Brief communications/Notes

Development of resistance to carbamates and organophosphorus compounds in *Anopheles albimanus* in nature

G. P. Georghiou, [1] V. Ariaratnam, [1] & S. G. Breeland [2]

Carbamate and organophosphorus insecticides are currently being studied in the global malaria eradication effort as alternative chemicals of choice for use against DDT- and dieldrin-resistant *Anopheles* mosquitos. Although they are more expensive and less persistent than DDT as residual sprays for the walls of buildings, many of these chemicals have outstanding toxicity for adult and larval mosquitos. Such toxicity remains largely unaffected by existing DDT or dieldrin resistance, and until recently it appeared that selection pressure on *Anopheles* species did not result in resistance to these chemicals. This paper reports the appearance of high levels of resistance to several carbamate and organophosphorus insecticides in a natural population of *Anopheles albimanus* in coastal El Salvador. The observed resistance was found in an area that has been under intensive treatment with organophosphorus insecticides for several years for cotton insect control and may be attributed to indirect selection pressure by these treatments.

The first signs of impending resistance to organophosphorus compounds in field populations of *Anopheles* were reported by the World Health Organization in 1965 and consisted of suspected slight increases in tolerance to malathion in *A. albimanus* in Guatemala and Nicaragua. [3] Subsequently, Breeland et al. (1970) demonstrated that aerial applications of this chemical in the area of La Libertad, El Salvador, in March 1969 were significantly less toxic to caged wild-caught adult *A. albimanus* than to similarly exposed adults of a laboratory colony. Assays with the standard WHO testing kit verified these conclusions. Breeland and his co-workers pointed out that the decrease in susceptibility to malathion in El Salvador occurred only in areas of intensive cotton cultivation. These areas are being subjected during six months of the year, at almost weekly intervals, to heavy applications of several organophosphorus insecticides, including trichlorfon, parathion, parathion-methyl, and malathion (Breeland et al., 1970).

Organophosphorus insecticides have been employed extensively on cotton in El Salvador for more than 10 years. Since *A. albimanus* breeds also in the vicinity of cotton fields (Breeland et al., 1970), it may be assumed that it is subjected to indirect selection pressure by these chemicals. The reported field resistance to malathion in *Anopheles* is of significance in the global malaria eradication programme since this compound, the organophosphorus compound fenitrothion, and the carbamate propoxur are the only compounds among more than 1 400 candidate materials to have been tested successfully through most stages of the WHO international collaborative programme for evaluating and testing new insecticides (Wright et al. 1969).

For almost a decade, studies have been carried out in our laboratory at the University of California, Riverside, on the potentialities of promising new insecticides and on mechanisms of resistance to them in mosquitos. Intensive selection pressure in the laboratory on *A. albimanus* from Panama and Haiti using *m*-cumenyl methylcarbamate, propoxur, and fenitrothion has resulted in only minimal resistance to these insecticides, not exceeding 3 times the LC_{50} for larvae, despite the presence of DDT- and dieldrin-resistant genes in the parental populations (Georghiou, 1963, 1969; Georghiou & Calman, 1969; Georghiu & Metcalf, 1963). Such results have been considered to be encouraging indications that high resistance to carbamates or organophosphorus compounds in *Anopheles* would, if it ever developed, require extremely prolonged selection pressure.

Following publication of the WHO reports of impending resistance of *Anopheles* to organophosphorus compounds, we obtained a collection of this

[1] Department of Entomology. University of California, Riverside, Calif. 92502, USA.

[2] Central America Malaria Research Station, San Salvador, El Salvador.

[3] WHO (1965) *Information circular on insecticide resistance, insect behaviour and vector genetics*, No. 56, p. 24.

[1] Also: Ariaratnam, V. & Georghiou, G. P. (1971) unpublished document WHO/VBC/71.277.

