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VARIATION IN THE PROTECTION PERIODS OF REPELLENTS ON INDIVIDUAL HUMAN SUBJECTS: AN ANALYTICAL REVIEW[1]

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ABSTRACT. Mosquito repellent test data from the literature were analyzed to estimate mean protection periods and among-subjects standard deviations. Standard deviations were a linear function of the means. Numbers of subjects needed to determine mean protection periods of 1–8 h with confidence limits of ±0.5, 1.0, 1.5, and 2.0 h at the 99 and 95% levels of confidence were computed from regression values of the standard deviation, and a table of sample sizes was constructed for use in planning repellent tests.

KEY WORDS Repellents, insect repellents, mosquito repellents

INTRODUCTION

Wadley (1946) reported that 5 subjects differed significantly in periods of protection obtained from 6 repellents in tests against *Aedes aegypti* (L.). The among-subjects standard deviation was 2.0 h. However, review of the literature shows that the among-subjects standard deviation differs among studies. This is to be expected, because sample standard deviations are themselves variable, with the standard error of a sample standard deviation from a normal population being $\sigma/\sqrt{(2n)}$.

Because the size of sample needed to estimate the mean of a normal population with a specified degree of precision at a specified level of confidence is determined by the standard deviation, it is desirable to estimate the among-subjects standard deviation of protection periods as accurately as possible for efficient planning of repellent tests.

The present study analyzed data from previous studies to estimate mean protection periods and among-subjects standard deviations. The estimates so obtained were further analyzed to estimate the numbers of subjects needed for selected degrees of precision and levels of confidence in the determination of protection periods.

MATERIALS AND METHODS

Computation of means and standard deviations: Twenty-two estimates of mean and standard deviation were obtained from 19 source studies (Table 1). Relevant parameters of the data analyzed are given in Table 2. Because the data reported and the experimental designs employed in the source studies were variable, methods of computation employed in the study will be described here in general terms only. Specifics of the methods employed are documented in the Appendix.

Walker and Lev (1953) provided formulas for computing the mean of a total group, sum of squares among groups, and sums of squares within groups, when only group means, number of cases, and variance or standard deviation are given. Fisher and Yates (1963) provided formulas and tables for estimating the standard deviation from the range and sample size. Langley (1970) provided formulas for combining means or standard deviations of random samples of the same statistical population. Mandel (1984) provided formulas for pooling the means of samples having different standard deviations or the standard deviations of samples having different means. In most cases, these formulas and tables were sufficient for purposes of the study.

Protection period is defined as the period between the time of application of the repellent and the time of occurrence of a specified end point, commonly the 1st or 2nd observed bite. If the test is terminated before the end point is reached, the result is reported as an inequality (e.g., >120 or "120+" min). Although the standard deviation can not be computed from data containing inequalities (Rutledge 1988), deletion of the inequalities introduces bias, because the values deleted are larger than those retained. Therefore, in the present study, repellents for which inequalities were reported were excluded from analysis. Repellents having long protection periods may be correspondingly underrepresented.

Because each source study was unique and may or may not have common factors with any other, mean protection periods were computed as the means of the observed protection periods, without adjustment for specific factors or variables operating in the source study. Protection periods and standard deviations reported in minutes were converted to hours for comparative purposes.

To simplify computations, among-subjects standard deviations were computed without adjustment for correlation of means and standard deviations within source studies. This approximation exaggerates the estimate of among-subjects standard deviation, although variation within studies is usually smaller than variation among studies. The bias is

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Table 1. Sources of the data analyzed.

