

**LABORATORY AND FIELD EVALUATIONS OF A REPELLENT SOAP
CONTAINING DIETHYL TOLUAMIDE (DEET) AND PERMETHRIN
AGAINST PHLEBOTOMINE SAND FLIES (DIPTERA: PSYCHODIDAE)
IN VALE DEL CAUCA, COLOMBIA**

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Abstract. The repellency and insecticidal efficacy of Nopikex®, a soap formulation containing 20% diethyl toluamide and 0.5% permethrin, was evaluated against a laboratory colony of phlebotomine sand flies (*Lutzomyia longipalpis*). The repellency of Nopikex soap was also compared with that of a placebo soap against another species (*Lu. Youngi*) in a forest near Tulua, Valle del Cauca, Colombia. In laboratory trials of the soap, no reduction in repellency was seen 4 hr after application, but within 8 hr, repellency decreased significantly to 67.0% of the initial value ($P < 0.05$) based on calculations of the coefficient of protection (CP). Under field conditions, the soap gave up to 100% protection immediately after application, but within 4 hours its CP value had decreased to a median value of only 44.3%. The placebo soap was also found to be somewhat repellent when compared with no treatment, giving a median CP value of 67.7 immediately after application. No significant mortality was seen in sand flies within 24 hr of exposure to the soap in the laboratory, even in those that had fed on protected volunteers.

As the only known vectors of *Leishmania*, phlebotomine sand flies (Diptera: Psychodidae) are of considerable public health importance in the neotropics, where cutaneous leishmaniasis is acquired principally in rural occupational situations or in areas where human dwellings are surrounded by forest.¹ Most New World sand flies of the genus *Lutzomyia* do not breed in periurban situations and have a relatively low propensity to enter houses compared with their Old World relatives (genus *Phlebotomus*). While this ensures that autochthonous urban leishmaniasis is almost unknown in the Americas, transmission of *Leishmania* in rural foci is harder to prevent. The breeding sites of vectors are generally difficult to locate, and the diurnal resting sites of the adults may also be hard to find or possess physical characteristics not conducive to spraying with residual insecticides.

For the above reasons, measures taken to combat leishmaniasis in the New World are generally restricted to diagnosis and treatment of the disease rather than control of the vectors. Treatment is somewhat costly and requires a series of daily or twice daily injections of Glucantime® (Rhone Poulenc, Paris, France) or another pentavalent antimonial that must be given by properly trained personnel. As such, chemotherapy is often impractical, particularly if the patient has to travel long distances and take time off from work to be treated. The pentavalent antimonials are among the safest drugs in current use. Only two deaths have been attributed to these compounds in 40 years of

administration.² However, the treatment may produce unpleasant side effects, particularly in very old patients or those with cardiac or renal problems. Measures by which infection with *Leishmania* can be prevented rather than treated are obviously to be preferred and several studies have been performed to evaluate the efficacy of repellents in various formulations against the bites of phlebotomine sand flies. Most of these are based on N,N-diethyl-*m*-toluamide (DEET), a chemical of proven efficacy against many other biting insects.³

Nopikex®, a repellent soap formulation manufactured under license in Cali, Colombia by Salder Ltd., was first developed in Australia in 1982⁴ and has been tested against disease vectors, particularly anopheline mosquitoes, in several countries.⁵⁻⁷ This formulation preserves the repellent effect of DEET, which is often neutralized by the alkalinity of soap.⁶ It also has the advantages of being relatively inexpensive and is easy to apply. Unlike the more commonly used lotion repellents, Nopikex does not react with plastic and appears to be less damaging to the skin (Salazar R, unpublished data). While no side effects have been reported from the use of this soap, medical problems have been associated with the use of other DEET-based repellents, particularly if the concentration exceeds 50%.⁸⁻¹⁰ A major disadvantage is that once applied, the soap must be left to dry on the skin, eliminating the repellent effect against mosquitoes once it is rinsed off.¹¹

The purpose of this study was to evaluate the efficacy of Nopikex against the bites of phlebotomine sand flies from a laboratory colony and in a forested area near Tulua, Colombia.

