EFFICACY OF DIETHYL METHYLBENZAMIDE (DEET) AGAINST *AEDES DORSALIS* AND A COMPARISON OF TWO END POINTS FOR PROTECTION TIME[1]

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ABSTRACT. The repellent deet (*N,N*-diethyl-3-methylbenzamide) was tested against the mosquito *Aedes dorsalis* in a coastal salt marsh in California. The experimental design incorporated a multiple regression model, sequential treatments and a proportional end point (95%) for protection time. The ED_{95} (95% effective dose) and 4-h ED_{95} were estimated at 0.05 mg/cm^2 and 0.09 mg/cm^2, respectively. The 0.05 mg/cm^2 protection time and 0.10 mg/cm^2 protection time were estimated at 0.2 h and 4.4 h. The decay constant and half-life were estimated at 0.17 h^{-1} and 4.1 h. The design and analysis of repellent field trials are discussed.

INTRODUCTION

Deet (*N,N*-diethyl-3-methylbenzamide) and ethyl hexanediol (2-ethyl-1,3-hexanediol) are arguably the most important mosquito repellents in world commerce today. Deet was discovered in 1953 by the Department of Agriculture under funding by the Department of the Army (McCabe et al. 1954). Ethyl hexanediol was discovered in the early 1940s by Rutgers University under funding by the National Carbon Company (Granett and Haynes 1945). These are the only mosquito repellents for which Pesticide Registration Standards have been issued by the United States Environmental Protection Agency to date.

One objective of the present study was to determine the performance characteristics of deet against *Aedes dorsalis* (Meigen) in the field for comparison with equivalent data obtained separately for ethyl hexanediol (Rutledge et al. 1989). *Aedes dorsalis* is a common day- and night-biting mosquito of fresh- and saltwater marshes in North America, Europe and Asia. The only previous comparison of deet and ethyl hexanediol against *Ae. dorsalis* was that of Gilbert (1957), who reported that the protection time of 1 ml of 50% deet on the forearm was 6.7 h in Oregon while that of ethyl hexanediol was only 5.1 h. Spencer and Akers (1976) reported that deet applied to the forearms at 0.40 mg/cm^2 provided 10–12 h of protection against *Ae. dorsalis* in Colusa County, California. Lutta et al. (1966) reported that deet applied to the face, neck, hands and legs in a thin layer provided complete protection against *Ae. dorsalis* in Karelia from the time of application (in the morning?) until testing was discontinued at darkness.

A second objective of the study was to demonstrate the use of proportional end points for protection time (Buescher et al. 1983) in lieu of the fixed end points (commonly the first or second bite) employed in conventional field trials of repellents.

MATERIALS AND METHODS

The study was conducted in the field at Skaggs Island U.S. Naval Reservation, Sonoma County, California, August 23–30, 1977. Skaggs Island is a partially reclaimed marshy area to the north of San Pablo Bay, a northern extension of the San Francisco Bay. Prior surveillance had indicated that *Ae. dorsalis* was abundant in this area. The field testing site was at the edge of an oat field adjacent to the marsh. Typical plants of the coastal salt marsh community of California are arrow grass (*Triglochin concinna* and *Triglochin maritima*: Juncaginaceae), salt grass and cord grass (*Distichlis spicata* and *Spartina foliosa*: Gramineae), pickle weed and seep weed (*Salicornia virginica* and *Sueda californica*: Chenopodiaceae), frankenia (*Frankenia grandifolia*: Frankeniaceae) and sea lavender (*Limonium californicum*: Plumbaginaceae).

Eight volunteers (five male and three female) and one alternate (male) participated in the study. Each volunteer participated on two of the four test days (August 23, 24, 25 and 30, 1977). Four volunteers participated on each test day. This arrangement provided 16 replications of

[1] Opinions and assertions contained herein are the private views of the authors and should not be construed as reflecting the views of the Department of the Army or the Department of Defense. Use of a trade name does not imply official approval or indorsement of the product mentioned. All volunteers gave free and informed consent, and the investigators complied with Army Regulation 70-25 and Army Medical Research and Development Command Regulation 70-25 governing the use of volunteers in research.