Reference no.[1]	Citation	Data analyzed
1	Gilbert et al. (1966)	Table 1, men
2	Gilbert et al. (1966)	Table 1, women
3	Traub and Elisberg (1962)	Table 3, repellent M-2020
4	Altman (1969)	Tables 1–3
5	Applewhite and Smith (1950)	Tables 1 and 2
6	Dua et al. (1996)	Page 407
7	Gouck and Bowman (1959)	Table 3
8	Smith et al. (1963)	Tables 5–9, 11, 12, 15, 19
9	Pijoan et al. (1946)	Table 1
10	Schreck and Smith (1977)	Table 2, Series 1
11	Travis (1950)	Table 1
12	Whittemore et al. (1961)	Table 2
13	Traub and Elisberg (1962)	Table 3, deet
14	Wadley (1946)	Page 31
15	Spencer et al. (1977)	Table 1
16	Wiesmann and Lotmar (1949)	Table 1
17	Spencer et al. (1976)	Table 3
18	Wiesmann and Lotmar (1949)	Page 299
19	Spencer and Akers (1976)	Table 1
20	Rietschel and Spencer (1975)	Table
21	Skinner et al. (1977)	Table 1
22	Reifenrath and Akers (1981)	Table 2

[1] Identifies corresponding entries in Tables 2 and 3 and the Appendix.

conservative in the sense that it maximizes the estimate of the among-subjects standard deviation and leads to a larger estimate of the number of subjects required.

The number of subjects employed in certain source studies was unclear because of uncertainty as to whether the same or different subjects were employed in tests conducted at different times and places. In such cases, the number of subjects was taken to be the minimum number needed to account for the data analyzed. This approach is conservative in the sense that it maximizes the estimate of the among-subjects standard deviation.

Where the source study reported observed or

Table 2. Relevant parameters of the data analyzed.

Reference no.[1]	State or country	Setting	Mosquito species	Test materials	Test subjects
1	Florida	Laboratory	1	1	50
2	Florida	Laboratory	1	1	50
3	Malaysia	Field	3	1	10
4	Panama	Field	1	2	5
5	Alaska	Field	4	13	9
6	India	Laboratory	1	1	5
7	Florida	Laboratory	1	3	3
8	Florida	Laboratory	1	3	8
9	Maryland	Laboratory	1	2	3
10	Florida	Field	1	2	5
11	Florida	Laboratory and field	4	12	6
12	Texas	Field	1	2	10
13	Malaysia	Field	3	1	10
14	Florida	Laboratory	1	6	5
15	California	Laboratory	1	4	8
16	Argentina	Field	8	2	8
17	California	Laboratory	1	7	16
18	France	Field	2	1	6
19	Florida	Field	1	3	4
20	California	Laboratory	1	1	16
21	California	Laboratory	1	1	11
22	California	Field	1	2	4

[1] See corresponding entry in Table 1 for identification of source study.

Table 3. Mean protection periods and standard deviations.

Reference no.[1]	Mean (h)	Standard deviation (h)
1	0.48	0.65
2	0.65	0.52
3	1.06	0.21
4	1.06	0.59
5	1.38	0.94
6	1.90	0.40
7	2.14	2.97
8	2.20	3.09
9	2.71	0.51
10	3.23	0.58
11	3.32	4.08
12	3.41	0.44
13	3.98	1.71
14	4.44	1.99
15	4.75	2.54
16	5.50	2.77
17	5.70	2.55
18	5.72	0.85
19	6.37	1.84
20	6.45	1.69
21	6.93	4.41
22	8.50	4.40

[1] See corresponding entry in Table 1 for identification of source study.

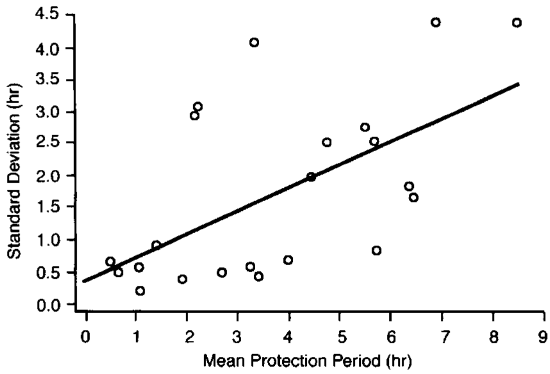

Fig. 1. Linear regression of standard deviations on mean protection periods: $Y = 0.3705 + 0.3596X$.

mean protection periods obtained on individual subjects, the among-subjects mean square was computed by analysis of variance, and the standard deviation was obtained as the square root of the among-subjects mean square. One-way, 2-way, or other conventional statistical designs were employed where possible.

