Effects of in vivo exposure to DEET on blood feeding behavior and fecundity in *Anopheles quadrimaculatus* (Diptera: Culicidae)

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Abstract

This study determined the effects of contact with DEET on guinea pig skin on mortality, probing time, blood feeding rate, engorgement time, and fecundity responses in female *Anopheles quadrimaculatus* Say. Exposure, in this manner, to 10% DEET (in ethanol) for 5 min, resulted in 98% mortality in mosquitoes after 24 h. The median probing time (PT 50) required by females, when exposed to 0.1%, 1.0%, and 10% DEET, was significantly (*P* < 0.0001) longer (12.5, 12.1, and 19.1 s, respectively) than the 6.8 s required by females to probe ethanol-treated skin (control). Similarly, mean blood feeding rates in populations of females exposed to 1.0% DEET for 6 min (14.4%) was £ lower (*P* < 0.001) (85.5%) than in females exposed to ethanol-treated skin, whereas the mean engorgement time on skin treated with 1.0% DEET (66.3 s) was significantly shorter (*P* < 0.0001) than for females feeding on the control guinea pigs (105.9 s). The mean number of mature oöcytes per female (fecundity) in treatment (1.0% DEET) and control mosquitoes was not significantly different. The responses to DEET observed in this study suggest that repeated exposure of female *A. quadrimaculatus* populations to this repellent, in laboratory bioassays, could result in confounding of toxicant and repellent effects and inaccurate estimates of DEET repellency.

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Index Descriptors and Abbreviations: Mosquito; Repellent; Mortality; Probing time; Engorgement; Malaria vector mosquitoes; Blood feeding; Fecundity

1. Introduction

An important facet of the protection of humans from mosquito attack and mosquito-transmitted disease agents (including the *Plasmodium* spp. that cause human malaria) may involve the use of DEET (*Durrheim and Govere, 2002*). The efficacy of DEET in this capacity is problematic because of high variance in the protection times provided against *Anopheles* mosquitoes. In laboratory studies, Schreck (*1985*) obtained an average of 129 min of protection from *A. albimanus* Weidemann bites using 100% DEET but only 35 min of protection using 75% DEET (in ethanol). In contrast, in the field in Panama, Altman (*1969*) obtained 90 min of protection from the same species using 25% DEET.

Dose-response studies in the laboratory (*Rutledge et al., 1978*) have shown that the range of intra-generic sensitivity to DEET in *Anopheles* spp. (measured as the median effective repellent dose [ED 50]) (0.022–0.076 mg/cm²) exceeds that for *Aedes* (0.015–0.042 mg/cm²) and *Culex* (0.011–0.022 mg/cm²) mosquitoes by 1.2–1.7 ×. Moreover, only a third (*R*² = 0.30) of the inter-generic variation in the responses of *A. stephensi* Liston or *A. albimanus* Weidemann to 31 different repellents (*Rutledge et al., 1983*) could be explained using responses to the same repellents by the other species.

Because the protection times afforded by DEET against many *Anopheles* spp. are relatively short, one could argue that the chemical lacks biological activity in any regard against malaria vectors. But casual observations in
laboratory repellent bioassays suggest that exposure to DEET induces morbidity (“tiredness”; unresponsiveness) and mortality in *A. albimanus* and *A. quadrimaculatus* Say test populations (Xue et al., 2003). This apparent biocidal effect presents a conceptual alternative to repellency as a basis for characterizing the biological activity of DEET. Consequently, in this study, we examined the effects of induced contact with DEET by *A. quadrimaculatus* on the blood feeding process and on reproduction and survival. To do this, we measured blood feeding rates, the time expired prior to the commencement of probing by female mosquitoes on host skin, and the length of time required for blood engorgement in test populations of mosquitoes that were induced to contact and/or feed upon the DEET-treated skin of live guinea pigs. We also measured acute mortality in DEET-exposed females and fecundity in the survivors. To date, detailed information of this type is lacking in the scientific literatures of this mosquito species.

