NEW REPELLENT COMPOUND SHOWS PROMISE AGAINST BITING MIDGES


ABSTRACT

Repellent field trials using human volunteers were conducted at Stansbury Island, Great Salt Lake, Utah, to compare the efficacy of four candidate repellent compounds against ceratopogonid midges of the genus Leptoconops. Leptoconops at Stansbury Island were very abundant, but their biting activity was closely related to weather conditions at the time of collections. In a direct comparison of efficacy of repellent candidates AI-37220 and DEET, the former outperformed the latter.

KEYWORDS: repellents, DEET, Leptoconops.

INTRODUCTION

Biting midges that most often attack humans fall into two genera, Leptoconops and Culicoides. These pests are not commonly associated with transmission of human pathogens, but they are nuisance biters and achieve such high populations that they can severely restrict efficient accomplishment of outdoor duties. The fact that soldiers have adopted a descriptive vocabulary for these biting flies (e.g., no-sees-ums, screaming mimis, teeth with wings) reflects the severity of the problem. Leptoconops are abundant during the spring along the shores of the Great Salt Lake and elsewhere in the Salt Lake Valley (Rees and Winget, 1970, Rees et al., 1971). The larvae develop in vast areas of briny oolitic sand that border the lake. As a result, this area provides a convenient location for field testing repellents.

The Department of Entomology, Walter Reed Army Institute of Research, is specifically funded for advanced development of an improved repellent product that protects against biting midges and outperforms the 34%-DEET extended-duration repellent formulation (EDRF) currently in the Army inventory. To

* The views of the authors do not purport to represent the views of the Department of the Army or of the Department of Defense. For the protection of human subjects, the investigators adhered to policies of applicable Federal Law 4SCFR46.
that end, this field study was designed to compare the inherent repellency of four compounds against ceratopogonid midges of the genus *Leptoconops*.

**METHODS AND MATERIALS**

Prior to actual conduct of repellent trials, a two-day presurvey was conducted to find a suitable location and to determine the best way to expose volunteers to the *Leptoconops*. The western shore of Stansbury Island in the Great Salt Lake (Tooele County, 37 miles NW of Tooele Army Depot), was selected as the trial site based on abundant flies found there.

Our original plan was to place the repellent material on forearms, adjusting the quantity to the size of the arm in order to achieve 400 micrograms/cm² (Coleman et al. 1993). However, during the presurvey, it was found that most of the biting occurs on the ears, not on the arms. In order to modify the procedure to test the repellents on the ears, the long and short dimensions of the ear were measured on each volunteer and the product multiplied by 2 to approximate the ear surface area.

Four repellent products were tested (Coleman et al. 1993). Recent work by the U.S. Department of Agriculture, the U.S. Army Environmental Hygiene Agency, and by Coulston International Inc. resulted in the identification of three compounds with repellent activity that are approved for testing on human volunteers. Two of these compounds, AI-37220, and AI-32765, are structurally related to the standard Army repellent, DEET. The third is a proprietary product consisting of bicyclic lactones and is designated CIC-4. The fourth compound tested was DEET.

There were two levels of treatment: (1) the type of repellent, and (2) time between application and exposure to fly bites. Ethanol solutions of the four test repellents (12.5%) and ethanol alone were applied to the ear at a dosage equal to 400 micrograms/cm² (American Society for Testing and Materials 1983; Coleman et al. 1993). The head was then covered with a nylon stocking with the ears protruding from small holes. Biting rates on the ears were recorded at 0, 2, 4, 6, and 8 hours after application. It was suspected that populations would vary with time of day, hence applications of repellents were timed so that each delay after application occurred once at 1000, 1200, 1400, 1600 and 1800 hours. For example, on one day repellents were applied at 0200, then tested at 1000 following an 8-hour delay. Repellents could then be washed off with ethanol and new applications made for tests at 1200 (0 hr.), 1400 (2 hr), 1600 (4 hr), and 1800 (6 hr).

The five treatments were rotated through all volunteers in random order. Two volunteers were also designated as controls (untreated) for each day of the study;
the order of this assignment was also random. Control individuals made 15-minute collections on the hour and half hour from sunrise until sunset, with concurrent measurements of dry and wet bulb temperatures, wind speed, light level, and sky conditions. This procedure was repeated on 9 days of the study.

Flies in the process of biting (judged by pain and by posture of the fly) were collected from the ears for 15 minutes or until 20 flies had bitten, whichever came first. If 20 or more were collected before a full 15-minute period had elapsed, the time was recorded and the data normalized to the number of flies that would have bitten during the complete interval. Flies were collected by a second person whose head was protected by a net. They were collected with mouth aspirators, transferred to permethrin-treated cartons, which quickly immobilized the insects, then counted and preserved in 80% ethanol in an individual vial for each collection for later identification at the Walter Reed Biosystematics Unit. Great care was taken to insure accurate identification, since it was possible that more than one species was present. To support identification, male *Leptoconops* were collected from swarms and emerging from pupae in sand.