Multivariate methods were employed to analyze data compiled from disparate experiments on the same subjects and to analyze data from experiments with asymetrical structure and/or missing or excluded observations. Because order of effects is important in multivariate statistical analyses (Mead 1990), effects attributable to subjects were given priority over other factors. This approach is conservative in the sense that it maximizes the estimate of the among-subjects standard deviation.

Where the source study reported among-subjects ranges and/or standard deviations of protection periods separately for 2 or more tests, the combined standard deviation was computed as described by Mandel (1984) from pooled sums of squares obtained by back-calculation from the among-subjects ranges or standard deviations (Fisher and Yates 1963, Mandel 1984).

Analysis of means and standard deviations: A linear regression of standard deviations on mean protection periods was computed. In computing the regression, observations were weighted by the number of subjects tested, as shown in Table 2. Means, standard deviations, and residuals from regression were tested for outlying observations by Grubb's test (Dunn and Clark 1974).

Sampling table: A table was constructed to provide numbers of subjects needed to determine protection periods of 1–8 h with confidence limits of ±0.5, 1.0, 1.5, and 2.0 h at the 99 and 95% levels of confidence. Estimates of required sample sizes were computed from the standard deviation as described by Martin and Bateson (1993).

RESULTS AND DISCUSSION

Means and standard deviations

Mean protection periods computed from the data identified in Table 1 ranged from 0.48 h (data of Gilbert et al. 1966) to 8.50 h (data of Reifenrath and Akers 1981) (Table 3). The extreme values were not significant by Grubb's test for outliers ($T_1 = 1.419$, $T_{22} = 2.092$, $n = 22$, $P > 0.05$).

Standard deviations computed from the data identified in Table 1 ranged from 0.21 h (data of Traub and Elisberg 1962) to 4.41 h (data of Skinner et al. 1977) (Table 3). The extreme values were not significant by Grubb's test for outliers ($T_1 = 1.114$, $T_{22} = 1.905$, $n = 22$, $P > 0.05$).

Analysis

The linear regression of standard deviations on mean protection periods was

$$Y = 0.3705 + 0.3596X,$$

where Y is the standard deviation and X is the mean protection period (Fig. 1). The residuals from regression ranged from −1.58 h (data of Wiesmann and Lotmar 1949) to +2.52 h (data of Travis 1950). The extreme values were not significant by Grubb's test for outliers ($T_1 = 1.461$, $T_{22} = 2.209$, $n = 22$, $P > 0.05$).

The coefficient of correlation was significant ($r = 0.60$, df = 20, $P < 0.05$). The coefficient of determination ($r^2 = 0.51$) indicated that 51% of the observed variation in the standard deviations was attributable to variation in mean protection periods. The remaining variation can be attributed to variation in species, climate, season, weather, materials and methods, and other variables associated with the respective source studies (Table 2).

Note added in revision: At the suggestion of an anonymous reviewer (Reviewer 1), additional analyses were performed to determine if the effects of locale (state/country) or setting (laboratory/field) (Table 2) on protection periods were significant. Neither factor was significant when included in the analysis ($F = 0.65$, df = 9,9, $P > 0.05$ and $F = 3.13$, df = 2,9, $P > 0.05$, respectively).

The original version of this paper included chi-square tests for goodness of fit of the observed distributions of means, standard deviations and residuals to the normal distribution (Steel and Torrie 1980). Values of χ^2 were not statistically significant ($\chi^2 = 0.95$, df = 3, $P > 0.05$ for means; $\chi^2 = 7.15$, df = 3, $P > 0.05$ for standard deviations; $\chi^2 = 4.65$, df = 3, $P > 0.05$ for residuals).

