MATHEMATICAL MODELS OF THE EFFECTIVENESS AND PERSISTENCE OF MOSQUITO REPELLENTS[1]

L. C. RUTLEDGE, R. A. WIRTZ[2], M. D. BUESCHER[3] AND Z. A. MEHR
Letterman Army Institute of Research, Presidio of San Francisco, CA 94129

ABSTRACT. Two models of the effectiveness and persistence of mosquito repellents on the skin were developed from published functions and data. The probit plane model, $Y = a + b_1X_1 + b_2X_2$, relates the response (Y, in probits) of the mosquito test population to the log dose (X_1) of repellent applied and the test period, or elapsed time from the time of application (X_2). The exponential decay model, $Z = X_1 + (b_2/b_1)X_2$, estimates the repellent residue ($\log^{-1} Z$) from X_1 and X_2. The models were validated with original data from tests of deet (*N,N*-diethyl-3-methylbenzamide) and ethyl hexanediol (2-ethyl-1,3-hexanediol) on the forearm against the yellow fever mosquito, *Aedes aegypti*. The probit plane model was evaluated as $Y = 8.83 + 1.56 X_1 - 0.69X_2$ for deet and $Y = 8.67 + 1.68X_1 - 0.92X_2$ for ethyl hexanediol when X_1 is in log mg/cm² and X_2 is in hours. The exponential decay model was evaluated as $Z = X_1 - 0.45X_2$ for deet and $Z = X_1 - 0.55X_2$ for ethyl hexanediol. The decay constant (λ) and half-life ($t_{1/2}$) were estimated as 1.03 hr^{-1} and 0.67 hr for deet and 1.26 hr^{-1} and 0.55 hr for ethyl hexanediol from the slope parameter (b_2/b_1) of the decay model. Applicable correlation coefficients, standard errors and confidence limits are given. The introduction of these models of the pharmacodynamics of mosquito repellents is a step toward establishing a rational basis for the research, development, testing and evaluation of repellents and for their regulation by the government. In addition, the mathematical functions employed may provide important clues to the basic physiological, ecological and behavioral mechanisms of repellent activity.

INTRODUCTION

In 1943 D. J. Finney showed that the aversion response of honey bees to lime sulfur, in probability units (= probits), was proportional to the logarithm of the dose (Finney 1943, 1947)[4]. Since that time this basic dose-response relationship has been established for many additional repellents and insect species in a variety of test systems. In particular, Buescher et al. (1982), working with the yellow fever mosquito, *Aedes aegypti* (L.), demonstrated its validity for several commercial mosquito repellents applied to the arms of volunteer test subjects.

In a subsequent study (Buescher et al. 1983) it was shown that, for a given probit level, the period of protection provided by deet (*N,N*-diethyl-3-methylbenzamide) was also proportional to the logarithm of the dose. It follows that, for a given dose, the level of protection provided will be directly proportional to the period of time that has passed from the time of application. Since the level of protection is known to fall off with time, the proportionality constant will, in this case, be negative. It is a curious circumstance that no prior statement of this relationship has been made, although several authors (notably Shannon 1951, Quraishi et al. 1958, Traub and Elisberg 1962 and Weaving and Sylvester 1967) have reported data suitable for analysis.

The degradation of protection with time reflects the loss of repellent from the skin through evaporation, absorption, sweating and wear (Maibach et al. 1974a, 1974b; Skinner and Johnson 1980). Notwithstanding the consider-

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

[2] Present address: Walter Reed Army Institute of Research, Washington, DC 20307.

[3] Present address: U. S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, MD 21701.

[4] In this paper the term "dose" is used to mean the amount, concentration, or rate of application of active ingredient applied in a given treatment or replicate of a repellent experiment, test, or trial. The term "response" is used to mean the response of the mosquito test *population*. This is defined on the basis of the percentage of the population that is repelled by the applied dose or its residue and is expressed in probability units, or probits.

