

DESSION IN .115 White Section 3 acti Section 0 DRESEARCH AND DEVELOPMENT BRANCH 12.05.00 H. A. 4. 10%. DEPARTMENT OF NATIONAL DEFENCE SALAR ANALABILITY CODES CANADA and/or SPECIAL A DEFENCE RESEARCH ESTABLISHMENT OTTAWA TECHNICAL NOTE NO. 76-28 5 PERSONAL PROTECTION AGAINST BITING FLIES: THE RELATIVE EFFECTIVENESS OF OVERJACKETS TREATED WITH VARIOUS INSECT REPELLENTS . by J.M. McAndless and G.P. Underwood **Environmental Protection Section NBC** Defence Division Nov 76 DDC JAN 21 197 John M. /McAndless LUDIL D **RECEIVED NOVEMBER 1976** Greg P./Underwood PROJECT NO. **PUBLISHED NOVEMBER 1976** 79-01-04 OTTAWA 76-204 404576,3

ABSTRACT

Wide-mesh overjackets treated with six different insect-repellent formulations were field tested against a local biting-fly population. The overjacket treatments included N,N-diethyl-m-toluamide (deet), two mixtures containing deet and vanillin, two morpholine derivatives and an oxazolidine derivative.

The overjackets treated with deet and deet-vanillin mixtures provided the most protection against biting flies. In protecting the facial area, these overjackets were as effective as applying deet liquid directly to the skin.

RESUME

On a éprouvé sur place, contre les insectes de l'endroit, l'efficacité d'un veston de tissu mou imprégné de six produits insectifuges distincts. Les différentes préparations comprenaient l'isomère de N-N-diéthyl-m-toluamide (<u>deet</u>), deux mélanges d'isomère de N-N-diéthyl-mtoluamide et de vanilline, deux dérivés de morpholine et un dérivé d'oxazolidine.

Ce sont les vestons traités a l'isomère de N-N-diéthyl-m-toluamide (<u>deet</u>) et au mélange de <u>deet</u> et de vanilline qui ont offert la meilleure protection contre les piqures de moustiques. Ces vestons protègent aussi bien la figure qu'un liquide à base de <u>deet</u> appliqué directement sur la peau.

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INTRODUCTION

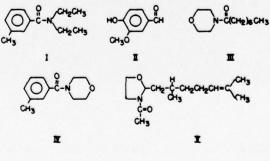
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As an alternative to applying insect repellents directly on the skin, repellent-treated overjackets show considerable promise in providing personnel with relatively long lasting protection against biting flies (1, 2, 3). One such overjacket, developed by the US Navy Medical Field Research Laboratory (NMFRL) consists of a lightweight, wide-mesh polyester netting with cotton strands running through it. The cotton component acts as a reservoir for insect repellent liquid and releases repellent vapour slowly, while the polyester provides strength and abrasion resistance. The garment takes the form of a waist-length jacket with extra long sleeves and a hood which covers the head, but not the face (Figure 1).