MATERIAL AND METHODS

Laboratory experiments. Laboratory trials were conducted using a colony of *Lu. Longipalpis* (Lutz & Neiva) originating in Melgar, Colombia. Five volunteers (four males and one female) were used in the laboratory assays. In each assay, one of the arms of the volunteers was rinsed under cold water, then rubbed with soap from the hand to the elbow for 2 min. Soap was applied by an assistant so that the volunteer's other arm remained uncontaminated. The lather was left to dry for 10 min, after which the volunteer placed his or her arms in two identical cotton barraud cages (75 mm x 35 mm x 35 mm) placed side by side, which were supported by metal frames and enclosed within plastic bags. Each cage contained 7-13 (mean 9.83 ± 0.87), unfed, 3-5-day-old female *Lu. Longipalpis*. Exactly 10 insects were used in 73% of the assays.

Insects were exposed to the arms of the volunteers for 30 min, after which the insects were maintained on a standard sugar/water diet for 24 hr. The mortality of the insects was recorded one and 24 hr after the exposure, and the exposure, and the number of blood-engorged sand flies in each cage was counted. Although the average number of bites per sand fly was also calculated for each cage (corresponding to the number of erythemas visible on the skin and the result of vasodilation by sand fly saliva), it was not possible to determine exactly how many times each sand fly had bitten, since these insects may probe the skin of a host several times before biting. The volunteers were re-exposed to different sets of insects 4 and 8 hr after application of the soap, and the treated arm was not washed during these intervals. Two replicates of the above experiment were conducted with each volunteer. In a second set of experiments, the arms of volunteers were encased in double plastic bags for 30 min before the 4- and 8- hr assays to induce sweating. Two replicates were also performed for each of the five volunteers.

Four additional experiments were also performed under similar conditions, with the exception that volunteers applied a placebo soap (that lacked both DEET and permethrin

but was otherwise identical in composition, appearance, and smell) onto one arm and no soap to the other. Three volunteers participated, one of whom was tested twice.

Field experiments. These were performed in the Juan María Cespedes Botanical Garden at Mateguadua, near Tuluá, Valle del Cauca, Colombia (4°5'N, 76°12'W). The study site consists of an area of secondary forest at an altitude of 1,150 m above sea level. The anthropophilic sand fly fauna of the area comprises four *Lutzomyia* species, of which *Lu. Youngi* constitutes at least 95% of most collections made with protected human bait, with smaller numbers of *Lu. columbiana* (Ristorcelli & Van Ty), *Lu. Lichyi* (Flock & Abonnenc), an *Lu. Shannoni* (Dyar). Although *Lu. Youngi* belongs to the *Lu. Verrucarum* (Townsend) species group and is a suspected vector of *Leishmania* in certain parts of its range,¹² cutaneous leishmaniasis has never been recorded in Mateguadua.

Either four or two pairs of volunteers participated in each of the nine field-exposure experiments in which the repellency of Nopikex was compared with that of the placebo soap. One member of each pair was randomly assigned the former and the other the placebo soap. Participants were not informed which soap they had been assigned. Both members of a pair applied the soaps at the same time, covering the lower legs from the knees to the ankles for two min. During each of the nine assays, two of the pairs applied soap immediately before beginning the exposure and the other two pairs 4, 8, or 12 hr before exposure.

All assays were done during the peak period of sand fly biting activity, within two hours of sunset (7:30 pm). Each exposure lasted 1 hr, during which volunteers aspirated all of the sand flies that landed on the treated surface of their legs. Insects landing on clothing or other parts of the body were not collected.

All wild-caught sand flies were killed, cleared in lactophenol, and identified on the basis of the morphology of the spermathecae. Three additional assays using two pairs of volunteers were performed in which the placebo soap was compared with no soap. One member of each pair applied placebo soap in the manner described above immediately before the assay, which was also carried out for 1 hr after sunset. The other member of each pair did not apply soap to the lower legs.

