of chlorpyrifos (Dursban) in pipelines

Operational Time	Calculated Time
Minutes	Minutes
COVERED	
28.8	28.9
28.4	28.9
25.2	25.9
24.3	25.9
23.2	26.9
22.2	26.9
UNCOVERED	
7.2	28.9
7.3	28.9
6.8	28.9
6.2	25.9
6.1	25.9
6.7	25.9
4.7	26.9
4.6	26.9
4.6	26.9

demonstrate good larvicidal action. lculations were made to determine ime needed to treat 1 ft. of 30 in. pipe. rtunately the calculations were only for a completely enclosed system. to the fact that a vacuum was being ed when an open system was used that previous air currents existed, it me impractical to state that a particu- liameter and length of pipe could be ed in a specific period of time. he best method of determining when nsecticide has cleared a pipeline is by

pyrifos (Dursban) in premeasured pipelines

	Speed of Dispersal (Average)
ft	Ft/Min
NDPIPES COVERED	
240	46.6
109	44.7
155	50.8
NDPIPES UNCOVERED	
240	174.6
109	176.3
155	251.9

visual observation. Physical observation of the insecticide cloud as it emerges from the end standpipe is a positive indication that the pipeline has been satisfactorily treated.

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AN *IN VITRO* BLOOD-FEEDING SYSTEM FOR QUANTITATIVE TESTING OF MOSQUITO REPELLENTS [1]

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ABSTRACT. A new test system was developed to assess the intrinsic repellency of chemical compounds and formulations to mosquitoes. The system incorporates an *in vitro* blood-feeding device which allows unrestricted "free choice" feeding on various repellent-treated membrane surfaces, thereby providing comparative feeding data on a quantitative basis. Three configurations of the blood-feeding device permit the use of a variety of experimental designs. Examples of experiments employing factorial and dose-response designs are presented. The *in vitro* repellent test system was proved in more than 250 repellent tests utilizing a variety of chemicals and mosquito species and strains.

Investigators concerned with the development of new and improved mosquito repellents have employed various methods of evaluating the intrinsic repellency of chemical compounds and formulations to mosquitoes. In one approach to the problem, an olfactometer such as that of Schreck et al. (1967) is employed to measure the repellency of candidate materials relative to that of a control or of a repellent standard such as N,N-diethyl-m-toluamide (deet). Although the olfactometer method is convenient and productive, it does not closely simulate the natural conditions of host-seeking and blood-feeding and it does not measure tactochemical repellent effects.

A more natural test is provided by those methods in which a repellent-treated area of the skin of a human test subject is directly exposed to bites of the test mosquitoes (Smith et al. 1963). For reasons of comfort and safety the untreated control is customarily neglected and the test is terminated when one or a few bites have been received on the treated area. The parameter estimated is the minimum effective dosage, or the minimum amount of repellent per unit of surface area required to protect the skin from bites of the

[1] The 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. Mention of a commercial or proprietary product does not constitute a recommendation or endorsement by the Department of the Army or the Department of Defense.

mosquito test population. Since, in effect, only the least sensitive of the mosquitoes in the test population (i.e., those representing the insensitive tail of the population distribution) are tested, the data obtained in this kind of test are essentially semiquantitative, and the statistical advantages of working with the population mean are lost. In addition, the potential hazard of new compounds and formulations applied to the skin effectively limits the use of human test subjects to the later stages of repellent development.

Methods that employ an experimental animal in lieu of the human test subject circumvent many of the problems associated with the use of humans. However, Smith et al. (1963) have demonstrated that results obtained in repellent tests may vary with the animal species employed as the test subject. It is believed that the differential results obtained relate to mosquito host preference and to specific characteristics of the host such as hairiness, skin temperature, perspiratory and sebaceous factors, and anti-mosquito behavior.

In this report we describe an *in vitro* mosquito blood-feeding system designed and developed specifically for use in evaluating the intrinsic repellency of chemical compounds and formulations. The system represents a refinement and extension of that of Bar-Zeev and Smith (1959), and is based on our previous experience in the design and use of *in vitro* mosquito blood-feeding systems (Rutledge et al. 1964). More than 250 repellent tests have been conducted with this test system, including tests of a variety of repellent chemicals and mosquito species and strains. Detailed results of the tests conducted will be presented in subsequent reports. In the present paper we include representative examples intended to demonstrate the utility and adaptability of the test system and to illustrate the test procedure and the methods of data analysis.