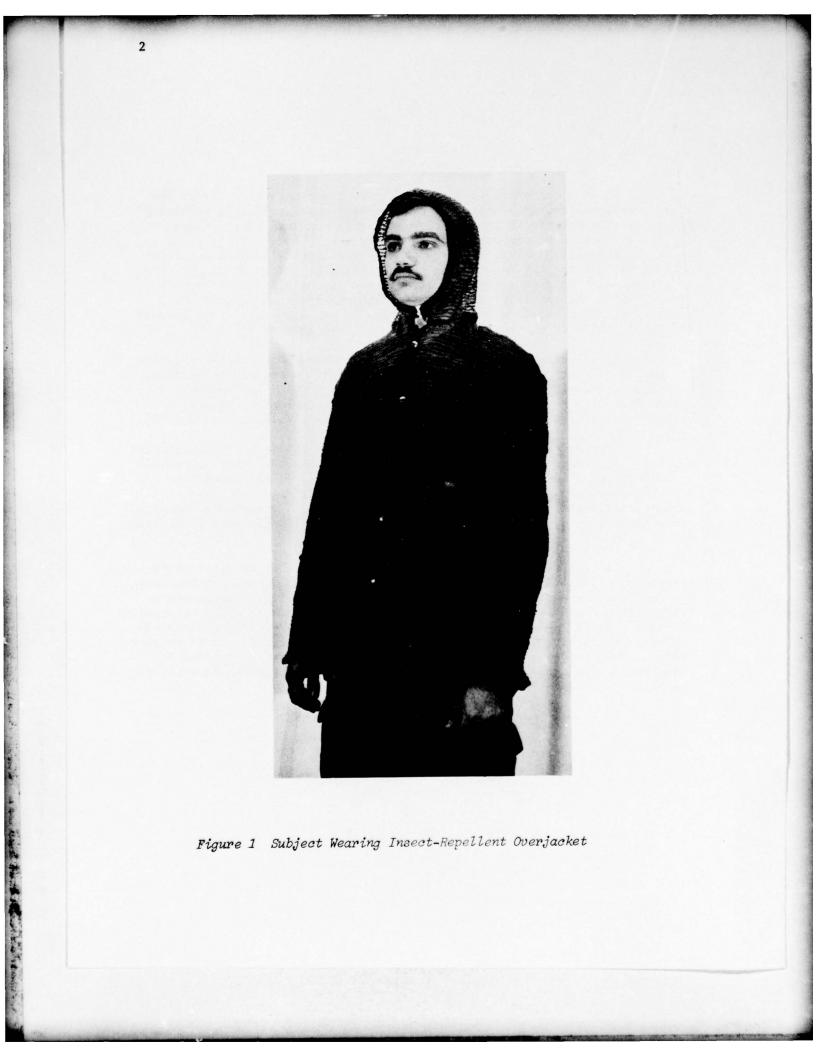
Prior work carried out in Canada (4, 5) has shown that repellenttreated overjackets provide a high level of protection against mosquitoes, tabanids and blackflies. These tests, and others carried out in the United States, have usually been restricted to overjackets treated at various strengths with the repellent N,N-diethyl-meta-toluamide (deet) (I).

Several other chemicals, including some morpholine and oxazolidine derivatives, are potentially more effective repellents than deet, as shown by limited tests against certain mosquito species (6, 7). In addition, deet formulations containing additives such as vanillin (4-hydroxy-3methoxybenzaldehyde)(II) when applied to the skin provide markedly increased protection times against yellow fever mosquitoes in comparison to deet alone (8, 9).

In this report, a small field trial is described in which overjackets treated with deet (I) and several promising compounds and formulations were compared with respect to their effectiveness against a local population of biting flies. The other compounds tested on the overjackets were 4-caprylylmorpholine (III), 4-(m-toluoyl)morpholine (IV), 3-acetyl-2-(2,6-dimethyl-5heptenyl)oxazolidine (V) and mixtures of deet and vanillin (I and II respectively). The treated overjackets found to provide the best protection in these tests were then compared in effectiveness to deet applied directly to the skin.



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MATERIALS AND METHODS

Insect Repellents

N,N-diethyl-m-toluamide (deet) was procured as a 75% solution in isopropanol^a and was used without further purification. Caprylylmorpholine (CM) (III), m-toluoylmorpholine (TM) (IV) and 3-acetyl-2-(2,6-dimethyl-5-heptenyl)oxazolidine (OX) (V) were synthesized according to the procedures given in Appendix A. Following purification, each compound was dissolved in isopropanol to give a 75% (w/v) solution. Vanillin (II) was obtained commercially and used as received. Equal weights of deet and vanillin were dissolved in isopropanol to give a 75% solution (DV). For comparison, a 37.5% isopropanol solution of deet-vanillin mixture was also made up (DV/2).

Overjackets

Jackets were constructed of polyester and cotton netting^b and were patterned after the final design used for the NMRFL overjacket (2). The garments were weighed and then treated at the level of 1/4 g of repellent per g of netting by immersing them in appropriate repellent-isopropanol solutions. In the case of the jackets treated with DV/2, the level of treatment was 1/8 g of deet (and 1/8 g of vanillin) per g of netting.

Following treatment, the jackets were air-dried for 12 hours to permit evaporation of isopropanol solvent. The garments were stored in foillined paper bags when not in use.

