TESTS OF COMMERCIAL REPELLENTS ON HUMAN SKIN AGAINST *AEDES AEGYPTI*[1]

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ABSTRACT. A comparison of 9 commercial repellents was made on human volunteers against *Aedes aegypti* using dose-response methods. In the first series of tests measuring intrinsic repellency (0 hour), Stabilene® and MGK® Repellent 326 were significantly inferior ($p < 0.05$) to deet, dibutyl phthalate, Indalone®, dimethyl phthalate, MGK® Repllent 11, ethyl hexanediol, and Citronyl™ (ranked by ED_{50}). A second series of tests conducted to measure the persistence of these compounds showed Stabilene, MGK Repellent 326 and dibutyl phthalate were ineffective after 4 hours. Efficacy ranking by 4-hour ED_{50} was Indalone, Citronyl, dimethyl phthalate, ethyl hexanediol and deet. The relative superiority of deet in comparison to other standard repellents is discussed with references to the literature.

Scientific investigations involving efficacy testing require relevant and accurate baseline data by which to measure the potencies of the experimental materials being tested. In particular these data should be acquired by quantitative, standardized methods that reasonably address the variables and conditions in which the ultimate product will be utilized. In the past, up to and including the present, the comparative efficacy of insect repellents has been determined by a variety of methods including in vitro and in vivo techniques that have generally used a "time protection" test, or repellent efficacy as measured by time to first bite(s) (Grannet 1940). A problem with this criterion is that insects responding in this system are only those in the least sensitive tail of a population, and measurements taken from the extremes of a population distribution are less reliable than those taken from the median (Bar-Zeev 1962). In addition, this method has been modified for reasons of comfort and convenience using olfactometers (Schreck et al. 1967), cloth or membrane systems (USDA 1967) as substitutes for skin, and as Feldman and Maibach (1970), and Reifenrath et al. (1980) have pointed out, significant quantities of repellent are lost through percutaneous absorption. Hence, tests utilizing human skin substitutes may skew results optimistically and may be more appropriate for initial screening rather than advanced or final evaluation.

To circumvent these problems, dose-response type testing may be used as an alternative to evaluate repellent efficacy (Bar-Zeev and Smith 1959). Test results may be reported as the median effective dosage required to repel 50% of the insect test population (ED_{50}). The ED_{95} may also be reported to give an estimation of the concentration needed to give protection against 95% of the population (Dremova et al. 1976).

The present paper rates the efficacy of 9 commercially available repellents against *Aedes aegypti* (Linn.), both intrinsically (0 hour) and at the 4 hour persistence level on human volunteers. In addition to providing a data base for comparison with experimental compounds, we believe these data constitute the most inclusive and quantitative testing of proprietary repellents ever conducted against this species.

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[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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MATERIALS AND METHODS

TEST INSECTS. All tests were conducted using nulliparous *Ae. aegypti* (UCSF strain), 5–15 days of age. Mosquitoes were maintained at 27°C and 75% RH under a 12:12 hour photoperiod incorporating 1 hour of simulated sunrise and 1 hour simulated sunset. Daytime illumination was held at 30 fc. 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*.

REPELLENTS TESTED. The following materials were tested against *Ae. aegypti* using human volunteers: (1) deet, (*N,N*-diethyl-*m*-toluamide) 75% in ethanol, Federal stock no. 6840–753–4963, Airsol Company Inc., Neodesha, KS. (2) Dimethyl phthalate, technical grade, FMC Corporation, Middleport, NY. (3) ethyl hexanediol (2-ethyl-1,3-hexanediol), technical grade, FMC Corporation. (4) Stabilene® (butoxy polypropylene glycol), technical grade, Union Carbide Chemical Co., New York, NY. (5) MGK® Repellent 326 (di-*n*-propyl 2,5-pyridine-dicarboxylate), technical grade, McLaughlin Gormley King Co., Minneapolis, MN. (6) MGK® Repellent 11 [1,5*a*,6,9,9*a*, 9*b*-hexahydro-4*a* (4H)-dibenzofurancarboxaldehyde], technical grade, McLaughlin Gormley King Co. (7) Indalone®, (butyl 3,3-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate), technical grade, K & K Laboratories Inc., Plainview, NY. (8) di-*n*-butyl phthalate, technical grade, Union Carbide Chemical Co. (9) R-69, (Citronyl(TM)) [3-acetyl-2-(2-6-dimethyl-5-heptenyl)-oxazolidine], technical grade, S. C. Johnson and Son, Inc., Racine, WI.