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the test, distributed equally among volunteers and test days.

The test material was technical grade deet (McLaughlin Gormley King Co.). Treatments were made in the laboratory (Letterman Army Institute of Research, Presidio of San Francisco, California) in the morning and tested for residual repellency against *Ae. dorsalis* in the field (Skaggs Island U.S. Naval Reservation) in the afternoon of the same day. Deet was applied to the forearms and lower legs of the volunteers in 2.5% or 5% solution in ethanol. Prior to the study the forearms and lower legs of the volunteers had been measured to permit adjustment of the volume of solution applied for application rates of 0.05 mg/cm^2 (from 2.5% solution) and 0.1 mg/cm^2 (from 5% solution). The repellent was applied at 0.05 mg/cm^2 on two test days (August 25 and 30, 1977) and at 0.1 mg/cm^2 on two test days (August 23 and 24, 1977). This arrangement provided eight replications of the test for each dose of deet distributed equally among volunteers and test days.

The repellent solutions were applied at random to both arms and one leg or to one arm and both legs of each volunteer at 0800, 1000 and 1200 h. Solutions were measured and dispensed with a 2-ml hypodermic syringe and spread evenly over the treatment area with the tip of a glass rod. An equal volume of ethanol was applied to the repellent-free arm or leg at 1200 h as a control. The treatments were tested against *Ae. dorsalis* in the field at 1500 h (1445–1515 h) on August 23, 1977, and at 1430 h (1415–1445 h) on August 24, 25 and 30, 1977. Thus, the test periods were 3, 5 and 7 h posttreatment on August 23, 1977, and 2.5, 4.5 and 6.5 h posttreatment on August 24, 25 and 30, 1977.

At the start of the day's test each volunteer put on a head net and thick cotton gloves and sat on a camp stool with forearms and lower legs exposed. During the test each volunteer collected all mosquitoes biting his exposed arms and legs with an aspirator and placed them in prelabeled cages (one-pint cardboard cartons with screen covers). Separate cages were used for each arm and leg. At the end of the test the mosquitoes collected were returned to the laboratory for identification. *Aedes dorsalis* was the only species collected in the study.

The data from each day of testing (four replications) were pooled for analysis (Table 1). The pooled data were analyzed by multiple regression of percent repellency (probit transformation) on dose (logarithmic transformation) and test period (Rutledge et al. 1985). Confidence intervals were determined as described by Rutledge et al. (1985).

RESULTS

The multiple regression equation obtained in the tests of deet against *Ae. dorsalis* (Table 1) was

$$Y = 11.89741 + 3.98046\ X_1 - 0.28988\ X_2$$

in which Y is the estimated percent repellency in probits, X_1 is the logarithm of the applied dose in mg/cm^2, and X_2 is the test period in h. The multiple correlation coefficient ($R = 0.85$) was statistically significant ($P < 0.005$).

The dose required to provide 95% protection against *Ae. dorsalis* (ED_{95}) was estimated by substituting 0 h ($X_2 = 0$) and the probit value for 95% ($Y = 6.64975$) into the multiple regression equation and solving for the antilog of X_1.

Table 1. Test data for deet against *Aedes dorsalis* at Skaggs Island U.S. Naval Reservation, Sonoma County, California, during 1977.

Test day	Replications	Dose (mg/cm^2)	Log dose	Test period (h)	No. of bites		Percent repellency[a]	Probit value[b]
					Control	Treatment		
August 23	1–4	0.10	−1.0	3	24	0	97.9[c]	7.03
		0.10	−1.0	5	24	5	79.2	5.81
		0.10	−1.0	7	24	4	83.3	5.97
August 24	5–8	0.10	−1.0	2.5	32	0	98.4[c]	7.14
		0.10	−1.0	4.5	32	0	98.4[c]	7.14
		0.10	−1.0	6.5	32	4	87.5	6.15
August 25	9–12	0.05	−1.3	2.5	24	3	87.5	6.15
		0.05	−1.3	4.5	24	15	37.5	4.68
		0.05	−1.3	6.5	24	19	20.8	4.19
August 30	13–16	0.05	−1.3	2.5	4	0	87.5[c]	6.15
		0.05	−1.3	4.5	4	1	75.0	5.67
		0.05	−1.3	6.5	4	1	75.0	5.67

[a] Percent repellency = 100 (control − treatment)/control.
[b] Obtained in standard tables (Fisher and Yates 1963) from the percent repellency.
[c] Adjusted value for 100% observation (Armitage 1971).

