ORGANOPHOSPHOROUS INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES IN MARYLAND, 1985–89

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ABSTRACT. From 1985 to 1989, the susceptibility of the 9 major culicine mosquito species in Maryland to the larvicide temephos or the adulticide malathion was studied. The susceptibility of *Culex* spp. to temephos has declined in most areas of Maryland since 1967; however, only one strain of *Culex pipiens* was found to be temephos resistant in this study. *Aedes sollicitans*, *Ae. albopictus* and *Ae. taeniorhynchus* were temephos susceptible. All mosquitoes tested in the adult stage were susceptible to malathion.

INTRODUCTION

Concern over insecticide resistance as the cause of control failures has led many states to establish programs or conduct studies to detect resistance in mosquitoes (El-Khatib and Georghiou 1985, Thompson 1986, Boike et al. 1985, Khoo et al. 1987). These and other studies (Brown 1986, Lacey and Lacey 1990) have revealed resistance to many organophosphorus (OP) insecticides in mosquitoes as one cause of control failures that have led to the development of integrated pest management (IPM) programs. In 1985, the Maryland Department of Agriculture established an OP insecticide resistance surveillance program.

We determined the susceptibility status of the 9 major culicine species in Maryland to the larvicide temephos or the adulticide malathion, the 2 OP insecticides most widely used for mosquito control in Maryland. Test results were compared and contrasted between geographic strains and to susceptible strains.

MATERIALS AND METHODS

Mosquito strains: The 9 culicine species studied were: *Aedes albopictus* (Skuse), *Aedes canadensis* (Theobald), *Aedes cantator* (Coquillett), *Aedes sollicitans* (Walker), *Aedes taeniorhynchus* (Wiedemann), *Aedes triseriatus* (Say), *Aedes vexans* (Meigen), *Culex pipiens* Linnaeus and *Culex restuans* Theobald. All mosquitoes were field collected as larvae and tested in the F1 generation, except for *Ae. sollicitans*, *Ae. canadensis*, *Ae. cantator* and *Ae. vexans*. These latter species could not be colonized and were tested following field collection. Thirty-five geographic strains, comprising 60,000 larvae and 5,207 adults were tested in this study. Collection sites for each strain (Tables 1 and 2) are different and were not sampled more than once, except for the *Ae. albopictus* collections in Baltimore City. A susceptible SABAH strain of *Ae. albopictus* was obtained courtesy of George Craig of the University of Notre Dame. Arthur Boike of the John Mulrennan Sr. Research Laboratory in Panama City, FL provided the susceptible strain (JAMSRL) of *Ae. taeniorhynchus*.

Bioassays:

Larvae: Larval bioassays (Table 1) were conducted according to World Health Organization (1981a) procedures. Alcoholic temephos solutions supplied by WHO were employed in these tests. No difference in activity was detected between the WHO solutions and stock solutions of temephos prepared from analytical grade temephos (92.9%, American Cyanamid). Lots of 25 third or fourth instar larvae were exposed for 24 h in 16 oz. (473.5 ml) polyethylene plastic cups (Sweetheart cup) with 249 ml distilled water and one ml of the appropriate insecticide solution. Each test consisted of at least 3 replicates per concentration and 3–6 dosage levels. All tests were repeated 2–20 times, depending on the availability of larvae.

Adults: Adult mosquito bioassays (Table 2) were conducted according to WHO (1981b) protocol. Twenty-five adult females (3–5 days old) were blood-fed on days 1–3. Mosquitoes were transferred from rearing cages to plastic tubes of WHO kits lined with untreated paper. They were then transferred to the exposure tubes lined with either 5% malathion paper or olive oil control paper and exposed for up to 1 hour. The mosquitoes were returned to the holding tubes for 24 h and supplied with a solution of 10% sucrose. Mortality was recorded at the end of 24 hours. A minimum of 3 replicates was used for each dosage level. Tests were repeated one to 8 times for each strain.