In a final series of nine assays, the repellent was compared with no treatment, using the same procedure as before. Three assays each were performed in which the placebo was applied immediately before, 4 hr before, and 8 hr before exposure. Comparisons were made based on calculations of the coefficient of protection (CP) calculated from the formula $(A - B) \times 100/A$, with A being the number of insects biting the untreated arm and B the number biting the arm treated with the soap.³ Because volunteers were exposed to many more insects in field trials compared with laboratory assays, repellency in the former was assessed based on number of sand flies probing rather than the number that engorged. Individual flies could also be collected before they had a chance to engorge in the field, since this not being possible in the laboratory experiments.

Statistical analysis. For laboratory evaluations, CP values were compared between time intervals by Kruskal-Wallis analysis of variance of ranks. For field evaluations, the

numbers of sand flies caught on volunteers treated with Nopikex were compared with those on volunteers using a placebo soap by the Mann-Whitney U test. Differences between the mean number of bites were compared by Scheffe's multiple comparison procedure. The CP values of the four time intervals between application of soaps and exposure were compared by the Kruskal-Wallis test to determine whether these values changed significantly with time. Comparisons between numbers of sand flies landing on volunteers treated with Nopikex or the placebo soap were made with those landing on unprotected volunteers by the Mann-Whitney U test.

Acceptance of Nopikex was evaluated in a survey of 40 Centro Internacional de Entrenamiento e Investigaciones Médicas personnel divided into two groups and asked to apply the soap or a placebo to one arm and note whether or not they felt any discomfort 0, 10, and 30 min after application. The results were analyzed by the chi-square test with Yates' correction and the Fisher exact test.

RESULTS

Laboratory experiments. The mean numbers of engorged insects in the laboratory assays are shown in Table 1. This was used as a measure of repellency in addition to the mean number of bites each volunteer received per assay since the latter was variable and could not be determined for individual flies. The CP value is also shown for each assay. Changes in CP values with time for the two sets of experiments (under normal conditions and with induced sweating) are also shown in Table 1. Volunteers who applied the repellent soap immediately before the assay were completely protected from sand fly bites. In volunteers who had applied the soap 4 hr before exposure, the mean CP value decreased to 93.7, and in those in whom sweating had been induced, it decreased to 88.3. The mean CP values for volunteers who had applied the soap 8 hr before exposure decreased significantly ($P < 0.05$) to 67.0 in volunteers in whom no sweating was induced and to 66.8 in those who had sweated.

The results of assays in which the placebo soap was tested against no form of protection in the laboratory were highly variable, and negative CP values were obtained for two of the initial exposures. In exposures immediately after application of the placebo soap, the mean CP value was 61.1, decreasing to 37.8 in exposures 4 hr after application but increasing again to 82.5 in those made 8 hr after application. Although these differences were not significant at the $P < 0.05$ level, the results do suggest that even the placebo soap was repellent to some extent.

Although comparisons of the mean number of bites per assay (Table 2) did not reveal significant reductions in repellency with time for any of the treatments (repellent, repellent with induced sweating, placebo, and control), significant differences were seen among the four at 4 hr and 8 hr after application ($P < 0.05$), with the mean number of bites much greater on control volunteers than on those treated with the repellent soap.

TABLE 1

*Results of assays to determine repellency of the repellent soap Nopikex against Lutzomyia longipalpis sand flies in the laboratory**

	Interval (hr)	<u>Mean no. engorged</u>				<i>P</i>
		Soaped	Control	CP†	H‡	
A	0	0	63.7	100	6.6	0.037§
	4	3.0	66.7	93.7		
	8	19.4	56.4	67.0		
B	0	0	68.1	100	7.9	0.019§
	4	2.0	55.2	88.3		
	8	11.9	62.2	66.8		
C	0	27.8	44.8	61.1	2.43	NS
	4	13.9	24.2	37.8		
	8	18.9	48.6	82.5		

* A = normal; B = induced sweating; C = comparison of placebo versus no treatment and no induced sweating. NS = not significant. Comparisons of coefficient of protection (CP) values between time intervals from application of soap to exposure were made by Kruskal-Wallis analysis of variance of ranks.

