

**FINAL REPORT**

**Penta5USA Phase I –**  
***In Vitro* Repellent Bioassay**

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## ABSTRACT

All Penta5USA formulations maintained significantly fewer bites than controls and outperformed Cutter Skinsations 7% DEET at 0, 1, 2, 4, 6, and 8 hours post-treatment. Repellency for ST-S ranged up to 100%. NBNS-L, NBNS-S, and STDC-S peaked at 93% and maintained 85% repellency or greater throughout 8 hrs.

## Objective

Quantify and compare repellency of four Penta5USA formulations to a DEET standard, and a non-treated control against yellow fever mosquitoes, *Aedes aegypti*.

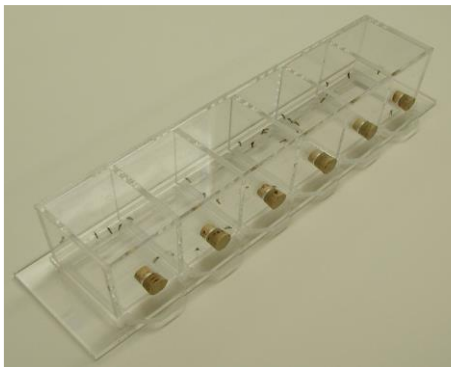
## Materials and Methods

1. This study was performed on November 12, 2019 in a temperature-controlled room following techniques developed by Klun et al (2008).
2. Bioassays were conducted in nine, six-chambered Plexiglas K&D modules interconnected with hoses to a water bath and a temperature-controlled inversion circulator (Fig. 1).
3. Sugar water was removed from colony reared *Aedes aegypti* 24 hrs. prior to testing to insure avid biting activity. Five females were aspirated into each K&D chamber (Fig 2).
4. Nine replications of the following treatments were randomly assigned to the chambers (Fig. 3):
  - a. Four Penta5USA repellent formulations:
    - i. No Bite No Scent Lotion (NBNS-L)
    - ii. No Bite No Scent Spray (NBNS-S)
    - iii. Still Tails for Dogs and Cats Spray (STDC-S)
    - iv. Secure Still Tails (ST-S)
  - b. Positive control - 7% DEET standard (Cutter® Skinsations Insect Repellent)
  - c. Negative control - Non-treated
5. Repellents were applied with a small paint brush over 12 cm<sup>2</sup> ink-demarcated rectangles drawn on 2"X2"X9" strips of organdy cloth. Liquid repellents were applied with at 27.6 ul with a micropipettor and the lotion was applied at 0.06g (Fig. 4 a-d).
6. Treated cloth strips were taped to flat plastic templates placed between the K&D modules and lower Plexiglas bases (Fig. 1). The templates had five 12 cm<sup>2</sup> rectangular holes cut into them to expose treatments to the lower Plexiglass bases (Fig. 5).
7. Surface wells in the lower Plexiglas bases were filled with a blood substitute (i.e., CDTA and ATP mixture) and were covered with moistened collagen membranes (Fig 6).
8. The blood substitute was heated to 38°C with water pumped through hose lines attached to the lower bases and the water bath (Fig. 6).
9. Mosquitoes were exposed to the treated surfaces by opening the sliding doors in the K&D modules for 90-second biting counts at six post-treatment time intervals (0, 1, 2, 4, 6, and 8 hrs.) (Fig. 1).
10. Fresh mosquitoes were aspirated into the chambers for each time interval.
11. Ambient temperature and humidity were continuously recorded throughout the study with a HOBO data logger.
12. Biting count means were the dependent variables in the statistical model. Time interval, module, column, row, chamber, and treatment were independent variables (i.e., main effect variables).
13. Residuals were tested for normality with SAS Proc Univariate and the Shapiro-Wilk Test.

14. Variances in biting count means by time and treatment were statistically analyzed with SAS Proc ANOVA and compared with Duncan's multiple comparisons test at  $p < 0.05$ . Means were charted with standard errors.
15. Mean % repellency was calculated by subtracting the mean biting count in the control from the treatment, dividing by the control, and multiplying by 100. This was performed for each time interval and charted.