The "test period" is the measured period between the time when the dose is applied and the time when the response is determined. This period should be distinguished from the "exposure period" and the "holding period" of insecticide susceptibility tests and from the "repellent time" or "protection time" of Rudolfs (1930) and his followers. The latter is a compound function of the effectiveness and persistence of the test material (Busvine 1971).

The "residue" is that part of the applied dose that remains on the skin at the end of the test period. This usage is equivalent to that of the Federal Food, Drug and Cosmetic Act, but our context and concerns are obviously different.

The terms "effectiveness" and "persistence" are used here in the way that they are customarily used in insecticide work (World Health Organization 1963). These two terms have been confused by most repellent workers in the past (Busvine 1971).

able knowledge of the modes of loss of repellents from the skin now available, no quantitative model for the decay of the repellent dose over time has been formulated. However, if we assume that the response of the mosquito test population to a repellent residue is the same as that to a freshly applied dose of the same strength, which seems reasonable, it is possible to derive the decay curve indirectly from the known relationships of dose, response and time. The central concept in this approach is that of classical bioassay, by which the strength of a substance is determined by its effect on the test organism.

In view of the foregoing the present study was undertaken to: (1) Develop and validate a biological model relating dose, time and the effectiveness of mosquito repellents on the skin and, (2) Derive and evaluate a physical model for the decay of repellent deposits on the skin over time.

MATERIALS AND METHODS

MOSQUITOES. The University of California at San Francisco (UCSF) strain of *Ae. aegypti* was used in the study. The source of the strain and a description of our standard rearing procedures were given by Rutledge et al. (1978). The mosquitoes used were nulliparous females of mixed age in the range of 5 to 15 days.

REPELLENTS. The repellents used were technical grade deet (McLaughlin Gormley King Co.) and technical grade ethyl hexanediol (2-ethyl-1,3-hexanediol, Eastman Organic Chemicals). All dilutions were prepared with absolute ethanol.

METHODS. The methods used in the study were adapted from those of Buescher et al. (1982, 1983) as follows: (1) For variable dose/fixed time experiments five 29-mm diam. circular test areas were outlined on the flexor region of the forearm with a fine-point felt-tip pen. The test areas were treated at random with 0.025 ml of four serial dilutions of the test repellent and a control (absolute ethanol). At the time of the test a 4 × 5 × 18 cm plastic test cage containing 15 mosquitoes was applied to the forearm and a slide was withdrawn to expose the five test areas through matching holes in the floor of the cage. The number of mosquitoes biting in each test area at the end of 90 seconds was then recorded. (2) For fixed dose variable time experiments four test areas were treated at random with a fixed dose 1, 2, 3 and 4 hr before the test cage was applied to the forearm. The fifth test area was treated with absolute ethanol 5 min before the test cage was applied, as a control. Data were recorded as in the variable dose/fixed time experiments.

The test dosages and test periods used in the study are shown in columns d and f of Tables 1 (deet) and 2 (ethyl hexanediol). Column b shows the number of trials performed in each experiment. All except 11 of the 219 trials were performed on the same 3 test subjects. All trials were performed under equal conditions. During the test period the test subjects performed their normal laboratory and office duties but did not wash or abrade the test areas. Participants in the study gave free and informed con-

Table 1. Test data for deet.

(a) Exp. No.	(b) No. Trials	(c) Treatment No.	(d) Dose (mg/cm²)	(e) Log Dose	(f) Test Period	No. of bites (g) Control	No. of bites (h) Treatment	(i) % Repellency*	(j) Probit
I	12	1	0.002	−2.70	0 hr	33	27	18.2	4.09
		2	.004	−2.40	"	"	14	57.6	5.19
		3	.008	−2.10	"	"	6	81.8	5.91
		4	.016	−1.80	"	"	1	97.0	6.88
II	28	5	0.16	−0.80	4 hr	65	20	69.2	5.50
		6	.32	−0.49	"	"	14	78.5	5.79
		7	.64	−0.19	"	"	12	81.5	5.90
		8	1.28	+0.11	"	"	4	93.8	6.54
III	45	9	0.20	−0.70	1 hr	126	5	96.0	6.75
		10	"	"	2	"	27	78.6	5.79
		11	"	"	3	"	78	38.1	4.70
		12	"	"	4	"	118	6.3	3.47
IV	22	13	0.30	−0.52	1 hr	56	0	99.1**	7.37
		14	"	"	2	"	8	85.7	6.07
		15	"	"	3	"	2	96.4	6.80
		16	"	"	4	"	13	76.8	5.73

* % Repellency = 100 X (Control − Treatment) ÷ Control.
** Adjusted value for 100% observation (Armitage 1971).