† Calculated from CP values in individual assays, not from overall mean values of engorged insects.

‡ The H statistic measures the extent to which *P* samples differ with respect to their relative ranks: $H = 0$ if all samples have the same mean rank and this becomes increasingly large as the distance between sample mean rank increases.

§ H values were significant at $P < 0.05$.

Field assays. Identification of insects captured during the field assays confirmed that 95% were *Lu. Youngi*. Landing activity was very variable among the sampling nights, ranging from one to 364 sand flies/volunteer/hr. Results of the field assays are shown in Table 3. Volunteers who applied repellent soap immediately before the assays attracted only 30.9% as many sand flies as those who applied the placebo at the same time. This difference was significant ($P < 0.05$). The median CP value obtained was only 73.5, which was considerably less than that seen in laboratory assays.

Volunteers who applied repellent soap 4 hr before the assays attracted 44.7% as many sand flies as those using the placebo, with a median CP value of 44.3. When soap was applied 8 hr before the assays, protected volunteers attracted 78.9% as many insects as those using the placebo, with a median CP value of 12.8.

Finally, volunteers who applied the repellent soap 12 hr before exposure attracted 1.07 times as many sand flies as those using the placebo, so that the protective effect at this time was effectively absent. No laboratory studies were done to examine repellency at this interval or to compare the attractiveness to sand flies in the field of volunteers who had applied the soap 12 hr before with those who had not used any treatment. Although it appears from the median CP value recorded for this interval (27.5) that Nopikex retains some repellent effect up to 12 hr after application, in three of the six pairs tested for this interval, protected volunteers attracted more sand flies than their partners who had applied placebo at the same time.

TABLE 2

Mean number of bites per sand fly calculated for each treatment, based on the numbers of erythemas on exposed skin of volunteers

Treatment	Time after application (hrs)		
	0	4	8
Repellent	0	0	0.90
Repellent, induced sweating	0	0.05	0.20
Placebo*	0.75	0.36	0.33
Control	1.62	1.09	1.41
F Probability†	0.068	0.006‡	0.025‡

* Soap lacking diethyl toluamide and permethrin, but otherwise identical to Nopikex.

† Between treatments; none of the within-treatment comparisons were significant at the $P < 0.05$ level.

‡ $P < 0.05$.

Results of assays in which repellent or placebo were compared against no treatment are shown in Table 4. Volunteers using the placebo always attracted fewer sand flies than those who applied no treatment, with the median CP value being 67.7 and the total number captured on those using the placebo being 32.0% of that attracted to volunteers using no soap of any kind. These results confirm those obtained in laboratory comparisons of placebo soap and no treatment. Although relatively few sand flies were captured in the assays in which repellent was compared with no treatment, the CP value obtained for all but one of the exposures was 100, with only three (4.6% of the total number captured being taken on a volunteer protected by Nopikex.

The mortality of sand flies exposed to soaped arms of volunteers immediately after application during our laboratory study never exceeded 7.3%, probably because the sand flies did not make physical contact with the repellent. However, mortality in insects that did feed on arms to which repellent had been applied and sweating was induced was also negligible. Several flies fed on arms treated with the placebo soap, but mortality here was again insignificant perhaps because perspiration had washed the soap from the area that were bitten.