However, Reviewer 1 found that the distribution of standard deviations differed significantly from normal in a computer simulation and by the Kolmogorov–Smirnov test, the Box–Cox procedure, and the plot of order statistics. On the basis of the Box–Cox analysis, Reviewer 1 reanalyzed the data using a logarithmic transformation of the standard deviations, concluding that "the sample sizes [so obtained] were not too different [from those of Table 4], so that the extra effort was not overly fruitful and the interpretation of the simpler model was lost."

Similarly, an in-house reviewer (Reviewer 3) found that the distribution of standard deviations differed significantly from normal by the Anderson–Darling test. On this basis, Reviewer 3 fitted a quadratic (2nd degree polynomial) curve to the data, concluding that "the fitted values for standard deviation based on quadratic fit to the smoothed data show[ed] little difference [from those based on linear regression] through 7 h [of protection]."

In an additional analysis, Reviewer 3 grouped source studies with similar mean protection periods to compute the bias error, pure error, and F value for lack of fit (Draper and Smith 1981). Because the value of F was not statistically significant, Reviewer 3 concluded that the F test for lack of fit provided no reason to doubt the adequacy of the linear regression model.

According to Draper and Smith (1981), the ratio of the F value for regression to the tabulated value must be ≥4 for the regression to be useful, as opposed to being merely significant. Reviewer 3 found that this ratio was 4.75 in the present study and concluded that the regression model was useful. In this connection, Martin and Bateson (1993) have suggested that the correlation observed in the study ($r = 0.60$) can be interpreted as moderate,

Table 4. Numbers of subjects needed to determine protection periods of 1–8 h with confidence limits of ±0.5–2.0 h at the 99 and 95% levels of confidence.[1]

Protection period (h)	Standard deviation[2] (h)	$D = 0.5$ h	$D = 1.0$ h	$D = 1.5$ h	$D = 2.0$ h
			$\alpha = 0.01$		
1	0.73	15	4	2	1
2	1.09	32	8	4	2
3	1.45	56	14	7	4
4	1.81	87	22	10	6
5	2.17	125	32	14	8
6	2.53	170	43	19	11
7	2.89	222	56	25	14
8	3.25	280	70	32	18
			$\alpha = 0.05$		
1	0.73	9	3	1	1
2	1.09	19	5	3	2
3	1.45	33	9	4	3
4	1.81	51	13	6	4
5	2.17	73	19	9	5
6	2.53	99	25	11	7
7	2.89	129	33	15	9
8	3.25	163	41	19	11

[1] Numbers of subjects were computed from the formula: $n = (s^2 z_{\alpha/2}^2)/D^2$, where n is the number of subjects, s is the standard deviation, $z_{\alpha/2}$ is the critical value of the cumulative normal variable z at the $\alpha/2$ level of significance, α is the level of statistical significance to be attached to the estimate, and D is the maximum acceptable difference between the sample mean and the true (population) mean (Martin and Bateson 1993). Results of computation were rounded to the next higher integer, as the number of subjects cannot be fractional.

[2] Standard deviations were computed from the regression equation $Y = 0.3705 + 0.3596X$, where Y is the standard deviation and X is the mean protection period (see text).

indicating a substantial relationship of means and standard deviations.

In a further analysis, Reviewer 3 identified observations 1 and 2 as particularly influential and reanalyzed the data with those observations deleted to determine their effect on the conclusions of the study. Reviewer 3 concluded that "The prediction equation was hardly altered by deleting these two observations, but the . . . ratio of F values fell . . . to 1.9." Because neither the X values (0.48 and 0.65, respectively) nor the Y values (0.65 and 0.52, respectively) of observations 1 and 2 were significant outliers (see above), we suggest that the relatively large influence of observations 1 and 2 reflects the relatively large weights assigned to those observations in the regression analysis (Table 2).