During the trial, the jackets were worn over field clothing with the hood drawn up over the head. The sleeves of the jacket were rolled up to the wrists to permit recording of data. Under circumstances not requiring manual dexterity, the sleeves can be rolled down to provide the hands with protection against biting flies. Therefore, in evaluating the overjackets, only the number of insect landings which occured in the facial area was used as a measure of their relative effectiveness; landing data for the hands has been included in the report for reference.

Liquid Repellent

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A 75% solution of deet in isopropanol was used for application to the skin.

Conduct of Field Trial

The trial was carried out during the months of June and July 1976. During this period, relatively hot and humid conditions prevailed, with temperatures ranging between 22°C and 31°C and relative humidities ranging between 55% and 85%.

a This solution is the standard issue repellent for Canadian Armed Forces personnel.

b S-1624 jacket netting, Polylox Corp., New York, N.Y.

The trial site was located in a heavily wooded area near a small stream which was constrained by a beaver dam. A large swamp was situated nearby. During the trial this area contained a population of biting flies made up mainly of mosquitoes and tabanidae. Small numbers of blackflies were also observed. The species of biting flies collected on subjects during testing are listed in Table I.

TABLE I

Genus	Species	% of Total Collected
<u>Culicidae</u> (mosquitoes)	
Aedes	spp.d	19.2
	stimulans (W1k.)	19.1
	intrudans (Dyar)	13.2
	punctor (Kirby)	13.2
	communis (DeG.)	10.3
	excrucians (Wlk.)	8.8
	implicatus (Vock.)	7.3
	vexans (Mg.)	1.5
	diantheus (H.D. and K.)	1.5
	cineurus (Mg.)	1.5
	impiger (W1k.)	1.5
<u>Tabanidae</u> (deer flies	, horseflies, etc.)	
Chrysops	indus (0.S.)	2.9

Biting Fly Species Collected During The Trial^C

^CThe insects were identified by Dr. J.M. McAndless, Defence Research Establishment Ottawa.

^dUnidentified species.

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A series of tests was first conducted with the aim of comparing the effectiveness of the various jacket treatments against the local population of biting flies. The most effective jackets were then selected and further tests were conducted to compare their effectiveness with that of deet applied to the skin. Each of these latter tests was conducted over a longer time interval so as to note whether the level of protection provided by the jackets and/or topically-applied repellent was diminishing during the test period.

Six men were used as subjects for the first part of the trial. Each subject in turn wore one of the six types of treated overjackets or acted as a control (no protective measures used). The control subject was only exposed to the biting flies for a short period of time, generally at the start of each

test. This subject then donned one of the jackets and continued with the test.

After six text periods, a comparison was made between the two most effective jackets and liquid repellent applied directly to the skin using five subjects. Three subjects applied liquid repellent to their faces and hands while the remaining two used the overjackets. This comparison was carried out on two separate days. At the conclusion of the trial each subject had tested all the available jackets at least once, had applied liquid repellent to his skin and had also acted as a control.

The time intervals during which subjects wearing jackets, control subjects, and those with topically-applied repellent were'exposed to the biting flies were recorded. Since some items were exposed for longer periods than others, data for each item was averaged to allow comparison in terms of landings per hour.

Each test period comprised the following routine:

- the subjects were transported to the site and were issued appropriate test items;
- 11) the subjects sat in pairs for a period of time and each subject recorded the number of insect landings which occurred on the face and hands of his partner (Figure 2). A landing was defined as one in which an insect alighted and began to probe or bite. Insect specimens were collected during this time using an aspirator;



Figure 2. Subjects recording insect landings

- iii) from time to time subjects went for short walks and exchanged sitting locations;
- iv) at the conclusion of the test, all equipment was collected and the subjects were transported from the site.

RESULTS

Relative Effectiveness of Overjacket Treatments

An evaluation of the relative effectiveness of six overjackets treated with different repellents was carried out over the first six test periods. A total of 2543 insect landings was recorded during this portion of the trial. Figure 3 shows the number of landings recorded for each test period and indicates that the insect population density remained reasonably constant throughout this period. For reference, the total number of landings recorded for each subject is given in Appendix B.

Table II shows the distribution of recorded landings which occurred on the hands and faces of subjects for each of the items tested. Over the six test periods, control subjects as well as those wearing overjackets were exposed to the biting fly population for differing lengths of time. To compare the various items directly, figures based on the number of landings per hour of exposure were calculated. The calculated data for the face and hands is shown in Table III.