TABLE 3

Results of assays to determine the repellency of Nopikex against phlebotomine sand flies (Lutzomyia youngi) under field conditions

Interval (hr)	Treatment*	No. of flies collected		Coefficient of protection†		Mann-Whitney U statistic‡
		Median	Range	Median	Range	
0	R	805	0-138	73.5	-160.0-100	31§
	P	62.5	5-251			
4	R	17.0	1-50	44.3	0-75.9	NS
	P	43.5	1-83			
8	R	35.5	0-123	12.8	-20-100	NS
	P	53.5	5-120			
12	R	65.5	20-364	27.5	-787.8-8-8-81.6	NS
	P	107.5	41-173			

* R = repellent; P = placebo

† Coefficient of protection values obtained from different intervals compared by the Kruskal-Wallis test, with $H = 2.18$ (not significant at $P < 0.05$).

‡ Nos. Of sand flies caught on volunteers protected by Nopikex compared with those using a placebo by the Mann-Whitney U test. NS = not significant.

§ Only significant difference seen immediately after application of soap. ($U_1 < U = 37$, $P < 0.05$).

TABLE 4

Results of assay to determine the repellency of Nopikex or placebo with no treatment against Lutzomyia youngi (treatment always applied immediately before assay) under field conditions

Treatment*	No. Of sand files captured		Coefficient of protection		Mann-Whitney U statistic†
	Median	Range	Median	Range	
R	0	0-3	100	25.0-100	0‡
N	8.0	4-26			
P	19.5	7-46	67.7	40.2-89.2	1‡
N	61.5	40-128			

* R = repellent; N = no soap; P = Placebo

† No. Of sand flies caught on volunteers protected by Nopikex or placebo compared with those landing on volunteers who had applied no soap by the Mann-Whitney U test.

‡ Significant differences seen in both cases ($U_1 < U = 5$, $P < 0.05$).

The results of the survey on the acceptance of Nopikex or the placebo are shown in Table 5. No significant differences were seen between the repellent and placebo with respect to the number of people reporting discomfort at 0, 10, or 30 min after application. The mild

discomfort recorded by eight of the volunteers using Nopikex and 11 of those using the placebo was described as a slight dryness, tightness, or stickiness of the skin. None of the respondents reported sensations that might have been attributed to the active ingredients of Nopikex (such as tingling, itching, or burning) rather than to the soap itself.

DISCUSSION

Based on the result of our laboratory and field studies, it appears that Nopikex is effective for up to 8 hr after its application in reducing the number of bites of phlebotomine sand flies. Its repellency is based not only on the active ingredients of DEET and permethrin, but also on the vegetable oils used in the manufacture of the soap itself. According to the model for repellent activity presented by Wright¹³ and subsequently modified by MacIver,¹⁴ substances such as DEET function by masking host odor, which prevents the insect from orienting correctly towards the source. Their effect may therefore be described as confusion rather than repulsion of the host-seeking response. The alkalinity of soaps such as the placebo used in the present study might neutralize lactic acid, which is known to be attractive to mosquitoes.¹⁵ This might explain the unexpectedly low numbers of sand flies captured on volunteers using the placebo soap, as well as the lower CP values obtained for comparisons of Nopikex and placebo relative to trials of the former against no soap. The effectiveness of the soap appears, however, to be limited to 8 hr after application, and after 12 hr, its repellency to sand flies is negligible. On one occasion, a volunteer who had applied repellent 12 hr before attracted 364 *Lu, youngi*, nine times as many as his unprotected partner. Rather than suggesting that Nopikex had rendered the protected individual more attractive to sand flies, it appears that the repellent effect of the soap had diminished completely, so that differential attractiveness of the two volunteers now depended on factors such as relative amounts of carbon dioxide and other stimulants they emitted and the air currents on which these were borne, rather than on any property of the soap. It should be noted, however, that at least one study reported DEET to be attractive to mosquitoes at low concentrations.¹⁶

TABLE 5

Relative proportions of volunteers noting discomfort 0, 10, and 30 min after having one arm soaped with Nopikex repellent or placebo under laboratory conditions (n = 20 in each of the two groups)

T (min)	Discomfort noted? (yes/no)		
	Nopikex	Placebo	P*
0	3/17	4/16	NS
10	8/12	8/12	NS
30	5/15	11/9	NS

* No significant differences were seen based on chi-square analysis with Yates' correction or Fisher's exact test (two-tailed) between the repellent and placebo groups for any of the three time intervals after applications. NS = not significant.