Table 2. Test data for ethyl hexanediol.

(a) Exp. No.	(b) No. Trials	(c) Treatment No.	(d) Dose (mg/cm²)	(e) Log Dose	(f) Test Period	No. of bites (g) Control	(h) Treatment	(i) % Repellency*	(j) Probit
I	12	1	0.002	−2.70	0 hr	25	23	8.0	3.59
		2	.004	−2.40	"	"	14	44.0	4.85
		3	.008	−2.10	"	"	10	60.0	5.25
		4	.016	−1.80	"	"	1	96.0	6.75
II	28	5	0.16	−0.80	4 hr	76	95	0.7**	2.54
		6	.32	−0.49	"	"	44	42.1	4.80
		7	.64	−0.19	"	"	34	55.3	5.13
		8	1.28	+0.11	"	"	22	71.1	5.56
III	58	9	0.20	−0.70	1 hr	235	17	92.8	6.46
		10	"	"	2	"	116	50.6	5.02
		11	"	"	3	"	193	17.9	4.08
		12	"	"	4	"	143	39.1	4.72
IV	14	13	0.32	−0.49	1 hr	59	1	98.3	7.06
		14	"	"	2	"	21	64.4	5.37
		15	"	"	3	"	36	39.0	4.72
		16	"	"	4	"	45	23.7	4.28

* % Repellency = 100 X (Control − Treatment) ÷ Control.
** Adjusted value for 0% observation (Armitage 1971).

sent, 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.

RESULTS AND DISCUSSION

Data base. The raw data obtained in the study are shown in columns g and h of Tables 1 and 2. Percent repellency (column i) was calculated as indicated in the footnotes to the tables. Probit values for percent repellency (column j) and logarithms of the doses applied (column e) were obtained from standard tables. The data used in the analyses were the log dose (column e), test period (column f) and probit value (column j)[5].

[5] The "free choice" experimental design used in this and prior studies (Rutledge et al. 1978; Buescher et al. 1982, 1983) is considered to simulate the natural conditions of repellent use more closely than a "no choice" design, since in nature mosquitoes are free to seek an untreated or poorly treated part of the host or an alternate, untreated host. Since the free choice design does not yield quantal data, a reviewer has questioned whether the probit transformation is appropriate for use in the study. Although the probit transformation is derived from quantal theory, several authorities, including Goldstein (1964) whom we have cited in previous papers, have pointed out that it is equally appropriate for the analysis of non-quantal data if the dose-response function is sigmoidal in form. In this case standard errors and confidence limits are based on Fieller's Theorem rather than on quantal theory. The confidence limits shown in Fig. 3 of the present paper are based on Fieller's Theorem; however, our evaluation of the probit plane model (Tables 3 and 4, Figs. 1 and 2) is a straightforward multiple regression analysis, for which well-known standard formulas are available (Steel and Torrie 1980). The standard errors and correlation coefficients given in the tables apply to the transformed data (log dose and probit) rather than to the original data (mg/cm² dose and percent repellency).

Probit plane model. This model employs the probit plane function of Busvine (1971) and Finney (1971):

$$Y = a + b_1X_1 + b_2X_2 \quad (1)$$

In our case Y is the response of the mosquito test population in probits, X_1 is the logarithm of the applied dose, X_2 is the test period and a, b_1 and b_2 are constants determined by the repellent, the mosquito species and the test conditions. When the test period (X_2) is held constant, equation (1) becomes

$$Y = c_1 + b_1X_1 \quad (2)$$

where $c_1 = a + b_2X_2$. In the case where $X_2 = 0$, this reduces to

$$Y = a + b_1X_1 \quad (3)$$

which is the classical dose-response function. When the dose (X_1) is held constant, equation (1) becomes

$$Y = c_2 + b_2X_2 \quad (4)$$

where $c_2 = a + b_1X_1$. In this case the observed response is proportional to the test period as postulated in the introduction.