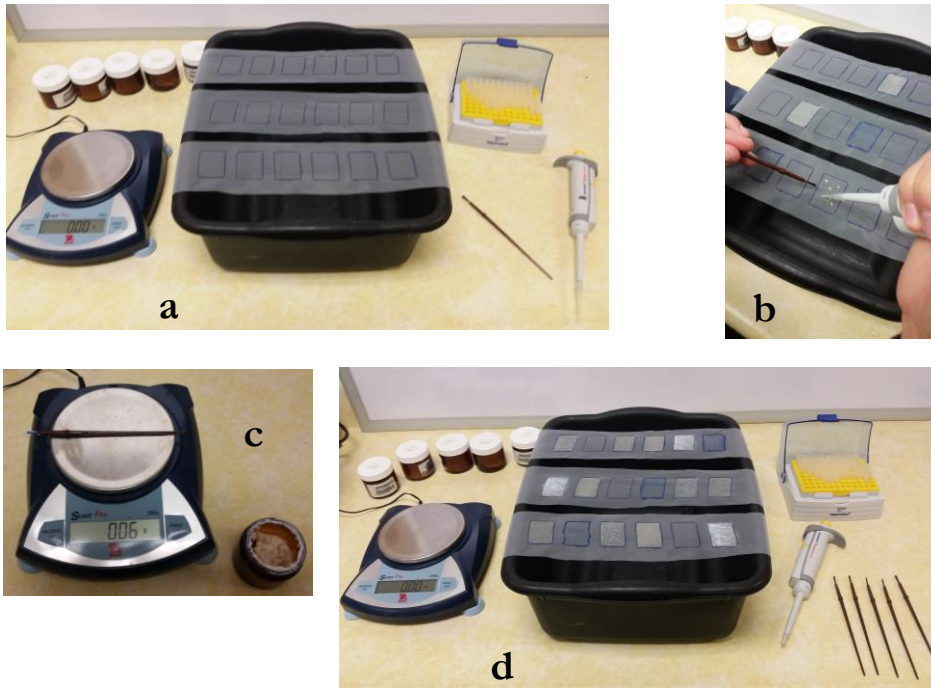
**Fig. 1. Upper K&D modules stacked on lower bases and treated cloth-covered templates. Doors opened to expose mosquitoes to treatment surfaces.**



**Fig. 2. A K&D module equipped with a sliding door beneath each of six chambers stocked with 5 female mosquitoes.**



**Fig. 3. Repellent treatments (left to right): No Bite No Scent Lotion (NBNS-L), Still Tails for Dog and Cat Spray (STDC-S), No-Bite No Scent Spray (NBNS-S), Still Tails Spray (ST-S), and Cutter Skinsations DEET 7%)**



**Fig. 4.** Process for applying repellents to cloth strips, a. ink-demarcated cloth strips taped to dish pan, b. spray repellents applied with micropipettor and paint brush, c. lotion repellent weighed and applied with paint brush, d. cloth strips with treatments applied.



