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METHODS OF TESTING AND ANALYZING EXCITO-REPELLENCY RESPONSES OF MALARIA VECTORS TO INSECTICIDES[1]

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ABSTRACT. A new test system that includes an excito-repellency test box, test procedures, and statistical treatment of data is described. The method consists of enclosing 25 mosquitoes in an exposure chamber lined with insecticide-treated or untreated (control) test papers. Each chamber has a single portal for mosquitoes to escape to a receiving cage, and numbers escaping are manually recorded at 1-min intervals. The exposure chamber accommodates a screened, 2nd chamber that, when placed in the exposure chamber, prevents the mosquitoes from making physical contact with test papers. A full assay utilized one exposure chamber that permits physical contact with insecticide-treated papers, one chamber that permits physical contact with control papers, one chamber that prevents physical contact with insecticide-treated papers, and a 4th chamber that prevents contact with control papers. After insecticide exposure, test populations are held for observations on 24-h mortalities. A survival analysis approach is described for estimating mosquito escape rates and for comparing differences in mosquito escape rates, with or without physical contact with insecticide, among populations, insecticides, and doses of insecticide.

INTRODUCTION

Assays for evaluating behavioral responses of malaria vectors to insecticide residues have been reviewed by Muirhead-Thomson (1960), Coluzzi (1963), Busvine (1964), and Elliott (1972). The test of greatest value for studies of insecticide avoidance was described by Coluzzi (1963) as a box with slits for escaping. Such a box was described by Rachou et al. (1973) and is referred to as the excito-repellency test box. Similar excito-repellency test boxes are described by Rachou et al. (1973), Charlwood and Paraluppi (1978), Roberts et al. (1984), Rozendaal et al. (1989), and Evans (1993). In excito-repellency tests, mosquitoes are released inside a box lined with sprayed paper. Outlets in the form of out-projecting baffles permit the mosquitoes to escape into 2 separate cages. The baffles prevent the mosquitoes from reentering the box and the numbers escaping are counted by time postrelease. The difficulties of working with test boxes were described by Roberts et al. (1984). Major problems relate to the difficulties in introducing specimens into the boxes, removing live specimens at the end of test periods, and providing a standardized insecticide dose. The lack of an appropriate method of data analysis has been another shortcoming of the test method. Earlier methods did not test for behavioral responses without physical contact with insecticide-treated papers.

Described herein are improved boxes for testing behavioral responses of adult *Anopheles* mosquitoes with or without physical contact with insecticide residues. Survival analysis methods are described for the statistical treatment of test data.

MATERIALS AND METHODS

The test method consists of enclosing 25 mosquitoes in an exposure chamber lined with insecticide-treated or untreated (control) papers. Each exposure chamber has a single portal for mosquitoes to escape to a receiving cage. The exposure chamber accommodates a screened, 2nd chamber (inner chamber) that, when placed in the first chamber, prevents the mosquitoes from making physical contact with test papers. Under test conditions, mosquitoes are enclosed within the exposure chamber and the only source of light comes from the exit portal. A full assay consists of 4 exposure chambers of 2 treatment chambers and 2 control chambers, as shown in Table 1. Treatment chambers are lined with test papers impregnated with insecticide and an oil-based carrier. Control chambers are lined with papers impregnated with carrier alone. One treatment chamber permits tarsal contact with insecticide. The second treatment chamber includes the inner chamber, so mosquitoes cannot make tarsal contact with insecticide. For brevity, tests with or without the inner chambers, for either treatment or control papers, are referred to as contact trials (no inner chamber) or noncontact trials (with an inner chamber).

Components of the excito-repellency chambers are illustrated and numbered in Fig. 1. Except for a inner panel (No. 1), the exposure chamber is constructed of metal and can be chemically cleaned. The exposure chamber (No. 4) is constructed of stainless steel and each chamber is 34 × 32 × 32

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