TABLE II

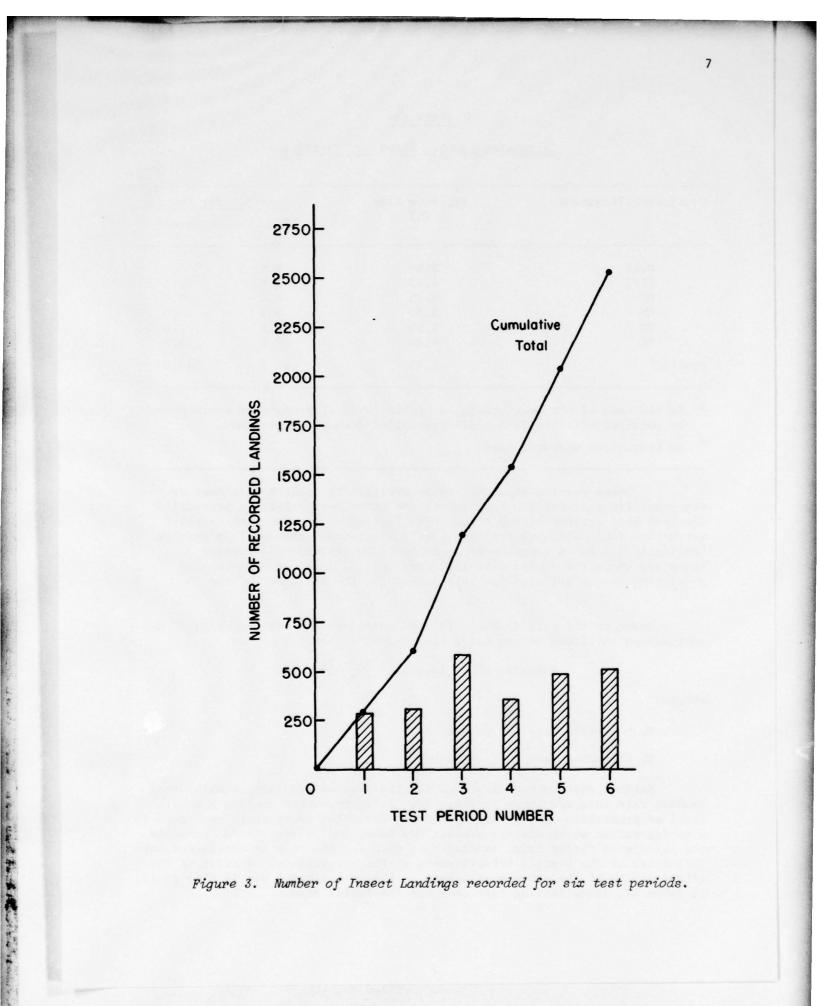
Overjacket Treatment	Exposure Time	Number of Landings		ngs
	(h)	Face	Handse	Total
deet	9.60	8	68	76
DV/2	4.47	8	181	189
DV	8.25	36	246	282
CM	3.50	22	84	106
TM	4.50	76	594	670
ox	4.30	80	557	637
control ^f	1.95	97	486	583

Distribution of Recorded Landings For Six Test Periods

In the case of the overjackets, a higher level of protection would probably be attained with the jacket sleeves rolled down over the hands.

No protective measures used.

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TABL	E	II	Ι
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Overjacket Treatment	Exposure Time (h)	Landings Per Hour	
		Face	Hands€
deet	9.60	0.8	7.1
DV/2	4.47	1.8	40.5
DV	8.25	4.4	29.8
CM	3.50	6.3	24.0
TM	4.50	16.9	132.0
OX	4.30	18.6	129.5
control ^f	1.95	49.7	249.2

Calculated Landing Rates For Test Items

^e In the case of the overjackets, a higher level of protection would probably be obtained with the jacket sleeves rolled down over the hands.

f No protective measures used.

These results show that those overjackets treated with deet or deet-vanillin mixtures are superior to the other overjackets in protecting the head area against biting flies. The overjacket treated with caprylyl morpholine (CM), although relatively effective, was withdrawn from further testing at 3.5 hours exposure after subjects complained of a burning sensation where the jacket material contacted the skin. A deet-treated overjacket was substituted for this jacket for the remainder of the trial.