**Fig. 5.** Treated cloth strips attached to plastic templates placed on top of lower Plexiglas bases.

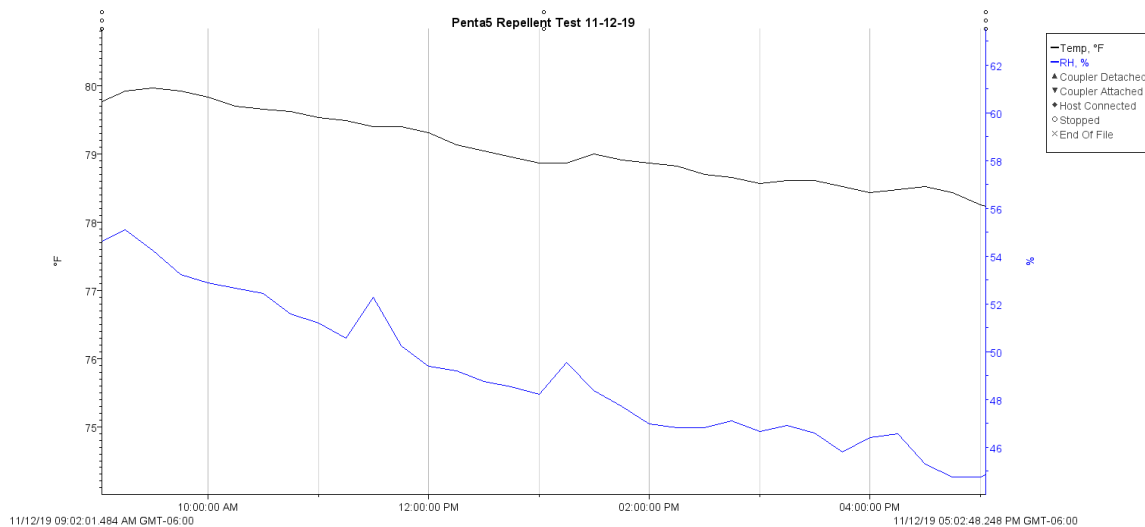


**Fig. 6. Lower bases containing surface wells filled with blood substitute covered with collagen and interconnected to temperature-controlled water bath.**

## Results

### *Environmental Data:*

The bioassays were conducted on November 12, 2019 beginning at 9:03 a.m. (CDT) and ending at 5:05 p.m. (Fig. 7). Ambient conditions were relatively stable throughout post-treatment intervals ranging 78-80°F and 45-55% R.H.



**Fig. 7. Ambient temperature and relative humidity during bioassays.**

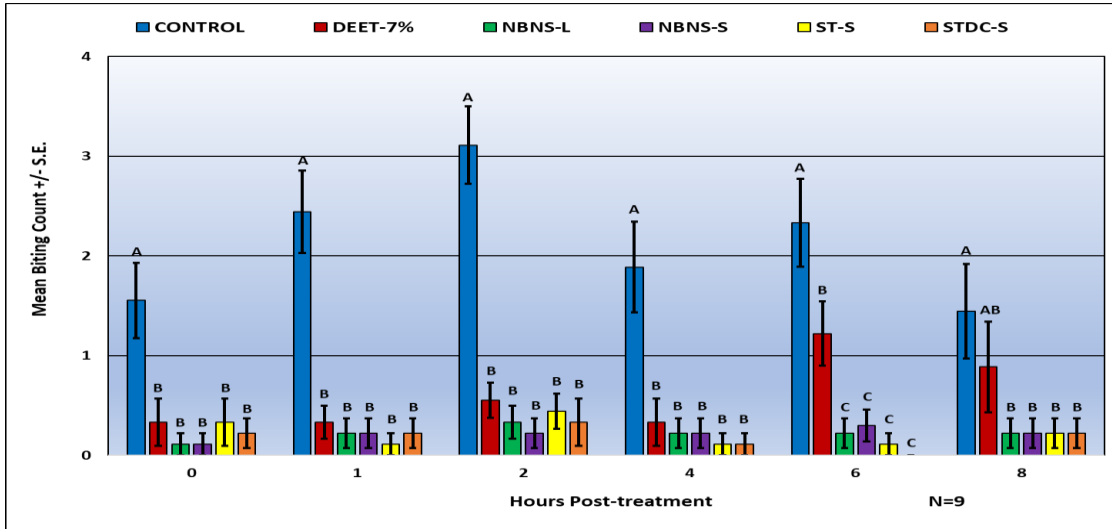
### *Normality Tests:*

There was no difference in data distribution between non-transformed and log or square root transformed data. Consequently, all statistical analysis was conducted on non-transformed data.

