Hocking, B. 1953. The intrinsic range and speed of flight of insects. Trans. R. Entomol. Soc. (Lond.) 104:223–345.
Magnarelli, L. A. 1980. Bionomics of *Psorophora ferox* (Diptera:Culicidae): Seasonal occurrence and acquisition of sugars. J. Med. Entomol. 17:328–332.
Percival, M. S. 1961. Types of nectar in angiosperms. New Phytol. 60:235–281.
Van Handel, E. 1967. Determination of fructose and fructose-yielding carbohydrates with cold anthrone. Anal. Biochem 19:193–194.
Van Handel, E. 1968. Direct microdetermination of sucrose. Anal. Biochem. 22:280–283.
Van Handel, E. 1972. Detection of nectar in mosquitoes. Mosq. News 32:458.
Van Handel, E., J. S. Haeger and C. W. Hansen 1972. The sugars of some Florida nectars. Am. J. Bot. 59:1030–1032.

THE DOSE-PERSISTENCE RELATIONSHIP OF DEET AGAINST *AEDES AEGYPTI*[1]

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Since its introduction (Gilbert et al. 1957), deet has become the most widely used insect repellent in both the civilian and armed forces inventories. At present there are over 250 different commercial formulations of this compound marketed in the U.S., with concentrations of active ingredient ranging from 2 to 100% (United States Environmental Protection Agency 1980). The formulation of deet currently issued to the armed forces consists of 71.25% deet and 3.75% other isomers in an ethanol base. This concentration was apparently chosen as a compromise between user acceptability and repellent efficacy. A 100% concentration was found to be excessively greasy or sticky at room temperature, and this was presumably lowered to the nearest point of user toleration (Shambaugh and Pratt 1959).

Dose-response methods can be used to determine the correlation between the dose of a repellent and the response it elicits (Rutledge et al. 1976). In particular, a threshold dose may often be determined after which additional increments in the dose of a repellent administered elicit little and eventually no increase in response at all. From a practical standpoint such information is useful in optimizing the cost/benefit ratio of a product or for determining a more acceptable and efficient formulation for the user.

The following experiment was conducted to determine the optimal correlation between persistence and dosage of deet applied on human skin using the U.S. Army standard deet formulation and *Aedes aegypti* (Linn.).

The test method used in these experiments is similar to the ED_{50} arm test reported by Buescher et al. (1982). The flexor region of a test subject's forearm is outlined with five 29 mm diameter circles with a plastic template and a felt tipped pen. A control (ethanol) and four serial dilutions of deet (*N,N*-diethyl-*M*-toluamide, 75% in ethanol, Federal Stock No. 6840-753-4963, Airsol Company Inc., Neodesha, KS) in ethanol were assigned at random to the five test areas. Dosages were calculated in mg/cm^2 using a constant application volume of 0.025 ml and spread evenly within the outlined areas with the tip of a glass rod. The plastic test cage containing 15 mosquitoes was then positioned over the treated areas using Velcro® strips, the slide at the bottom withdrawn, and the number of bites received after 90 seconds was recorded. In subsequent test trials the range of dosages applied was adjusted to estimate the ED_{95} of deet at 0, 1, 2, 3, 4, 5 and 6 hours after the repellent was applied. During the 1–6 hour test interval, four male volunteers conducted normal activities but were not allowed to wash, abrade, or conduct vigorous physical activities that might affect the treated areas. Volunteers were used in all testing.

All tests were conducted with nulliparous *Ae. aegypti* (UCSF strain), 5–15 days of age. Mosquitoes were maintained at 27°C and 75% RH under a 12:12 hr photoperiod incorporating 1 hr of simulated sunrise and 1 hr of simulated sunset. Daytime illumination was held at 30 f.c. Larvae were reared on a diet of Purina Guinea Pig Chow® (ground to 40 mesh), brewer's yeast, and undefatted, desiccated, powdered liver (ratio by weight, 4:4:1). Adult mosquitoes were maintained on 10% sucrose *ad libitum*.

Data were analyzed on a Data General Eclipse 330 computer by the method of probit analysis.

