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RESPONSE OF *ANOPHELES DIRUS* AND *AEDES ALBOPICTUS* TO REPELLENTS IN THE LABORATORY[1]

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ABSTRACT. Laboratory tests were conducted to study the response of *Anopheles dirus* and *Aedes albopictus* to repellent formulations containing diethyl methylbenzamide (deet) and dimethyl phthalate. *Anopheles dirus* was tolerant of low concentrations of deet (5–20%), and formulations containing ≤35% deet provided protection for ≤90 min. In contrast, *Ae. albopictus* was sensitive to these formulations, which provided ≥180 min protection from bites.

Anopheles dirus Peyton and Harrison is the major vector of malaria in Thailand (Rosenberg et al. 1990), and other countries in mainland Southeast Asia, including Burma, Cambodia, and Bangladesh (Rosenberg and Maheswary 1982). Despite the importance of this species as a vector of malaria, there are no published records of its response to repellents. The use of repellents and other personal protection measures to minimize contact with malaria vectors in Southeast Asia is especially important for 2 reasons. Firstly, the region is the center for *Plasmodium falciparum* drug resistance, making chemoprophylaxis problematic in many areas (Looareesuwan et al. 1992). Secondly, *An. dirus* is largely exophilic and therefore unaffected by interior spraying of houses with DDT and fenitrothion (Prasittisuk 1985). In this note we report laboratory test results on skin comparing the response of *An. dirus* and *Aedes albopictus* (Skuse), a potential vector of dengue and other arboviruses (Hawley 1988), to several commercially available and laboratory prepared repellent formulations.

Four commercial formulations containing diethyl methylbenzamide (deet) were tested. These were the lotions Autan (Bayer, Germany) containing 20% deet, and Extended Duration Repellent Formulation (U.S. Army EDRF, 3M Corp.) containing 35% deet in a polymer; and stick formulations containing 33% deet (Intergrade Trading Co., Bangkok), and 33% deet (U.S. Army). Solutions were prepared in 95% ethanol from 95% deet (Colbar, Australia), and technical grade dimethyl phthalate (DMP) (Bacto Laboratories).

Tests were with 3 human volunteers (2 Thai females and a Caucasian male) and based on a method described by Schreck (1985). Mosquitoes tested were 6–7-day-old laboratory-reared nulliparous females. *Anopheles dirus* was from a colony originally established from Chonburi, Thailand, in 1968, and *Ae. albopictus* was from a colony established from Hawaii in 1989. Both colonies were reared at 27 ± 2°C, 75–80% RH, in natural light. For each test 200 mosquitoes were placed into a screen wire cage measuring 30 × 30 × 30 cm. Two to 3 h prior to testing the sugar/water pad was removed from the cage.

Tests were conducted by exposing untreated and repellent-treated human forearms to the mosquitoes. A surgical glove was worn during the tests to prevent biting on the untreated hand. To ascertain mosquito avidity, an untreated forearm was exposed in the cage for up to 1 min, and the number of mosquitoes attempting to bite was recorded. The mosquitoes were blown from the arm before any blood was taken. The formulation to be tested was then applied evenly to the same forearm, between wrist and elbow. For liquid formulations, 1 ml of the solution was applied because this volume evenly covered the surface area. For stick and lotion formulations, the repellent container was weighed before and after application. Between 0.4 and 1.0 g of each formulation was applied for each test. The treated arm was then exposed in the test cage for 5 min, then subsequently exposed at 30-min intervals for *An. dirus*, and hourly intervals for *Ae. albopictus*, until 3 bites were recorded, terminating the test. To ensure that test mosquitoes were still avid after the repellent was tested, the untreated forearm was exposed to the same mosquitoes, and the number attempting to bite in up to 1 min was recorded.

The response of *An. dirus* to the repellents is shown in Table 1. The results indicate that *An. dirus* was tolerant of the lower concentrations of deet and DMP, with less than 90 min protection provided in these tests. Higher concentrations of deet, namely 50 and 75%, provided protection

[1] The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense. Mention of a commercial product does not constitute an endorsement of the product by the Department of Defense. The volunteers gave informed consent to participate in the study.

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Table 1. Response of *Anopheles dirus* and *Aedes albopictus* to forearms treated with various repellent formulations.[1]

Formulation	*An. dirus* No. tests	*An. dirus* Mean minutes ± SE of protection[2]	*An. dirus* Range	*Ae. albopictus* No. tests	*Ae. albopictus* Mean minutes ± SE of protection[2]
deet					
Autan (20% deet)	6	5.0 ± 4.6	0–30	3	>180
EDRF (35% deet)	3	40.0 ± 8.2	30–60	2	>240
U.S. Army Stick (33% deet)	6	5.0 ± 4.6	0–30	2	>240
Thai Stick (33% deet)	9	13.3 ± 8.3	0–60	3	>240
35% deet	2	90.0 ± 42.4	30–150		—
50% deet	5	n.c.[3]	60–>180	5	>210
75% deet	4	n.c.[4]	180–>300		—
Dimethylphthalate					
10% DMP	5	18.0 ± 6.6	0–30	2	150 ± 26.3
20% DMP	4	37.5 ± 16.4	0–90	2	180 ± 42.4
30% DMP	2	120	120	2	>240
50% DMP	2	150	150		

[1] Mean biting rate for untreated arm: *An. dirus* 21.5 ± 1.3 bites/min ($n = 48$) and *Ae. albopictus* 101.4 ± 9.7 bites/30 sec ($n = 21$).
[2] Forearms protected until 3 bites were recorded.
[3] Not calculated because some tests terminated after 180 min without 3 bites.
[4] Not calculated because some tests terminated after 300 min without 3 bites.

for up to 180 min, and 50% DMP provided 150 min protection. In contrast *Ae. albopictus* was sensitive to all repellents tested. All deet formulations prevented bites for 180 min or more, and DMP for 150 min or more. Earlier studies also showed that *Ae. albopictus* is sensitive to deet (Schreck and McGovern 1989, Curtis et al. 1990).

