CONCLUSIONS

A single application of WE-48, at a dosage of 0.009 ppm, gave 3 weeks of effective mosquito control, while a single application of M-3570, at a dosage of 2.5 ppm. gave at least 21 weeks of effective control. The formulations had significant quantitative effects only on larval and adult populations. However, the effectiveness of the formulations could not be adequately evaluated by monitoring only naturally-occurring larval and adult populations, since the overall natural population did not remain at a high level during the entire test period. The monitoring of insecticide residue levels, or the use of mosquito larvae as bioassay organisms, provided a consistent measure of larvicidal effectiveness.

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MALATHION RESISTANCE IN *AEDES AEGYPTI* FROM PRESSURE ON ADULTS[1]

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Detailed information on the development of resistance to malathion in *Aedes aegypti* larvae has been obtained by malathion pressure on larvae (Brown and Pal, 1971), but similar studies with adults, of equal or more importance, have not been made. Brown and Abedi (1960) found that malathion pressure on larvae causes only a small increase in adult resistance.

During the *Aedes aegypti* eradication campaign of 1969 in Puerto Rico, strains resistant to malathion occurred, and the adults of one such strain from the town of Arecibo were resistant by a factor of about five when compared with the most susceptible Puerto Rican strains (Fox and Bayona, 1972). The purpose of the present experiments was to find out (a) whether resistance in the Arecibo strain would increase by pressure on the adults in the laboratory for a dozen generations and (b) whether the Arecibo strain allowed to breed freely in the laboratory over a long period of time away from malathion would lose its resistance.

MATERIALS AND METHODS. Using the World Health Organization standard kit, I tested caged adults of the Arecibo, Puerto Rico strain and saved the survivors to provide eggs for breeding the subsequent generations. For the early generations it was sometimes necessary to save survivors of 0.4 percent and 0.8 percent malathion to get enough eggs, but in later generations survivors of 1.6 percent and 3.2 percent were sufficiently numerous for exclusive use. After pressure on the parents and pressure on each of 12 filial generations, I made comparative tests using the same set of impregnated papers on (a) a malathion suscepti-

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ble laboratory strain, the Camp Detrick strain, colonized in our laboratory since 1962 from eggs received through the courtesy of Dr. H. F. Schoof; (b) the Arecibo Parents strain which was originally collected by Dr. Jaime Moya on July 28, 1969 at the Old Cemetery, Road No. 2, Arecibo, Puerto Rico, and has been breeding freely in our laboratory ever since; and (c) the Arecibo F_{12} generation which finally resulted by pressure on adults originating from the Arecibo Parents strain.

To make a test, I exposed 15 to 20 adult females to a concentration for one hour, read the mortality after a 24-hour recovery period, and calculated the final mortality percent from the average of four replicates. I also made tests based on increasing times rather than concentrations by exposing 15 to 20 females to 0.4 percent malathion for times in multiples of 60 minutes, making the readings after 24 hours, and basing the final mortality percent on four replicates. The experiments began in November, 1969 and ended about 2½ years later in May, 1972, and involved six sets of WHO impregnated papers used in turn.

RESULTS. The different sets of papers did not give uniform results, particularly at 0.4 percent malathion, and further, papers lost their effectiveness as they grew older. Table 1 gives the results of tests against the parents and the third to the twelfth filial generation of pressure at 0.8 percent, 1.6 percent, and 3.2 percent malathion and also indicates the approximate age of the papers. Results with 6.4 percent malathion when used, were as follows: F_6, 99 percent mortality; F_7, 98 percent; F_9 and F_{10}, 100 percent; and F_{12}, 97 percent. The data in Table 1 referring to F_{11} and F_{12} and all my data in Tables 2 and 3 derive from use of the same set of papers, Number 6, impregnated in February, 1972 and expired in June, 1972. Table 2 gives the results of tests involving exposure times with 0.4 percent malathion against the Camp Detrick, the Arecibo Parents, the Arecibo F_{11} and the Arecibo F_{12} strains, and includes for comparison published results of similar tests with the Kongolekan (Africa) strain. Table 3 gives the LC_{50}, LC_{90}, and LC_{95} values for the Camp Detrick, Arecibo Parents, and Arecibo F_{12} strains, and Figure 1 shows the dosage mortality relationships of these strains. Table 4 shows the resistance ratios obtained, in each instance, by dividing the LC_{50} of a resistant strain

Table 1.—Effect of malathion pressures on adult *Aedes aegypti* of the Arecibo, Puerto Rico strain.

Generation	Percent mortality at concentration (%) 0.8	1.6	3.2	Age of papers (months)
Parents	34	72	100	2–3
F_3	23	73	ND	1–2
F_4	10	52	91	3–4
F_5	10	33	91	1–2
F_6	10	18	66	3–4
F_7	20	38	83	2–3
F_8	32	46	95	2–3
F_9	22	38	78	3–4
F_{10}	8	29	77	2–3
F_{11}	6	31	83	1–2
F_{12}	3	14	57	2–3

ND=Not done.