### *Biting Count and Repellency:*

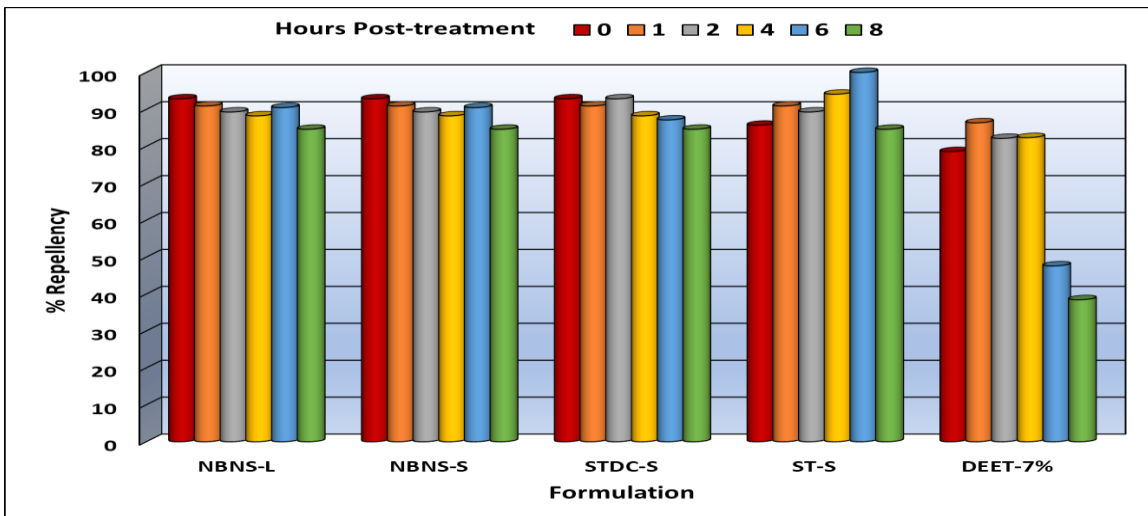
There were no significant differences in average biting counts among the nine K&D modules. Biting counts in the controls were significantly greater than all Penta5USA

formulations at 0, 1, 2, 6, and 8 hrs. post-treatment (Fig. 8). Cutters 7% DEET lost effectiveness at 8 hrs. with biting counts not differing significantly than the control. There was no significant difference in biting counts among the four Penta5USA formulations at any of the post-treatment time periods.



**Fig. 8.** *Aedes aegypti* mean biting counts with standard errors at 6-time intervals for each treatment. Means designated with different letters within same time intervals were significantly different at  $p < 0.05$ .

As measured by percent repellency, all Penta5USA formulations performed better than the Cutter Skinsations 7% DEET at all time intervals (Fig. 9). Repellency for NBNS-L, NBNS-S, and STDC-S ranged from 93-85% at 0-8 hrs. post-treatment, respectively. ST-S ranged from 86-100% from 0-6 hrs., respectively, and decreased to 85% at 8 hrs. The greatest repellency for Cutter Skinsations was 87% at 1 hr. post-treatment. Repellency was slightly lower through 4 hrs. and then dropped to 48% and 39%, respectively, at 6 and 8 hrs. post-treatment.



**Fig. 9.** Percent repellency of experimental formulations compared to 7% DEET against *Aedes aegypti* at 0, 1, 2, 4, 6, and 8 hrs post-treatment.

## **Conclusions & Discussion**

All Penta5USA formulations performed as well as Cutter Skinsations 7% DEET out to 4 hrs and exceeded DEET at 6 hrs as measured by repellency post-treatment. Instances where repellency increased at later time intervals were attributed to normal variation expected in this type of bioassay.

This study screened experimental formulations under highly controlled conditions. It is important to note results may vary with different mosquito species, environmental conditions, and in human subject trials. Further research is warranted to substantiate these findings. These results, although preliminary, are encouraging.

## **Literature Cited**

Klun, J. A., M. A. Kramer, A. Zhang, S. Wang, and M. Debboun. 2008. A quantitative in vitro assay for mosquito deterrent activity without human blood cells. *J. Am. Mosq. Contr. Assoc.* 24:508-512.