TEST PROCEDURE. The test system used in these experiments is identical to the procedure first reported by Buescher et al. (1982) with the exception that 15 female mosquitoes were used per test (replicate) with a 90 second test period. As before, the flexor region of the test subject's forearm was used as the substrate. Using a plastic template as a guide, five 29 mm diameter circles were outlined with a felt tipped pen and each test area was labeled "A" thru "E" beginning with the area nearest the elbow. A control (ethanol) and 4 serial dilutions of the test repellent in ethanol were assigned at random to the 5 test areas. Dosages were calculated in mg/cm² using a constant application volume of 0.025 ml and spread evenly within the outlined area with the tip of a glass rod. The plastic test cage containing the mosquitoes was then applied over the treated areas using Velcro® strips, the slide at the bottom withdrawn, and the number of bites taken over time was recorded. In addition, a 4 hour test was executed to measure the persistence of the compound tested. This procedure was similar to that described above with the difference that the insects were applied 4 hours after the repellent and correspondingly higher dosages of the latter were utilized. During the 4 hour pre-test interval the volunteers conducted normal office activities, but were not allowed to wash, abrade, or conduct vigorous physical activities that might affect the treated areas. In subsequent 0 or 4 hour test trials the range of dosages applied was adjusted to bracket the median effective dosage (ED_{50}) of the test repellent. If a dose-response relationship could not be established for a particular repellent (i.e. an ED_{50} was not estimated), that compound was considered ineffective after a mininimum of 10 replicates were completed. In all testing 5 male volunteers were used.

STATISTICAL ANALYSIS. Data collected in this study were analyzed on a Data General Eclipse 330 computer by the method of probit analysis. The calculation of the confidence limits was based on the method of Goldstein (1964).

RESULTS AND DISCUSSION

The results of over 150 tests conducted to estimate the median effective dose of the 9 repellents tested are shown in Table 1. A minimum of 10 up to 30 replicates

Table 1. Effectiveness of commercial repellents against *Aedes aegypti* using human volunteers.

	mg/cm² ED_{50}	95% confidence limits	mg/cm² ED_{95}	95% confidence limits
Diethyl toluamide	0.0034	0.00281–0.00404	0.0145	0.01178–0.01948
Dibutyl phthalate	0.0037	0.00131–0.00590	0.0181	0.01269–0.03315
Indalone	0.0041	0.00212–0.00640	0.0156	0.00943–0.06058
Dimethyl phthalate	0.0042	0.00240–0.00625	0.0125	0.00819–0.03187
MGK repellent 11	0.0044	0.00257–0.00650	0.0151	0.00955–0.04460
Ethyl hexanediol	0.0052	0.00268–0.00925	0.0166	0.00935–0.11442
Citronyl	0.0052	0.00208–0.00836	0.0740	0.04937–0.16154
MGK repellent 326	0.0249	0.01144–0.03626	0.1425	0.09323–0.37697
Stabilene	0.3183	0.21713–0.44274	0.8745	0.59784–1.83501

(tests) were required to estimate an ED_{50}, depending on accuracy of the initial bracketing range. The ranking of the repellents is based on the point estimate of the ED_{50} for statistical accuracy. In this series of tests which may be said to measure intrinsic repellency (0 hour), MGK Repellent 326 and Stabilene showed significantly inferior repellency to the other compounds tested. Deet, dibutyl phthalate, Indalone, dimethyl phthalate, MGK Repellent 11, ethyl hexanediol and Citronyl ranked first thru seventh respectively based on the point estimate of the ED_{50}. However taken in conjunction with their respective confidence limits these differences were not statistically significant (Table 1, Fig. 1).

The results of a second series of tests conducted to measure the persistence of these compounds are found in Table 2. After 4 hours, MGK Repellent 326, Stabilene and dibutyl phthalate were found to be ineffective. Once again, no significant differences in efficacy among the remaining compounds could be determined at the 4-hour ED_{50} level. Further scrutiny of these data indicates the oftentimes theoretically large amounts of repellent required to obtain protection after 4 hours. The ED_{95} values can best be put into perspective by the fact that complete saturation and pooling of these compounds on the skin occurs in the 2–4 mg/cm² range (W. G. Reifenrath, personal communication). Indalone, deet, and MGK Repellent 11 provided the lowest estimates for 95% protection after 4 hours.

These data would seem to address 2

Table 2. Effectiveness of commercial repellents against *Aedes aegypti* after 4 hours using human volunteers.

	mg/cm² ED_{50}	95% confidence limits	mg/cm² ED_{95}	95% confidence limits
Indalone	0.1337	0.00418–0.27358	0.56921	0.28424–********
Citronyl	0.1464	0.04298–0.24031	2.56485	1.32989–15.22678
Dimethyl phthalate	0.1904	0.09744–0.27314	7.28383	3.20595–********
Ethyl hexanediol	0.1906	0.00495–0.42644	7.04690	3.10860–********
Diethyl toluamide	0.1976	0.11988–0.28221	1.14408	0.72255–2.61279
MGK repellent 11	0.2393	0.18081–0.40887	1.64437	0.74021–15.77367
MGK repellent 326	******	*******_*******	*******	******_********
Stabilene	******	*******_*******	*******	******_********
Dibutyl phthalate	******	*******_*******	*******	******_********

* Ineffective or not determined.