Table 1. Insecticide resistance levels in *Anopheles albimanus*, El Salvador, in June 1970 and February 1971

Insecticide	Haiti/Panama strain (susceptible)		El Salvador strains					
			June 1970			February 1971		
	LC_{50} (ppm)	slope	LC_{50} (ppm)	slope	RR[a]	LC_{50} (ppm)	slope	RR[a]
propoxur	0.39	7.7	0.53	7.1	1.4	64.1	0.43	164.4
carbaryl	0.89	6.6	1.02	5.9	1.1	15.7	0.8	16.9
parathion–methyl	0.0065	3.0	0.022	3.5	3.4	0.17	2.1	26.2
parathion	0.0031	6.8	0.0098	3.1	3.2	0.057	1.9	18.4
malathion	0.085	2.9	0.25	2.7	2.9	1.1	1.8	12.9
fenitrothion	0.025	3.8	0.045	5.3	1.8	0.23	2.9	9.2
dichlorvos	0.11	7.1	0.11	7.0	1.0	0.29	5.9	2.6
Dursban	0.0063	4.7	—	—	—	0.015	3.6	2.4
fenthion	0.023	6.0	0.026	5.8	1.1	0.028	4.1	1.2
Abate	0.005	2.6	0.0049	2.6	1.0	0.006	3.0	1.2

[a] Resistance ratio = (LC_{50} El Salvador strain)/(LC_{50}) susceptible strain.

species from the area of Hacienda Melara, La Libertad, El Salvador, in June 1970, and colonized the insects in our laboratory. Our studies provided definitive evidence of the appearance of tolerance to organophosphorus compounds in larvae, amounting to the following increases in LC_{50} values: 3.2× for parathion, 3.4× for parathion-methyl, and 2.9× for malathion (Ariaratnam & Georghiou, 1971).[1] Moreover, when this larval population was subjected to rigorous selection pressure by propoxur, resistance rose within three generations to more than 100× for propoxur, 74.8× for carbaryl, 35.5× for parathion, 36.9× for parathion-methyl, 20× for malathion, and 9.6× for fenitrothion (Ariaratnam & Georghiou, *op. cit.*). Thus it was apparent that *A. albimanus* was indeed capable of developing resistance to carbamates and organophosphorus compounds and that the population of this species in the La Libertad area had attained a tolerance level that would readily lead to high resistance upon rigorous selection pressure by propoxur.

We have continued to study the situation in El Salvador and can now report that additional samples collected from the same locality 8 months later in February 1971 show considerably increased resistance to carbamates and organophosphorus compounds. The samples consisted of approximately 500 gravid females obtained from the field and permitted to oviposit at the CAMRS laboratory.[1] Some 3 000 eggs from these females were then shipped to our Riverside laboratory.

Larvae of the F_1 and F_2 generations resulting from the shipment were tested for susceptibility to a variety of insecticides. Parallel tests were performed on larvae of a strain of Haiti/Panama origin, which were susceptible to carbamates and organophosphorus compounds but resistant to dieldrin and moderately resistant to DDT. The colonies were maintained in 30×30×30 cm screened cages at 29°C and 60–70% R.H. and provided with white mice for blood meals. The larvae were kept in enamelled pans at 29°C and fed on a 50:50 mixture of brewers' yeast and Purina Laboratory Chow.[2] Larvae were tested in the early fourth instar in groups of 20 in 250-ml waxed paper cups containing 100 ml of water. The insecticides used were technical or analytical grade samples in standard w/v acetone solutions of the desired concentrations, 1 ml of which was added to 100 ml of water. The results were observed 24 hours later. Tests were repeated on at least 5 different days and the data subjected to probit analysis (Finney, 1952) on an IBM 360/50 computer.

The results (Table 1) clearly indicate that the

[1] Central America Malaria Research Station, San Salvador, El Salvador.