Based on the data in Table III, a "relative effectiveness rating" was assigned to the items tested using the following formula:

Relative effectiveness = $\frac{N_{c} - N_{i}}{N_{c}} \times 100$

where

 N_c = landing rate for control and

 N_i = landing rate for test item.

Ratings which were calculated for the various overjackets using the landing rate data are shown in Table IV. A higher number implies a greater level of protection against biting flies. Since the overjackets were used in a configuration which did not protect the hands in the best manner possible, the calculated rating using landing rate data for the face is considered more indicative of the overall effectiveness of the overjackets. A ratio of the effectiveness of the other overjackets to the deet-treated one is also given in Table IV, using landing rate data for the facial area.

Overjacket Treatment	Effectiveness Rating		Ratiog
	Face	Hands	
deet	98	97	1.00
DV/2	96	84	0.98
DV	91	88	0.93
CM	87	90	0.89
TM	66	47	0.67
OX	63	48	0.64
control	0	0	0.00

Relative Effectiveness of Overjackets

^g As compared to the deet-treated overjacket; only data for the face is used in computing the ratio.

Comparison of Treated Overjackets and Liquid Repellent

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Based on the data shown in Table IV, the overjackets treated with deet and deet-vanillin mixture (DV/2) were chosen for comparison with deet liquid applied to the skin. This comparison was carried out on two separate days, once in late June and once near the end of July. No further tests were carried out as the insect population density was found to be much lower during the second test period. A total of 275 insect landings was recorded for this portion of the trial, including those landings recorded by the control subjects.

As was the case during the overjacket evaluation, the control subjects, those wearing overjackets and those who applied repellent to their faces and hands were exposed to the biting flies for differing time intervals. Recorded landing data was summed and divided by the exposure time to give results in terms of landings per hour. The comparative data is given in Table V along with relative effectiveness ratings computed using the rating formula described previously.

TA	B	LE	V
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Item	Repellent	Exposure Time	Landin	gs Per Hour	Effectiveness
		(h)	Face	Hands	Ratingh
overjacket	deet	7.72	0.6	3.6 ⁱ	98
liquidj	deet	27.15	1.0	0.3,	97
overjacket	DV/2	3.67	1.4	4.9 ¹	96
control	none	1.00	36.0	148.0	0

Effectiveness of Treated Overjackets and Liquid Repellent

h computed using landing rate data for the face only.

i a lower landing rate would probably be attained if the sleeves of the overjacket were rolled down over the hands.

j on the average, each subject applied a total of 2.4g of deet to his face and hands.

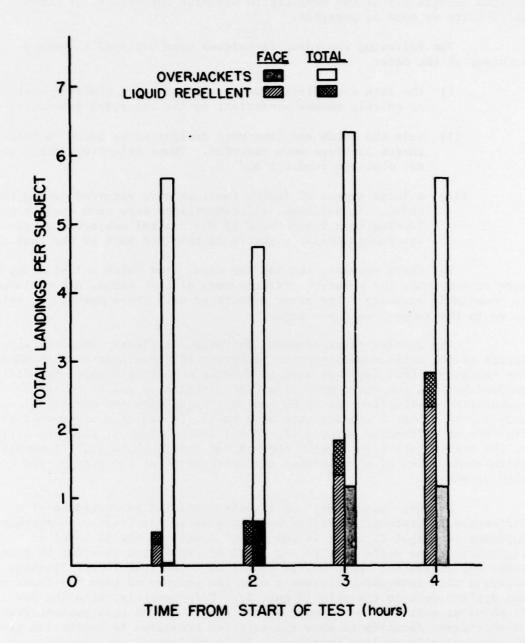
Although the results in Table V indicate that the two overjackets and the topically-applied repellent are equally effective against the biting fly population, the following points should be noted:

- i) the overjackets used in this comparison had been exposed previously for several hours during the earlier part of the trial, and
- ii) the effectiveness of the topically-applied repellent did not remain constant for the duration of each test period (approx. 4.5 hours). That is, subjects using deet liquid recorded relatively few landings over the first hour or two and then recorded increasingly more landings as time progressed.

