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NONCORRELATION OF INSECTICIDE AND REPELLENT TOLERANCES IN REPRESENTATIVE SPECIES AND STRAINS OF MOSQUITOES[1]

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ABSTRACT. Median effective dosages (ED50) of the repellent diethyl toluamide and median lethal concentrations (LC50) of the insecticides DDT and malathion were determined for 13 strains of *Anopheles albimanus, An. stephensi, An. quadrimaculatus, Aedes aegypti, Ae. taeniorhynchus, Culex tarsalis* and *Cx. pipiens*. Correlations of the ED50's of diethyl toluamide with the LC50's of DDT and malathion were not statistically significant. The results indicate that diethyl toluamide would be equally effective against insecticide-resistant and insecticide-susceptible mosquitoes in the field.

A number of thiocyanate, pyrethroid, organophosphate and chlorinated hydrocarbon insecticides have been shown to be repellent to different species of *Anopheles, Aedes, Armigeres, Culex* and *Mansonia* (Rudolfs 1930, Roy et al. 1942, Metcalf et al. 1945, Ribbands 1946, Johnson 1947, Kennedy 1947, Wharton and Reid 1950, Vigueras and Corzo 1960, Busvine 1964, Hudson and Esozed 1971). Conversely, Sarkaria and Brown (1951) demonstrated significant knockdown of *Aedes aegypti* (Linnaeus) by 32 repellents, including dimethyl phthalate, dimethyl carbate, Indalone® (butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate) and citronella oil. Elliott (1964) demonstrated that dimethyl phthalate and ethyl hexanediol were weakly insecticidal to *Anopheles gambiae* Giles, and Kuraishy et al. (1962) reported that a repellent cream containing diethyl toluamide and cetyl trimethyl ammonium bromide produced 7 to 9% mortality in unspecified field-collected anopheline and culicine mosquitoes.

[1] Opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Studies of several species of *Anopheles* and *Aedes* have indicated that the insecticidal and insect-repellent properties of DDT are unrelated (Coluzzi 1958, Hecht and Corzo 1960, Hecht et al. 1960, Cullen and DeZulueta 1962). Similarly, Sarkaria and Brown (1951) found no correlation between repellency and knockdown potential in a series of 42 compounds tested against *Ae. aegypti*. On the other hand, it is known that selection for increased tolerance to one insecticide can significantly increase the tolerance of a species to unrelated insecticides (Brown and Abedi 1960, Plapp and Hoyer 1968, Rongsriyam and Busvine 1975, Ayad and Georghiou 1979, Omer et al. 1980). The form of low-level resistance known as "vigor tolerance" is, in fact, defined on the basis of non-specificity (Hoskins and Gordon 1956, World Health Organization 1963).

The foregoing considerations suggest that it is possible for cross-resistance between insecticides and repellents to occur in mosquito populations. Linkages of this kind could be an important factor in the selection of repellents for use against insecticide-resistant mosquito populations in the field. The purpose of the present research was to compare the tolerances of several available species and strains of mosquitoes to diethyl toluamide, DDT and malathion.

MATERIALS AND METHODS

CHEMICALS. Commercial grade diethyl toluamide, containing 71.25% *N,N*-diethyl-*m*-toluamide and 3.75% other isomers of diethyl toluamide in ethanol (Airosol Company, Inc.); technical grade malathion, 95% (Chem Service, Inc.); analytical grade *p,p'* DDT, 100% (Nutritional Biochemicals Corp.).

MOSQUITOES. Thirteen laboratory strains of *Anopheles albimanus* Wiedemann, *An. stephensi* Liston, *An. quadrimaculatus* Say, *Aedes taeniorhynchus* (Wiedemann), *Ae. aegypti*, *Culex tarsalis* Coquillett and *Cx. pipiens* Linnaeus were used in the study (Table 1). The provenance of the strains and the laboratory rearing procedures and conditions were given by Rutledge et al. (1978). The Hartwell Dam strain of *An. quadrimaculatus*, not listed in that report, was obtained from Dr. D. E. Weidhaas, Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida.

TEST METHODS. The median effective dosages (ED50's) of diethyl toluamide for the different species and strains of mosquitoes were determined by the method of Rutledge et al. (1976). The method employs an *in vitro* test system which permits unrestricted ("free choice") blood-feeding through any of 5 membrane surfaces, 4 of which are treated with serial dilutions of the test repellent. The 5th membrane surface is treated with diluent alone, as a control. The ED50 of the test repellent is calculated by probit analysis from feeding counts made during the test. The results obtained for diethyl toluamide have been reported previously (Rutledge et al. 1978), except for those pertaining to the Hartwell Dam strain of *An. quadrimaculatus*. They are

Table 1. Median effective dosages of diethyl toluamide and median lethal concentrations of DDT and malathion for 13 strains and species of mosquitoes.

Species/Strain	ED50 Diethyl Toluamide (mg/cm²)	LC50 DDT (%)	LC50 Malathion (%)
Anopheles albimanus	0.076	0.9	0.16
Anopheles stephensi	.034	1.6	.27
Anopheles quadrimaculatus			
USDA	.022	1.2	.27
Hartwell Dam	.009	6.1	.13
Aedes aegypti			
Moyo Indoor	.042	7.9	.24
Amphur	.036	—	.18
UC San Francisco	.031	1.1	.12
Masaka	.024	2.3	.18
USDA	.030	2.6	.22
Aedes taeniorhynchus	.015	2.0	.22
Culex tarsalis	.022	—	.29
Culex pipiens			
USAEHA	.017	5.2	.25
UC Davis	.011	—	.25

repeated here to facilitate comparison with the corresponding results obtained in the insecticide susceptibility tests.