[1] The opinions and 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. Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

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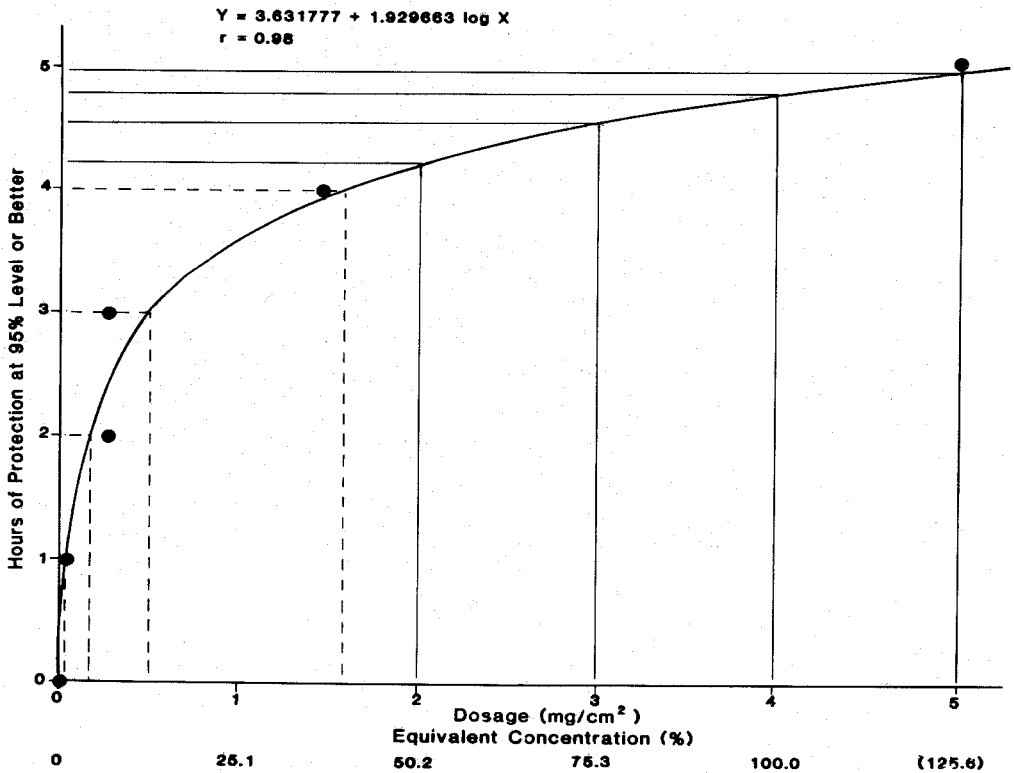

Fig. 1. Graph of dosage vs hours of 95% protection for deet against *Ae. aegypti*. The equivalent percent concentration for 5 mg/cm² (125.6) is a hypothetical estimate. The regression equation Y = 3.63177 + 1.919663 log X may be used to estimate length of protection (Y) for any given dosage (X).

The calculation of the confidence limits was based on the method of Goldstein (1964).

Figure 1 illustrates the hours of protection at the 95% level or greater versus the dosage of deet applied to the skin. Dosages between 0.01 to 2.0 mg/cm² (Table 1) provided the greatest increases in persistence with respect to the dosage of repellent applied. Dosages greater than 2.0 mg/cm² yielded comparatively insignificant increases in persistence. Thus, a concentration of approximately 50% deet gave slightly more than 4 hr of protection against *Ae. aegypti*, while doubling this dose to a maximum of 100% further increased persistence by less than 1 hr. Numerous protection times have been reported for deet and *Ae. aegypti* (Gilbert et al. 1957, Schreck 1977); however, this would appear to be the first comprehensive dose-persistence correlation for this compound. As such, these data suggest that even under controlled laboratory conditions deet may offer far less protection against species such as *Ae. aegypti*, than previously recorded. Possibly of greater interest is the implication that more efficient formulation and use of the 75% US Army standard deet formulation could be accomplished by simply

Table 1. ED_{95} at 0–6 hours posttreatment for deet against *Ae. aegypti*.