The lower CP values obtained for repellent-placebo comparisons (where volunteers using the placebo were also protected to some extent) than for repellent-no soap comparisons (Table 4) also illustrate a common problem involved in the use of repellents, i.e., that of the deflection of biting insects by repellents to unprotected individuals in the vicinity. These unprotected people may therefore run an even greater risk of contracting a vector-borne disease such as leishmaniasis than they would if neighbors or family member did not use a repellent.

Since permethrin is a contact insecticide with low vapor pressure, its effects would be limited to those insects that were not repelled by DEET and lit on the protected individual to feed. Similar to other synthetic pyrethroids, permethrin also has an irritant effect on contact that affects the ability of blood-feeding insects to probe the skin of the host. Curtis and Hill found that the same permethrin concentration used in Nopikex (0.5%) was sufficient to cause 31% mortality in unfed *Anopheles gambiae* that had brushed against the skin of treated volunteers.¹¹ Interestingly, this effect was not seen in fed mosquitoes. Although sand flies are much smaller than mosquitoes, our results suggest that the insecticide content of Nopikex is not effective in these insects, although it may contribute to repellency.

Under laboratory conditions, protection by the repellent was effectively complete for up to 4 hr, and the soap remained more than 60% effective for at least an additional 4 hr. In the field, protection was less long-lasting, and the median number of flies captured on protected individuals doubled within 4 hr of application. Although there is no evidence that the chemical components of sweat reduce the repellency of Nopikex, it does appear to be less effective under field conditions than in the laboratory, and its repellency is likely to be lost even more rapidly if the wearer does manual work that produces copious sweating, causing the covering of soap to be washed off. However, work of this type is less likely to be done at night when sand flies are active and it would probably not normally be necessary to apply the soap as much as 12 hr before protection was needed, unless the activity required the wearer to remain outdoors all night.

Although there is no conclusive evidence to show that the use of repellents is effective in lowering the incidence of leishmaniasis, two similar evaluations of Nopikex have been carried out in Colombia with the participation of soldiers on active service in *Leishmania*-endemic areas (Rojas CA and other, unpublished data and Soto J, Silva R, unpublished data). Neither study was able to demonstrate differences in the incidence of leishmaniasis between groups supplied with the repellent soap and a placebo, perhaps because volunteers assigned placebo were provided with repellent soap by members of the other group or as a result of the information all participants were given for ethical reasons on leishmaniasis and its prevention. The low incidences of leishmaniasis (0-5.3%) recorded in soldiers given the repellent or placebo in these two studies could also indicate that the soap has significant repellent properties even without the active ingredients. To give complete protection against leishmaniasis, a repellent would need to prevent even brief probing of the skin by sand flies, since transmission is not dependent on the insects taking a blood meal.¹⁷

In summary, the repellent soap Nopikex is clearly active against phlebotomine sand flies. The slight discomfort felt by the wearer when the soap is left to dry on the skin is transitory. However, the ease with which Nopikex can be rinsed off the skin means that its use would be limited in certain situations. Repellency is significantly reduced after 4 hr and the soap should be reapplied if it is necessary to remain in an area where sand flies are biting. A 50-g bar of Nopikex costs approximately \$1 (US) and lasts for up to a month of regular use. Although the soap could be distributed at low cost to communities in areas in which sand fly biting is a constant problem (whether or not *Leishmania* is endemic), as with all repellents, its use should be restricted as much as possible to avoid health problems associated with long term use of DEET or permethrin. The toxic effects of both these compounds include irritation to the eyes or mucous membranes and disturbances of the central nervous system if ingested in sufficient quantities. The acute oral 50% lethal dose values of DEET and permethrin for rats are 2 and 430 g/kg of body weight, respectively.¹⁸

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