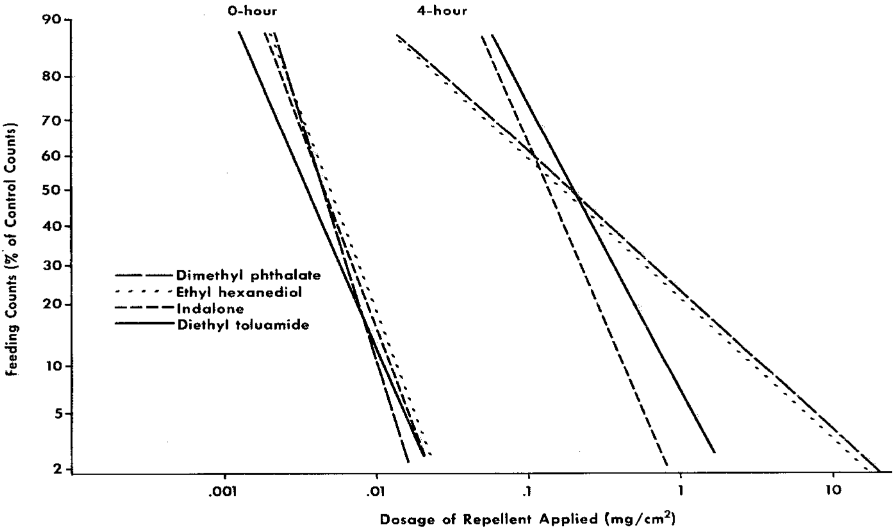

Fig. 1. Dose-response curves at 0 and 4 hours for 4 standard repellents against *Aedes aegypti* on human volunteers. Dosages are plotted on the logarithmic scale, while responses are plotted on the probit scale.

points: the difference between the concept of intrinsic repellency and persistence, and relatively and quantitatively, how much better is one repellent than another. As Busvine (1971) has pointed out the qualities of repellency and persistence have been confounded in the past. In this paper we have used the term intrinsic repellency as defined by Garson and Winnike (1968). While Schreck (1977) has objected to the use of this term, his argument is more pertinent to the limitations of the human language rather than to the question of whether these attributes do in fact exist for any and all repellents. We believe the data from this study would support the latter contention. In the first set of experiments all 9 compounds were demonstrated to show a constant degree of repellency (50%), but varying with the dosages of the different repellents tested. In the second series of tests 3 of these could demonstrate no repellency after 4 hours, in addition to the fact that the efficacy ranking of the compounds changed simply as a factor of time.

The question of the relative efficacy of deet in relation to other standard repellents when applied on the skin has been reported by Gilbert et al. (1955, 1957) and Schreck (1977). In the earlier studies deet was introduced as an "outstanding" new repellent "superior or equal" to the other repellent compounds tested. While this study would tend to confirm that deet is at least as good as the other standards tested against *Ae. aegypti*, the question of its degree of superiority would seem open to interpretation. Khan et al. (1973) found no significant differences in repellency between deet, ethyl hexanediol, and dimethyl phthalate against *Ae. aegypti*. Dremova and Markina (1975) reported deet was no more effective than dimethyl phthalate against *Ae. vexans* Meigen. Gilbert and Gouck (1955) claimed deet appeared only slightly more effective than ethyl hexanediol and dimethyl phthalate against *Anopheles albimanus* Wied. and *Mansonia titillans* (Walker). Mahadevand and Varma (1967) found deet to be only as effective as dimethyl phthalate against *Culex pipiens fatigans* Wied. (= *Cx. quinquefasciatus* Say). Whittemore et al. (1961) reported that 100% M-2020 (dimethyl phthalate 40%, ethyl hexanediol 30%, dimethyl carbate 30%) afforded equal protection to 75% deet against *Ae. scapularis* (Rondoni). Indeed, deet has been found inferior to some of these older repellents in other investigations. Schreck (1977) reported that dimethyl phthalate and ethyl hexanediol were more effective than deet against *An. quadrimaculatus* Say and that ethyl hexanediol was more effective than deet against *An. albimanus*. Further examples of these tendencies have been reported in the literature against other species of blood sucking arthropods as well. Schmidt (1977) reported ethyl hexanediol was more effective than deet against *Glossina morsitans* Westwood. Schmidt and Schmidt (1969) found Indalone was as effective as deet against *Phlebotomus papatasi* Scopoli while Buescher et al. (1982) found an identical result with *Lutzomyia longipalpis* (Lutz and Neiva). Gilbert et al. (1957) reported M-2020 was just as effective as deet against *Chrysops discalis* Williston during field tests in Oregon. Finally deet has been shown to be totally ineffective against still other species: *Dermacentor marginatus* (Sulz.), *Hyalomma asiaticum* Schulze (Dremova and Smirnova 1970), *Anopheles pulcherrimus* Theobald, (Zhogolev 1968), *Ornithodoros coriaceus* Koch (Loomis and Furman 1977), *Leptoconops kerteszi* (Kieffer) (Sjogren 1971). Thus, it would seem that while over 350 different reports on deet have been published (Rutledge et al. 1978) the question of the relative superiority of this compound remains to be definitively answered.

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