Figure 4 illustrates this latter effect, using data recorded during hourly intervals for the two test periods in which the overjackets and topically-applied repellent were compared. Since the two overjackets tested are considered to be equally effective, the data for these items has been combined. Figure 4 shows that, with the overjackets, the total number of landings per subject remained fairly constant throughout the test periods while the total for the liquid repellent began to increase after the second hour. Also indicated in this Figure is the distribution of landings which occurred between the face and hands of the subjects. In general, those subjects who applied repellent to their skin tended to experience more landings on the face than on the hands while the opposite was true for those subjects wearing overjackets.

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Figure 4 Effect of time on the level of protection provided by test items



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DISCUSSION

During the trial, test items and control responsibility were rotated amongst all of the subjects to minimize the effects of human variability as much as possible.

The following recording techniques were employed to ensure accuracy of the data:

- i) the data was obtained using subject pairs enabling each subject to quickly become proficient in the recording procedure;
- ii) only the hands and face were designated as areas on which insect landings were recorded. These relatively small areas are observed readily; and
- iii) a large number of insect landings were recorded during the trial. In addition, all comparisons were made against the landing rate experienced by the control subjects, a rate which was substantially higher than that for each of the test items.

For these reasons, the landing data, from which calculations were made to determine the relative effectiveness of test items, are believed to be reasonably accurate. The error associated with these numbers is estimated to be in the range 2 to 5 per cent.

The landing rates recorded in Table III clearly show the superiority of the various deet-treated jackets over those treated with OX and TM. The chi-square test (10) was used to determine whether significant differences existed between the three most effective overjackets and to verify the comparative ineffectiveness of OX and TM. Using data for the facial area only and the control landing rate as a basis, it was determined that no significant difference existed (P = 0.05) in the level of protection provided by the overjackets treated with the various deet formulations (Appendix C). Calculated values of x^2 confirmed the inferiority of the overjackets treated with OX and TM.

The chi-square test was likewise employed to determine if a difference in protection existed between wearing the treated overjackets and applying repellent directly to the skin. Again, at the 5% level of significance, no difference in the level of protection provided by these items was indicated. However, it should be noted that insect landings occurred with increasing frequency with the passage of time for those subjects who applied deet to the skin (Figure 4). Unfortunately, with the low level of physical activity on the part of the subjects, the test periods were not of sufficient duration to show the expected breakdown in protection provided by the topically-applied repellent. It is estimated that, depending on the level of activity, the application of 75% deet liquid to the skin gives between 2 and 8 hours of sufficient protection. By contrast, it has been

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estimated (1) that a jacket would be effective for at least 6 weeks with one treatment, essentially independent of work level. Both of these estimates depend on many variables, such as weather conditions and insect population density.

Since the overjackets treated with the various deet formulations did not differ appreciably in effectiveness, the deet plus vanillin mixture (containing half the usual amount of the toluamide) may be an acceptable substitute for deet alone. There are two possible benefits from this. First, a reduction of the amount of deet used is possible, a desirable situation especially if this repellent should be in short supply. Stocking tests carried out previously (11) on the effectiveness of vanillin as an extender for deet indicate that a mixture containing 82.5% deet and 17.5% vanillin gave the same level of protection as 100% deet against yellow fever mosquitoes. The other advantage may be in enhancing the protection time of deet itself. Recent tests (8,9) indicate that deet formulations containing vanillin, when applied to the skin, provide as much as a doubling in protection time against this same mosquito species, in comparison to deet alone. These results contrast with earlier reports (12), which indicated that no significant increase in protection time occurred with the addition of small amounts of vanillin. In view of the apparent discrepency in the effect of the addition of vanillin to deet, further tests will be necessary to determine whether deet-vanillin overjacket treatments enhance the protection time afforded by the garment treated with deet alone.

Caprylylmorpholine (CM) appears to be unsuitable for further use as a repellent even though it is only slightly less effective than deet. Earlier reports have pointed out the adverse effects of this compound and its homologues on the skin and respiratory tract (13,14); the present work confirms these results. Only a small amount of the compound, such as that which is transferred to the skin in the areas where the hood touches the face, is sufficient to cause an unpleasant burning sensation. This irritation can persist for several hours despite repeated washings, according to the experience of the trial subjects.

