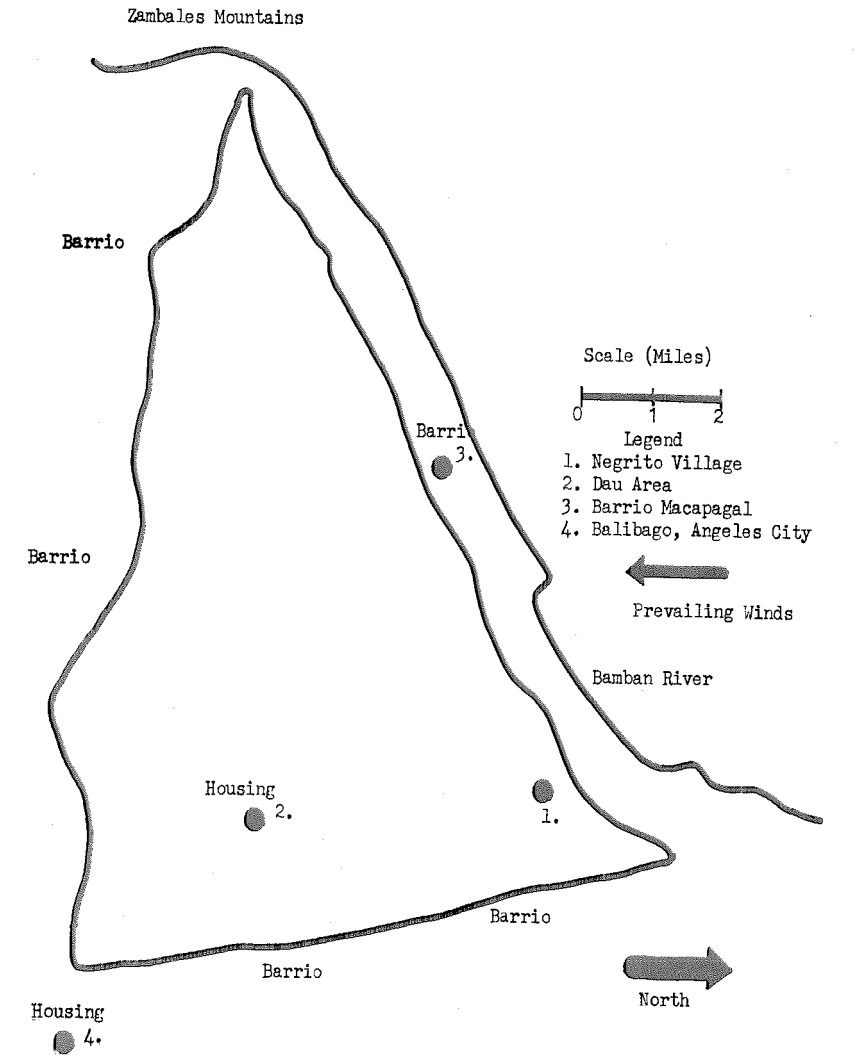

FIG. 1.—Diagram of Clark Air Base showing the approximate location of mosquito sources.

TABLE 2.—Average monthly weather data for the testing period.

Date	Temperature (°F)		Relative humidity (%)	
	Maximum	Minimum	Maximum	Minimum
Sept. 1963	86.0	74.0	96.0	70.0
Oct. 1963	88.0	74.0	87.0	56.0
Nov. 1964	82.7	72.3	87.4	65.5
Dec. 1964	82.4	69.8	80.7	56.2
Feb. 1965	87.1	68.9	75.8	41.5
Mar. 1965	88.6	71.1	77.8	42.2
Apr. 1965	92.6	74.6	73.3	40.6
May 1965	89.3	75.1	79.9	53.5
June 1965	87.0	74.0	85.7	65.0
Average	87.1	72.6	82.6	54.5

paper and reading the dosage level at the 50 and 90 percent intercepts. An approximation of the relative slopes of the regression lines can be obtained by using the formula $\frac{1}{LD90-LD50}$. An LD50 greater than 0.1 ppm for *Culex* and 1.0 ppm for *Aedes* is indicative of resistance in larvae. Since no LD50 exceeded these respective limits, no resistance was indicated for the larval tests. However, strain and species responses for each insecticide varied based on the LD50 value. Strain 1 of *C. pipiens* was the most susceptible to BHC and dieldrin, while the other strains of *C. pipiens* were similar in response. *A. aegypti* was considerably more tolerant of malathion than the four strains of *C. pipiens*. *A. albopictus* was more tolerant to DDT than the other species and strains. *C. fuscocephalus*, most commonly found in carabao wallows, was very highly susceptible to all the insecticides tested.

