## EFFECTS OF SUBLETHAL DOSAGES OF ABATE® UPON ADULT FECUNDITY AND LONGEVITY OF AEDES AEGYPTI

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ABSTRACT. Sublethal concentrations of Abate<sup>®</sup> (temephos) were applied to  $F_2$  generation Aedes aegypti larvae, and fecundity and longevity were recorded in the emerged adults. Females exposed to Abate oviposited only in the first 2 gonotrophic cycles, meanwhile control females laid a few eggs after taking the third blood meal. Dosages of 0.009, 0.013 and 0.015 mg/liter of Abate decreased the mean egg production per gonotrophic cycle 37, 47 and 69%, respectively, in relation to the control. Females that were exposed as larvae to Abate lived longer than the control females.

Abate<sup>®</sup> (temephos) is the larvicide recommended for use worldwide to control Aedes aegypti (Linn.), but unfortunately resistance to Abate by this vector has been reported from several parts of Asia and the Caribbean area (OMS 1980, Georghiou et al. 1987). Therefore this insecticide must be used wisely because it may be difficult to find another product of such low toxicity to man.

Levinson (1975) suggested the term "insectistatics" for those agents that interfere with normal processes of growth and reproduction without necessarily leading to mortality. Moriarty (1969) reviewed the available literature on the sublethal effects of synthetic insecticides on reproductive potential, behavior, enzyme induction, heart rate and excretion in insects. It may be advisable to use Abate as an "insectistatic' for integrated dengue control programs if its toxicity at sublethal concentrations significantly affects the fecundity and longevity of Ae. aegypti. When this mosquito was treated after blood feeding with a sublethal dose of dieldrin  $(0.0075 \,\mu g/\text{liter})$ , the first gonotrophic cycle was normal: however, subsequent feeding behavior was reduced, which resulted in diminished egg production during the gonotrophic cycles that followed (Duncan 1963). Firstenberg and Sutherland (1981) reported that Altosid® and methoprene applied at  $LC_{50-90}$  and  $LC_{50}$ , respectively, decreased egg production, but egg production increased when Ae. aegypti was treated with DDT (0.1 ppm) (Havertz and Curtin 1967) and with DDT, dieldrin and malathion (Sutherland et al. 1967). The purpose of this research was to investigate the effects of sublethal concentrations of Abate upon the fecundity, longevity and number of gonotrophic cycles of female Ae. aegypti, the vector of dengue fever.

 $F_2$  generation Ae. aegypti larvae were treated with sublethal concentrations of Abate. The colony used originated from 3rd and 4th instar larvae collected in Sabinas Hidalgo, Nuevo Leon, Mexico, a town 90 km north of Monterrey. Larvae were reared in  $35 \times 25 \times 5$  cm plastic pans containing 1.0 liter of deionized water and were fed a mixture of dog food (Apican<sup>®</sup>), chicken food (Alpesur®) and cereal (Gerber®) in a 2:1:1 ratio. Food was added evenly over the water surface every 3 days. Insectary conditions were kept at 25°C, 70-80% RH and 13:11 LD. Adults were held in a  $60 \times 60 \times 60$  cm screened cage and fed on 5% honey solution from moistened cotton pads placed inside the cage. Females also fed to satiety on rabbit blood and oviposited 2 to 3 days later. They laid their eggs upon a paper strip that covered the water surface of a plastic cup. The cup was removed from the cage 48 h after oviposition began. To stimulate hatching, the paper strips were immersed in 40°C sterile water and Brewer's yeast was lightly sprinkled on the water surface. To collect eggs for the  $F_2$  generation studies, the wet paper strips were removed from the cups, dried and placed in egg storage boxes. When larvae were needed for the bioassays, they were reared as above. A standard kit from the World Health Organization (WHO) was used for bioassays with several concentrations of Abate. Several preliminary bioassays determined the 15-85% mortality range. Each bioassay included 5 concentrations of Abate and the control (only deionized water). The definitive concentrations were 0.010, 0.015, 0.020, 0.025 and 0.030 mg/ liter. Twenty 4th instar larvae were placed in a 150-ml plastic container with each toxic solution; 4 replicates were run for each concentration tested. Probit analysis was applied to the mortalities obtained for the different concentrations of the larvicide to calculate the regression equation from which the sublethal concentrations  $LC_{10}$ ,  $LC_{30}$  and  $LC_{50}$  were calculated. Two hundred 4th instar larvae were placed in 125 ml of each sublethal concentration, and the emergent males and females were maintained together for 3 days in a  $20 \times 20 \times 20$  cm screened cage to ensure mating. Twenty females were then randomly selected and confined individually in a 1-liter plastic container closed with a screen and with a lateral square hole into which a screened cylinder was glued. The mosquito fed once per gonotrophic cycle through this hole on a rabbit's shaved back (Fig. 1). A metallic cup (5 cm diam) with water and a paper strip was placed in the container for oviposition. The paper strip was removed and the eggs were counted daily. Three days without ovipositional activity was arbitrarily considered the end for each gonotrophic cycle, and fecundity and/or longevity were recorded for each female.