After determining the tolerance of *An. dirus* to deet, further studies were made. The test method was modified to investigate the effect of exposing standard concentrations of deet to various numbers of mosquitoes. Solutions prepared in 95% ethanol containing 5, 10, and 20% of deet were applied to forearms of 2 female volunteers and then exposed in cages containing 25, 50, 100, or 200 mosquitoes each. Four replicates of each test concentration with each cage density were conducted. The results are shown in Table 2. The tolerance of *An. dirus* to lower concentrations of deet was again observed, and an overall trend of shorter duration of protection with increased mosquito density also observed. The data in Table 2 were transformed ($\log(x + 1)$) and subjected to ANOVA using a randomized complete block design. The effect of both repellent concentration ($F = 6.54$, $df = 2$, $P = 0.004$) and density of mosquitoes tested ($F = 7.39$, $df = 3$, $P = 0.001$) on the duration of protection were significant.

In another series of tests, the standard test described earlier using 200 mosquitoes/cage was extended to determine the duration of protection

Table 2. Duration of protection of 3 concentrations of deet applied to forearms and exposed in cages containing various densities of *Anopheles dirus*.[1]

Concentration of deet (%)	Mean minutes ± SE[2] protection in cages containing the indicated number of mosquitoes: 25	50	100	200
5	37.5 ± 12.4	22.5 ± 6.5	37.5 ± 16.3	7.5 ± 6.5
10	82.5 ± 6.5	37.5 ± 6.5	97.5 ± 6.5	15.0 ± 7.5
20	120.0 ± 18.4	105.0 ± 22.5	52.5 ± 14.4	22.5 ± 7.5

[1] Mean biting rate for untreated arms: 5.1 ± 1.1 (25 mosquitoes), 5.4 ± 1.2 (50), 10.2 ± 1.8 (100), and 23.5 ± 3.6 (200) bites/min, respectively.
[2] Mean of 4 replications. Forearm considered protected until 3 bites recorded.

provided by the 3 deet concentrations until 10 bites of *An. dirus* were recorded. Deet provided little overall protection at these concentrations, with 5% deet providing no protection ($n = 6$), 10% with 37.5 ± 16.4 ($n = 4$) min protection, and 20% with 162 ± 33.5 ($n = 5$) min protection. During these tests 27.9 ± 5.7 bites/min were recorded on untreated forearms.

These laboratory results show that *An. dirus* adults are tolerant of concentrations of deet at ≤35% regardless of formulation and DMP at <30%. A significant number of commercial repellent formulations that are currently available contain less than 30% deet, as in rare cases the prolonged and inappropriate use of higher concentrations of deet have caused some adverse effects, primarily adverse dermatological effects (Curtis 1992). The use of these repellents may not offer complete protection from biting *An. dirus*. Studies in other laboratories with vectors of malaria have also recorded tolerance for repellent compounds in some species. Curtis et al. (1990) reported that *Anopheles pulcherrimus* Theobald, *Anopheles albimanus* Wied., and *Anopheles gambiae s.l.* were less susceptible to deet than *Aedes aegypti* (Linn.) in laboratory tests. Schreck (1985) reported that 100% deet provided protection from *An. albimanus* for only 2 h in laboratory tests with 1,000–1,500 caged mosquitoes. He concluded that only limited protection could be expected by using deet, despite field reports that showed deet can protect individuals for 3 h or more (Schreck 1985). This note provides additional laboratory evidence of anopheline insensitivity to deet at levels that effectively repel other mosquito genera, warranting further studies with field populations of the respective species.

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REFERENCES CITED

Curtis, C. F. 1992. Personal protection methods against vectors of disease. Rev. Med. Vet. Entomol. 80:543–553.

Curtis, C. F., J. D. Lines, Lu Baolin and A. Renz. 1990. Natural and synthetic repellents, pp. 75–92. *In:* C. F. Curtis (ed.). Appropriate technology in vector control. CRC Press, Boca Raton, FL.

Hawley, W. A. 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 4(Suppl. 1):1–40.

Looareesuwan, S., T. Harinasuta and T. Chongsuphajaisiddhi. 1992. Drug resistant malaria, with special reference to Thailand. Southeast Asian J. Trop. Med. Public Health 23:621–634.

Prasittisuk, C. 1985. Present status of malaria in Thailand. Southeast Asian J. Trop. Med. Public Health 16:141–145.

Rosenberg, R. and N. P. Maheswary. 1982. Forest malaria in Bangladesh. II. Transmission by *Anopheles dirus*. Am. J. Trop. Med. Hyg. 31:183–191.

Rosenberg, R., R. G. Andre and L. Somchit. 1990. Highly efficient dry season transmission of malaria in Thailand. Trans. R. Soc. Trop. Med. Hyg. 84:22–28.

Schreck, C. E. 1985. The status of deet (*N,N* diethyl-*m*-toluamide) as a repellent for *Anopheles albimanus*. J. Am. Mosq. Control Assoc. 1:98–100.

Schreck, C. E. and T. P. McGovern. 1989. Repellents and other personal protection strategies against *Aedes albopictus*. J. Am. Mosq. Control Assoc. 5:247–250.