[2] Ralston Purina Co., St Louis, Mo., USA.

population of *A. albimanus* at Melara acquired considerable resistance to a variety of insecticides between June 1970 and February 1971. Resistance was highest to the carbamates propoxur and carbaryl and the organophosphorus compounds parathion, parathion-methyl, malathion, and fenitrothion, and only slight to dichlorvos and Dursban,[1] whereas there was no resistance to fenthion and Abate.[2] This is in agreement with the characteristic subgroup specificity of resistance to organophosphorus compounds observed in other insects (March, 1960; Georghiou & Hawley, 1971). A high degree of resistance is evident not only in increased LC_{50} values and the resultant resistance ratios, but also in characteristically low slope values (*b*) of the log-dose-probit-mortality regression lines. This is especially apparent with the carbamates, where *b* is 0.8 for carbaryl and 0.43 for propoxur. Similar results were obtained when resistance to propoxur and parathion was assayed in glass beakers instead of waxed paper cups: there was a small decrease in the LC_{50} for both the susceptible and the resistant strains but there was no significant change in the level of resistance obtained.

High resistance—especially in the aquatic stage, which is bioassayed by continuous exposure of the organisms for 24 hours—would suggest that a combination of increased metabolism and decreased penetration of the insecticide may be responsible for resistance. The role of reduced penetration in producing considerably magnified levels of resistance has been amply demonstrated in the housefly (Plapp & Hoyer, 1968; Georghiou, 1971). Until appropriate tests on metabolism are carried out in the present strain, it is not possible to offer a biochemical interpretation of the broad spectrum of resistance we have observed, especially in regard to the involvement of carbamates in such resistance. Studies with various synergists on the propoxur-selected strain mentioned earlier, whose resistance spectrum closely resembles that observed in the present study, suggest that mixed-function oxidases and carboxylesterases are involved in metabolism (Ariaratnam & Georghiou, *op. cit.*). Mixed-function oxidases and a reduced rate of penetration have been shown to be responsible for propoxur resistance in larvae of *Culex pipiens fatigans* (Shrivastava et al., 1969).

Although the precise mechanisms of resistance in the present case are unknown, it is evident that the observed pattern of resistance to organophosphorus compounds corresponds closely to the extensive use of parathion, parathion-methyl, and malathion on cotton in El Salvador during the past several years. Fenitrothion has not been employed in this area, however, and the 9.2-fold resistance to this insecticide may be true cross resistance [1] in view of the close similarity of its molecule to that of parathion-methyl. A similar situation exists in California, where *Aedes nigromaculis* resistant to organophosphorus compounds showed a 75-fold cross resistance to fenitrothion although this compound has not been used commercially in the State (Schaefer & Wilder, 1970). In the housefly, the coexistence of resistance to fenitrothion and to parathion-methyl has been shown to be due to enhanced degradation of the activation products—the oxygen analogues of these pesticides—by phosphatase action (Hollingworth et al., 1967). The involvement of carbamates in such resistance is less well understood, however, in view of the relatively limited use that has been made of these insecticides in the study area. It is possible that resistance to these materials has resulted from selection by carbaryl, which is applied to rice and occasionally to cotton and maize (corn), and may have been enhanced by the broad-spectrum resistance to organophosphorus compounds present in the population.

The coexistence of propoxur, malathion, and fenitrothion resistance in *A. albimanus* in El Salvador, and its possible effect on malaria eradication in Central America, require careful assessment, since these three chemicals have been considered to be the outstanding alternative insecticides for use against DDT- and dieldrin-resistant anophelines.

ACKNOWLEDGEMENTS

This investigation was supported in part by Public Health Service research grant CC 00301 from the Center for Disease Control, Atlanta, Ga., USA.

REFERENCES

Ariaratnam, V. & Georghiou, G. P. (1971) *Nature (Lond.)*, **232**, 642

Breeland, S. G. et al. (1970) *Bull. Wld. Hlth Org.*, **43**, 627

Finney, D. J. (1952) *Probit analysis*, London, Cambridge University Press

[1] *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl phosphorothioate.

[2] *O,O,O′,O′*-tetramethyl *O,O′*-thiodi-*p*-phenylene phosphorothioate.

[1] "Cross resistance" refers to those cases in which one mechanism confers protection against a variety of toxicants.