The value obtained was 0.05 mg/cm^2. Since the shortest test period was 2.5 h (Table 1), this estimate is an extrapolation beyond the range of the data on which it is based and must be regarded as approximate. The dose required to provide 95% protection against *Ae. dorsalis* for four hours (4-h ED_{95}) estimated in the same way ($X_2 = 4$) was 0.09 mg/cm^2 (95% confidence limits 0.076–0.154 mg/cm^2).

The 95% protection time for the 0.05 mg/cm^2 dose (i.e., the length of the period during which the 0.05 mg/cm^2 dose will provide ≥95% protection) was estimated by substituting the logarithm of 0.05 ($X_1 = -1.30103$) and the probit value for 95% ($Y = 6.64975$) into the multiple regression equation and solving for X_2. The value obtained was 0.2 h. Since the shortest test period was 2.5 h (Table 1), this estimate is an extrapolation beyond the range of the data on which it is based and must be regarded as approximate. The 95% protection time for the 0.1 mg/cm^2 dose ($X_1 = -1$) estimated in the same way was 4.4 h (95% confidence limits 2.27–5.93 h). The steep increase in the protection time of deet from 0.2 h at 0.05 mg/cm^2 to 4.4 h at 0.10 mg/cm^2 observed in this study is similar to that observed by Buescher et al. (1983) for low doses of deet against *Ae. aegypti*. This reflects the exponential relation of dose and protection time, in which the rate of increase of protection time with dose is large at low doses and small at high doses.[5]

The decay constant (λ, Rutledge et al. 1985) was estimated from the equation

$$\lambda = -(1/\log e)(b_2/b_1)$$

in which log e (the logarithm of the base, e, of the system of natural logarithms) is 0.43429 and b_1 and b_2 are the coefficients of regression (3.98046 and −0.28988, respectively) from the multiple regression equation. The value obtained was 0.17 h^{-1} (95% confidence limits 0.046 to 0.442 h^{-1}).

The half-life ($t_{½}$, Rutledge et al. 1985) was estimated from the equation

$$t_{½} = (1/\lambda)(\log 2/\log e)$$

in which λ is 0.16769, log 2 (from the negative logarithm of ½ in the definition of half-life) is 0.30103 and log e is 0.43429. The value obtained was 4.1 h (95% confidence limits 1.57–15.20 h).

[5] The exponential relation of dose and protection time (Buescher et al. 1983) holds only if protection time is based on a proportional end point. As shown in Table 2, the relation is more complex if protection time is based on a fixed end point.

Table 2. Comparison of test method of Granett (1938) and that of Buescher et al. (1983) in terms of the percentage function (Base × Rate = Percentage) and the standard normal distribution.

Base[a] (Bites/Min)	Rate[b] (%)	Percentage[c] (Bites/Min)	Standard deviation[d]
Method of Granett (1938)			
0		No result	
5	10.00	0.5	1.28
10	5.00	0.5	1.64
15	3.33	0.5	1.83
20	2.50	0.5	1.96
25	2.00	0.5	2.05
30	1.67	0.5	2.13
35	1.43	0.5	2.19
Method of Buescher et al. (1983)			
0		No result	
5	5	0.25	1.64
10	5	0.50	1.64
15	5	0.75	1.64
20	5	1.00	1.64
25	5	1.25	1.64
30	5	1.50	1.64
35	5	1.75	1.64

[a] Biting rate on the control at the time when the end point is reached. The range tabulated (0–35 bites/min) is the range observed by Granett (1938). End point of Granett (1938) was one bite in 2 min, or 0.5 bites/min (observed); end point of Buescher et al. (1983) was 5% of the base (computed).