2. Materials and methods

2.1. Mosquitoes

The *A. quadrimaculatus* we used were 5–7 day-old nulliparous females from a laboratory colony maintained [27 °C, 80% relative humidity, and 14:10 h (L:D) photoperiod] at the USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida. Larvae were reared according to the technique described by Gerberg et al. (1994). Adults emerged directly into screened stock cages and were provided continuous access to 10% sucrose/water solution. Blood meals (for colony maintenance) comprised defibrinated bovine blood warmed to 36–44 °C and presented to mosquitoes (in stock cages) in a lamb gut membrane. The laboratory colony of *A. quadrimaculatus* females can feed at any time. The experiment was conducted in the morning.

2.2. Testing procedure

In all studies, the experimental unit comprised a clear plastic cup (5 cm diam. × 4 cm long; volume 78.4 cm³) with 1.7-mm mesh cloth covering the open end. Each cup contained a single female or multiple female mosquitoes (Xue and Barnard, 1999) depending on the requirements of a test. The mosquitoes were introduced through a hole by a mouth aspirator. A typical observation comprised placement of the mesh-covered end of the cup against the shaved abdomen of a restrained (in supine position) guinea pig (Smith et al., 1963) until the designated response was observed (to the ethanol or DEET-treated skin), or the required period of exposure to such skin was achieved.

2.3. Mortality

Initial tests were made to determine the effects of exposure to DEET on mosquito mortality. This was accomplished by inducing female mosquitoes to contact three guinea pig’s skin separately treated with 0.1%, 1.0%, or 10.0% DEET (in ethanol) for 5 min using the above described procedure. Each of the four replicates in this series of tests comprised the exposure of 150 females to guinea pigs that had received a 0.5 ml dose of one of the three DEET concentrations (treatment), or the ethanol control, on a 10 cm² area of their abdomen (n = 16). At the end of the exposure period, each plastic cup (with the female mosquito inside) was placed mesh-end down into a shallow plastic tray (56 cm × 43 cm × 8 cm H) on a bed of paper toweling saturated with 10% sucrose solution. Percent mortality was observed and recorded after 24 h.

2.4. Time to probing

One-hundred plastic cups, each containing six females, were used in each of the four replicates of this experiment. Prior to a test, the cups were divided into four groups of 25. Each cup was then randomly selected and individually exposed to the control (ethanol) or one of the three DEET treatments (0.1%, 1.0%, or 10.0% DEET in ethanol) on three guinea pig’s skin.

To observe probing time responses to DEET concentration, we used a stopwatch to measure the time from placement of the cup on guinea pig skin until three of the six females in the cup engaged in probing. Previous to these studies, this measurement (time to probing by 50% of exposed females) has been termed the PT50 (Khan et al., 1965).

2.5. Blood feeding rate, engorgement time, and fecundity

The purpose of these experiments was to determine the rate of blood feeding in test populations of mosquitoes, as well as the time elapsed between commencement of blood feeding and engorgement. To determine blood feeding rate, we used two groups of 25 cups (one female per cup) in each of six replicates. Due to the high mortality of mosquitoes exposed to 10% DEET treated guinea pig, a 1% DEET treated guinea pig was used for this study. The female in each cup was exposed to guinea pig skin that had been treated with a 0.5-ml dose of either ethanol (control) or 1.0% DEET in ethanol (treatment). Each mosquito was exposed to the skin until feeding commenced (initial insertion of the mouthparts), or for 5 min, with the exposure period in the former case ending upon withdrawal of the mouthparts from the skin. Females that failed to commence feeding within 5 min were removed and recorded as non-blood fed.

The foregoing procedure was also used to determine engorgement time responses to DEET-treated skin. In this case, we measured (with a stopwatch) the time elapsed between insertion and withdrawal of the mouthparts on DEET-treated and ethanol-treated guinea pig skin. The observations were replicated six times. Fecundity responses were determined by holding blood fed females (exposed
during feeding to either 1.0% DEET-treated or ethanol-treated skin) in shallow trays for 72 h on a bed of paper towels saturated with 10% sucrose solution. After this time, the mosquitoes were sacrificed by freezing and the number of mature ovarioles in each female determined by dissection of the ovaries. The fecundity experiment was repeated four times.

2.6. Statistical analysis

Percent mortality, median probing time (Khan et al., 1965), blood feeding rate, engorgement time, and fecundity responses were observed and recorded separately on the basis of a completely randomized design and analyzed using analysis of variance procedures (SAS, 2003). Means were compared using the least significant difference (LSD) test ($P = 0.05$).

