IRRITABILITY TO DDT OF NATURAL POPULATIONS OF THE PRIMARY MALARIA VECTORS IN COLOMBIA

MARTA L. QUINONES AND MARCO F. SUAREZ

Ministerio de Salud, Programa Antimalarico, Apartado aereo 4851, Bogota, Colombia

ABSTRACT. Natural populations of *Anopheles albimanus*, *An. darlingi* and *An. nuneztovari* were tested for their irritability to DDT in different regions of Colombia. Individual anophelines were exposed to filter papers impregnated with 2% DDT and irritability was determined based on the number of flights a mosquito made in 15 min. All three species showed variability in their irritability to DDT. *Anopheles albimanus* showed irritability only in Bolivar, but not in three other regions. *Anopheles darlingi* showed no irritability in neither the Atlantico nor Llanos regions, while *An. nuneztovari* showed irritability in the Oriente but not in Uraba. The irritability to DDT shown in some populations of *An. albimanus* and *An. nuneztovari* may reduce the effectiveness of residual applications of this insecticide by causing the mosquitoes to seek untreated surfaces and/or leave the house to rest outdoors and thus avoid a lethal dose.

INTRODUCTION

Intradomiciliary application of DDT is used for malaria control in Colombia, and the effectiveness of the insecticide is dependent upon the postblood feeding behavior of anophelines. Anophelines after blood feeding must rest on the insecticide-treated internal surfaces of the house for a period of time sufficient to acquire a lethal dose. The mosquitoes can avoid contact with the insecticide by a natural tendency to rest outside houses (exophily) or due to irritation caused by contact with the insecticide (Pampana 1966). Mosquitoes may only absorb a sublethal dose of insecticide when the insecticide formulation causes a locomotor-stimulant effect on the mosquito (Elliott 1964). In some species of anophelines, the proportion which escapes the insecticidal action due to irritability can be high, limiting the effectiveness of indoor residual spraying with DDT (Muirhead-Thomson 1960).

This study was carried out to quantify the irritability to DDT of the primary malaria vectors (*Anopheles albimanus* Wiedemann, *An. darlingi* Root and *An. nuneztovari* Gabaldon) from various regions of Colombia.

MATERIALS AND METHODS

Various methods have been used to study the effect of DDT on the behavior of anophelines by quantifying the number of flights in irritability tests or measuring their ability to escape lethal contact with DDT surfaces in excito-repellency tests. At present there is no single, widely accepted method to measure insecticide irritability and its consequences (Coluzzi 1962, Brown 1964, Busvine 1964, Georghiou et al. 1972). The irritability tests were carried out during 1984 and 1985 following one of the steps (namely B) in a tentative method of determining the irritability of mosquitoes to insecticides proposed by the World Health Organization (WHO) Expert Committee on Insecticides (WHO 1960) and evaluated for efficacy by Brown (1964). Filter papers, one impregnated with 2% DDT, which according to Hecht and Hernandez (1960) correspond to 0.714 g/m^2, and a control paper without DDT, were attached to a wall and transparent plastic cones placed over the papers. The papers and cones were those supplied by the World Health Organization with their standard adult mosquito susceptibility and wall bioassay test kits, respectively (WHO 1981). Individual anophelines were simultaneously placed in each cone, and after a 3-min adaptation period, the number of flights during the subsequent 15 min was counted. This method was selected for its facility in carrying out the tests in the field without need of additional equipment. After the test, the anophelines were transferred to paper cups, killed and identified as to species. All tests were carried out between 0800 and 1100 hr. The walls were shaded, never sprayed with DDT or any other insecticide and were usually in a house used as a field laboratory.

The anophelines used in the tests were from human landing collections made at night (1800–2400 hr), inside and outside of the houses. They were held in clean paper cups (7 × 7 cm) until used in tests the following morning. Cotton pads containing a 10% sugar solution were provided to the mosquitoes. Only nonblood-fed mosquitoes were used in these tests because blood-fed mosquitoes show less flight activity (Hamon and Eyraud 1961, cited in Brown and Pal 1973).