**RESULTS**

All *Leptoconops* collected during these trials were of one species, *Leptoconops americanus* Carter. *Leptoconops* at Stansbury Island were very abundant, but their biting activity was closely related to weather conditions at the time of collections. During our brief visit to the area, temperatures ranged widely from a day-time low of 5°C to a day-time high of 29°C. Few flies bit at temperatures below 12°C (54°F) and numbers decreased at temperatures above 25°C (77°F) (Figure 1). Wind also influenced the activity of the flies, the number of bites decreasing when wind gusted above 6 mph at lower temperatures. Cloudiness and rain at the time of collection inhibited activity, but especially during periods of lower temperature. On some days, there was evidence of a minor peak of biting activity in late morning and a major peak in late afternoon (Figure 2).

The trials of repellents in ethanol showed that Al-37220 maintained 95% effectiveness four hours after application which was better than the other three repellents. With the exception of CIC-4, all repellent candidates provided greater than 95% reduction in bites for at least two hours (Table 1). The effectiveness of both CIC-4 and Al-32765 diminished to 71% after 4 hours and to less than 50% after eight hours.

Two separate statistical analyses indicated that Al-37220 outperformed the other repellent candidates. Analysis of variance of the number of bites for all trials resulted in a significant difference between repellents ($P=0.03$), with Al-37220 more effective than Al-32765 and CIC-4 (Table 2). In
contrast, reduction in biting attributable to treatment with DEET was not significantly different from reductions attributable to the other repellents. Although the analysis of variance did not show a significant difference between DEET and AI-37220, in a direct comparison between these two repellent treatments with a paired t-test (paired by trial), AI-37220 outperformed DEET ($P = 0.04$) (Figure 3).

DISCUSSION

_Leptoconops_ biting midges at Stansbury Island were sufficiently numerous to create a serious operational threat to unprotected troops. Biting rates as high as 840 bites per hour were recorded on the ears alone. The bites of these flies are painful when inflicted and create an inflamed induration with a central petechia, which in 3 of 6 volunteers persisted with itching and swollen lymph nodes for several days. Furthermore, these flies have the habit of crawling about the skin before biting, which is also extremely annoying.

This study provided a clear demonstration of the value of understanding the biology of biting arthropods for support of military operations. Stansbury Island would be a very challenging environment for outdoor duties because of biting flies, yet a survey conducted at the wrong time would have suggested only a minor problem. Biting activity is heavily influenced by weather conditions at the time of collection, so that a survey during a cool, windy, or rainy day might leave the impression that biting pressure was low, even though hundreds of bites per hour could be expected the next day or even later on the same day.

Of the repellent candidates tested, AI-37220 performed the best, providing 95% protection for at least four hours. DEET and AI-32765 provided two hours less protection and CIC-4 was 95% effective only immediately after application. Based on the Stansbury Island study alone, we would recommend further development of AI-37220.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Sammie Dickson and Mr. Bob Brand, managers of the Salt Lake City and Tooele Valley Mosquito Abatement Districts, respectively, for their cooperation and generous support. Both managers kept us informed as to weather conditions and earliest fly emergence, significantly facilitating accomplishment of our mission.

REFERENCES CITED


Table 1. Reduction in the number of bites received by repellent-treated volunteers as a percentage of the mean number of bites received by untreated (control) volunteers. Based on four replicates at 0 hours and 5 replicates at 2, 4, 6, and 8 hours. The mean number of bites on ears of control individuals for each interval after application were 30 at 0 hours, 37 at 2 hours, 53 at 4 hours, 47 at 6 hours and 47 at 8 hours.

<table>
<thead>
<tr>
<th>Repellent</th>
<th>0 hours</th>
<th>2 hours</th>
<th>4 hours</th>
<th>6 hours</th>
<th>8 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEET</td>
<td>98</td>
<td>96</td>
<td>80</td>
<td>68</td>
<td>69</td>
</tr>
<tr>
<td>CIC-4</td>
<td>95</td>
<td>92</td>
<td>71</td>
<td>60</td>
<td>47</td>
</tr>
<tr>
<td>AI-37220</td>
<td>100</td>
<td>99</td>
<td>97</td>
<td>77</td>
<td>74</td>
</tr>
<tr>
<td>AI-32765</td>
<td>99</td>
<td>98</td>
<td>71</td>
<td>36</td>
<td>45</td>
</tr>
</tbody>
</table>

Table 2. Results of comparative analysis on the number of bites received by all treatments for all trials using the Student-Newman-Keul’s test*. Means followed by different letters are significantly different.

<table>
<thead>
<tr>
<th>Repellent</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>SNK*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIC-4</td>
<td>16</td>
<td>27</td>
<td>a</td>
</tr>
<tr>
<td>AI-32765</td>
<td>15</td>
<td>23</td>
<td>a</td>
</tr>
<tr>
<td>DEET</td>
<td>10</td>
<td>18</td>
<td>ab</td>
</tr>
<tr>
<td>AI-37220</td>
<td>6</td>
<td>12</td>
<td>b</td>
</tr>
</tbody>
</table>

DF = 3/72; F = 31; P = 0.03
Figure 1. Biting activity of *Leptoconops americanus* in relation to dry-bulb temperature at Stansbury Island, Great Salt Lake, Utah.
Figure 2. Temporal distribution of diurnal biting activity of *Leptoconops americanus* at Stansbury Island, Utah.
Figure 3. Results of a direct comparison of AI-37220 and DEET, using a paired t-test (paired by trial; n = 25; p = 0.04).