[b] Percent of the test population biting on the repellent treatment at the time when the end point is reached (base = 100%). In the method of Granett (1938) this is variable; in the method of Buescher et al. (1983) it is constant at 5% (proportional end point).

[c] Biting rate on the treatment (percentage of the base) at the time when the end point is reached. In the method of Granett (1938) this is set at one bite in 2 min, or 0.5 bites/min (fixed end point); in the method of Buescher et al. (1983) it is variable.

[d] Position of the rate on the horizontal axis of the standard normal curve as given in tables of the area of the normal curve (Fisher and Yates 1963). Unit of measurement is the standard deviation (SD). In the method of Granett (1938) this is a variable corresponding to the variable rate; in the method of Buescher et al. (1983) it is a constant (1.64 SD) corresponding to the constant rate. Cf. Table A.2 of Steel and Torrie (1980), which gives values of the range in unit standard deviations for sample sizes from 20 to 1,000.

DISCUSSION

In conventional repellent tests all the treatments are applied to the test participants at the same time. The time at which the treatments are applied is chosen on the basis of the expected protection time of the repellent to be tested and the time during which biting by the target species is expected to occur. After the treatments have been applied, the test participants are exposed to the test insects intermittently or con-

tinuously until the end point of the test (commonly the first or second bite) is reached. During this period the weather and the biting activity and/or population density of the target species may vary significantly.

If the biting cycle of the target species is short, as in many crepuscular species, several outcomes are possible: 1) If the treatment decays to the threshold of the target species before the biting cycle begins, the end point of the test will not occur until biting begins, and the observed protection time will be an overestimate. 2) If the treatment decays to the threshold of the target species during the biting cycle, the observed protection time will be an accurate estimate of the true protection time. 3) If the treatment decays to the threshold of the target species after the biting cycle ends, the end point of the test will not occur until the next succeeding biting cycle begins, and the observed protection time will be an overestimate. 4) In the latter situation the test may be terminated before the end point is reached, an outcome known as "plussing out." For a discussion of the consequences of plussing out see Rutledge (1988).

The present study demonstrates an experimental design intended to reduce the variance of protection time attributable to variation in the weather and the biting activity and/or population density of the target species by permitting the evaluation of treatments of varying age within a relatively short time frame. In the sequential treatment design the treatments are applied to the test participants at different times. The times at which the treatments are applied are chosen to bracket a range of possible values of protection time during the period when biting by the target species is expected to occur. After the treatments have been applied, the test participants are exposed to the test insects for a relatively short time, during which the weather and the biting activity and/or population density of the target species will be relatively constant.[6]

This kind of experimental design was pioneered by R. C. Shannon (see Travis 1951) in tests against *Anopheles*, *Culex*, and *Mansonia* mosquitoes in Trinidad in 1943 and has been used by Findlay et al. (1946) in tests against *Glossina palpalis* (Robineau-Desvoidy) (Diptera: Muscidae) in Africa, Traub and Elisberg (1962a, 1962b) in tests against *Anopheles*, *Aedes*, *Armigeres*, *Culex*, and *Mansonia* mosquitoes in Malaya, and Shimmin et al. (1974) in tests against unspecified mosquitoes in California and *Anopheles* and *Aedes* mosquitoes in North Carolina.

Granett (1938) demonstrated that protection time as defined by a fixed end point (the first bite) is inversely related to the biting rate of the mosquito test population. For example, the protection time of Repellent No. 1 (composition not stated) against *Aedes cantator* (Coq.) and *Aedes sollicitans* (Walker) in New Jersey decreased smoothly from 126 min when the biting rate was 1–2 per min to only 75 min when the biting rate was 11–15 per min. Dethier (1956) related this effect to the normal distribution of repellent tolerances in the mosquito test population.[7] On this basis Buescher et al. (1983) introduced a new definition of protection time based on a proportional end point (95% of the control) instead of the fixed end point (commonly the first or second bite) of conventional tests.[8] The purpose of this change was to reduce the variance of protection time attributable to variation in the density and/or biting activity of the insect test population.