VALIDATION OF THE PROBIT PLANE MODEL. The multiple and partial regression and correlation of Y (column j, Tables 1 and 2) on X_1 (column e) and X_2 (column f) are given in Tables 3 and 4 for deet and ethyl hexanediol, respectively. All multiple and partial correlation coefficients were significant at the 1% level. The deviations of the observed values from their respective regression values are shown in Figures 1 and 2. The deviation shown for treatment 12 with deet (−1.49 probits) was the only significant ($P < .05$) deviation from regression observed.

On the original (percent repellency) scale of measurement the deviations from regression ranged from 1.0 to 42.1% for deet and from 1.0 to 27.4% for ethyl hexanediol. Mean deviations were 14.5 and 13.8%, respectively.

The problem of variation in the responses of mosquitoes to repellents is closely related to that of the variability of animal behavior in general. The responses of animals to stimuli can be altered by intercurrent factors acting at any point in the receptor-effector pathway, including the receptor, the afferent, efferent and connector neurons, the synapses and nerve endings, and the effector itself. In particular, interconnections within the central nervous system provide opportunities for interaction of the neurons of the receptor-effector pathway with other events in the central nervous system, and thereby with other external and internal stimuli. Kennedy (1977, 1978) has recently reemphasized the importance of the central nervous

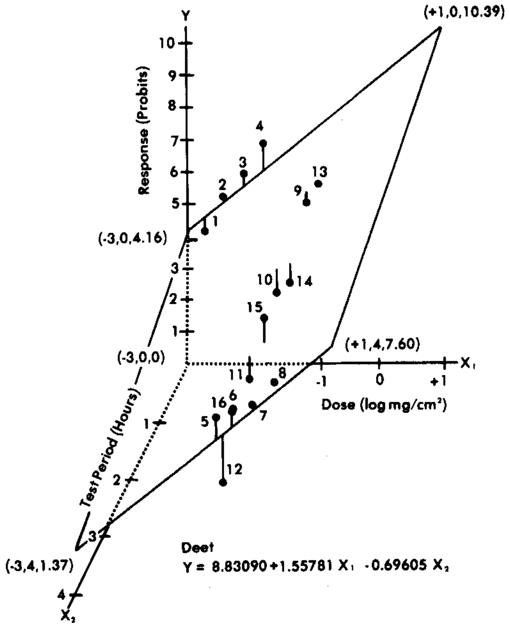

Fig. 1. Multiple regression of the response of *Aedes aegypti* on dose and test period for deet. Observations are numbered to correspond with the respective treatment numbers of Table 1. Vertical lines indicate the direction and magnitude of deviations of observed values from the regression plane.

Table 3. Statistical data for validation of mathematical models of the effectiveness and persistence of deet on the skin.

Model	Regression equation	Standard error of estimate	Correlation coefficient	Probability
(1) $Y = a + b_1X_1 + b_2X_2$	$Y = 8.83090 + 1.55781X_1 - 0.69605X_2$	0.72549	0.760	<0.01
(2) $Y = c_1 + b_1X_1,\ X_2 = 0$	$Y = 8.8309_1 + 1.5578_0X_1$	"	0.753	"
(2) $Y = c_1 + b_1X_1,\ X_2 = 4$	$Y = 6.04670 + 1.55781X_1$	"	"	"
(4) $Y = c_2 + b_2X_2,\ X_1 = -0.70$	$Y = 7.74043 - 0.69605X_2$	"	−0.726	"
(4) $Y = c_2 + b_2X_2,\ X_1 = -0.52$	$Y = 8.02084 - 0.69605X_2$	"	"	"

Table 4. Statistical data for validation of mathematical models of the effectiveness and persistence of ethyl hexanediol on the skin.