Our decision to retain the original (linear regression) analysis was based on several considerations. In our opinion, a point exists beyond which increasingly refined and sophisticated statistical analyses yield diminishing returns in terms of clarity and credibility of presentation. Many phenomena result in data distributed in a manner sufficiently normal to provide the basis of theory in biology and other fields of application (Steel and Torrie 1980). In the present case, neither logarithmic transformation (Reviewer 1) nor quadratic curve fitting (Reviewer 3) materially changed the outcome of the analysis. Testing for lack of fit, useful regression, and influential observations (Reviewer 3) tended to support the linear regression model.

Sampling table

Because the among-subjects standard deviation of protection periods is a function of the mean, it is necessary to know an approximate value of the mean to compute the number of subjects needed to determine the mean precisely. This requirement for advance knowledge of the parameter to be estimated is common in repellent studies (Rutledge et al. 1989) and in bioassay studies in general (Finney 1978).

Table 4 provides estimated among-subjects standard deviations for mean protection periods of 1–8 h and the corresponding numbers of subjects needed to determine the mean protection period with confidence limits of ±0.5, 1.0, 1.5, and 2.0 h at the 99 and 95% levels of confidence. Given the uncertainty in the standard deviations from which the sample sizes were derived, the values shown should be regarded as guidelines only. However, uncertainties in the source studies were interpreted conservatively (see the Materials and Methods section and the Appendix), and we believe that the values given will be found useful in practice.

This paper is the 1st published attempt to determine the number of subjects needed in repellent tests. Additional research is needed to refine and extend Table 4, taking into account variation in species, climate, season, weather, materials and methods, and other variables present in repellent tests.

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APPENDIX

This Appendix documents the methods used in computing the means and standard deviations shown in Table 3 from the data identified in Table 1. The information provided is not essential for understanding the body of the report. Section numbers of the Appendix correspond with the reference numbers of Tables 1–3. Symbols and terms follow Steel and Torrie (1980). The terms "mean square" and "variance" are equivalent.

Methods of computing means from data that do not include the observed values were described by Walker and Lev (1953) and Langley (1970) and will not be repeated here. For brevity, the methods described for computing among-subjects standard deviations are considered complete when the variance attributable to subjects is obtained, with further computation of the standard deviation as the square root of the variance being understood.

Where source studies reported among-subjects ranges and/or standard deviations of protection periods separately for 2 or more tests, the combined standard deviation was computed as described by Mandel (1984) from pooled sums of squares obtained by back-calculation from the among-subjects ranges or standard deviations (Fisher and Yates 1963, Mandel 1984). For brevity, this procedure is referred to as "pooling."

1) Gilbert et al. (1966, Table 1, men) reported the among-subjects range of means of 4 "readings" of the protection period of deet on 50 men in tests against *Ae. aegypti*. The standard deviation corresponding to the stated range was obtained from Table XX of Fisher and Yates (1963) and multiplied by $\sqrt{4}$ to obtain the among-subjects standard deviation on a per-observation basis (Steel and Torrie 1980:142).

2) Gilbert et al. (1966, Table 1, women) reported the among-subjects range of means of 4 "readings" of the protection period of deet on 50 women in tests against *Ae. aegypti*. The among-subjects standard deviation was obtained as described in Section 1.

3) Traub and Elisberg (1962, Table 3, repellent M-2020) reported among-subjects standard deviations obtained in 6 determinations of the protection period of repellent M-2020 on 10 subjects in tests against a natural association of mosquitoes in Malaysia. The 6 standard deviations were pooled to obtain the combined among-subjects standard deviation.

4) Altman (1969, Tables 1–3) reported the observed protection periods of various concentrations of 6 repellents on 5 subjects in tests against *Anopheles albimanus* Wiedemann in Panama. The present analysis was limited to 50% *N,N*-diethylbenzenesulfonamide (Table 1: 2 subjects, 1 replication) and 25% dimethyl phthalate (Table 3: 4 subjects, 2 replications), because certain tests of the other repellents were terminated before completion.

