

Modification of the K&D Module Technique for Efficacy & Duration Evaluation of Commercial Repellents

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ABSTRACT

The protocol using the K&D module for mosquito repellent testing was modified to screen commercial and experimental repellents for efficacy and duration. 18 repellents were tested against *Culex quinquefasciatus*. Repel Lemon Eucalyptus®, Mosiguard® and some of the experimental formulations provided long-term repellency equal or surpassing 24% DEET. Other botanicals (Walk About® & Buzz Off®) also provided repellency comparable to 6-10% DEET.

INTRODUCTION

Several methods have been employed to evaluate mosquitoes repellents. Although field efficacy is the ultimate indicator on how well a product works, environmental and biological variability make these studies difficult to perform and interpret. Consequently, laboratory methods have been developed under more controlled conditions. Of these, the hand-in-cage test is probably the most common. This technique is quite labor and time intensive. It limits the number of repellents that can be tested and the amount of replication. Another method developed in the 80's (Anonymous 1983), involved strapping a small multi-chambered, plastic screened cage containing mosquitoes to evaluators' arms. More recently, this technique was improved with advent of the K&D module and testing technique (Klun & Debboun 2000). The K&D module is similar to the previous-mentioned cage except it is completely enclosed with sliding doors located beneath each of six mosquito-holding chambers (Fig. 1). It reduces potential repellent interaction and the testing protocol used allows for more replication. We found the K&D module technique to be superior to outdoor and hand-in-cage methods. However, we did modify the technique to further reduce repellent interactions and to optimize a system for testing *Culex quinquefasciatus*.

OBJECTIVES:

1. Modify K&D repellent testing protocol for duration and efficacy evaluation against *Cx. quinquefasciatus*.
2. Evaluate several commercial and experimental repellents using the modified technique.

MATERIALS & METHODS

The following alterations were made to the K&D technique:

1. 10 mosquitoes/chamber was determined to be ideal for testing *Cx. quinquefasciatus*.
2. Clear packing tape was applied to the base of the modules and cut open to expose the sliding doors. The tape was removed, alcohol swabbed and replaced between replications to reduce module contamination.
3. Randomly assigned treatments were applied at 28.6 ul to 12 cm² rectangles drawn on the skin surface with a template aligning with the door openings.
4. Three treatments each separated by one chamber width were tested simultaneously on the surface of the leg. By rotating the chamber 180° to the opposite leg, we were able to test three

additional treatments utilizing all six chambers. The top and both sides of each leg were used so that the evaluator could perform three reps of each treatment

5. Six treatments consisting of five test repellents and a non-treated control were tested simultaneously by three evaluators. Each evaluator conducted two-minute biting counts, three treatments at a time, three times on three surfaces of both legs for each time interval.
6. Tests were repeated at 0, 1, 2, 4 & 6 hrs. post-treatment with freshly stocked mosquitoes between assays. Each evaluator was supplied with six modules to rotate between assays. Modules were cleaned and restocked with mosquitoes between time intervals.
7. Each treatment was tested nine times/day at each time interval and the tests were repeated over three days so that repellency means were based on 27 observations per time interval.
8. 18 commercial and experimental repellents were tested in four separate studies (Fig. 2a-d).
9. Percent repellency was calculated by subtracting the number of bites in treatment from control divided by control multiplied by 100.



Fig. 1. K&D Repellent Test Module



Fig. 2a. Repel Lemon Eucalyptus, Mosiguard, Bug Guard, Homola E (Experimental) & OFF! Deep Woods 24% DEET.



Fig. 2b. ShooBug, Buzz Away, Royal Neem, OFF! Skintastic 6.7% DEET & Walk About



Fig. 2c. Buzz Off, Alternative-Experimental, Comparable-Experimental, Cutter 6.7% DEET, Cutter 9.5% DEET



Fig. 2d. Mookies Insect Repellent, Skin-So-Soft bath oil & BVA Conceal

RESULTS & DISCUSSION:

Comparative repellency for the 18 products tested are presented in Fig. 3-6. Each chart displays the least to the most effective products from left to right. Overall, the best performing repellents are presented in Fig. 3. There was very little difference in repellency among the five products. Mosiguard® and Repel Lemon Eucalyptus® were the most effective DEET-alternative botanical or "natural" repellents competing favorably with the higher DEET-containing formulations, Homola E and OFF! Deep Woods®. Although duration was slightly lower, BugGuard® containing IR3535 performed quite satisfactorily as well.

Other botanicals shown in Figs. 4 & 5 performed well for the first time interval, but diminished considerably thereafter. The better products provided 95%+ repellency for at least 2 hrs post-treatment comparing similarly to the lower DEET containing repellents (Cutters® and OFF! Skintastic®). Walk About® and Bug Off® were the best repellents in this category.