Data analysis: The data were analyzed by probit analysis using version 5 of SAS/STAT (SAS Institute 1985). Data presented in Tables 1 and 2 provide chi-square values that indicate the goodness of fit of the data to the probit model. The LC values were read from the ProcProbit printout for data conforming to the probit model (chi-square values not significant (NS) at $P = 0.05$). For strains with responses not con-

Table 1. Susceptibility of *Aedes* and *Culex* larvae to temephos*

County	Strain	Year	n[1]	LC_{50}	LC_{90}	χ^{2} [3]	df	Resistance ratio[2] LC_{50}	Resistance ratio[2] LC_{90}
			Aedes albopictus						
Baltimore	Balt. City	1988	500	0.00952	0.01445	NS	3	1.5	1.20
Baltimore	Balt. City	1988	1,000	1.2% mortality at 0.005 ppm				—	—
			Aedes canadensis						
Anne Arundel	Selby	1985	475	0.00261	0.00726	NS	1	—	—
Prince George's	Beltsville	1985	2,025	0.00840	0.03000	NS	3	—	—
Queen Anne's	Stevensville	1985	375	—	0.01000	S	2	—	—
			Aedes cantator						
Queen Anne's	Kent Point	1989	265	0.0003	0.0069	NS	1	—	—
			Aedes sollicitans						
Anne Arundel	Rosehaven	1985	750	0.00319	0.00517	NS	2	1.4	1.6
Cecil	Courthouse Pt.	1985	750	0.00356	0.00529	NS	3	1.5	1.7
Dorchester	Becker	1986	1,075	0.00540	0.01400	S	2	2.3	4.4
Dorchester	Elliot	1987	525	96% mortality at 0.005 ppm				—	—
Dorchester	Elliot	1987	1,625	100% mortality at 0.005 ppm				—	—
Dorchester	Fishing Bay	1985	2,000	0.00442	0.00691	NS	3	1.8	2.2
Dorchester	Fishing Bay	1986	400	0.00348	0.01919	NS	3	1.5	6.0
Dorchester	Fishing Bay	1988	400	98.0% mortality at 0.005 ppm				—	—
Dorchester	Pokata Ck.	1987	650	99% mortality at 0.005 ppm				—	—
Queen Anne's	Grasonville	1986	425	0.00766	0.01408	NS	2	3.3	4.4
Queen Anne's	Grasonville	1987	400	100% mortality at 0.005 ppm				—	—
Queen Anne's	Grasonville	1987	240	<0.00001	0.00008	NS	3	<0.1	0.3
Queen Anne's	Grasonville	1987	2,250	0.00046	0.00196	NS	4	0.2	0.6
Queen Anne's	Grasonville	1988	1,000	0.0056	0.00950	S	3	2.4	3.0
Somerset	Fairmount	1987	1,925	99.6% mortality at 0.005 ppm				—	—
Somerset	Crisfield	1988	1,000	0.00323	0.00589	NS		1.4	1.8
			Aedes vexans						
Anne Arundel	Selby	1985	600	0.00401	0.00539	NS	2	—	—
Queen Anne's	Kent Point	1987	1,050	100% mortality at 0.005 ppm				—	—
Queen Anne's	Stevensville	1985	3,775	0.00700	0.01600	S	3	—	—
			Aedes taeniorhynchus						
Worcester	Assateague Is.	1985	9,575	0.00700	0.02200	S	3	1.0	2.4
			Culex pipiens						
Anne Arundel	Annapolis	1987	1,000	0.00006	0.00240	NS	1	<.1	1.2
Baltimore	Balt. Highlands	1986	764	0.02100	0.02300	NS	3	17.5	1.2
Prince George's	College Park	1985	2,675	0.01100	0.04100	NS	3	9.2	20.5
Prince George's	Hyattsville	1985	11,050	0.00740	0.03300	S	3	6.2	16.5
Prince George's	Upper Marlb.	1985	1,200	0.00804	0.06100	S	3	6.7	30.5
Prince George's	Bowie	1985	1,200	0.00350	0.11500	S	3	2.9	57.5
Prince George's	Bladensburg	1985	750	0.00804	0.02260	NS	3	6.7	11.3
Somerset	Crisfield	1988	300	0.00611	0.07147	NS	1	5.1	35.7
Wicomico	Salisbury	1985	1,500	0.00710	0.01650	NS	3	5.9	8.3
Wicomico	Salisbury	1985	3,375	0.00700	0.01700	NS	3	5.8	8.5
Wicomico	Salisbury (Lab)	1986	1,300	0.01500	0.10000	S	2	12.5	50.0
			Culex restuans						
Prince George's	Beltsville	1985	3,550	0.01100	0.41000	S	3	—	—
Queen Anne's	Grasonville	1989	98% mortality at 0.01 ppm			—	—	—	—

[1] n = Number of individuals tested excluding controls.