3. Results

3.1. Mortality

Mean mortality in *A. quadrimaculatus* females was significantly increased ($F_{3,12} = 5255.4, P < 0.0001$) by exposure to DEET (Table 1). Differences in mortality between the 0.1% and 1.0% DEET concentrations (2.2% and 3.5%, respectively), were not significant but 5 min of exposure to 10% DEET killed 98% of adult females within 24 h. Mean mortality in control females was 0% and was significantly different from that for females exposed to any concentration of DEET.

3.2. Time to probing

Probing time responses in DEET-exposed female mosquitoes were significantly ($F_{3,396} = 19.2, P < 0.0001$) longer than in females exposed only to ethanol. Responses to the 0.1% and 1.0% concentrations were significantly shorter (12.5 and 12.1 s, respectively), than the mean response of 19.1 s for females exposed to the 10% DEET concentration. The average median probing time for control females was 6.8 s, which was significantly less (by 2–3×) than that for females exposed to any concentration of DEET.

3.3. Blood feeding rate, engorgement time, and fecundity

Exposure of *A. quadrimaculatus* females to 1.0% DEET for 5 min significantly reduced the mean rate of blood feeding in the test population ($F_{1,6} = 93.2, P < 0.0001$) as well as the mean time required for blood engorgement ($F_{1,208} = 59.8, P < 0.0001$). Approximately 6× fewer females fed in populations exposed to DEET (14.4%) on guinea pig skin for ≤5 min, compared with populations exposed to ethanol (85.5%). The females that did feed spent less time (66.3 s) doing so (by 37%) than females exposed to ethanol (105.9 s), although differences in mean fecundity between DEET-exposed (129.9 eggs) and control females (134.4 eggs) were not significant ($F_{1,179} = 0.5, P = 0.49$).

4. Discussion

Xue et al. (2003) showed that the application of aliquots of commercially available aerosol spray-formulated repellents containing DEET to a fixed volume of space occupied by adult mosquitoes, resulted in knockdown and mortality of *Aedes albopictus* Skuse, *A. aegypti* L. and *A. quadrimaculatus*. The findings of the present study corroborate the acute adulticidal activity of DEET.

Yet other aspects of the blood feeding process in *A. quadrimaculatus* are affected by exposure to DEET. Fewer DEET-exposed females attempted to feed and required more time to commence probing, than females exposed only to ethanol. The former group also required less time to ingest a blood meal and matured fewer eggs than ethanol-exposed females. These same types of effects on host-seeking and blood feeding behavior, as a result of exposure to insecticides, their residues, and/or repellents, have been noted elsewhere for other *Anopheles* spp. (Muirhead-Thompson, 1960; Wilson et al., 1973) and for *Aedes* mosquitoes (Kalmus and Hocking, 1960; Khan, 1965).

The significantly shortened mean engorgement time responses in DEET-exposed *A. quadrimaculatus* are consistent with lower mean fecundity responses in the same treatment group although, in the latter case, the slight difference is difficult to explain given significant differences in mean engorgement times (and the expectation that less engorgement time will result in a smaller blood meal size). One explanation for this observation may be that DEET stimulates the rate of blood engorgement and/or induces a physiological shift to a ‘fast-feeding’ mode, such as has been noted for some *Aedes* species (Chadee et al., 2002).

The data gathered in this study are unlikely to be directly useful with respect to enhancing the efficacy of...
DEET against *Anopheles* mosquitoes. Nevertheless, they may help us develop improved methods for assessing DEET efficacy in laboratory bioassays against malaria vectors. In particular, the mortality responses observed here raise concern for the effect(s) of DEET-induced morbidity in *Anopheles* spp. in laboratory repellent bioassays in which the same population of female mosquitoes is exposed, several times in succession, to DEET on the skin of a human subject. Although the typically short protection times afforded by DEET against *Anopheles* spp. minimize this source of variation, one could argue that such shortened times are (at least in part) an artifact induced by DEET toxicity. A desirable alternative in such bioassays would be to test females from the same cohort, but without previous exposure to DEET, in successive observations.

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**References**