Model	Regression equation	Standard error of estimate	Correlation coefficient	Probability
(1) $Y = a + b_1X_1 + b_2X_2$	$Y = 8,67053 + 1.67983X_1 - 0.92035X_2$	0.66731	0.839	<0.01
(2) $Y = c_1 + b_1X_1,\ X_2 = 0$	$Y = 8.67053 + 1.67983X_1$	"	0.805	"
(2) $Y = c_1 + b_1X_1,\ X_2 = 4$	$Y = 4.98913 + 1.67983X_1$	"	"	"
(4) $Y = c_2 + b_2X_2,\ X_1 = -0.70$	$Y = 7.49465 + 0.92035X_2$	"	−0.836	"
(4) $Y = c_2 + b_2X_2,\ X_1 = -0.49$	$Y = 7.84741 - 0.92035X_2$	"	"	"

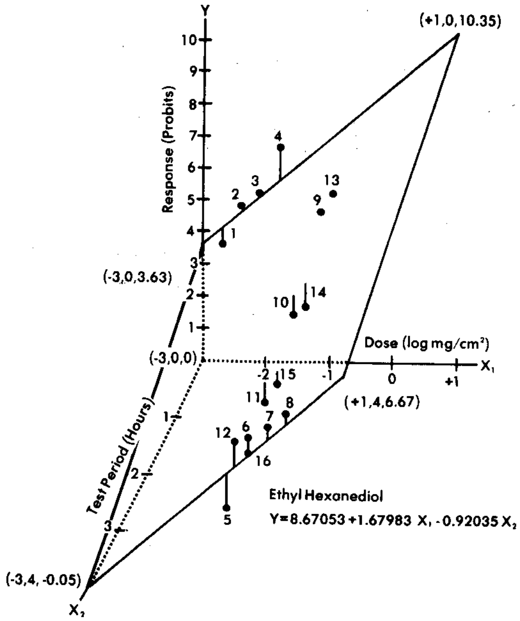

Fig. 2. Multiple regression of the response of *Aedes aegypti* on dose and test period for ethyl hexanediol. Observations are numbered to correspond with the respective treatment numbers of Table 2. Vertical lines indicate the direction and magnitude of deviations of observed values from the regression plane.

system in defining the observed responses of insects to chemical stimuli. From this point of view the behavioral plasticity of the test species is a major source of experimental error in repellent studies.

Exponential decay model. If the response of the mosquito test population to a repellent residue is the same as that to a freshly applied dose of the same strength, then equation (3) can be rewritten as

$$Y = a + b_1 Z \quad (5)$$

where Z is the logarithm of the residue of dose X_1 at time X_2. Substituting the right member of equation (5) for Y in equation (1) gives

$$a + b_1 Z = a + b_1 X_1 + b_2 X_2$$

which simplifies to

$$Z = X_1 + (b_2/b_1)X_2 \quad (6)$$

This function relates the residue ($\log^{-1} Z$) to the applied dose ($\log^{-1} X_1$) and the test period or elapsed time (X_2) as an exponential decay function.

Evaluation of the decay model. Evaluation of the decay curve (equation 6) in terms of the observed values for b_1 and b_2 (Tables 3 and 4) gives $Z = X_1 - 0.44681X_2$ for deet and $Z = X_1 - 0.54788X_2$ for ethyl hexanediol. The values obtained for the decay constant (λ) and the half-life ($t_{½}$) were 1.03 hr^{-1} and 0.67 hr for deet and 1.26 hr^{-1} and 0.55 hr for ethyl hexanediol. Graphs of the decay function for the case X_1 = log 0.20 mg/cm² are shown in Fig. 3.