[2] Resistance Ratio = $\dfrac{LC_{50} \text{ or } LC_{90} \text{ of field strain}}{LC_{50} \text{ or } LC_{90} \text{ of susceptible strain}}$

[3] NS = Not significant, LC Values from probit analyses.
S = Significant, LC Values from plotted data.

* Lethal concentration in μg AI/ml (ppm).

Table 2. Susceptibility of *Aedes* spp. and *Culex pipiens* adults to malathion

County	Strain	Year	Lethal time estimates (min) following exposure to 5% malathion paper n[1]	LT$_{50}$	LT$_{90}$	χ^2 [2]	df
			Aedes triseriatus				
Anne Arundel	Crofton	1985	1,125	9.400	18.000	NS	2
Washington	Smithsburg	1986	425	4.490	7.410	NS	2
Baltimore	Balt. City	1988	125	83% mortality at 15 min exposure			
			Aedes sollicitans				
Dorchester	Becker	1986	450	3.682	6.569	NS	2
Queen Anne's	Grasonville	1987	99	90% mortality after 10 min exposure			
Queen Anne's	Grasonville	1986	550	3.440	6.620	NS	2
Somerset	Crisfield	1988	175	66% mortality at 10 min exposure			
Somerset	Crisfield	1988	150	73% mortality at 10 min exposure			
Somerset	Crisfield	1988	150	69% mortality at 10 min exposure			
			Aedes taeniorhynchus				
Worcester	Assateague Is.	1985	1,200	5.470	9.053	NS	2
			Aedes albopictus				
Baltimore	Balt. City	1988	200	100% mortality at 1 h exposure			
Baltimore	Balt. City	1988	150	100% mortality at 1 h exposure			
Baltimore	Balt. City	1988	150	47% mortality at 15 min exposure			
Baltimore	Balt. City	1988	150	58% mortality at 15 min exposure			
Baltimore	Balt. City	1988	50	49% mortality at 15 min exposure			
			Culex pipiens				
Prince George's	Bladensburg	1985	3,675	16.520	34.500	NS	2
Washington	Smithsburg	1986	550	4.170	7.360	NS	2

[1] n = Number of individuals tested excluding controls.
[2] NS = Not significant, LC Values from probit analyses.
S = Significant, LC Values from plotted data.

Table 3. Baseline susceptibility of *Culex* and *Aedes* larvae to temephos*

Species	Strain	Year	LC$_{50}$	LC$_{90}$	References
Aedes albopictus	Sabah	1990	0.0065	0.012	Sweeney (1990)
Aedes albopictus	Sabah	1990	—	0.01(LC95)	Wesson (1990)
Aedes sollicitans	Cambridge	1967	0.0023	0.0032	Joseph and Berry (1967)
Aedes taeniorhynchus	JAMSRL	1990	0.007	0.009	Sweeney (1990)
Culex pipiens	Bowie	1974	0.0015	0.0021	Joseph (1974)
Culex pipiens	Crisfield	1967	0.0012	0.002	Joseph and Berry (1967)
Culex pipiens	Crisfield	1974	0.0009	0.003	Joseph (1974)

* Lethal concentration in μg AI/ml (ppm).

forming to the probit model (chi-square values indicating a significant difference (S) at P = 0.05), the LC values were derived from plots of the raw data. This is characteristic of a population where 10–15% of the individuals are highly tolerant or resistance to the tested insecticide (B. Khoo, unpublished data).