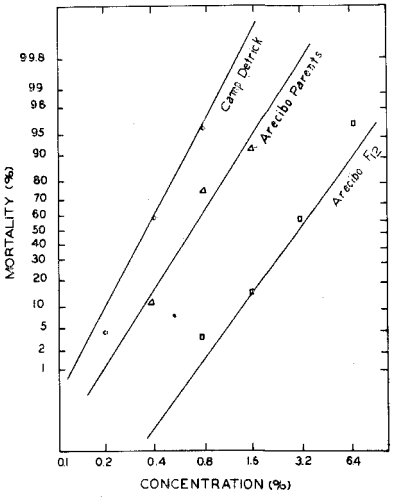

Fig. 1.—Dosage-mortality relationships of malathion and the adults of laboratory strains of *Aedes aegypti*.

by the LC_{50} of a less resistant or susceptible one.

DISCUSSION. Early in the 1963–1969 *Aedes aegypti* eradication campaign, Public Health Service authorities found that adults from southern United States and Puerto Rico succumbed 92–100 percent after one hour's exposure to 3.2 percent malathion in laboratory tests, and assumed from such kills the effectiveness of malathion in the field (Flynn and Schoof, 1965; 1967; Flynn, Schoof, *et al,* 1964). But Table 1 shows that after the fifth generation of pressure on adults 3.2 percent malathion no longer killed completely, and against the F_{12} generation, resulted in only 57 percent mortality.

World Health Organization experts recommend that workers try for lethal times rather than lethal concentrations, and Hamon and Sales (1970) obtained LT_{50} =153.3 minutes when they exposed the Kongolekan strain to 0.4 percent malathion. After exposure for 360 minutes, the Arecibo F_{12} strain resulted in less than 50 percent mortality (Table 2), and so it was impossible to obtain the LT_{50} value, or at least inconvenient, because of the long exposure period necessary. A serious drawback to obtaining LT values of malathion-resistant strains is that routine exposure periods of 6 or more hours may be impractical for some laboratories.

Ziv, Brown and Brown (1969) found that pressure for seven generations on the larvae of two strains already resistant in the field, Albina, Surinam and Montego Bay, Jamaica, increased the resistance of one slightly and decreased the resistance of the other. The Arecibo, Puerto Rico strain resistant by a factor of about five at the beginning of pressure on the adults continued to increase in resistance with each generation of pressure (to a factor of about seven at F_7) and by the twelfth generation of pressure reached a resistance factor of about 12 (Tables 1 and 4); nor were there any strong signs of reversion to susceptibility. These data indicate that adults have greater potential to become resistant than do larvae.

Adults, just as larvae (Matsumura and Brown, 1961) lose resistance when malathion pressure is relaxed. After 2½ years inbreeding in the laboratory away from malathion, the Arecibo strain decreased in LC_{50} value from 1.18 percent to 0.65 percent (Table 4). Although there is less than a two-fold difference in the LC_{50} values, the dosage-mortality lines of the Camp Detrick laboratory strain and the Arecibo Parents strain are not parallel indicating the latter strain's potential to become resistant (Figure 1).

Table 2.—Percent mortality of strains of *Aedes aegypti* after exposure to 0.4 percent malathion.

Exposure (minutes)	Kongolekan Upper Volta, West Africa *	Camp Detrick laboratory susceptible	Arecibo, Puerto Rico, parents	Arecibo, F_{11} pressure	Arecibo, F_{12} pressure
60	6	58	11	ND	ND
120	20	92	65	12	ND
180	75	100	78	26	ND
240	94	ND	91	49	ND
300	ND	ND	ND	51	23
360	ND	ND	ND	ND	43

ND=Not done.
* Data from Hamon and Sales (1970).

SUMMARY AND CONCLUSION. The present work shows that after 12 generations of continuous selective malathion pressure adult *Aedes aegypti* of the Arecibo, Puerto Rico strain became resistant by a factor of 12, and in laboratory tests 40 or 50 percent survived exposure to 3.2 percent malathion for 1 hour, or exposure to 0.4 percent malathion for 6 hours. However, caged adults inbreeding over a period of about 2½ years away from malathion lost most but not all of their resistance. The impli-

Table 3.—Toxicity of malathion to *Aedes aegypti* adult females as shown by LC values in percent and confidence limits in parentheses.

Strain	LC_{50}	LC_{90}	LC_{95}
Camp Detrick	0.35 (0.30–0.40)	0.64 (0.52–0.78)	0.75 (0.60– 0.94)
Arecibo parents	0.65 (0.55–0.77)	1.28 (1.00–1.64)	1.56 (1.16– 2.09)
Arecibo F_{12}	3.10 (2.56–3.75)	6.75 (5.09–8.94)	8.40 (6.09–11.59)

Table 4.—Adult *Aedes aegypti* resistance to malathion as shown by resistance ratios.

Strains	LC_{50} in percent	Resistance ratio
Arecibo parents/ Camp Detrick	0.65/0.35	1.86
Arecibo F_{12}/ Camp Detrick	3.10/0.35	8.86
Arecibo F_{12}/ Arecibo parents	3.10/0.65	4.77
Arecibo F_7/ Guayama *	1.80/0.25	7.20
Arecibo F_{12}/ Guayama *	3.10/0.25	12.40
Arecibo F_{12}/ Arecibo *	3.10/1.18	2.63
Arecibo */ Arecibo parents	1.18/0.65	1.82
Arecibo */ Guayama *	1.18/0.25	4.72

* LC_{50} value during the control campaign in Puerto Rico in 1969 (Fox and Bayona, 1972).

cations are that in future control projects in Puerto Rico, malathion must eradicate *Aedes aegypti* within 2 or 3 years or lose effectiveness because of adult resistance.

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