MATERIALS AND METHODS

MOSQUITOES TESTED. The following mosquito species and strains have been tested with the repellent test system to be described: (1) *Anopheles albimanus* Wiedemann and *An. quadrimaculatus* Say. Repellent test strains obtained from Dr. D. E. Weidhaas, USDA Insects Affecting Man Research Laboratory, Gainesville, Florida. (2) *An. stephensi* Liston. Strain obtained from Col B. F. Eldridge, Walter Reed Army Institute of Research, Washington, D.C. (3) *Culex pipiens* L. Autogenous strain obtained from Dr. G. A. H. McClelland, University of California, Davis, California. Anautogenous strain obtained from Mr. R. D. Wells, U.S. Army Environmental Hygiene Agency, Edgewood Arsenal, Maryland. (4) *Cx. tarsalis* Coquillett. Autogenous strain obtained from Mr. P. T. Rigby, U.S. Army Environmental Hygiene Agency, Regional Division, Fort Baker, California. (5) *Aedes aegypti* L. Eight geographic strains (AMPHUR, CARRIZAL, DHOW, MASAKA, MOYO INDOOR, NEWALA HOUSE, OCALA and TEKELIT) obtained from Dr. G. B. Craig, University of Notre Dame, Notre Dame, Indiana. Repellent test strains obtained from Dr. Weidhaas and from Dr. A. A. Khan, University of California School of Medicine, San Francisco, California. (6) *Ae. taeniorhynchus* (Wiedemann). Strain obtained from Mr. Jere Downing, Rutgers, the State University, New Brunswick, New Jersey.

The mosquitoes were reared and maintained at 80°F. and 80% R.H. under a 12:12 hr photoperiod incorporating 1 hr of simulated sunrise and 1 hr of simulated sunset. Daytime illumination was held at 30-foot candles. The larvae were reared on a diet consisting of equal parts by weight of Purina Guinea Pig Chow (ground to the 40-mesh grade), active dry yeast or brewer's yeast and desiccated, powdered liver. The adult mosquitoes were maintained on 10% sucrose solution prior to testing. All tests were conducted with nulliparous females in the age range of 5 to 15 days.

REPELLENTS TESTED. The following materials have been tested with the system to be described: (1) Deet, 75% in ethanol, Federal stock no. 6840-753-4963, Airosol

Company, Inc. (2) Deet, technical grade, McLaughlin Gormley King Company (MGK). (3) Dipropyl pyridine-2,5-dicarboxylate (MGK Repellent 326), technical grade. (4) 1,5a,6,9,9a,9b-Hexahydro-4a (4H)-dibenzofurancarboxaldehyde (MGK Repellent 11), technical grade. (5) N-(2-ethylhexyl)-5-norbornene-2,3-dicarboximide, technical grade, MGK. (6) Triethylene glycol monohexyl ether, MGK samples X-3000-75, X-3001-75 and X-3002-75. (7) Ethyl hexanediol, practical grade, Eastman Chemical Company. (8) 4-Hydroxy-3-methoxy-benzaldehyde (vanillin), m.p. 82-83.5°C., J. T. Baker Chemical Company. (9) Twenty-eight coded repellent chemicals furnished by Dr. W. A. Skinner, Stanford Research Institute, Menlo Park, California.

REPELLENT TEST SYSTEM AND TEST PROCEDURE. The central component of the repellent test system is a mosquito feeding device, which consists of a hollow plastic box having an assembly of circular wells or blood-reservoirs (the feeding stations) on its upper face. The box is fitted with inlet and outlet ports for connection to a constant-temperature water circulator and with 1 or more thermometer ports for use in monitoring the water temperature. This device was fabricated in 3 configurations (Figures 1A-1C). The 4-well mosquito feeding device (Figure 1A) measures 3x9x9 cm. The wells are 7 mm deep and 3 cm in diameter with a capacity of 5 ml. The 5-well device (Figure 1B) measures 3x5x21 cm. The wells are identical to those of the 4-well device. The 17-well device (Figure 1C) measures 3x11x23 cm. The wells are 3 mm deep and 2 cm in diameter with a capacity of 1 ml.

In use, the wells are filled with outdated human blood for transfusion, which can be obtained from local blood banks. Blood preserved with either anticoagulant citrate dextrose solution U.S.P. (ACD solution) or anti-coagulant citrate phosphate dextrose solution U.S.P. (CPD solution) is suitable for this purpose. Prior to use, the blood is replenished with 5 mM adenosine triphosphate (ATP), without which the mosquitoes will not feed freely (Rutledge et al. 1964). The blood is maintained at 37°C. with water from the constant-temperature water circulator.