The least effective botanical repellents are presented in Fig. 6. These products never provided 100% repellency even immediately after application.

Our studies demonstrate how the K&D module can be effectively used to screen commercial repellents to include duration data under standardize conditions producing repeatable, reliable results. Note however, results presented here may not be the same for other species or under field conditions.

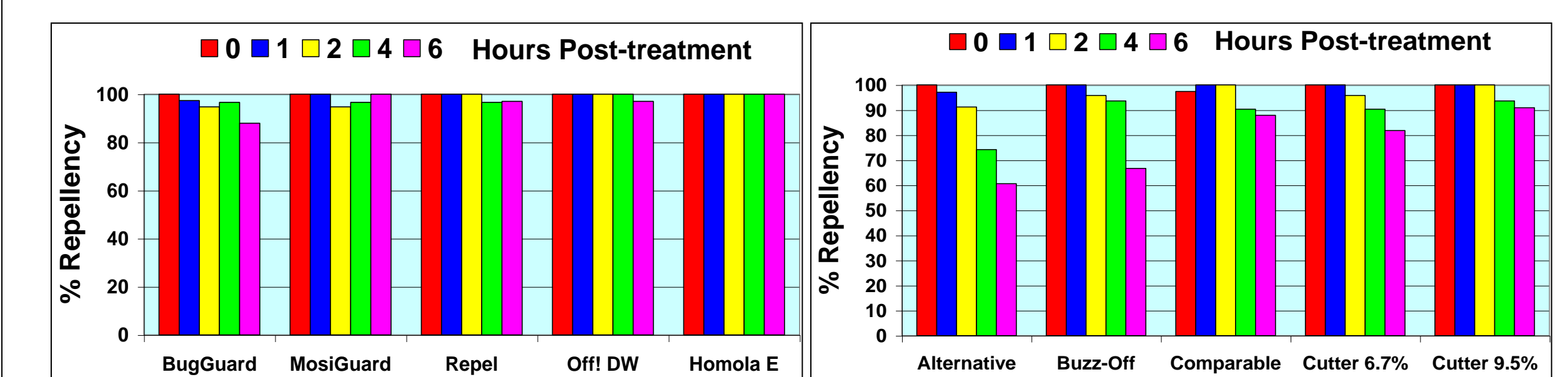


Fig. 3. Comparative repellency-Study 1. Fig. 4. Comparative repellency-Study 2.

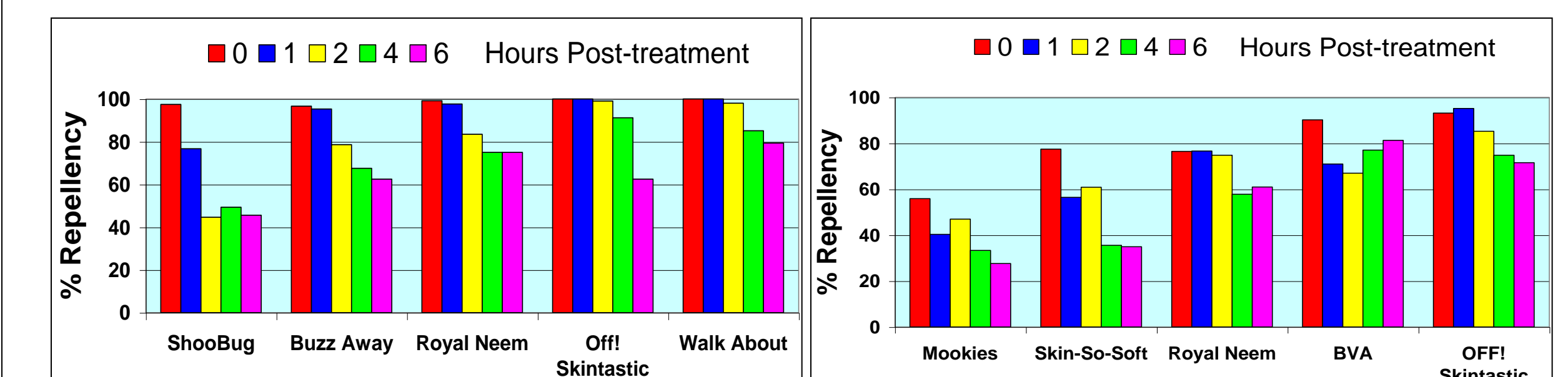


Fig. 5. Comparative repellency-Study 3. Fig. 6. Comparative repellency-Study 4.

LITERATURE CITED:

Klun, J. A. & M. Debboun. 2000. A new module for quantitative evaluation of repellent efficacy using human subjects. *J. Med. Entomol.* 37(1): 177-181.

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