Since Z was not measured directly in the study, it is not possible to calculate the standard error of estimate of the decay curve. However, the decay constant and the half-life depend solely on the slope of the decay curve, which is estimated by the quantity b_2/b_1. The variance of b_2/b_1 can be obtained by the method for b_T/b_S in slope ratio assays (Finney 1978). This leads to the confidence limits of b_2/b_1 and thereby to confidence limits for the decay constant and half-life. The respective limits are shown in Fig. 3.

According to Fig. 3 the variability to be expected in estimating a repellent residue is proportional to its age. This reflects the cumulative effects of such variables as sweating, abrasion, air temperature and wind speed that come into

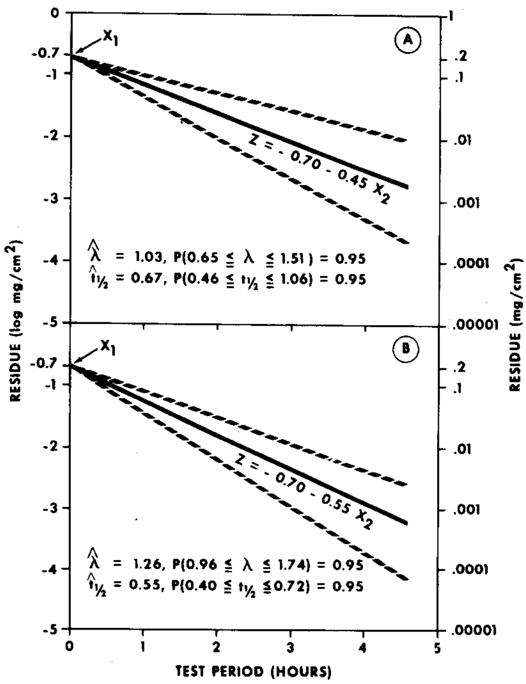

Fig. 3. Decay curves for 0.20 mg/cm² doses of deet (A) and ethyl hexanediol (B) on the skin. Broken lines indicate 95% confidence limits for the slope (b_2/b_1) of each curve.

play during the test period. One result of this effect is that the ED_{50} or ED_{95} of a repellent can be determined with greater certainty than its 4-hr ED_{50} or 4-hr ED_{95} (cf. Tables 1 and 2 of Buescher et al. 1982). The extreme variability of repellent "protection times" (see Busvine 1971) can also be interpreted on this basis.

CONCLUDING REMARKS. This study demonstrates that, within present limits of error, the mosquito population response (in probits) to an applied dose of repellent (logarithmic) over time can be represented by a simple linear model (equation 1). This model in turn leads to the decay curve (equation 6) of the actual deposit on the skin. These models will no doubt be modified, extended and refined in future studies, and we can not yet claim to have achieved a full understanding of the pharmacodynamics of insect repellents. Nonetheless, much has been learned from the effort.

The probit plane model and the exponential decay model are a step toward establishing a rational basis for the research, development, testing and evaluation of repellents and for their effective regulation by the Environmental Protection Agency and the state governments. An understanding of the interrelations of dosage, effectiveness and persistence is needed to break the mind-set of "protection time" introduced by Rudolfs (1930). In addition, the mathematical functions employed in the models provide important clues to the basic physiological, ecological and behavioral mechanisms of repellent activity. For example, the dose-response function (equation 3) reflects the underlying tolerance distribution of the test population. As will be shown in a later paper, the parameters of the tolerance distribution are important determinants of the "protection time" of repellents.

The biological and physical models developed in the study employ several common functional relationships that have been firmly established in other fields of study. The probit plane model (equation 1) is included in standard textbooks of insecticide evaluation (Busvine 1971) and probit analysis (Finney 1971). According to Tsutakawa (1982), the dose-response function (equation 3) can be traced back to the 1920's. It is now widely used in entomology, microbiology, pharmacology, toxicology and other fields of study. The exponential decay function (equation 6) and the associated concepts of decay constant and half-life were introduced in 1902 by the physicist Ernest Rutherford. They are now widely used in the environmental and life sciences, including health physics, pharmacodynamics and pesticide residue studies. Accordingly, it is our hope that the present study will help to advance repellent research from its present status as an obscure specialty into the main streams of biology and medical entomology.

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