The blood-filled wells are covered with squares of commercial Baudruche membrane (Long and Long Company, Bellville, New Jersey) through which the mosquitoes feed and to which the repellents are applied. This material, which is derived from bovine cecum, is strong, flexible, wettable and nearly as acceptable to mosquitoes as fresh animal skin (Rutledge et al. 1964). A film of silicone stopcock grease applied to the rim of the blood reservoir is used to seal the membrane to the blood reservoir.

All treatments, including the control, are assigned at random to the wells of the feeding device. The repellent dilutions are prepared in ethanol and are calculated so that a constant volume of the diluted material will provide the desired dosage of repellent (on a mg/cm^2 basis) when spread over the test area. An Eppendorf pipette is used to deliver the precise volume of material. A 0.025 ml pipette is used with the 4-well and 5-well feeding devices (blood surface area=7.1 cm^2 per well), and a 0.010 ml pipette is used with the 17-well feeding device (blood surface area = 3.1 cm^2 per well). The repellent is spread evenly on the membrane with a clean glass rod and allowed to dry for 5 minutes prior to admitting the mosquitoes to the test surfaces. Figure 1F illustrates the 5-well feeding device prepared for use, together with the constant-temperature water circulator and other equipment used in the test procedure.

Three standard 1x1x1 foot mosquito cages (American Biological Supply Company, Baltimore, Maryland) were specially modified for use in the repellent test system. The modified cage (Figure 1D) has 3 sides of clear plastic and 1 side of stockinet, a wire-screen top and a clear plastic floor incorporating a machined cut-out to receive the mosquito feeding device and a sliding door to admit the mosquitoes to the test surfaces (Figure 1E). The design