ED_{95} (mg/cm²)	95% Confidence limits	Posttreatment time (hr)
0.0111	0.00654–0.05770	0
0.0544	0.03715–0.13113	1
0.2586	0.14016–4.70181	2
0.2634	******–******	3
1.4547	1.22895–1.79646	4
5.0461	1.85611–******	5
39.0605	6.20691–******	6

* Not determined.

lowering the deet concentration to approximately 50%. It is likely that such reduction would bring savings in acquisition costs and increase user acceptability of the product, a serious problem among U.S. Army field personnel (Hooper and Wirtz 1983). By comparison it is useful to note that of over 250 deet formulations marketed by private industry, which has always been sensitive to consumer acceptability, only a few contain deet concentrations greater than 50% (United States Environmental Protection Agency 1980).

Further laboratory and field tests may be advisable against more deet tolerant species such as *Anopheles albimanus* Wied. before such a reduction is effected; however, if disease prevention is the purpose of utilizing insect repellents, greater overall use due to a more acceptable formulation may easily compensate for a slight reduction in the spectrum of efficacy.

References Cited

Buescher, M. D., L. C. Rutledge and R. A. Wirtz. 1982. Tests of commercial repellents on human skin against *Aedes aegypti*. Mosq. News 42:428–433.

Gilbert, I. H., H. K. Gouck and C. N. Smith. 1957. New insect repellent. Soap Chem. Spec. 33:115–117, 129–133.

Goldstein, A. 1964. Biostatistics, an introductory text. Macmillian Co., New York. 272 p.

Hooper, R. L. and R. A. Wirtz. 1983. Insect repellent used by troops in the field: Results of a questionnaire. Mil. Med. 148:34–38.

Rutledge, L. C., M. A. Moussa and C. J. Belletti. 1976. An *in vitro* blood-feeding system for quantitative testing of mosquito repellents. Mosq. News 36:283–293.

Schreck, C. E. 1977. Techniques for the evaluation of insect repellents: A critical review. Annu. Rev. Entomol. 22:101–119.

Shambaugh, G. F. and J. J. Pratt. 1959. Development of insect repellents for personal use. I. Diethyltoluamide. Pesticide Section Report No. 1. Chemical and Plastics Division, U. S. Army Quartermaster Research and Engineering Center, Natick, Massachusetts.

United States Environmental Protection Agency. 1980. Registrants with pesticide products containing the active ingredient *N,N*,diethyl-*m*-toluamide and other isomers. Washington, D.C. 146 p.

FIELD ISOLATIONS OF FILARIAL WORMS PRESUMED TO BE *DIROFILARIA IMMITIS* FROM MOSQUITOES IN KENTUCKY[1]

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Fifty-two species of mosquitoes have been recorded in Kentucky (Darsie and Ward 1981), and 29 of these species are known to occur in Calloway County (Courtney and Christensen 1982). Several species collected in Calloway County have been incriminated elsewhere as potential vectors of dog heartworm (*Dirofilaria immitis*) (Ludlam et al. 1970, Hu 1931); therefore, live trapping of mosquitoes for *D. immitis* isolation was done in 1981 as part of an attempt to determine which species might be natural vectors of dog heartworm in western Kentucky.

Calloway County is situated in the southwestern portion of Kentucky, and mosquitoes were collected on May 29 and 30, June 10, 16 and 24, and August 11, 13 and 19, with CO_2-baited CDC traps near the city of Murray. Trapping was done near a dog kennel that contained at least one dog with a microfilaremia of *D. immitis*. Collected mosquitoes were examined for filarial worm infections by previously described methods (Christensen and Andrews 1976).

A total of 1,871 adult females were collected comprising 14 species in 6 genera (Table 1). Of these, 1,544 were pooled by species in an attempt to isolate infective-stage *D. immitis*, but all pools were negative (Table 1).

Over half of the species collected in this study have been reported as potential vectors of *D. immitis* (Ludlam et al. 1970, Bemrick and Sandholm 1966, Christensen and Andrews 1976). But of 317 mosquitoes dissected in our study, only two were positive for filarial worms (Table 1). Twenty 2nd-stage juveniles were recovered

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