The choice of 4-(m-toluoy1)morpholine (TM) as an overjacket treatment was based, in part, on expectations related to its chemical structure. That is, TM contains both toluamide and morpholine moieties; each of these belongs to a class of compounds of which certain derivatives are highly effective biting-fly repellents. However, under the given conditions, the combination of these inherently repellent moieties in toluoy1morpholine did not result in a highly effective repellent.

As a result of the fact that those subjects with topically-applied repellent experience more bites on the face and those wearing jackets more on the hands, a highly effective means for providing protection may be a combination system. In areas such as the Canadian North the wearing of an overjacket and application of repellent directly to the hands may be very effective against large concentrations of biting flies.

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In areas of more moderate insect activity it may be possible to eliminate the body of the jacket, leaving only the hood to protect the face and apply liquid repellent to protect the hands. The benefits of this approach would be a substantial cost savings and a reduction in the amount of snagging which occurs with the overjacket while moving through the bush. Tests to evaluate this concept will be carried out in the future.

CONCLUSIONS

- 1. Under the test conditions, overjackets treated with deet or deet plus vanillin mixtures were equally effective against biting flies and provided the same level of protection to the facial area as applying deet directly to the skin.
- During each test period, the level of protection provided by the treated overjackets remained fairly constant, while the effectiveness of the topically-applied repellent showed signs of diminishing after a few hours.
- The compounds 4-(m-toluoy1)morpholine and 3-acety1-2-(2,6-dimethy1-5hepteny1)oxazolidine are less effective repellents than the deet formulations when used as overjacket treatments.
- 4. Although relatively effective, 4-caprylylmorpholine is unsuitable for use as an insect repellent because of its irritative effects.

ACKNOWLEDGEMENTS

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APPENDIX A

Synthesis of Insect Repellents

All chemicals used as synthesis intermediates or solvents were obtained commercially as the highest purity grade available and were used as received. Reaction products were characterized by infrared spectrophotometry, nuclear magnetic resonance spectroscopy and by reference to published physical data.

4-caprylylmorpholine (III)

A 1-litre 3-necked reaction flask equipped with condenser, dropping funnel and stirrer was charged with ether solution (500 ml) containing 50.5 g of triethylamine (0.5 mole) and 43.5 g of morpholine (0.5 mole). The solution was cooled in an ice-water bath and a solution of 81.3 g of octanoyl chloride (0.5 mole) in 100 ml of anhydrous ether was added slowly with stirring. The mixture was stirred for an additional hour while warming up to room temperature. The mixture was filtered and the solid residue was washed several times with ether. The filtrate and washings were combined and treated in succession with 5% HCl, 5% sodium bicarbonate and water. The ether extract was dried over anhydrous sodium sulfate and then filtered. The ether solvent was removed on the rotary evaporator and the remaining oil was fractionally distilled under reduced pressure.

> Yield: 4- caprylylmorpholine 70.0 g (66%) bp₁₂ 170-177°C (lit. (15) bp_{0.2} 87-97°C) n²⁵_D 1.4724 (lit. (15) n²⁵_D 1.4712)

4-(m-toluoy1)morpholine (IV)

A 150-ml round-bottom flask was charged with 68.1 g (0.5 mole) of m-toluic acid and then fitted with a condenser and gas collection trap. Thionyl chloride (100 ml) was added slowly through the condenser and the mixture was refluxed for 2 hours (gas evolution ceased). Excess thionyl chloride was removed by distillation. Dry benzene (50 ml) was added to the flask and the mixture was again distilled to remove traces of thionyl chloride. The crude toluoyl chloride was then added slowly with cooling and stirring to a solution of 87 g (1.0 mole) of morpholine in 500 ml of anhydrous ether. The mixture was refluxed for three hours, cooled and added directly to 150 ml of dilute hydrochloric acid to neutralize the reaction. The ether phase was separated, washed in turn with 5% sodium bicarbonate and water and then dried over anhydrous sodium sulfate. The ether solvent was removed on the rotary evaporator and the remaining oil was fractionally distilled under reduced pressure.

yield:	4-(m-toluoy1)morpho	line 60g (59%)	
	bp0.9 151-152°C	(lit. (16) bp7 191-194°C)	
	n_D^{21} 1.5542	(lit. (16) n_{D}^{20} 1.5548)	

