

Technical Insights: Method for mosquito repellent tests on candles

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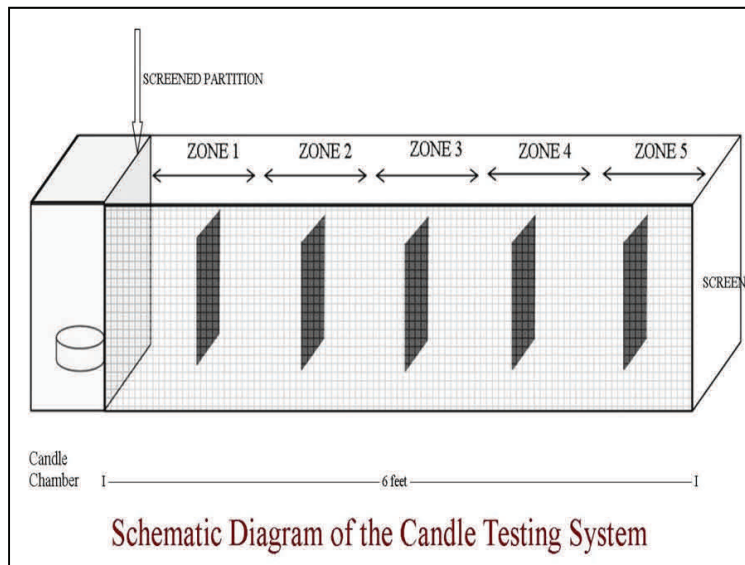
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Poseidon Sciences is a research and development company specializing in biological tests of materials and formulations in a wide range of products, including marine, cosmetics, pharmaceuticals and related industries.

There has been a proliferation of candles with claims of mosquito protection. However, objective data on the performance of such products are often lacking. Human tests conducted during the biting period of mosquitoes in an outdoor setting are the most objective method of testing candle repellent performance. But, when in the process of developing optimum formulation, human testing can be expensive and time consuming. For this reason, scientists at Poseidon have developed a convenient screening method to show the performance of experimental candle formulations.

This document describes procedures for the laboratory evaluation of candles submitted to Poseidon Sciences (Poseidon) to evaluate the effects against *C. quinquefasciatus*. The test samples are typically prepared by the project sponsor and submitted for evaluation to Poseidon. All studies were conducted at the Poseidon Sciences Insect Control Laboratory in Miagao, Iloilo (Philippines).



The test system comprises an enclosed candle chamber attached by a screened wall to a 6 ft tunnel (1 ft wide, 4 ft high; covered with solid white cloth) through which the candle 'smoke' is permitted to travel. The mosquitoes (100+)

as late stage pupae were introduced into the tunnel area in the evening prior to the start of the study on the following day at 10 AM. By morning, these pupae had emerged into adult mosquitoes and fed on sugar solution and distribute throughout the tunnel. Not all the mosquitoes emerge successfully so that there will be variability on the total number of mosquitoes on each run. Each test comprises new mosquitoes introduced to the tunnel. The numbers are normalized as percentage of the total mosquitoes. Inside the tunnel are five pieces of 6 inch x 28 inch black cloth hanging from the top of the chamber at 1 ft. intervals. Mosquitoes prefer to perch on darker surfaces, but do tend to fly from one area to another.