The among-subjects mean square was estimated by multivariate statistical analysis. The model employed in the analysis included the response variable, PROTECTION PERIOD (quantitative), and 2 explanatory variables, SUBJECT (qualitative) and REPELLENT (qualitative). SUBJECT included 5 classes: subjects PB, RA, VA, VB, and WL. REPELLENT included 2 classes: 50% *N,N*-diethylbenzenesulfonamide and 25% dimethyl phthalate.

5) Applewhite and Smith (1950, Tables 1 and 2) determined the protection periods of 10 repellents on 9 subjects in tests against natural associations of mosquitoes at Anchorage (June 29–July3, 1948) and Big Delta (July 8–12), Alaska. Six of the 10 repellents were retested on 5 subjects at Big Delta (July 16–18), and one of the 6 was retested in comparison with 3 additional repellents on 3 subjects at Big Delta and Eilsen Field (July 16–18). In each case, each repellent was tested once on each subject, and the among-subjects range of protection periods was reported.

For purposes of analysis, it was assumed that 9 subjects were employed in the tests and that groups of 3 and 5 subjects were chosen at random from the 9 for the July 16–18 tests. Data from tests of 3 repellents at Anchorage were excluded from analysis, because certain tests of those repellents at that location were terminated before completion. Standard deviations corresponding to the remaining among-subjects ranges were obtained from Table XX of Fisher and Yates (1963) and pooled to obtain the combined among-subjects standard deviation.

6) Dua et al. (1996:407) reported the among-subjects standard deviation of protection periods of an extract of flowers of *Lantana camara* (Verbenaceae) on 5 subjects in tests against *Aedes albopictus* (Skuse). No additional analysis was needed in this case.

7) Gouck and Bowman (1959, Table 3) reported subject means obtained in 4 determinations of the protection periods of 3 repellents on 3 subjects in tests against *Ae. aegypti.* Subject means were converted to totals, and the among-subjects mean square was obtained as in the analysis of variance.

8) Smith et al. (1963, Tables 5–9, 11, 12, 15, 19) reported mean protection periods of varying doses of 3 repellents on 8 subjects in tests against *Ae. aegypti* under varying experimental conditions.

The among-subjects mean square was estimated by multivariate statistical analysis. The model employed in the analysis included the response variable, PROTECTION PERIOD (quantitative), and 5 explanatory variables, DOSE (quantitative), SUBJECT (qualitative), REPELLENT (qualitative), END POINT (qualitative), and SKIN TREATMENT (qualitative). SUBJECT included 8 classes: subjects A–H. REPELLENT included 3 classes: dimethyl phthalate, ethyl hexanediol, and deet. END POINT included 2 classes: the 1st bite and the 5th bite. SKIN TREATMENT included 4 classes: sweated (Table 5), disinfected (Table 6), shaved (Table 15), and normal.

In the source study, doses were reported in terms of concentration (%) and volume (ml) of material applied per forearm (Table 5), weight (g) of material applied per forearm (Table 6), or weight (mg) of material applied per unit area (in.^{2}) of forearm (Tables 7–9, 11, 12, 15, 19). In the present study, doses were converted to mg/cm^2 using appropriate conversion factors and the surface areas of the forearms of the subjects as given in Table 1 of Smith et al. (1963). Because protection periods are proportional to the logarithm of the dose applied (Rutledge et al. 1989), values of DOSE were entered as log mg/cm^2.

Means reported in the source study were based on 1 (Tables 6, 7, 9, 12), 2 (Table 15), 4 (Tables 5, 11, 19), or 6 (Table 8) repetitions of the test procedure. In the present analysis, entries were weighted by the number of repetitions to obtain the among-subjects mean square on a per-observation basis.

9) Pijoan et al. (1946, Table 1) reported the observed protection periods of 2 repellents on 3 subjects in tests against *Ae. aegypti.* Tests were conducted in 4 blocks defined by ambient temperature and humidity and level of physical activity of the subjects. Protection periods were recorded separately for the left and right forearms. In the present study, the data were analyzed as a $2 \times 3 \times 4$ (2 treatments $\times$ 3 subjects $\times$ 4 blocks) experimental design with duplicate observations (left and right arms) to obtain the among-subjects mean square.