Median lethal concentrations (LC50's) of DDT and malathion were determined by the standard methods of the World Health Organization (1970), except that fresh test papers were prepared for use in the WHO test kit, as follows: Solutions of 8% DDT and 0.5% malathion were prepared in olive oil-acetone (1:2) and diluted serially for treatment of the test papers. Test papers were cut from Whatman No. 5 filter paper stock (W. & R Balston, Ltd.), saturated with the required solutions, drained, blotted and dried for 24 hours prior to use. Extra papers were stored in aluminum foil wrappers in a refrigerator for subsequent use.

RESULTS AND DISCUSSION

The results obtained in the study are shown in Table 1. ED50's of diethyl toluamide ranged from 0.009 mg/cm^2 for the Hartwell Dam strain of *An. quadrimaculatus* to 0.076 mg/cm^2 for *An. albimanus*. LC50's of DDT ranged from 0.9% for *An. albimanus* to 7.9% for the Moyo Indoor strain of *Ae. aegypti*. LC50's of malathion ranged from 0.12% for the UC San Francisco strain of *Ae. aegypti* to 0.29% for *Cx. tarsalis*.

The correlations of the ED50's and LC50's were calculated as Spearman's coefficient of rank correlation (r_s), since this test does not depend on specific assumptions regarding the statistical distribution of the variables tested (Steel and Torrie 1960). Values of (r_s) obtained in the analysis were −0.36 for the correlation of ED50 of diethyl toluamide with LC50 of DDT, −0.17 for the correlation of ED50 of diethyl toluamide with LC50 of malathion, and +0.14 for the correlation of LC50 of DDT with LC50 of malathion. None of the correlations were significant at the 5% level.

These results are similar to those of Prasittisuk and Busvine (1977), who found that median lethal times (LT50's) for exposure to 4% DDT and 0.033% permethrin were not significantly correlated in 13 strains of *Ae. aegypti, An. gambiae* and *An. quadrimaculatus*. Similarly, Sun et al. (1980) found that a strain of *An. albimanus* and 4 strains of *Cx. pipiens* representing the principle mechanisms of resistance to organophosphorus, carbamate, DDT, dieldrin and pyrethroid insecticides did not differ significantly from the control strains in tolerance to *Bacillus thuringiensis* var. *israelensis* de Barjac toxin. However, the question of whether insecticide and repellent tolerances are genetically or physiologically linked in particular strains or species will have to be resolved separately for each. In the present study, *An. stephensi* was relatively tolerant to both diethyl toluamide and malathion and the Moyo Indoor strain of *Ae. aegypti* was exceptionally tolerant to diethyl toluamide and DDT (Table 1), but the biological significance of these findings is not known.

Although the coefficients of correlation obtained in the study were not statistically significant, it should be noted that two of the three were negative rather than positive. The possibility of negatively correlated tolerances to pesticides has been raised in several reports in connection with the WARF "antiresistant" for DDT (N,N-di-n-butyl-p-chlorobenzenesulfonamide) and other compounds. Our results should not be interpreted as support for that thesis.

The occurrence of cross-resistance between insecticides and repellents has not been conclusively disproved by this study because of the possibility of Type II error (Steel and Torrie 1960). However, the likelihood of that event seems slight in view of the results obtained, which indicate that levels of sensitivity to diethyl toluamide are unrelated to levels of susceptibility to DDT and malathion in mosquitoes.

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ANOPHELES CULICIFACIES: THE EFFECTS OF ADULT BODY WEIGHT AND TROPHIC STATUS ON DIELDRIN LT_{50} DETERMINATIONS

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ABSTRACT. The live weight of adult *Anopheles culicifacies* was affected by variation in rearing conditions and using either unfed or blood fed females for insecticide tests. Mosquitoes homozygous resistant to dieldrin were the main genotype used on 4% dieldrin treated papers for exposure times from 5 to 16 hours. LT_{50} determinations showed significant heterogeneity but clearly heavier blood fed adults showed less susceptibility to dieldrin. All the laboratory reared adult sizes could be found in the field as shown by collections from one village during 1976. The implication of such weight and trophic status variation is discussed with respect to discriminating dosages and the degree of resistance involved.

INTRODUCTION

Insecticide tests with WHO test kits are generally used to detect the presence and measure the frequency of resistance genes in mosquito populations. However, as pointed out by Davidson and Zahar (1973), the results may be affected not only by genetic factors but also by environmental factors influencing the size and physiological status of the mosquitoes used for testing. Such variability might be particularly important when laboratory rearing of the mosquitoes for testing is not feasible and when wild collected specimens must therefore be used. Great variability in the size and other characteristics of wild mosquitoes is to be expected in areas such as Punjab when ambient temperatures may vary more than 40°C during the year.

The objectives of the present study were to describe the effects in the laboratory of variation in body-size and trophic status on dieldrin LT_{50} and try to extrapolate these findings to field conditions by monitoring seasonal changes in adult female body-size.

MATERIALS AND METHODS

STRAINS. Colonies of homozygous susceptible (SS) and resistant (RR) *An. culicifacies* (Giles) were selected respectively, from Sattoki and Multan, Punjab Province, parent strains colonized originally by Ainsley (1976) and studied genetically for the inheritance of resistance to dieldrin by Sakai et al. (1979). Dieldrin resistance in *An. culicifacies* was found to be semi-dominant, with exposure to 0.4% for 1 hr killing all SS and to 4.0% for 2 hr killing all SS and heterozygotes (RS), but not the RR.

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