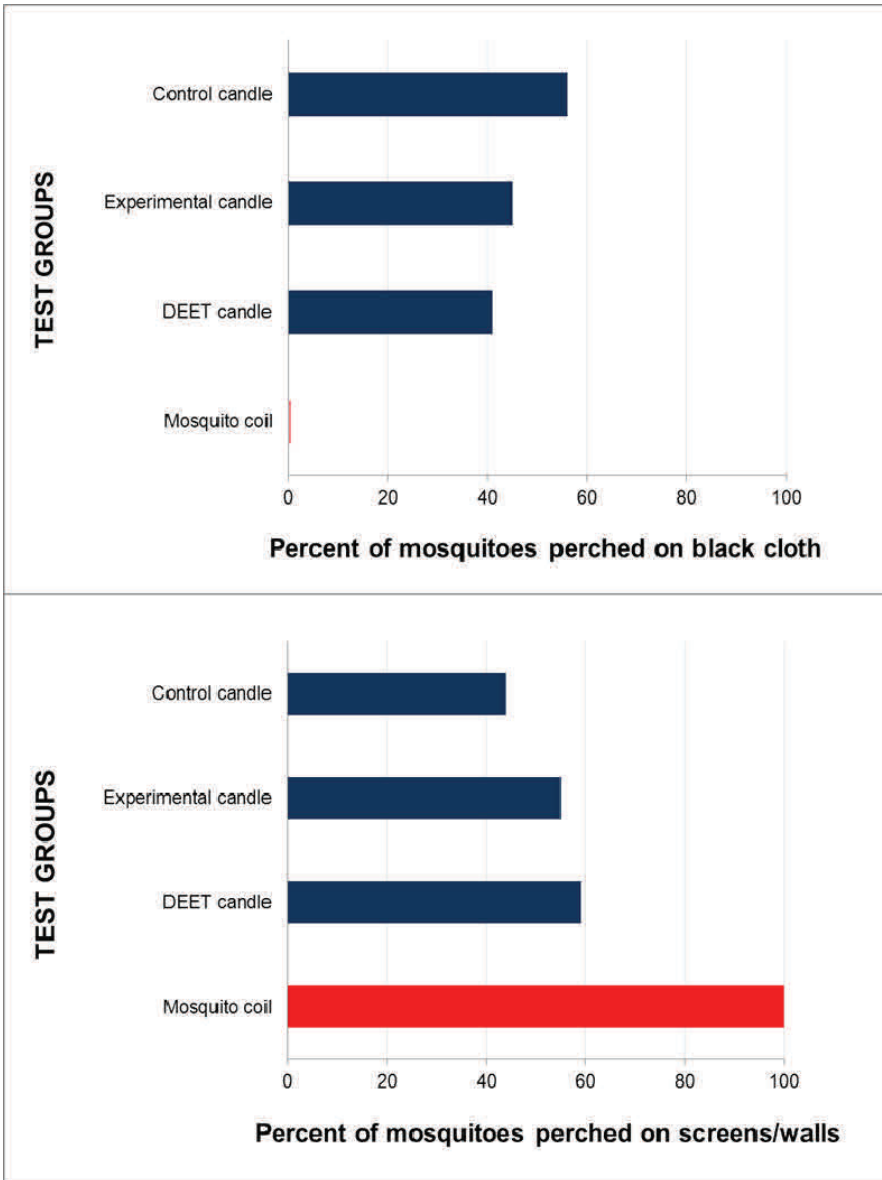
The candle is introduced into the chamber and lit for a total of 20 minutes and withdrawn. The time was sufficient to have the smoke permeate by passive movement through the tunnel and exit on the screened portion at the end of the tunnel. If a repellent effect is observed, the mosquitoes will move as far away from the candle chamber, preferentially perching on the ceiling walls and screen at the end of the tunnel. This behavior can occur in a matter of seconds. The number of mosquitoes perched on the cloth and the walls of chamber are counted after the 20 min exposure by opening a screened side of one wall.

EXAMPLE OF TYPICAL EXPERIMENTAL RESULTS

The data show the following trends:

1. The mosquitoes equally perched on cloth and walls. However, there were no dramatic differences between control and experimental candle summarized as percentage perched according to zones. The experimental candle uses low risk EPA listed compounds of marginal repellency effects.
2. The smoke permeated through the tunnel as evidenced by the fragrance emerging from the end of screened tunnel confirming that there was adequate movement of the smoke from the candle chamber to the end of the 6 ft tunnel. Mosquitoes remained on same locations and appeared not bothered by the candles.
3. Preliminary tests were conducted in which exposure at 10, 20 and 30 minutes did not make any significant difference in the results (data not shown here)
4. An additional test was conducted by inclusion of 5% DEET in candle wax and similarly exposed to the same test regimen.

The data above shows that DEET also had marginal effect at the low concentration used here. An additional reference test was also conducted to re-validate the test using Lion Tiger mosquito coil (regular size, containing methofluthrin; see



http://www.lion-tiger.com/products/mosquito_coils/index.html). The data show that mosquito coil drove the mosquito away from the dark cloth, with 100% of the mosquitoes perched on the walls and mostly at the ceiling of the tunnel. The exposure was only for a period of 3 seconds and sufficient to exhibit extreme avoidance behavior by the mosquitoes in the tunnel.