10) Schreck and Smith (1977, Table 2, Series 1) reported among-subjects ranges of the protection periods of 2 repellents on 5 subjects in tests against *Aedes taeniorhynchus* (Wiedemann) in Florida. Standard deviations corresponding to the ranges were obtained from Table XX of Fisher and Yates (1963) and pooled to obtain the combined among-subjects standard deviation.

Note: Ranges reported by Schreck and Smith (1977) in Series 2 and 3 of Table 2 include 2 observations on each subject. Because the ranges refer to observations, not subjects, they could not be used in the study.

11) Travis (1950, Table 1) reported mean protection periods of dimethyl phthalate, butopyronoxyl, and a set of 10 unspecified repellents on 3 (dimethyl phthalate), 6 (butopyronoxyl), or 4 (10 repellents) of 6 subjects in tests against *Anopheles quadrimaculatus* Say (dimethyl phthalate), *Ae. aegypti* (dimethyl phthalate), *Aedes sollicitans* (Walker) (butopyronoxyl), or *Ae. taeniorhynchus* (10 repellents).

The among-subjects mean square was estimated by multivariate statistical analysis. The model employed in the analysis included the response variable, PROTECTION PERIOD (quantitative), and 3 explanatory variables, SUBJECT (qualitative), REPELLENT (qualitative), and SPECIES (qualitative). SUBJECT included 6 classes: subjects 1–6. REPELLENT included 3 classes: dimethyl phthalate, butopyronoxyl, and 10 repellents. SPECIES included 4 classes: *An. quadrimaculatus, Ae. aegypti, Ae. sollicitans,* and *Ae. taeniorhynchus.*

Means reported in the source study were based on 28 (*An. quadrimaculatus*), 20 (*Ae. aegypti*), 4 (*Ae. sollicitans*), or 10 (*Ae. taeniorhynchus*) repetitions of the test procedure. In the present analysis, entries were weighted by the number of repetitions to obtain the among-subjects mean square on a per-observation basis.

12) Whittemore et al. (1961, Table 2) reported means ($\bar{Y}$) and standard deviations (s) of the protection periods of 2 repellents obtained in paired observations on 10 (n) subjects in tests against *Aedes scapularis* (Rondani) in Texas. The value of Student's t was also reported. In the present study the data were reanalyzed by 2-way (2 treatments $\times$ 10 subjects) analysis of variance. Validity of the reanalysis was verified by performing the same op-

erations on a worked example of the t-test provided by Steel and Torrie (1980:103).

The sums of the squares of the observations in each treatment were obtained by back calculation from s as $(n - 1)s^2 + (n\bar{Y})^2/n$. The total sum of squares (total SS) was then obtained by combining the sums of squares so obtained and subtracting the correction term, $C = [\Sigma(n\bar{Y})]^2/\Sigma n$.

The treatment mean square (treatment MS) was obtained from the treatment totals $(n\bar{Y})$ as in the analysis of variance. The error MS was obtained as treatment MS/F, where F is the variance ratio (treatment MS/error MS) obtained as $F = t^2$ (Steel and Torrie 1980:144). Treatment MS and error MS were multiplied by the respective numbers of degrees of freedom to obtain the treatment SS and error SS, and the among-subjects mean square was obtained as (total SS − treatment SS − error SS)/$(n - 1)$.

13) Traub and Elisberg (1962, Table 3, deet) reported the among-subjects standard deviations obtained in 6 determinations of the protection period of deet on 10 subjects in tests against a natural association of mosquitoes in Malaysia. The combined among-subjects standard deviation was obtained as described in Section 3.

14) Wadley (1946:31) reported the among-subjects sum of squares and its associated degrees of freedom in a balanced incomplete block test of 6 repellents on 5 subjects against *Ae. aegypti.* The among-subjects mean square was obtained by dividing the sum of squares by the number of degrees of freedom.

