European Review for Medical and Pharmacological Sciences 2010; 14: 443-448

Laboratory evaluation of Dimethyl phthalate treated wristbands against three predominant mosquito (*Diptera: Culicidae*) vectors of disease

K. KARUNAMOORTHI*,§,#, S. SABESAN§

*Unit of Vector Biology & Control, Department of Environmental Health Science, College of Public Health & Medical Sciences, Jimma University, Jimma (Ethiopia); §Vector Control Research Center (Indian Council of Medical Research), Indira Nagar, Pondicherry (India); #Research and Development Center, Bharathiar University, Coimbatore, Tamil Nadu (India)

Abstract. – *Objectives and Materials and Methods:* **The repellent efficacy of dimethyl phthalate (DMP) treated wristband was determined against mosquitoes, *viz Anopheles stephensi* Liston, Aedes aegypti Linnaeus, *Culex quinquefasciatus* Say at two concentrations viz., 1.5 and 2.0 mg/cm² under the laboratory conditions. DMP treated wristband had shown variable degrees of repellency impact against different mosquito species.**

Results: **Its offered higher reduction of man landing rate against *Anopheles stephensi* at both concentrations of 1.5 (81.1%) and 2.0 mg/cm² (87.0%). 79.8% and 84.8% of protection achieved against *Culex quinquefasciatus* at concentrations of 1.5 and 2.0 mg/cm², respectively. 74.4 and 86.5% of reduction of man landing rates were obtained against *Aedes aegypti* at concentrations of 1.5 and 2.0 mg/cm² respectively. The reduction of man-landing rate evaluations were confirmed by t-test compared between control group and each experimental group. The t-test result shows at 1.5 ($p = 0.0026$; t = 19.2; df = 2) as well 2.0 mg/cm² ($p = 0.0025$; t = 19.8; df = 2) are extremely significant to reduce the man vector contact.**

Conclusion: **The present data suggest that DMP treated wristbands are most promising against both day and night-biting mosquitoes and significantly reducing the man-vector contact. Therefore, it could serve as a potential as means of personal protection device against insect nuisance biting and insect-borne disease when and where other kinds of personal protection measures are impossible and impracticable.**

Key Words:

Man landing rate, Repellent treated wristbands, Mosquitoes, Dimethyl phthalate, Personal protection.

Introduction

Vector-borne diseases continue to inflict high morbidity and mortality and are an important cause of poverty and underdevelopment[1]. Mosquitoes are known vectors of several disease-causing pathogens, which affect many millions of people all over the world. *Aedes aegypti* is known to carry dengue, yellow fever and Chikungunya. Malaria is carried by *Anopheles stephensi*; and filarial disease by *Culex quinquefasciatus*[2,3]. One to two million deaths worldwide are reported annually due to malaria. Lymphatic filariasis affects at least 120 million people in 73 countries including India and in the remaining countries in Africa, Southeast Asia, and Pacific Islands[4].

Insect repellents are widely used as a means of personal protection against biting arthropods, the main motive usually being avoidance of nuisance and discomfort. Personal protection measures, including the use of repellents, are also important in reducing the risk of contracting insect vector borne disease[5]. The first practical synthetic insect repellent was dimethyl phthalate[6]. The widely used synthetic insect repellent chemicals are dimethyl phthalate (DMP) and dimethylbenzamide (also known as diethyltoluamide, DEET). The chemicals are effective against other biting insects as well as many species of mosquitoes[7,8].

Personnel of military forces have close contact with the environment and can be bitten by insects and exposed to the related diseases, more than other people[9,10]. During last few years particular attention has been given to formulation and testing of various combination of some effective repellents (such as DMP) that have been declared safe for application on skin[11,12].