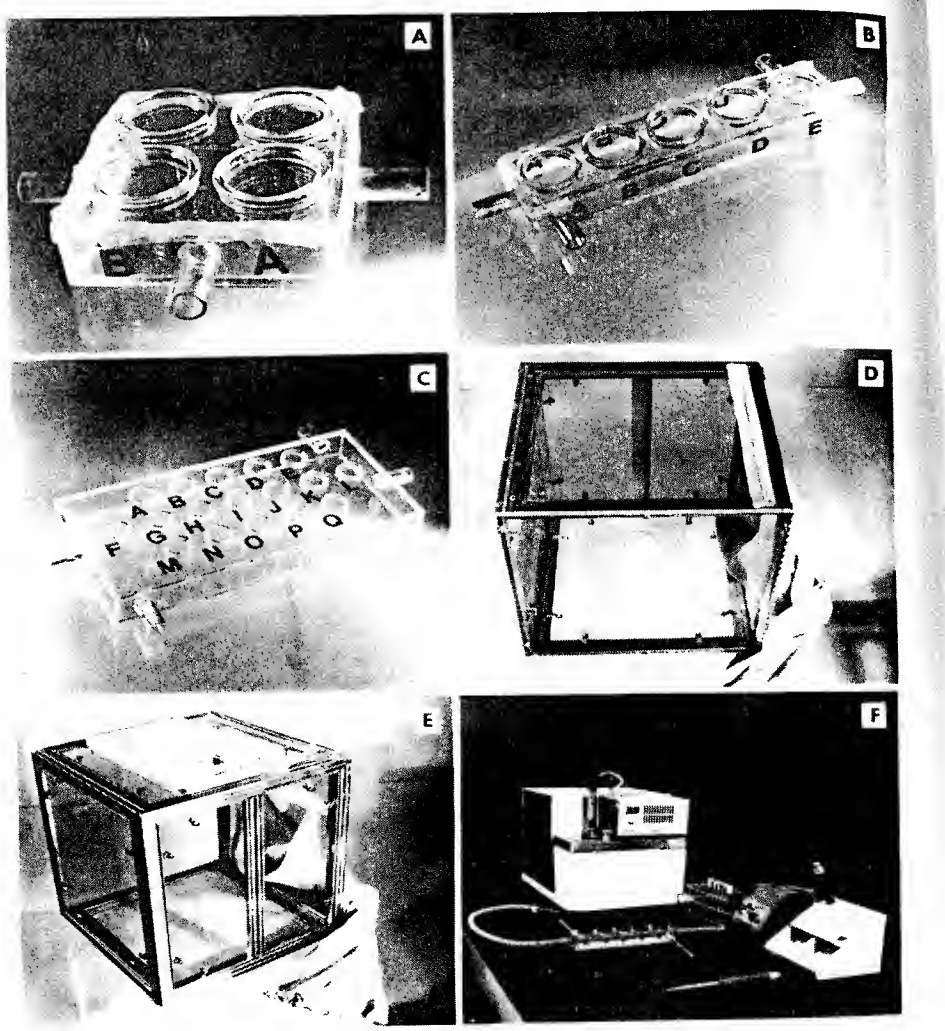

Fig. 1. Components of the *in vitro* mosquito repellent test system. A-C, details of the 4-, 5-, and 17-well mosquito blood-feeding devices. D, test cage for use with the 5-well feeding device. E, details of sliding door mechanism in bottom of test cage. F, 5-well feeding device prepared for use and connected to a constant-temperature water circulator.

of the test cage is intended to minimize interaction between the observer and the mosquito test population (the plastic sides) and to permit simultaneous access of the test mosquitoes to all the repellent treatments (the sliding door).

Figure 2 illustrates the complete repellent test system in use, with an observer recording the data obtained. In our test procedure the numbers of mosquitoes feeding on each treatment are recorded at 2-minute intervals for a period of 20 minutes. The totals of the 10 feeding counts obtained for each treatment represent the test results. However, in certain experimental designs (notably the dose-response analysis), the feeding counts are converted to percentages of the control count prior to analysis. In our procedure, the test population ordinarily consists of 250 female mosquitoes. However, data will be presented to demonstrate that the test results are essentially independent of the mosquito population density. The 2-minute, 20-minute, 250 mosquito procedure described represents a compromise between the need for economy in mosquito rearing expenses and the need for an adequate data base to insure reliability of the test results.

RESULTS

4-WELL CONFIGURATION. The results of 3 tests utilizing the 4-well mosquito blood-feeding device (Figure 1A) to evaluate the repellency of vanillin and deet at 0.04 mg/cm^2 to the AMPHUR strain of *Aedes aegypti* are presented in Table 1. In each test the following treatments were assigned at random to the 4 feeding stations:

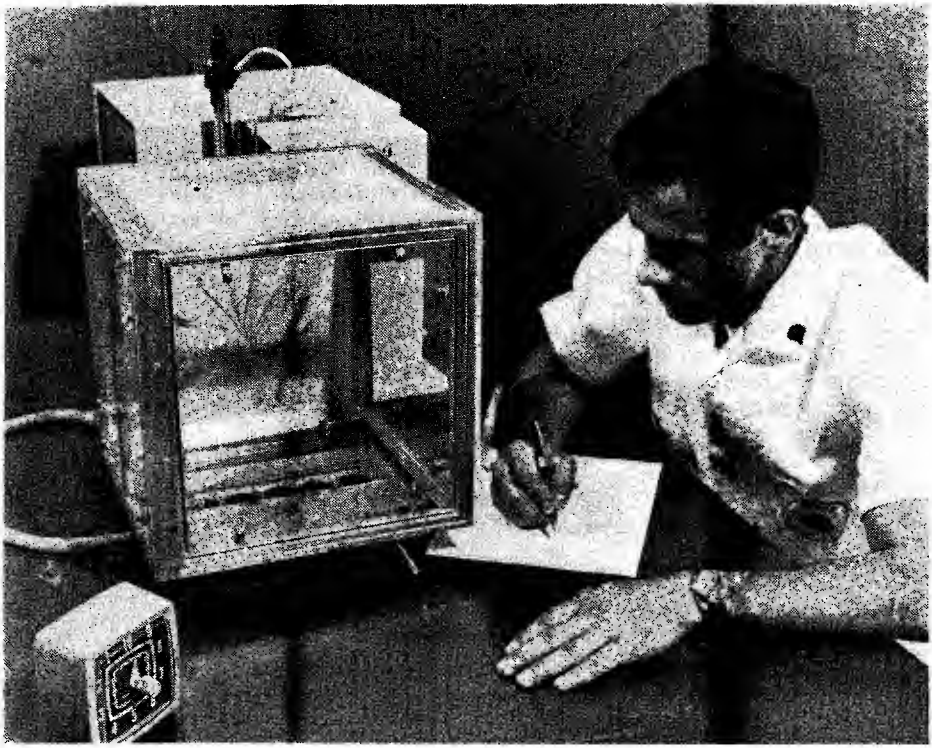

Fig. 2. *In vitro* mosquito repellent test system in use with observer recording the feeding counts.