The anophelines tested were from areas with malaria transmission and where indoor residual treatments of DDT are applied in 6-month cycles. The number of tests done in each area depended on the availability of anophelines. The data were analyzed using the *t* test for paired samples (Sokal and Rohlf 1981) to compare the number of flights of the mosquitoes exposed to DDT with the number of flights of the controls.

RESULTS AND DISCUSSION

Tests with *An. albimanus* were carried out in four regions: Guajira-Magdalena, Bolivar, Uraba and Pacifico (Table 1 and Fig. 1). The *An. albimanus* population from Bolivar showed a high degree of irritability, whereas the populations from the other regions showed no significant differences. The population of this species in Bolivar also differs from the other populations sampled here in that it has shown mortalities compatible with the presence of resistant individuals in DDT-susceptibility tests, whereas the others have shown susceptibility (Quinones et al. 1987). These results disagree with studies where DDT-resistant populations show less DDT-irritability (Brown and Pal 1973).

Irritability of DDT to *An. albimanus* has been found previously in Mexican and Central American populations. Experiments with colonized *An. albimanus* in Mexico showed irritability when exposed to a dose of 0.5 g/m^2 of DDT for 20 min, using transparent plastic cylinders with impregnated papers at each end (Mancera and Hernandez 1960). In El Salvador, Rachou et al. (1973), using excito-repellency boxes which allow mosquitoes to escape, found that 82–92% left the boxes when exposed to a dose of 2 g/m^2 DDT for 1 hr.

Although the different methods used prevent any direct comparisons of these results, the data indicate that there is a difference in DDT-irritability behavior between *An. albimanus* populations from Central America and Colombia, with the exception of the Bolivar population. In addition to the differences in irritability and susceptibility, Elliott (1968) reported that distinct differences existed in the pattern of biting periods and the biting peaks between populations of *An. albimanus* from the Magdalena Valley and Caribbean coast area, although differ-

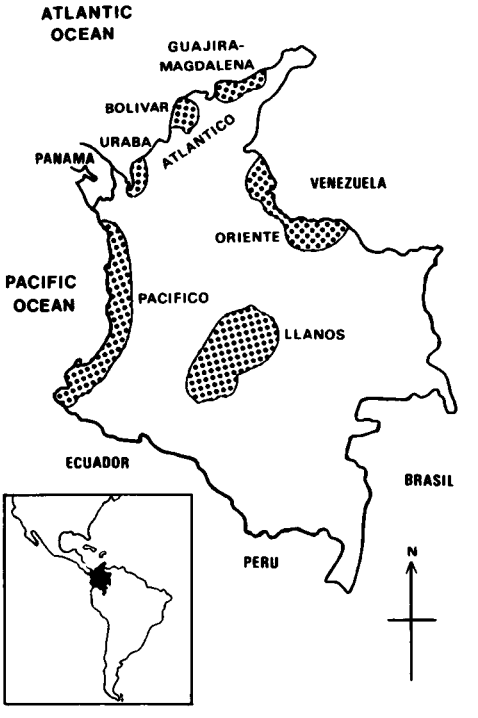

Fig. 1. Map of Colombia. Shading indicates areas of tests.

Table 1. Irritability of DDT on three anopheline species from various regions in Colombia.[1]

Species/region	Treatment	No. of tests	Mean no. flights (±SD)	*t* value
An. albimanus				
Guajira-Magdalena	Exposed	23	13.5 (18.4)	1.55
	Control		6.7 (8.2)	
Bolivar	Exposed	172	172.6 (65.8)	8.42*
	Control		36.4 (39.0)	
Uraba	Exposed	33	18.2 (18.6)	0.59
	Control		16.1 (19.4)	
Pacifico	Exposed	108	18.5 (12.0)	1.66
	Control		15.7 (15.9)	
An. darlingi				
Atlantico	Exposed	23	12.2 (10.7)	0.86
	Control		10.8 (7.6)	
Llanos	Exposed	50	24.3 (26.2)	1.94
	Control		16.9 (16.9)	
An. nuneztovari				
Uraba	Exposed	26	11.8 (10.9)	0.71
	Control		9.7 (9.2)	
Oriente	Exposed	194	11.3 (8.3)	5.98*
	Control		7.4 (9.1)	

[1] Irritability determined from exposing mosquitoes to 0.714 g/m^2 DDT impregnated filter paper for 15 min.
* Significant ($P < 0.01$; *t* test).

ences have not been found in polytene chromosomes (Ramirez et al., unpublished data) and isozymes (Monje et al., unpublished data).