The resistance ratio (RR) is defined as the LC$_{50}$ or LC$_{90}$ values of the field collected strain divided by the LC$_{50}$ or LC$_{90}$ values, respectively, of the susceptible strain. This ratio was used to compare the susceptibility of larvae from field collected strains with that of a susceptible strain for the same species.

Baseline susceptibility: The results of larval bioassays with temephos were compared with the susceptible strains listed in Table 3. A strain was resistant to temephos if the resistance ratio for the LC$_{50}$ was more than 10× that of the susceptible strain (Brown and Pal 1971). Resistance to malathion in the adult mosquito bioassays was determined by survival of a 1 h exposure to 5% malathion papers, as described by the WHO (1981b) protocol.

RESULTS AND DISCUSSION

The results of larval bioassays are listed in Table 1. All resistance ratios for larvae of *Aedes* spp. were less than 10×, indicating susceptibility to temephos, as defined by Brown and Pal (1971). However, the dosage-mortality profile of the *Ae. sollicitans* strains tested in 1986 from Becker and Fishing Bay Marshes indicates that populations in these areas have a significant number of temephos resistant individuals. A similar dosage-mortality profile was exhibited by the *Ae. taeniorhynchus* strain from Assateague Island in Worcester County. The *Ae. canadensis* Beltsville strain was probably resistant but this was not proven since no data exist for susceptible strains. The Baltimore City strain of *Ae. albopictus* was very susceptible to temephos with a $LC_{90} = 0.01445$ ppm. This value was close to the baseline susceptibility concentration of $LC_{95} = 0.01$ ppm (Wesson 1990) and $LC_{90} = 0.012$ ppm (Sweeney 1990) reported for the SABAH strain (Table 3).

Culex pipiens strains collected from Prince George's, Somerset and Baltimore counties were temephos susceptible but had high LC_{90} values (Table 1). The LC_{90} of the Bowie strain increased 54-fold from 1974 (Joseph 1974) to 1985, from $LC_{90} = 0.0021$ to $LC_{90} = 0.1150$ ppm. The Crisfield strain from Somerset County had a $LC_{90} = 0.002$ ppm in 1967, in 1974 the $LC_{90} =$ 0.003 ppm and in 1985 the LC_{90} increased to 0.07147 ppm. On the other hand, the LC_{50} value of the Crisfield strain increased only five-fold in the same period. The Baltimore Highlands strain was resistant as defined by Brown and Pal (1971).

All of the mosquito strains tested in the adult stage (Table 2) were susceptible since no individuals survived beyond the diagnostic dose of 1 h as defined by WHO (1981b). All LT_{90} values were low and the populations were homogeneous in their dosage mortality profile, except for the *Ae. triseriatus* population from Crofton and the *Cx. pipiens* population from Bladensburg.

The susceptibility of mosquito populations in Maryland to temephos has declined in the past 20 years. The results of this study show that this insecticide should not be used to control *Cx. pipiens* in Maryland. The decreased sensitivity of *Cx. pipiens* strains to temephos may be the result of repeated exposure to runoff from OP insecticide treated residential and agricultural areas and 5–8 treatments with temephos each season. In Maryland from 1967 to 1986, larvicide treated acreage increased annually and 90% of the larvicide used was temephos. *Aedes sollicitans* is still susceptible, but results for strains collected from Dorchester County indicate the need to test these populations annually for temephos susceptibility. Malathion, the most widely used mosquito control insecticide in Maryland, is still a highly effective adulticide. No evidence of resistance to malathion was detected in this study.

Research by Joseph (1974) and the results of the present study were contributing factors in the establishment and revision of the IPM program in Maryland, which employs source reduction, biological and chemical control methods. The mosquitofish, *Gambusia holbrooki*, is reared in multiple locations and distributed to hundreds of breeding areas each season. It has been very effective in sewage lagoons and stormwater retention areas as an alternative to pesticides for the larval control of mosquitoes. The insect growth regulator (IGR), methoprene, and the bacteria, *Bacillus thuringiensis* var. *israelensis* are replacing temephos to control mosquito larvae, especially in wetlands. These trends are expected to continue into the future as temephos resistance develops and environmental scientists and the public increase the demand for nonchemical control methods.

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