The straight line fitted by Probit analysis for the percentage mortality observed for each concentration of Abate tested had the equation Y = -2.3935 + 6.2637X. The LC<sub>50</sub> calculated was 0.015 mg/liter (Fig. 2). From this equation, X values were obtained as follows: X = a - Y/-b, where a and b are the coefficients of the equation and Y is the empirical probit for 10 and 30% of kill. Using this procedure, the  $LC_{10}$  and  $LC_{30}$ were 0.009 and 0.013 mg/liter, respectively. Females exposed to Abate oviposited only during the first 2 gonotrophic cycles. Control females, however, laid a few eggs after taking a third blood meal. The  $LC_{10}$ ,  $LC_{30}$  and  $LC_{50}$  of Abate decreased the mean egg production per gonotrophic cycle 37, 47 and 69%, respectively, compared with the control females (Table 1). Although a reduction in oviposition could indicate other adverse effects, including effects on matelocating, courtship and associated physiological events such as spermatogenesis and sperm motility (Haynes 1988), the most direct effect of Abate apparently was behavioral, on the females' propensity to feed, which may have resulted in less blood ingestion.

A linear regression analysis relating the number of eggs laid per female to the concentration of Abate used quantitatively evaluated the effect of Abate on fecundity (Fig. 3). The regression



Fig. 1. A. Screen closing the plastic container; B, plastic container with the mosquito inside; C, screened cylinder closing the lateral hole of the container; D, shaved back of the rabbit.



Fig. 2. Dose-mortality line for Abate applied to 4th instar Aedes aegypti larvae.



Fig. 3. Linear regression of mean number of eggs per female with doses of Abate applied to *Aedes aegypti* 4th instar larvae.

equation was Y = 79.28 - 3,332.42X with r = 0.99. The average fecundity decreased by 3.33 eggs for each  $\mu$ g/liter. This relationship is useful when considering control measures as it shows that emerged females will not oviposit when larvae are treated with 23  $\mu$ g/liter of Abate. Our recommendation is to use this concentration of Abate where resistance in *Ae. aegypti* is known to be significant, e.g., on the Tortola and Antigua islands in the Caribbean (Georghiou et al. 1987).

Exposure to Abate completely inhibited oviposition in the third gonotrophic cycle (Table 1). Females blood fed but did not oviposit. A few

	Mean no. of eggs laid per female			Total no. of eggs	Mean no. of eggs
Treatment	1	2	3	female	cycle*
Control	48	26	5	79	26a
$LC_{10}$	35	14	0	49	16b
$\mathbf{LC}_{30}$	27	15	0	42	14b
$LC_{50}$	18	6	0	24	8b

Table 1. Effect of sublethal concentrations of Abate applied to 4th instar larvae upon the fecundity of Aedes aegypti females (n = 20).

\* Means followed by different letters are significantly different (Duncan's multiple range test, P < 0.05).

of the control females, however, oviposited after taking a third blood meal. Females emerging from larvae exposed to Abate lived longer than the control females, because the average longevity in days ( $\pm$  SE) for females treated as larvae with sublethal doses of Abate was 22.3 $\pm$ 5.0, 21.3 $\pm$ 4.8, 24.9 $\pm$ 5.6 and 20.1 $\pm$ 4.5 for the LC<sub>10</sub>, LC<sub>30</sub>, LC<sub>50</sub> and the control, respectively. Similarly, Knutson (1955) found that *Drosophila melanogaster* Meig. that survived treatment with LC<sub>66.99</sub> of dieldrin lived longer than the untreated controls. Causes of this effect are unknown.

This research was supported by grant No. 88– 01–0175 from the Dirección General de Investigación Cientifica y Superación Academica of Secretaria de Educación Pública, México. The authors appreciate the help of Francisco Lopez-Antuñano from PAHO who provided the Abate Kit.

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