Table 1. Comparative effects of vanillin, deet and vanillin + deet on feeding of the AMPHUR strain of *Aedes aegypti*

Test No.	Feeding Counts			
	Control (Ethanol)	Vanillin (.04 mg/cm²)	Deet (.04 mg/cm²)	Vanillin+Deet (.04+.04 mg/cm²)
140	220	167	104	114
141	264	225	120	86
142	236	211	115	67
Total	720	603	339	267

(1) Ethanol only, (2) 0.04 mg/cm² vanillin, (3) 0.04 mg/cm² deet and (4) 0.04 mg/cm² vanillin + 0.04 mg/cm² deet. The experiment represents the 2x2 factorial design, in which 2 factors (vanillin and deet) were tested, each at 2 levels (0.00 and 0.04 mg/cm²). The 3 replications provide the basis for testing the statistical significance of the interaction of the 2 compounds.

The analysis of variance (Table 2) indicated that both vanillin and deet were significantly repellent to the test mosquitoes at the dosage used and that there was no significant interaction between the two. In this case the repellent effects of the compounds tested were additive. In the case of a statistically significant interaction, one compound is either more effective in the presence of the other (synergism) or less effective in the presence of the other (interference). This type of test is particularly useful as a rapid screening method for repellent synergists and as a test for compatibility in repellent combinations. In principle at least, it could also be used in evaluating the effects of spreaders, stickers and other additives on the repellent efficacy.

5-WELL CONFIGURATION. The results of 6 tests utilizing the 5-well mosquito blood-feeding device (Figure 1B) to determine the median effective dosage (ED_{50}) of deet for the TEKELIT strain of *Aedes aegypti* are presented in Table 3. The experiment was a dose-response determination analogous to those employed to determine the median lethal concentration (LC_{50}) of an insecticide. In each test the following treatments were assigned at random to the 5 feeding stations: (1) Ethanol only, (2) 0.02 mg/cm² deet, (3) 0.04 mg/cm² deet, (4) 0.08 mg/cm² deet and (5) 0.16 mg/cm² deet. While 4 such tests are sufficient for determination of an LC_{50} (World Health Organization 1970), we recommend additional replication for determination of a repellent ED_{50}, since behavioral traits (response to repellent) are characteristically more variable than physiological traits (response to insecticide).

The observed data and the calculated

Table 2. Analysis of variance for effects of vanillin, deet and vanillin+deet on feeding of the AMPHUR strain of *Aedes aegypti*.

Source	df	SS	MS	F
Blocks (Replicates)	2	1,086.00	543.00	1.09
Vanillin	1	2,976.75	2,976.75	5.97*
Deet	1	42.840.75	42,840.75	85.97**
Interaction	1	168.75	168.75	0.34
Error	6	2,990.00	498.33	—
Total	11	50,062.25	—	—

* Significant at the 5% level.
** Significant at the 1% level.

Table 3. Results of 6 tests to determine the median effective dosage (ED_{50}) of deet for the TEKELIT strain of *Aedes aegypti*.

Test No.	Concentration of Deet (mg/cm²)				
	0.00	0.02	0.04	0.08	0.16
	Feeding Counts				
54	124	72	49	25	7
55	139	73	52	16	6
56	202	147	96	49	21
57	227	155	84	47	12
58	132	94	59	39	4
75	121	69	67	19	6
	Feeding Counts as % of Control Count				
54	100	58.1	39.5	20.2	5.6
55	100	52.5	37.4	11.5	4.3
56	100	72.3	47.5	24.3	10.4
57	100	68.3	37.0	20.7	5.3
58	100	71.5	44.7	29.5	3.0
75	100	57.0	55.4	15.7	5.0

dose-response line are graphed in Figure 3. In this case the calculated ED_{50} was 0.031 mg/cm² (95% confidence limits 0.027 to 0.035 mg/cm²), and the calculated ED_{90} was 0.120 mg/cm² (95% confidence limits 0.106 to 0.138 mg/cm²). The coefficient of correlation for the data illustrated was −0.96. The calculations employed in the analysis were those customarily used in dose-response analysis (Wadley 1967), except that the calculation of confidence limits was based on the '*g*" statistic, as described by Goldstein (1964).