The estimated LD50 values for DDT and dieldrin with the various adult mosquito populations are presented in Table 4. These values were obtained in the same manner as previously discussed. It is evident that the various strains of *C. pipiens* are quite tolerant to DDT and dieldrin. Strain 1 was the most susceptible strain to both insecticides. Strain 2 and the strains from Balibago and the Dau area were similar in being very tolerant to both DDT and dieldrin. Exposures of 240 minutes were required to obtain a 50 percent or more mortality using 4 percent test papers.

A. aegypti and *A. vexans* adults were much more susceptible to DDT and dieldrin than were the *C. pipiens* populations. The susceptibilities of these *Aedes* species were similar with DDT, but *A.*

TABLE 3.—Estimated LD50 and LD90 values for larvae.

Species and location	LD50 and LD90 in PPM							
	BHC		DDT		Dieldrin		Malathion	
	LD50	LD90	LD50	LD90	LD50	LD90	LD50	LD90
A. albopictus Clark Air Base	0.035	0.014	0.095	0.21	0.0054	0.045	—[a]	—[a]
A. aegypti Clark Air Base	0.035	0.073	0.023	0.041	0.0040	0.0077	0.090	0.14
C. pipiens Strain 1	0.021	0.12	0.026	0.070	0.0083	0.13	0.021	0.030
C. pipiens Strain 2	0.072	—[b]	0.025	0.15	0.033	0.17	0.018	0.029
C. pipiens Dau Area	0.068	0.14	0.031	0.084	0.050	0.10	0.032	0.082
C. pipiens Balibago	0.060	—[b]	0.049	0.16	0.063	0.33	0.024	0.031
C. fuscocephalus	0.0076	0.011	0.004[c]	0.004[c]	0.0008	0.0015	—[d]	—[d]

[a] Tests were not run.
[b] Tests did not exceed 90% mortality.
[c] Tests were not less than 90% mortality.
[d] Tests were not less than 50% mortality.

TABLE 4.—Estimated LD50 values in percent concentration of insecticide for adult resistance tests.

Species and Location	Exposure Time (Minutes)	LD50 DDT	Exposure Time (Minutes)	LD50 Dieldrin
C. pipiens Strain 1	120	3.8	120	1.6
C. pipiens Strain 2	240	2.2	240	1.2
C. pipiens Dau Area	240	2.0	240	2.0
C. pipiens Balibago	240	2.8	240	1.6
A. aegypti Clark Air Base	60	1.2	60	0.28
A. vexans Macapagal	60	0.8	60	0.13

vexans was considerably more susceptible to dieldrin than was *A. aegypti.*

The regression lines of the original data for dieldrin of the Dau area and Balibago populations of *C. pipiens* were not linear. Rather they exhibited a pronounced downward bending at increased concentrations of dieldrin, which is indicative of partial resistance in the population (Anonymous 1963c).

The indication of partial resistance for the two above-mentioned adult *C. pipiens* strains may be explained as follows. These mosquito strains showed partial resistance to dieldrin and not to DDT although all showed a high tolerance level. Dieldrin has not been known to have been in use prior to 1964 on Clark Air Base, and not at all in off-base areas. The one thing they had in common was that they originated in the midst of American housing areas. So, this resistance could possibly be due to the use of commercial aerosol insecticide preparations containing dieldrin, available to American personnel. The high tolerance of the *C. pipiens* strains to DDT was probably due to the use of DDT in the malaria eradication program for an eight-year period.

SUMMARY AND CONCLUSIONS

Resistance tests, employing WHO Test Kits, were conducted on *A. aegypti, A. albopictus, A. vexans,* and four strains of *C. pipiens.* Both larval and adult tests were conducted on each population with the exception of *A. albopictus.*

No evidence of resistance was found for the larvae, although responses were quite varied between populations. The adult tests showed that three of four *C. pipiens* strains were highly tolerant to DDT and dieldrin. The exception was *C. pipiens* strain 1. Partial resistance was indicated for the adult *C. pipiens* strains from the Dau area and Balibago. The results of adult tests of *A. aegypti* and *A. vexans* showed that they were both susceptible to DDT and dieldrin. *A. vexans* was very susceptible to dieldrin.

ACKNOWLEDGMENTS

The author is indebted to Major F. H. Dowell for his critical appraisal and editing of this paper, and for his and Colonel M. Y. Kremers' continuous encouragement. Without them, this study would not have been accomplished.

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