15) Spencer et al. (1977, Table 1) reported among-subjects standard deviations of the protection periods of 4 repellents on 8 subjects in tests against *Ae. aegypti.* Two of the repellents were tested once on each subject, and 2 were tested twice on each subject. The 6 standard deviations were pooled to obtain the combined among-subjects standard deviation.

16) Wiesmann and Lotmar (1949, Table 1) reported the numbers of bites observed 2, 4, 6, 9, 11, and 13 h after application of 2 repellents to 1–8 subjects at a normal rate, a half-normal rate, or as needed for coverage. Tests were conducted against a natural association of mosquitoes in Argentina.

For purposes of the present study, the end point of the protection period was considered to be the midpoint in time between the last recorded negative observation and the 1st recorded positive observation (Rutledge 1988). For example, where 0, 0, 0, 3, 0, and 3 bites were reported at 2, 4, 6, 9, 11, and 13 h, the protection period was considered to be (6 + 9)/2 = 7.5 h.

Protection periods were analyzed as a 2 × 3 (2 repellents × 3 application rates) experimental design with unequal replication (1–8 subjects) using multivariate statistical analysis. The model employed in the analysis included the response variable, PROTECTION PERIOD (quantitative), and 2 explanatory variables, REPELLENT (qualitative) and APPLICATION RATE (qualitative). REPELLENT included 2 classes: epellent 6–2–2 and Kik–Geigy. APPLICATION RATE included 3 classes: normal, half-normal, and as-needed.

In this analysis, the error (within-treatments) mean square represents the among-subjects variance. The estimate is a conservative approximation, because it includes experimental error and is an overestimate.

17) Spencer et al. (1976, Table 3) reported among-subjects standard deviations of the protection periods of 7 repellents on 4–16 subjects in tests against *Ae. aegypti.* Subjects were chosen at random from a pool of 30 males. The standard deviations were pooled to obtain the combined among-subjects standard deviation.

18) Wiesmann and Lotmar (1949:299) reported among-subjects ranges of protection periods of Kik–Geigy repellent obtained in 6 tests against a natural association of mosquitoes in France. The number of subjects employed in the tests was stated to be 5 or 6, but the numbers employed in specific tests were not given. As a conservative approximation, the number of subjects was considered to be 5 in each test. Standard deviations corresponding to the among-subjects ranges were obtained from Table XX of Fisher and Yates (1963) and pooled to obtain the combined among-subjects standard deviation.

19) Spencer and Akers (1976, Table 1) reported among-subjects standard deviations of protection periods of 3 repellents on 4 subjects in tests against *Ae. taeniorhynchus* in Florida. The standard deviations were pooled to obtain the combined among-subjects standard deviation.

Note: Data of Spencer and Akers (1976, Table 2) were not analyzed, because testing of certain (unspecified) repellents was terminated before completion.

20) Rietschel and Spencer (1975, Table) reported among-subjects standard deviations of the protection periods of 0.16 mg/cm^2 and 0.32 mg/cm^2 deet on 16 subjects in tests against *Ae. aegypti.* The standard deviations were pooled to obtain the combined among-subjects standard deviation.

21) Skinner et al. (1977, Table 1) reported mean protection periods of deet on 11 subjects in tests against *Ae. aegypti.* The test procedure was repeated 2–8 times on each subject. Subject means were converted to totals, and the among-subjects mean square was computed as in the analysis of variance.

22) Reifenrath and Akers (1981, Table 2) reported the observed protection periods of 4 repellents on 4 subjects in tests against *Anopheles freeborni* Aitken in California. Data for 2 repellents were excluded from the present analysis, because testing of those repellents was terminated before completion. Data for 1-(butylsulfonyl)-hexahydro-1*H*-azepine and triethylene glycol monohexyl ether were analyzed by 2-way (4 subjects × 2 repellents) analysis of variance to obtain the among-subjects mean square.