Results of irritability tests with *An. darlingi* (Table 1) showed no irritability in the two populations tested, one from Atlantico and the other from the Llanos (Fig. 1). However, it has been reported that populations of this species in Brazil show evasion when tested with DDT in excito-repellency boxes (Charlwood and Paraluppi 1978, Hayes and Charlwood 1977, Roberts et al. 1984). As mentioned above for *An. albimanus*, the different methods for measuring irritability to insecticides makes it difficult to compare results. However, these differences in DDT irritability behavior between *An. darlingi* from Colombia and Brazil could be related to the highly polymorphic larval polytene chromosomes (Tadei et al. 1982, Tadei 1985) as well as isozyme polymorphism (Narang et al. 1979).

Anopheles nuneztovari showed no irritability in the Uraba region, but the populations from the Oriente, along the border with Venezuela, showed a significant degree of response (Table 1 and Fig. 1). In this region the species has been reported to have natural exophilic and exophagic tendencies (Gabaldon 1981), and in Colombia this species has been reported to have a 50:50 ratio of endophagy to exophagy (Elliott 1968). Therefore, DDT residual spraying probably has a role in these behavioral characteristics. DDT evasion by this species has also been documented in excito-repellency tests near Manaos, Brazil (Charlwood and Paraluppi 1978). Elliott (1972) suggested that there are at least two species under the name, *An. nuneztovari*, because of the variation in its biting behavior. In the same way Kitzmiller et al. (1973) point out that this species could be a complex because of the differences in the X chromosomes of various populations.

The irritability to DDT shown in some populations of *An. albimanus* and *An. nuneztovari* may reduce the effectiveness of residual applications of this insecticide by causing the mosquitoes to seek untreated surfaces, leave the house to rest outdoors, or both, and thus avoid a lethal dose. However, any direct estimates of such effects would require carefully designed field studies on biting habits, resting habits and related behavior. Also, these data indicate that the DDT irritability behavior could be different between populations of the same species and the residual insecticides may vary in effectiveness from one region to another because of these differences. It is necessary to carry out a surveillance of the level of irritability in natural populations in order to interpret the effects of this phenomenon on the control measures with DDT in malaria control programs. The methodology used in this study is a simple method that could be used to carry out that surveillance.

ACKNOWLEDGMENTS

The cooperation of the Campanas Directas, Ministry of Public Health, Colombia, is gratefully acknowledged. We thank all the field personnel of the malaria control program who assisted, with special thanks to Mr. Moises Cortes, Mr. Aquileo Graciano, Mr. Lalo Garcia, Mr. Patrocinio Guarin, Mr. Uriel Ibarra, Mr. Rafael Lafont, Mr. Argemiro Hernandez, Mr. Guillermo Berruecos and Mr. Henry Jimenez for data collection. We also thank Dr. G. A. Fleming for his review of the manuscript and Mrs. Ismenia Aponte and Mr. Alvaro Lopez for assistance in processing the data. Financial support (Grant MVR-CO-2-85-46), in part, for this research was provided by the National Research Council, U.S. National Academy of Sciences, through a grant from the U.S. Agency for International Development.

REFERENCES CITED

Brown, A. W. A. 1964. Experimental observations governing the choice of a test method for determining the DDT irritability of adult mosquitoes. Bull. W.H.O. 30:97–111.

Brown, A. W. A. and R. Pal. 1973. Resistencia de los artropodos a los insecticidas. Organizacion Mundial de la Salud, Ginebra, Serie de monografias No. 38.

Busvine, J. R. 1964. The significance of DDT-irritability test on mosquitoes. Bull. W.H.O. 31:645–656.

Charlwood, J. D. and N. D. Paraluppi. 1978. O uso de caixas excitorepelentes com *Anopheles darlingi* Root, *Anopheles nuneztovari* Gabaldon e *Culex pipiens quinquefasciatus* Say obtidos em areas perto de Manaos, Amazonas. Acta Amazonica 8:605–611.