3-acety1-2-(2,6-dimethy1-5-hepteny1) oxazolidine (V)

A 1-litre 3-necked flask equipped with stirrer, condenser and dropping funnel was charged with 61 g (1.0 mole) of ethanolamine, 34 g of potassium hydroxide and 300 ml of benzene. The flask was cooled in a cold water bath while 77.1 g (0.5 mole) of citronellal was added dropwise with stirring. The mixture was stirred for 2.5 hours following the citronellal addition and then 102 g (1.0 mole) of acetic anhydride was added slowly while cooling and stirring was maintained. The mixture was stirred overnight at room temperature and then added to a large excess of water. The benzene phase was separated, and washed several times in turn with saturated sodium bicarbonate solution and water. Some benzene was added to facilitate the separation of aqueous and organic phases. The benzene solvent of the organic phase was removed on the rotary evaporator and the remaining oil was fractionally distilled through a vigreux column under reduced pressure.

Yield:	3-acety1-2-(2,6-	dimethy1-5-he	ptenyl)oxazolidine	78g (65%)
			bp0.1 120-122°C)	
	n_D^{22} 1.4802	(lit. (17)	n_D^{25} 1.4790).	

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APPENDIX B

Distribution of Recorded Landings Amongst Subjects

Subject	Exposure Time	Number of Rec	orded Landings Fo	r 6 Test Periods
F	(h)	Face	Hands	Total
A	6.3	75	372	447
В	6.2	66	412	478
С	6.8	56	320	376
D	5.6	31	324	355
E	5.7	50	467	517
F	6.0	49	321	370

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APPENDIX C

Chi-Square Analysis of Landing-Rate Data

The chi-square test was used to determine if the differences in landing rates between the overjackets treated with deet and with the two deet and vanillin formulations were significant. In addition, calculations were performed to verify the apparently significant difference between these three garments and the other two treated with OX and TM. Finally, x^2 values were used to determine if a significant difference existed between applying liquid repellent to the skin and wearing a deet or DV/2 treated jacket. As previously mentioned, data for the facial area only was used in these calculations.

Contingency tables (2 X 2) were constructed using the effectiveness ratings shown in Tables IV and V. The null hypothesis tested in all comparisons was that no difference existed between the items in the level of protection provided. The 5% level of significance was chosen (P = 0.05) with a correspondingly tabulated value of x^2 of 3.84, for one degree of freedom (18). Calculated values of x^2 greater than 3.84, therefore, signalled rejection of the hypothesis, while those less than this number indicated no significant difference between the items being compared. One such contingency table for the comparisons of the deet and TM overjackets is shown below.

Overjacket Treatment	Effective	Non-effective	Total
deet	98	2	100
TM	66	34	100
Total	164	36	200

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The Yates correction for 2 \times 2 tables (which improves the chisquare approximation for the 2 \times 2 table and for low frequencies) was employed in all cases. In this example the table becomes:

Overjacket Treatment	Effective	Non-effective	Total
Deet	97.5	2.5	100
TM	66.5	33.5	100
Total	164	36	200

Using the simplified formula for a 2 \times 2 table (19), x^2 was calculated as follows:

- $x^{2} = \frac{n (a'd' b'c')^{2}}{(a+b)(a+c)(c+d)(b+d)}$
 - $= \frac{200[(97.5)(33.5) (2.5)(66.5)]^2}{(100)(164)(100)(36)}$
 - = 32.55

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Since the calculated value of x^2 is greater than 3.84 (P= 0.05), it is concluded that the deet-treated and TM-treated overjackets are significantly different. The results for the remainder of the jacket comparisons are shown in the following table. The shaded area indicates values of x^2 in excess of 3.84. The overjackets, giving rise to these shaded values of x^2 are significantly different.

The comparison between wearing a deet or DV/2 treated overjacket and applying deet directly to the skin gave values of $x^2 = 0.00$. Again, no significant difference between these items is indicated. Chi-Square Values (P = 0.05) Calculated For Overjacket Treatments

deet				
0.17	DY2	en sterryt i	11912 2011	
3.46	1.32	DV		
32.55	27.32	17.06	ТМ	
36.82	31.42	20.58	0.09	ох

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