Although insecticide-treated bed nets protect against mosquitoes and malaria in many parts of the world, people may contract disease in the early evening hours[13]. Thus, there is the need to find supplemental personal protective measures to

Corresponding Author: Kaliyaperumal Karunamoorthi, M.Sc., M.Phil DPHM;
e-mail: k_karunamoorthi@yahoo.com

avoid insect/mosquito nuisance and prevent the disease transmission. Many species of blood-sucking insects bite predominantly around the ankles and wrists. Strips of cotton fitted around the extremities and impregnated with a repellent reduce insects/mosquitoes biting significantly. Impregnation of the repellent into cotton fabric strips is a more reasonable way for minimizing skin contact with repellent and they are greatly reducing the man-vector contact[14].

Generally, vector control is considered the most effective measure to prevent transmission of vector-borne disease[1]. However, currently vector-borne diseases remain serious threat to human kind due to emergence of the drug resistant parasites, insecticide resistant vectors and non-availability of suitable and effective vaccines. In this context, repellents are playing key role in order to reduce the man-vector contact eventually reduce the vector-borne diseases.

The purpose of the present investigation was to evaluate the repellent efficacy of Dimethyl phthalate (DMP) treated wristbands against three important vectors *Anopheles stephensi* Liston, *Culex quinquefasciatus* Say and *Aedes aegypti* (L.) at two concentrations viz., 1.5 and 2.0 mg/cm^2 under the laboratory conditions.

Materials and Methods

Repellent

The first practical synthetic insect repellent Dimethyl phthalate was selected for this present investigation. Dimethyl phthalate (DMP) 25% (EC) was obtained from Division of insecticides-chemistry, Vector Control Research Centre (Indian Council of Medical Research), Pondicherry 605 006, India.

Preparation of Mosquitoes

Anopheles stephensi a principal vector of urban malaria, *Aedes aegypti* a principal vector of dengue hemorrhagic fever and *Culex quinquefasciatus* a principal vector of bancroftian filariasis were selected for laboratory evaluation. The colonies of these mosquitoes were cultured and maintained in the laboratory at 27 ± 1°C and 85% relative humidity. The larvae were fed with dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. The mosquitoes were starved for 3-4 days before the beginning of the each experiment. Conditions during the test followed a standard diel cycle, with air temperature 27 ± 0.2^{0}C, 47 6 3% RH, and light intensity of 290 6 45 lux.

Experimental Cage and Wristbands

Insect cage was modified suitably to carry out the laboratory experiment. Three cages, each with a size of 46 × 46 × 46 cm^2 were kept in array, linked to each other through a muslin cloth passage. Therefore, the released female mosquitoes in the middle chamber can easily pass through to left (test) and right (control) chambers. The test cage was specially designed and provisions were also made to insert fore-arms on one side, and to collect the landing mosquitoes from the opposite side. The wristbands were provided with press buttons at both ends to fasten.

Repellent Treatment Procedure

Initially, each of the wristbands was soaked in water and the quantity of required repellent to wet the armbands thoroughly was measured. The quantity of water thus determined for each armband was used to mix with the desired repellents at two different concentrations 1.5 and 2.0 mg/cm^2. The repellent treatment of wristbands was made in a non-absorbent plastic container. Uniform distributions of repellents were ensured by rubbing squeezing of wristbands and subsequently, were flattened on a polythene sheet for shade drying. After thorough dry, each set of repellent treated wristbands were kept in separate plastic bags, in order to avoid the mixing with other concentration.

Human Volunteers and Use of Wristbands

In the present study, selected five healthy male human volunteer subjects (three males and two females) were recruited from the Medical Entomology Division, Vector Control Research Centre, Pondicherry, India. The study was reviewed and approved by the Institutional Review Board of the Vector Control Research Centre, Pondicherry, and subjects gave written informed consent before participating. Volunteer arms were washed and cleaned with ethanol solvent. The human volunteer left forearm was maintained as test and tied with repellent treated wristband while the right forearm tied with wristband treated with ethanol to serve as control. Allocation to test subjects was alphabetical and in that manner indiscriminate. Test materials were

given anonymous code designations to blind the study subjects and staff during application and testing.