The factor of test population density did not significantly affect the ED_{50} values obtained when population levels of 125, 250, 500 and 1000 females of the OCALA strain of *Aedes aegypti* were tested in 2 replicates each. In this case, the ED_{50} values obtained were 0.041, .034, .027 and .038 mg/cm² for test population levels of 125, 250, 500 and 1000 mosquitoes respectively. Thus, the values agreed to within 14 micrograms and the variation observed did not relate to the test population levels used.

The 5-well mosquito blood-feeding device was also employed to determine whether repellent applied to one of the feeding stations might affect the feeding counts obtained on the adjacent or farther stations. A high dosage (0.16 mg/cm²) of deet was applied to one of the end wells of the feeding device, and feeding counts were made on the 4 remaining (untreated) wells. Four replicates of the test were performed with *Anopheles stephensi* as the test species. The feeding counts obtained at the station adjacent to the treated one did not differ significantly from those obtained at any of the stations more distant. However, we routinely randomize the assignment of repellent treatments, in the event that a repellent more volatile than deet might have such an effect.

17-WELL CONFIGURATION. The results of 2 tests utilizing the 17-well mosquito blood-feeding device (Figure 1C) to compare the repellent effects of MGK Repellent 11, MGK Repellent 326, deet and ethyl hexanediol on *Anopheles stephensi* are presented in Table 4. In each test the 4 repellents were applied to the feeding stations at random in concentrations of 0.02, 0.04, 0.08 and 0.16 mg/cm². The 17th feeding station was treated with ethanol only, as a control. This control was included to provide a means of monitoring the level of blood-feeding activity in the mosquito test population; feeding counts obtained at this station were not utilized in the statistical analysis. The experiment represents the 4x4 factorial design, in which 2 factors (repellents and concentra-

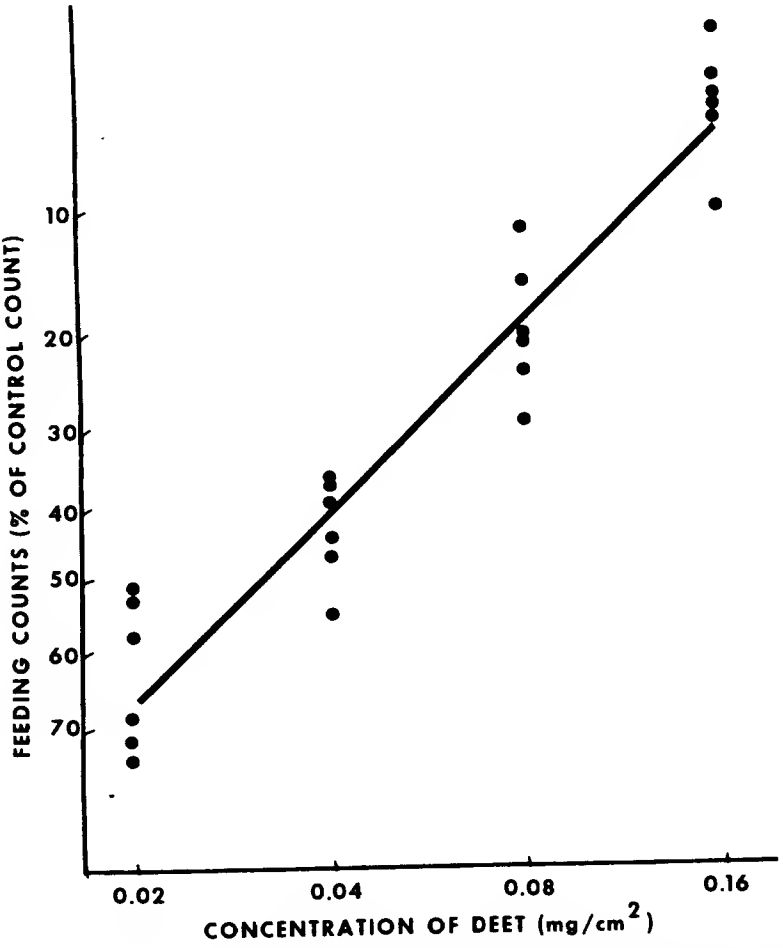

Fig. 3. Dose-response curve for 6 tests of deet with the TEKELIT strain of *Aedes aegypti*. Dosages are plotted on the logarithmic scale, and responses (feeding counts as percentages of the untreated control) are plotted on the probit scale.

tions) were tested, each at 4 levels (4 types of repellent, each in 4 concentrations). The 2 replications provide the basis for testing the significance of the statistical interaction of compounds and concentrations.