Coluzzi, M. 1962. An experimental method for determining the irritability of adult mosquitoes to insecticides. W.H.O. document MAL/329: 15 p.

Elliott, R. 1964. Studies on the kinetic response of mosquitoes to chemicals. Bull. W.H.O. 31:657–667.

Elliott, R. 1968. Studies on man-vector contact in some malarious areas in Colombia. Bull. W.H.O. 38:239–253.

Elliott, R. 1972. The influence of vector behavior on malaria transmission. Am. J. Trop. Med. Hyg. 21:755–763.

Gabaldon, A. 1981. *Anopheles nuneztovari* importante vector y agente de malaria refractaria en Venezuela. Bol. Dir. Malariol. Saneamiento Ambiental 21:28–38.

Georghiou, T., E. M. Ungureanu and C. Garrett-Jones. 1972. An apparatus for the study of the behavior patterns of mosquitoes under the influence of insecticides. Bull. W.H.O. 46:122–126.

Hayes, J. and D. Charlwood. 1977. O *Anopheles darlingi* evita o DDT numa área de malária resistente a drogas. Acta Amazonica 7:289.

Hecht, O. and J. Hernandez C. 1960. Resena de las investigaciones sobre la irritacion de anofelinos por

el contacto con superficies cubiertas con DDT. Boletin CNEP (Mexico) 4:93–106.
Kitzmiller, J. B., R. D. Kreutzer and E. Tallaferro. 1973. Chromosomal differences in populations of *Anopheles nuneztovari.* Bull. W.H.O. 48:435–455.
Mancera, V. O. and J. Hernandez. 1960. El efecto irritante en mosquitos de algunos insecticidas de contacto. Pruebas con *Anopheles albimanus* Wied. Boletin CNEP (Mexico) 4:82–85.
Muirhead-Thomson, R. C. 1960. The significance of irritability, behavioristic avoidance and allied phenomena in malaria eradication. Bull. W.H.O. 22:721–734.
Narang, S., J. M. M. Santos, J. G. Garcia, H. D. Cristakou and N. Narang. 1979. Genetica de populacoes de anofelinos IV. Estudos electroforeticos das populacoes naturais de *Anopheles nuneztovari* e *Anopheles darlingi.* Correlacao genetica entre especies. Acta Amazonica 9:529–542.
Pampana, E. 1966. Erradicacion de la malaria. Ed. Limusa-Wiley S. A. Mexico.
Quinones, M. L., M. F. Suarez and G. A. Fleming. 1987. Estado de la susceptibilidad al DDT de los principales vectores de malaria en Colombia y su implicacion epidemiologica. Biomedica 7:81–86.
Rachou, R. G., A. L. Schinazi and M. Moura-Lima. 1973. An intensive epidemiological study of the causes for the failure of residual DDT spraying to interrupt the transmission of malaria in Atalaya and Falla, two villages on the coastal plain of El Salvador, Central America. Rev. Bras. Malariol. Doencas Trop. Publ. Avulsas 25:5–293.
Roberts, D. R., W. D. Alecrim, A. M. Tavares and K. M. McNeill. 1984. Influence of physiological condition on the behavioral response of *Anopheles darlingi* to DDT. Mosq. News 44:357–362.
Sokal, R. R. and F. J. Rohlf. 1981. Biometry. 2nd ed. San Francisco: W. H. Freeman and Co.
Tadei, W. P. 1985. Biology of Amazonian mosquitoes, IX. On chromosome polymorphism of *Anopheles* (*Nyssorhynchus*) *darlingi* and a new arrangement in the X-chromosome. Cienc. Cult. 37:1330–1331.
Tadei, W. P., J. M. M. Santos and G. M. Rabbani. 1982. Biologia de anofelinos amazonicos. V Polimorfismo cromossomico de *Anopheles darlingi* Root (Diptera: Culicidae). Acta Amazonica 12:353–369.
World Health Organization. 1981. Instructions for the bioassay of insecticidal deposits on wall surfaces. W.H.O. Document VBC/81.5: 3 p.
World Health Organization. Expert Committee on Insecticides. 1960. Tentative method for determining the irritability of adult mosquitoes to insecticides. W.H.O. Tech. Rep. Ser. 191:46–49.