Georghiou, G. P. (1963) *Effects of carbamate insecticide selection pressure on* Anopheles albimanus *Wied.* In: *Proceedings and papers of the thirty-first annual conference of the California Mosquito Control Association*, Visalia, Calif., p. 80

Georghiou, G. P. (1969) *Wld Rev. Pest Control*, **8**, 86

Georghiou, G. P. (1971) *Proceedings of the second International Congress of Pesticide Chemistry, Tel Aviv*, London, Gordon & Breach, Vol. 2, p. 77

Georghiou, G. P. & Calman, J. R. (1969) *Bull. Wld Hlth Org.*, **40**, 97

Georghiou, G. P. & Hawley, M. K. (1971) *Bull. Wld Hlth Org.*, **45**, 43

Georghiu, G. P. & Metcalf, R. L. (1963) *Science*, **140**, 301

Hollingworth, R. M. et al. (1967) *J. agric. Food Chem.*, **15**, 250

March, R. B. (1960) *Misc. Publ. ent. Soc. Amer.*, **2**, 139

Plapp, F. W. Jr., & Hoyer, R. F. (1968) *J. econ. Ent.*, **61**, 1298

Schaefer, C. H. & Wilder, W. H. (1970) *J. econ. Ent.*, **63**, 1224

Shrivastava, S. P. et al. (1969) *Bull. Wld Hlth Org.*, **42** 931

Wright, J. W. et al. (1969) *Bull. Wld Hlth Org.*, **40**, 67

Suppression of a field population of *Aedes aegypti* by malathion thermal fogs and Abate larvicide

Y. H. BANG,[1] N. GRATZ,[2] & C. P. PANT[3]

In previous field studies, a field population of *Aedes aegypti* was suppressed by larviciding water containers with 1% Abate[4] sand granules.[5] However, the decline in the population following initial treatment was gradual, a factor that might limit the use of a larvicidal treatment by itself during an epidemic of dengue haemorrhagic fever. Immediate control of adult populations can be achieved by the application of insecticidal thermal logs, although the application must be repeated several times, as it has been found in previous field trials at the ARU (unpublished data) that the mosquito density returns to the pretreatment level within a week. A combined treatment by the two different methods i.e., adulticiding and larviciding, appears to be desirable in combining an emergency operation and a long-term control programme. The exact rate of the reduction in adult mosquito populations and their recovery in an area where all larval habitats are treated with Abate is not known.

The investigation reported in this paper was carried out in Bangkok by the World Health Organization *Aedes* Research Unit (ARU) to determine the efficacy of malathion thermal fogs, applied soon after larviciding by 1% Abate sand granules, on the suppression of a field population of *Ae. aegypti* and its recovery. This was one of a series of field studies undertaken by the ARU to devise emergency control measures that can be recommended when an epidemic occurs.

Description of the area

The study was carried out in a housing area of Bang Sue, in the northern part of Bangkok, which had an area of approximately 7 ha, was 830 m in length and 85 m in width, and was divided into two unequal parts by a playground (0.5 ha); the two parts were used for evaluation of the two different treatments. There are 52 apartment buildings, and 304 houses in the treated area: 172 in site A and 132 in site B. The structure of the houses at the two sites were alike and similar to those described by Bang et al.[5]

To determine the rate of reduction of the adult mosquito population in an area where there was no mosquito infiltration from untreated neighbour-

[1] Entomologist, WHO *Aedes* Research Unit, Bangkok, Thailand. Present address: WHO East Africa *Aedes* Research Unit, Dar es Salaam, Tanzania.

[2] Entomologist, Vector Biology and Control, World Health Organization, Geneva, Switzerland.

[3] Project Leader, WHO *Aedes* Research Unit, Bangkok, Thailand.

[4] *O,O,O′,O′*-tetramethyl *O,O′*-thiodi-*p*-phenylene phosphorothioate.

[5] Bang, Y. H. et al. (1969) *Pilot studies of Abate (OMS 786) as a larvicide for controlling* Aedes aegypti *in Bangkok* (Unpublished document WHO/VBC/69.164).