The analysis of variance (Table 5) indicated significant differences in the responses of the mosquitoes to the 4 repellents, and as might be expected, to the concentrations of repellent applied. Duncan's new multiple-range test (Steel and Torrie 1960) indicated that both MGK Repellent 11 and MGK Repellent 326 were more effective than ethyl hexanediol. Such comparisons of repellent effectiveness are valid for the entire range of repellent concentrations tested. The importance of this type of information lies in the fact that in actual use the dosage of repellent applied for protection may vary widely. The case in which one repellent is superior to another at one dosage but inferior to it at another dosage is indicated by statistical significance of the repellents x concentrations interaction. It should be noted that block effects were statistically significant in the present experiment, indicating that the mosquitoes fed more freely in one of the

Table 4. Comparative effects of four repellents at four dosage levels on the feeding of *Anopheles stephensi* (totals of 2 replicates)

Repellent	Feeding Counts				
	.02 mg/cm²	.04 mg/cm²	.08 mg/cm²	.16 mg/cm²	Total
MGK Repellent 11	2	1	0	0	3
MGK Repellent 326	37	10	0	1	48
Deet	60	43	5	0	108
Ethyl hexanediol	101	43	39	1	184
Total	200	97	44	2	343

two replicates than they did in the other. The analysis of variance adjusts for this source of variation, and the conclusions reached are valid for range of mosquito feeding activity actually occurring in the course of replication.

While the foregoing example will serve to illustrate the use of the 17-well mosquito feeding device, other types of experimental design are possible. In general, this instrument will accommodate any experiment that calls for 16 or fewer treatments, exclusive of the untreated control. This capability includes the Latin square design to size 4x4 and multiway experiments such as the 2x2x2x2 factorial. For example, the investigator could simultaneously compare 4 repellents singly and in all possible combinations of 2, 3, or 4. Alternatively, he could compare the combinations of a repellent and an additive, each in 4 concentrations or dosages. Numerous other experimental designs are available in standard statistical texts.

DISCUSSION

From the entomological viewpoint (i.e. excluding consideration of economy, toxicity, etc.) the 2 parameters of effectiveness and duration are of primary interest in the testing of candidate mosquito repellents. The *in vitro* repellent test system described in this report was designed to measure and compare the intrinsic repellencies of repellent compounds and their combinations and formulations. Work is now in progress on the development of an analogous test system designed to measure and compare the effective durations of these materials on treated surfaces.

As indicated in the "Materials and Methods" section, we have extensively evaluated the *in vitro* repellent test system. Tables 1 through 5 and Figure 3 indicate the degree of precision and reproducibility to be expected in tests made with the system. The analyses of variance (Tables 2 and 5) illustrate the techniques available for statistical control of experimental and sampling error.

None of the chemical compounds tested to date with the *in vitro* repellent test system have offered any particular or special problems with regard to the conduct of the test or interpretation of the results obtained. All the mosquito colonies tested have responded satisfactorily to the

Table 5. Analysis of variance for the effects of 4 repellents, each in 4 concentrations, on the feeding of *Anopheles stephensi*.

Source	df	SS	MS	F
Blocks (Replicates)	1	1696.53	1696.53	9.04**
Repellents	3	2302.59	767.53	4.09*
Concentrations	3	2742.09	914.03	4.87*
Interaction	9	1539.28	171.03	0.91
Error	15	2815.97	187.73	——
Total	31	11096.47	——	——

* Significant at the 5% level.
** Significant at the 1% level.

in vitro blood-feeding device. The species *An. albimanus, An. stephensi, Ae. aegypti* and *Cx. tarsalis* tend to feed more freely, while the species *An. quadrimaculatus, Ae. taeniorhynchus* and *Cx. pipiens* tend to feed less freely. Specific differences of this kind were also observed among 6 species of *Anopheles, Aedes, Armigeres* and *Culex* by Rutledge et al. (1964). Analogous effects affect the choice of laboratory feeding conditions and animal hosts for colony maintenance (Gerberg 1970). We have attempted the testing of field-collected specimens of several local species of *Aedes, Culex* and *Culiseta* with the *in vitro* repellent test system. The results obtained to date have been generally unsatisfactory due to failure of the mosquitoes to feed under laboratory conditions, either on the *in vitro* blood-feeding device or on a living host. It is anticipated that this problem can be overcome either by adapting the test system to field use or by more closely simulating actual field conditions in the laboratory.