Table 2 compares the positions of the fixed end point (first bite) of Granett (1938) and the proportional end point (95%) of Buescher et al. (1983) on the normal curve and gives the corresponding deviations from the mean of the stand-

[6] Both of the experimental designs discussed here provide any ability to determine a "protection time" for any repellent against any target species even if that "protection time" is longer than the biting cycle of the species. For example, it is possible to demonstrate a "protection time" of four hours against a species that bites for a period of only two hours. In such cases, however, it would seem that we only need to know that the protection time of the repellent is longer than the biting cycle of the target species.

[7] The tolerance distribution is actually lognormal (Rutledge et al. 1978), but this complication does not affect the present discussion.

[8] In response to this an anonymous reviewer stated: "What is the value in estimating when a material provides 95% protection? As a user of repellents, I want to know when the material fails to give 100% protection. When total protection ceases, disease transmission can start occurring." This opinion is badly out-of-date: 1) It has been known for many years that there is a critical level of the vector biting rate below which transmission of a vector-borne disease is interrupted (Macdonald 1957). Malaria and other vector-borne diseases have in fact been eradicated in many areas world-wide by reduction of the vector biting rate below the critical level with insecticides and other vector control measures. 2) The objective of 100% protection from mosquitoes ignores the basic principles of integrated pest management, by which the need for the application of control measures is determined by the economic threshold and economic injury level (Stern et al. 1959). 3) Dethier (1956), Busvine (1971), and others have shown that proportional end points such as 95% or 99% are statistically superior to fixed end points such as the first bite or the second bite. This point is discussed at length in the present paper.

ard normal curve for each method over a representative range of mosquito biting rates. In the method of Granett (1938) (fixed end point method) the magnitude of the deviation of the least repellent-sensitive individual of the mosquito test population (whose bite marks the end point of the test) from the mean *increases* with increasing density and/or biting activity of the mosquito test population. As Dethier (1956) has pointed out, this means that the time required for the treatment to decay to the threshold of the least repellent-sensitive individual (i.e., the protection time) *decreases* with increasing density and/or biting activity of the mosquito test population. In the method of Buescher et al. (1983) (proportional end point method) the magnitude of the deviation of the fifth percentile individual (whose bite marks the end point of the test) from the mean is constant at 1.64 standard deviations. This means that the time required for the treatment to decay to the threshold of the fifth percentile individual (i.e., the protection time) does *not* decrease with increasing density and/or biting activity of the mosquito test population.

As demonstrated in this study, protection time is not observed directly in the proportional end point test method. It is computed from a regression equation in the same way as the effective dose and is subject to the same rules of interpolation and extrapolation. Our estimates of the ED_{95} and the 0.05 mg/cm^2 protection time of deet were obtained by extrapolation to points between 0 and 2.5 h on the X_2 (test period) axis of regression. Such estimates are regarded as valid if "the regression relation used is known to be absolutely, or very nearly, correct in algebraic form" (Finney 1971). In the present case this point would seem to have been adequately established by prior work (Buescher et al. 1982, 1983; Rutledge et al. 1985). However, the relative accuracy of the extrapolated values remains uncertain. While procedures to establish confidence limits for extrapolated values of the dependent variable Y (the response) are available (Steel and Torrie 1980), we know of none to establish confidence limits for extrapolated values of the independent variables X_1 and X_2 (dose and test period). In general, however, such values will be more accurate for shorter rather than longer extrapolations.

As Finney (1978) has stated: "If the investigator knew the doses that would give the desired responses, he would have no need of an assay." In practice, however, the choice of dose and test period is always more or less subjective, depending on the extent of existing knowledge of the dose-response relation. In this connection one noted authority has wondered "whether the subject of repellent testing techniques should be treated as a science or as an art" (Schreck 1977). However, the scientific method of problem-solving by observation and experiment in no way excludes the process of trial and error.

ACKNOWLEDGMENTS

The authors thank C. A. Lowe, M. A. Moussa, G. N. Piper, H. S. Semey, R. K. Sofield and L. L. Young for participation in this study.

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