

# IN VIVO TEST METHOD for REPELLENT EFFECTIVENESS ON MOSQUITOES:

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**A placebo controlled trial comparing 30% DEET, Ticks-N-ALL  
All Purpose and Avon Skin So Soft**

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*Background:* The introduction of West Nile virus and other insect borne disease and illness is changing the perception that insects are nothing more than nuisance pests. Interest is increasing in ways to protect against them. One of the commonest is the use of insect repellents.

*Methods:* Tests were undertaken to determine the effectiveness of three insect repellents: 30% DEET, Avon Skin So Soft bath oil, and Ticks-N-All All Purpose. The repellents were compared with each other and against a placebo in eight separate test sessions. During each session, the three repellents and placebo were tested simultaneously. Each session lasted 180 minutes. After every session, the repellents were thoroughly washed from the test site and a minimum of 72 hours elapsed between tests. There were four test sites: right arm, left arm, right leg, and left leg. All test sites were of similar surface area. Both insect bites and landings were counted as events. Measures were taken to ensure that events were not counted twice.

*Results:* A total of 109 events occurred. The event distribution was as follows: placebo—62 events; Avon Skin So Soft —42 events; DEET—4 events; Ticks-N-All All Purpose — 1 event.

*Conclusions:* When tested against a placebo, both Ticks-N-All All Purpose ( $P = .00016$ ,  $NNT < 1$ ) and DEET ( $P = 0.004$ ,  $NNT < 1$ ) were found to provide significantly better protection, while Avon Skin So Soft did not ( $P = 1.5$ ). When tested against Avon Skin So Soft, both Ticks-N-All All Purpose ( $P = .0008$ ,  $NNT < 2$ ) and DEET ( $P < 0.004$ ,  $NNT < 1$ ) were found to provide better protection. When DEET and Ticks-N-All All Purpose were compared with each other, DEET ( $P = 0.0115$ ,  $NNT = 1.75$ ) was found to be 30% as effective as Ticks-N-All All Purpose.

## **Background**

Insects are mainly considered to be nuisance pests—their bites typically causing a pruritic irritation that itches for a day or two and then subsides. Until recently, there were no short- or long-term sequelae from such bites, aside from the rare case of cellulitis or scarring. However, the recent spread of insect borne disease has increased concerns about insect control. Government agencies have states that even though “the risk of illness from insect bites is low, and the risk of serious health effects is even lower... it is important to know the symptoms of illness related to infection and how to minimize your risk, especially if disease activity is reported in an area near you.”[1]

On a global basis insect-transmitted disease is responsible for a significant amount of morbidity and mortality. It is estimated that more than 700 million people become ill each year from insect-transmitted disease, and that 3 million people die from malaria, including one child every 30 seconds.[2] In response, numerous measures have been used to protect people from insects. On an international level, attempts have been made to create genetically modified mosquitoes that are

resistant to malaria,[3] and to produce vaccines against yellow fever, Japanese B encephalitis, dengue, and West Nile virus.[4] On a municipal level, many communities spray insecticide. On an individual level, people adopt numerous personal protective measures to reduce the risk of mosquito bites.[5] These measures include:

- Avoiding perfumed cosmetics
- Avoiding insect habitat during peak feeding times
- Remaining in completely enclosed, well-screened, or air-conditioned areas
- Sleeping under mosquito nets impregnated with permethrin
- Wearing loose-fitting, light-colored clothing
- Using insect repellents

In many areas, permethrin is not registered for use on clothing and permethrin-impregnated mosquito nets are not available.[5] And remaining in completely enclosed, well-screened, or air-conditioned areas is simply unacceptable for the outdoor enthusiast. Of the various insect repellents available, those containing DEET have become the gold standard. Many consumers, however, are reluctant to use DEET. It is an artificial chemical and is known to cause unwanted or unknown side effects. Indeed, DEET is a plasticizer, capable of dissolving watch crystals, the frames of glasses, and some synthetic fabrics.[6] In addition, many DEET products have an unpleasant odor and leave skin feeling dry. As a result, “natural products,” often derived from mineral oil or plant-based essential oils, have been gaining popularity as alternatives to DEET. Avon Skin So Soft bath oil and Ticks-N-All All Purpose are two such alternatives.

## Methods

This study compared the effectiveness of three different insect repellents: 30% DEET, Avon Skin So Soft (ASSS), and Ticks-N-All All Purpose (TNAAP). The three repellents were compared with each other and against a placebo.

Four test sites were used: subject’s right arm, left arm, right leg, and left leg. Each arm was exposed from the proximal deltoid to the end of the digits; each leg was exposed from the distal fifth of the thigh to the proximal medial malleolus. The exposed surface area of each arm was 2125 cm<sup>2</sup> and of each leg was 2080 cm<sup>2</sup>. The surface area difference between upper and lower limbs was negligible: the surface area of each limb was within a 1% variance of the mean surface area of 2102.5 cm<sup>2</sup>.