Our test system differs from that of Bar-Zeev and Smith (1959) in a number of technical design features and, in addition, in at least one basic principle of the design. In our system a single mosquito test population is exposed, as a unit, to the complete set of repellent test surfaces, while in that of Bar-Zeev and Smith separate test populations are exposed separately to each of the test surfaces. The design of Bar-Zeev and Smith allows the numbers of fed and un-fed mosquitoes to be determined separately for each treatment at the end of the test period. However, the present system offers obvious advantages in the control of sampling error and experimental error, since the test population is not segregated into separate subpopulations. In addition, we feel that the "free choice" feature of our design is more comparable to the natural situation, in which the mosquito is free to seek an alternate, untreated host, or at least an untreated or thinly treated part of the same host.

Logically, the ED_{50} measured by a "free choice" test system should be smaller than the corresponding parameter by a "no measured choice" test system, and in fact the value of 0.03 mg/cm^2 reported above for ED_{50} of deet for *Ae. aegypti* bears this relation to the equivalent value of 0.26 mg/cm^2 reported for the "no choice" test system of Bar-Zeev and Smith (1959). In effect, the "free choice" feature of our test system has shifted the point of reference for the ED_{50} to a lower level. We recognize that most of the repellent test procedures in current use are based on the "no choice" principle. However, for reasons stated in the preceding paragraph, we regard the "free choice" feature of our test system as being more appropriate for repellent testing purposes. Similarly, it appears to us that, in view of the capricious nature of mosquito blood-feeding behavior, the inclusion of an untreated control in each experiment is essential. Our tests therefore differ basically from those in which a repellent standard is substituted for the untreated control.

It is recognized that an *in vitro* repellent test system can not precisely simulate a test system utilizing human test subjects. It is necessary, however, that the *in vitro* test system give results that are comparable to those given by an equivalent system utilizing human test subjects. To date, our results indicate that the test system described in this report meets this requirement. For example, the ED_{90} of deet for *Ae. aegypti* reported above (0.12 mg/cm^2) compared favorably with the range of 0.07 to 0.17 mg/cm^2 reported by Smith et al. (1963, Table 11) as the deet residual recovered from the skin of 5 human test subjects at the time that *Ae. aegypti* test mosquitoes initiated feeding on skin surfaces initially protected with higher dosages. In more general terms, the *in vitro* repellent test data accumulated to date conform to a number of well-known principles established by investigators utilizing human test subjects, including, for example, the principle that ethyl hexanediol is generally less effective than deet and the principle that *An. albimanus* is generally less sensitive to repellents than *Ae. aegypti*.

The advantages of the *in vitro* test sys-

tem discussed in this paper include: (a) Appropriately designed *in vitro* blood-feeding systems closely simulate the natural conditions of mosquito host-seeking and blood-feeding. (b) Such systems can be standardized, thereby eliminating individual and specific differences attributable to the test subject, as well as differences in test conditions such as temperature, humidity and light intensity that may affect the test results. (c) The untreated control may receive any number of bites, permitting unbiased estimates of the population mean response and statistical adjustment for variation in mosquito test population density and avidity. (d) A number of repellents, concentrations or formulations can be tested simultaneously against a single mosquito test population under ambient standard test conditions, thereby permitting the use of standard experimental designs such as the dose-response analysis or the analysis of variance. (e) The results obtained are quantitative and represent the absolute repellency of the material tested (in terms of a control) rather than its relative repellency in terms of an arbitrary repellent standard. (f) Materials of unknown irritancy, toxicity, mutagenicity or carcinogenicity can be evaluated safely. (g) Repellent dosages can be precisely controlled, since they are applied to surfaces of standard size and conformation. (h) The method is economical of expensive candidate repellents or those in short supply.

The development of new or improved mosquito repellents necessarily involves the testing of selected formulations on human test subjects prior to their adoption for general use. Since this type of test is relatively expensive in terms of manpower and toxicological safety, it is best employed in the later stages of repellent development on formulations that have been thoroughly evaluated by other means. In this connection, we believe that the *in vitro* repellent test system described in this report will find its use primarily in the preliminary evaluation of repellent compounds and formulations prior to their testing on human subjects. In particular, we recommend its use in rapid-screening programs and in exploratory experiments of the kind described in the "Results" section, above.

CONCLUSIONS

The *in vitro* blood-feeding system developed for repellent testing purposes in the present study offers a number of distinct advantages which particularly favor it for use in rapid-screening programs and in the evaluation of the effectiveness of repellent combinations and formulations.

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