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DEET microencapsulation: a slow-release formulation enhancing the residual efficacy of bed nets against malaria vectors

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Summary Textile materials treated with synthetic repellents have the potential to provide protection against insect disease vectors but lack the residual activity necessary to achieve a prolonged effect or to be cost-effective. DEET MC is a formulation of DEET (N,N diethyl-m-toluamide) in which the repellent is gradually released from a capsule that binds the repellent. An experiment carried out on DEET-treated mosquito netting showed that the formulation repels, inhibits blood-feeding and kills mosquitoes for a period of at least 6 months under laboratory conditions. Such formulations may have the potential for use on nets against pyrethroid-resistant mosquitoes or on clothing or bedding materials distributed in disasters, emergencies or refugee camp situations.

1. Introduction

Resistance to pyrethroid insecticides has become increasingly widespread in the malaria vector *Anopheles gambiae* in western and eastern Africa and in *A. funestus* in southern Africa (Chandre et al., 1999; Hargreaves et al., 2000; Vulule et al., 1999). The recent failure of insecticide treated nets (ITNs) and indoor residual spraying (IRS) to kill or protect against pyrethoid-resistant *A. gambiae* in southern Benin (N'Guessan et al., 2007a) means that identifying alternative insecticides and repellents to supplement or replace the pyrethroids has become very urgent (Zaim and Guillet, 2002). A recent study involving impregnation of nets with DEET repellent conducted in experimental huts in Ivory Coast indicated that this is a promising approach to overcome the problems associated with pyrethroid-resistant

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mosquitoes and potentially prevent malaria (N'Guessan et al., 2006). When deployed on fabric rather than as a topical skin treatment, the DEET acted not only as a conventional insect repellent but also as a toxicant, killing the majority of pyrethroid-resistant *A. gambiae* and *Culex quinquefasciatus* mosquitoes that entered the huts. The formulation of DEET tested was a water-miscible lotion suitable for clothing or topical treatment. Being inherently volatile, any effect of DEET on mosquitoes was lost after 2—3 weeks. In the current era of long-lasting insecticidal nets, any formulation that needs such frequent replenishment is unlikely to find favour even in places where pyrethroids are no longer effective. Advances in formulation technology have been important in leading to long-lasting insecticidal nets. Microencapsulation technology, in which the active ingredient is enclosed within a polymer capsule and gradually leaches to the outside is one way in which residual activity may be prolonged. Microcapsule suspensions of pyrethroids are now entering the market as long-lasting indoor residual spray treatments (WHO, 2007). In order for DEET to become viable as a textile treatment, the repellent will need to be bound within some kind of long-lasting formulation. SDS Biotech K.K., Tokyo, Japan has recently developed a microencapsulated formulation of DEET in which the active ingredient diffuses slowly through a polymer membrane over a period of months. Human contact or friction with the treated fabric is believed to accelerate the diffusion process. To examine its potential as a fabric or net treatment, the microencapsulated DEET was applied to polyester netting and tested against *A. gambiae* in laboratory tunnel tests over several months (WHO, 2006). A standard topical formulation of DEET on netting served as a control.

2. Materials and methods

2.1. DEET MC

DEET MC is a 30% aqueous suspension of N,N diethyl-m-toluamide enclosed in a melamine microcapsule, supplied by Sumitomo Corporation, Tokyo, Japan. Median particle size is 4—5 μm. A standard topical, water-miscible formulation of DEET 30% was produced by Osler®, Paris, France. Both formulations were diluted in water and applied at 8 g DEET/m^2 on 100 denier polyester netting (N'Guessan et al., 2006; Pennetier et al., 2007). The netting samples were first tested 72 h after treatment and re-tested at intervals over 6 months. The netting samples were left unwrapped between tests.

2.2. Tunnel tests

Tunnel tests were undertaken with an insecticide-susceptible laboratory strain Kisumu of *A. gambiae* in Benin (Chandre et al., 2000; N'Guessan et al., 2007b, 2007c). The tunnel test is a laboratory system designed to allow many of the behavioural and toxicological actions that occur with host-seeking mosquitoes in the presence of treated materials. Tunnel tests are done as a forerunner to experimental hut trials, and provide information on repellency, blood-feeding inhibition and mortality. The equipment consists of a square glass cylinder (25 cm high, 25 cm wide, 60 cm long), which is divided into two compartments by a netting-covered frame that slots across the tunnel (WHO, 2006). In one of the compartments, a guinea pig is housed unconstrained in an open meshed cage and in the other compartment, 100 unfed female anopheline mosquitoes aged 2—5 d are released at dusk and left overnight. The netting is deliberately holed with nine 1 cm holes to provide opportunity for mosquitoes to pass into the baited compartment. The following morning, the number of mosquitoes found live or dead, fed or unfed in each compartment is scored. Live mosquitoes are given access to sugar solution, and monitored up to 24 h to score delayed mortality. For each repellent formulation, two replicate tunnel tests involving 100 mosquitoes per test were conducted on each sample of treated netting at 6 monthly intervals.

The procedure for use of guinea pigs in our tunnel experiments conformed with criteria established in EC Directive 86/609/ECC regarding protection of animals used for experimental purposes.

2.3. Data analysis

A χ^2 test was performed to assess trend in residual efficacy of treatments over time.

3. Results

The effects of the DEET treatments on penetration, blood-feeding and mortality rates are shown in Figure 1.

The unencapsulated formulation inhibited 80% of the mosquitoes from penetrating the holed netting when freshly applied, and over 3—6 months the proportion penetrating decreased significantly from 40 to 10% ($P = 0.001$), which was the same rate observed in the untreated control (Figure 1A). With the microencapsulated formulation, passage inhibition was only 40% initially and remained at this level over the full 6 months ($P = 0.11$).

Initially, inhibition of blood-feeding was 100% with the unencapsulated formulation, decreasing to 70% at 3 months and to complete loss of activity between 3 and 6 months (Figure 1B). Protection from the microcapsule increased after 1 month and reached a maximum at 6 months, suggesting that a higher concentration of active ingredient was present on the surface of the capsules after this interval.

With the unencapsulated formulation, mortality was 100% initially but showed exponential decay over the 6 months ($P < 0.001$) (Figure 1C). Mortality rates with DEET MC remained between 82 and 65% throughout, showing a gradual though significant decay in performance ($P = 0.03$).

4. Discussion

When applied to skin, conventional formulations of DEET persist for no more than several hours. When applied to textiles or netting, topical formulations may persist to good effect for 1—3 months. Evaporation or absorption rates on textiles are much slower than on skin. The mode of interaction with host-seeking mosquitoes may differ too. With skin application, mosquitoes are deterred from alighting on the