Each repellent was systematically rotated between the upper and lower test sites on one side of the body. Specifically, DEET and the placebo were applied only to the left limbs. When the left arm received DEET, the left leg received placebo. On the subsequent test, the application sites were reversed so that the left arm received placebo and the left leg received DEET. Similarly, ASSS and TNAAP were applied only to the right limbs. Each repellent was applied as a liquid. After every test, the repellents were thoroughly washed from the test sites and a minimum of 72 hours elapsed before the next session.

There were a total of eight separate sessions. The three repellents and placebo were all tested simultaneously. Each test session lasted 180 minutes. All sessions were conducted under field conditions in Southwest Florida, United States. The tests all took place in the late afternoon or early evening during the spring of 2015.

An event was judged to have occurred if a pruritic papule appeared on the test limb within 12 hours of the test period. An event was also judged to have occurred if a mosquito landed on the test limb and was destroyed. The site of each mosquito landing was marked with an ink pen so that if a pruritic papule later appeared it would not be counted as a second event.

## Results

A total of 109 events occurred. The event distribution was as follows: placebo test sites—62 events; Avon Skin So Soft test —42 events; DEET test sites—4 events; Ticks-N-All All Purpose sites—1 event. This is not a random distribution ( $P<.0001$ ). Further, there was no difference between the event distribution for each repellent when upper and lower limbs were compared: placebo: 30, 32 ( $P>0.775$ ); ASSS 22, 20 ( $P>0.75$ ); TNAAP: 0, 1 ( $P>0.83$ ); DEET: 2, 2 ( $P>1$ ).

Comparing each repellent against the placebo, it was found that both TNAAP and DEET (62 vs 1,  $P<.0001$ ; 62 vs 4,  $P<.0001$ ) provided significantly better protection than placebo, but ASSS did not (62 vs 42,  $P<.30$ ). The use of TNAAP, DEET, and ASSS resulted in a 97%, 30%, and 14% reduction in event occurrence, respectively, and the number needed to treat to prevent one event was 1.00 (=1) for TNAAP and 1.17 (~1) for DEET (Table 1).

Comparing the repellents with each other, it was found that both TNAAP and DEET protected better than ASSS and that TNAAP protected better than DEET (Table 2), which was 30% as effective as TNAAP.

## Conclusions

On any given day, numerous confounding variables affect insect bite frequency. These include the ambient temperature, wind speed, and humidity; the species of insect, the insect's level of hunger, and the density of the insect population; and the test subject's age, sex, activity level, and biochemical attractiveness to the insect.[6] In this study, measures were taken to minimize or eliminate these variables. For instance, the potential effect of variations in test subject age, sex, activity level, and biochemical attractiveness to the insect were eliminated by using the same test subjects. Further, the effect of variations in ambient temperature, wind speed, and humidity, as well as variations in insect species, hunger, and density were minimized by testing all four body sites, all three insect repellents, and the placebo simultaneously.

The systematic rotation of each insect repellent between the upper and lower test sites was designed to minimize any potential bite frequency variation between upper and lower limbs, and to negate the potential effect of the 1% surface area difference between the arm and leg mean surface area. In fact, analyses of the results show that there was no difference in bite frequency between upper and lower limbs ( $P>.05$ ). This is significant in that future studies of similar design will not need to rotate test limbs.

This study was carefully designed to eliminate any potentially confounding effects of previously applied repellent. After every session, the test site was thoroughly washed and a minimum of 72 hours was allowed to elapse before the next testing. A 72-hour interval was chosen based on the pharmacodynamics of DEET and the fact that after five half-lives, the active effect of any substance is negligible.[7] Thirty percent DEET is claimed to be effective for 6 hours,[1,5,8,9] so if any repellent remained on the test area despite thorough washing, a minimum of 12 half-lives would have passed before the next testing. Thus, the 72-hour period should have been more than enough to nullify any potential effect from residual repellent.

Our findings that TNAAP offers better protection than 30% DEET supports the claim that TNAAP may be used as an effective insect repellent for up to three hours where there is risk of infection by insect-transmitted disease.

Overall, the results indicate that both TNAAP and 30% DEET protect better than ASSS or placebo, and that TNAAP protects better than 30% DEET. These results suggest that TNAAP may be used as an alternative to DEET. However, use caution and reapply as often as necessary in areas where mosquito-transmitted disease results in significant morbidity or mortality.

Competing interests

None declared.

**Table 1. Effectiveness of repellents compared with placebo.**

	<b>Event distribution</b>	<b>P-value</b>	<b>Absolute risk reduction</b>	<b>Number needed to treat</b>
Placebo vs DEET	62 vs 4	P<.0001	30%	1.17 (~1)
Placebo vs TNAAP	62 vs 1	P=.0001	97%	1.13 (~1)
Placebo vs ASSS	62 vs 42	P=.30 (ns)	14%	*

\* Not statistically different from placebo

**Table 2. Effectiveness of repellents compared with each other.**

	<b>Event distribution</b>	<b>P-value</b>	<b>Absolute risk reduction</b>	<b>Number needed to treat</b>
DEET vs ASSS	4 vs 42	P<.0001	50%	2.14 (~3)
TNAAP vs ASSS	1 vs 42	P<.0048	30%	2.3 (~3)
TNAAP vs DEET	1 vs 4	P<.046	60%	1.4 (~1)

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