Carroll-Loye Biological Research
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Preliminary Laboratory Investigation of the Repellency of NO MAS Insect Repellent to the Phlebotomine Fly, *Lutzomyia longipalpis*

Test report prepared by Scott P. Carroll, Ph.D, for

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Information Summary

1) Objective

The objective of this study was to measure the Complete Protection Time afforded by the Test Material against the Phlebotomine sand fly, *Lutzomyia longipalpis* in the laboratory.

2) Test Material

The Test Material was NO MAS insect repellent formulated by Sam Darling.

Table 1. Test Material and Comparison Article information.

Repellent	Description	Active ingredients
NO MAS Insect Repellent 8 NM 1012	White lotion	PMD, Lemongrass oil

3) Control

Untreated skin.

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Testing Materials and Methods

1) Test Site and Date

Repellency testing was conducted in the Arthropod Behavior Laboratory at Carroll-Loye Biological Research on 31 January 2008 – 1 February 2008. Exposure began at 1600 h and concluded at 0030 h the next day.

2) Environmental Conditions

Temperature and relative humidity were recorded at 30 min intervals during efficacy testing.

3) Human Study Subject

One human volunteer participated in efficacy testing. This adult male was highly experienced in insect repellent testing. The level of risk associated with exposure to the bites of laboratory-reared *L. longipalpus* was judged to be low. The volunteer's graduate training in microbiology made him well suited to understand the methods used to assure the disease-free status of the flies. The subject was compensated financially for his participation.

4) Sand flies

Adult sand flies were reared and provided by Claudio Meneses, in the laboratory of Dr. Greg Lanzaro, College of Veterinary Medicine, University of California, Davis. Flies were less than one week old when provided. They were descendants of wild-caught flies from Callejon, Columbia. They were hand-carried from the laboratory sand fly rearing facility of Dr. Lanzaro to the Carroll-Loye laboratory on 31 January 2008.

5) Test Material and its application

NO MAS was couriered to Carroll-Loye Biological Research and received at the letterhead address on 29 January 2008. It was stored at the Carroll-Loye Offices in a closed cabinet at room temperature (20-27°C).

Dosing was based on the rate provided by Sam Darling (2 ml per 1000 cm^2 of skin surface), adjusted for the surface area of the subject's forearm (563 cm^2). The total dosage was therefore 1.126 ml.
Before repellent was applied, the subject washed his forearms carefully with a fragrance-free cleanser in tap water, rinsed them with tap water and then rinsed them again with 35% ethanol in water, and then dried them with a clean towel. Repellent was then applied with 1 ml syringes (0.01 ml measurement increment) and two fingertips in a surgical glove. The subject wore clean surgical gloves, secured at the wrist, throughout testing.

6) Exposure to sand flies

Approximately 500 adult sand flies were contained in the ca. 24 cm^3 nylon mesh cage in which they were received. We presumed that approximately 250 were females. That cage was inserted intact into a 25 cm^3 wire mesh cage that was covered on 5 sides with clear plastic wrap to maintain high humidity. Both cages had sleeves made from a tube of elastic fabric, and these were aligned to permit insertion of a forearm while nearly eliminating escape by flies. After treatment with NO MAS at 1600 h, the first exposures of the treated and untreated control arms was at 1630, and at one-half hour intervals thereafter until failure. Exposures were for 1 min (treated) and approximately 30 sec (untreated). We employed reduced exposure duration for the untreated arm because doing so permitted assessment of avidity while containing the number of bites to which the volunteer was subjected, and prevented a substantial proportion of the flies from feeding to repletion (and thus losing avidity) during the test. The untreated control arm was always exposed first.

7) Stopping Rule

Failure of the treatment was defined as that event in which a bite was *followed by another bite* in the same or in the next exposure period (i.e., the 'first confirmed bite'). The subject was instructed to cease exposure after failure.

8) Data recording

Landings were regarded as equivalent to bites when a fly landed on, or climbed through arm hair to, the skin surface, and remained stationary with its ventral head pressed to the skin surface. A dim light source was used to permit visualization. Landing/bite data, along with environmental data, were recorded on a data sheet every 30 minutes, after each one-minute exposure.

9) Data analysis

Use of only a single subject leads us to rely mainly on the presentation of raw data. Complete Protection Time is defined as the elapsed time between treatment and failure.

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Test Results

Environmental Conditions

Efficacy data were collected under suitable environmental conditions for foraging sand flies. In 17 records from 1600-0030 hrs, temperature ranged narrowly, from 25.6-26.6 °C. Relative humidity ranged from 40-54%.

Ambient Landing Pressure

The number of landings accrued in each 1-min exposure of the untreated arm ranged from 2-5 (Table 1).

Influence of Test Material on Probability of LIBes

Sand flies were strongly affected by NO MAS, and alighted on the subject in only a small minority of exposures (Table 1).

Table 1. Number of bites by individual *L. longipalpis* on the treated and untreated forearms of a the volunteer[1].

Exposure time	Bites on Treated	Bites on Control
1630	0	2
1700	0	3
1730	0	5
1800	0	3
1830	0	2
1900	0	3
1930	0	5
2000	0	2
2030	0	3
2100	0	4
2130	0	3
2200	1	5
2230	0	2
2300	0	3
2330	0	3
2400	1	3
2430	1	4

[1]Treated arms were exposed for 1 min, while untreated arms for approximately 30 seconds. Exposure intervals were 30 minutes, and commenced 30 minutes after the time of treatment.
Based on the data presented in Table 1, the Complete Protection Time spanned from 1600-2400 h, or eight hours.

Discussion and Conclusions

NO MAS provided approximately 8 h of protection against bites of *Lutzomyia longipalpis* of the Callejon, Columbia test population, in the laboratory. This result should be regarded as preliminary, due mainly to the use of only one volunteer, one laboratory-reared sand fly population, and the absence of replication. However, several factors suggest high value for this repellent.

First, phlebotomines are known to be repelled by other repellents to which mosquitoes are responsive, including those containing DEET, picaridin, and MS-220. Thus it is not surprising that NO MAS, which utilizes the powerful mosquito repellent molecule PMD, worked well in this study.

Second, the test conditions, though artificial, were challenging. Preliminary observations showed that exposures of greater than 30 seconds were met with dozens of bites. Consistent with this observation, during the first 30 seconds after the untreated arm was inserted to the cage, increasing numbers of flies oriented to the arm and began to land on it. The density of flies very near the arm, and evidently about to land on it, was many times greater at 30 seconds than near the time at which the arm was inserted, in every exposure.

Third, the three recorded bites, beginning with the first at 6.5 hrs, all took place above the wrist along the ridge of the radius bone. That area was the part of the forearm for which it was most difficult to avoid brushing the cage sock when inserting the arm for each exposure. The later two bites were along the same ridge of the radius within a couple of inches of the wrist. In contrast, the remainder of the arm, including the broadly hairless dorsal region, was completely protected throughout sampling. Accordingly, it is possibly that abrasion of the treated arm against the cage sock reduced the duration of repellency that we recorded.

These suggestions serve to increase confidence in the performance of the NO MAS repellent, and perhaps the representativeness of the results of this small study. Beyond

the limitations of sample size, setting and replication listed above, we made no other observations that suggested anything but near absolute protection throughout the study period. The volunteer reported the repellent to have a pleasant fragrance, to be easy to apply, non-greasy with excellent vanishing properties.

In conclusion, NO MAS shows excellent potential as a repellent of phlebotomine flies.