Repellency Test Procedure

Ahead of the experiments five hundred 3-4 day old blood-starved adult female mosquitoes were released into the middle chamber and now the mosquitoes can freely traverse test (Left) as well as control (Right) chambers. Before each test, the readiness of the mosquitoes to bite was confirmed by having subjects insert their untreated forearm into the test cage. Once subjects observed five mosquito landings on the untreated arm, they removed their arm from the cage. Human volunteers left forearm tied with repellent treated wristband (Test) while right forearm tied by armband treated with ethanol (Control). Subsequently, the study subject inserts the left and right forearm into the respective chambers. Mosquitoes landing on forearms of test and control were collected by insect collectors. The laboratory experiment with *Culex quinquefasciatus* and *Anopheles stephensi* were carried out during night h (18.00-6.00), while in contrast, the observations for *Aedes aegypti* was made during the day h (06.00-18.00).

Mosquitoes landing on the left and right forearms were captured by using the flash light and aspirator by two insect collectors and placed into containers covered with netting.. Insect collectors were rotated at an interval of ten minutes to avoid bias collections. Mosquitoes collected from the test and control were kept separately and counted. Experiments were replicated five times in each concentration for each species of mosquitoes. All experiments were carried out with temperature 28 ± 2^0 C and relative humidity (RH) 75±5% under the laboratory conditions.

Statistical Analysis

To measure the efficacy of DMP, percentage of repellency was calculated firstly as follows: % Repellency = 100 × (C-T)/C, where C is the bitten number by mosquitoes counted from control group and T is the number counted from the experimental group of volunteers[15-17]. The differences between control and experimental groups were statistically tested with student's *t*-test.

Results

DMP treated wristband offered higher reduction of man landing rate against *Anopheles stephensi* at both concentrations of 1.5 (81.1%) and 2.0 mg/cm^2 (87.0%). 79.8% and 84.8% of protection achieved against *Culex quinquefasciatus* at a concentration of 1.5 and 2.0 mg/cm^2, respectively. 74.4 and 86.5% of reduction of man landing rates were obtained against *Aedes aegypti* at concentrations of 1.5 and 2.0 mg/cm^2 respectively. The reduction of man-landing rate evaluations were confirmed by *t*-test compared between control group and each experimental group. The *t*-test result shows at 1.5 ($p = 0.0026$; t = 19.2; df = 2) as well 2.0 mg/cm^2 ($p = 0.0025$; t = 19.8; df = 2) are extremely significant to reduce the man vector contact (Table I, II and Figure 1). Over all, DMP treated wristbands shows 78.3% and 86.1% average reduction of man landing rates against all three tested mosquito species at 1.5 and 2.0 mg/cm^2 respectively.

It is an average reduction of man landing rate of both concentrations (1.5 mg/cm^2 and 2.0 mg/cm^2) against all three vector mosquitoes. It was calculated by applying the following procedure;

Table I. Efficacy of DMP treated wristbands at a concentration of 1.5 mg/cm^2 against mosquito vectors of disease under the laboratory conditions.

Mosquito species	No. of mosquitoes captured		% reduction of man lading rate	P(T<=t) two-tail
	Control	*1.5 mg/cm²*		
Anopheles stephensi	286	54	81.1	t-value = 19.2
Culex quinquefasciatus	258	52	79.8	df = 2
Aedes aegypti	263	68	74.4	$p = 0.0026$*

*Note: $p < 0.05$ is statistically significant.

Table II. Efficacy of DMP treated wristbands at a concentration of 2.0 mg/cm^2 against mosquito vectors of disease under the laboratory conditions.

Mosquito species	No. of mosquitoes captured		% reduction of man lading rate	P(T<=t) two-tail
	Control	*2 mg/cm^2*		
Anopheles stephensi	294	38	87.0	t-value = 19.8
Culex quinquefasciatus	298	45	84.8	df = 2
Aedes aegypti	252	34	86.5	p = 0.0025*

*Note: $p < 0.05$ is statistically significant.

1.5 mg/cm^2 = 81.1+79.8+74.4 = 235.3
= 235.3/3 = 78.3%
2.0 mg/cm^2 = 87.0+84.8+86.5 = 258.3
= 258.3/3 = 86.1%

Discussion

The control of vector-borne diseases represents one of the greatest global public health challenges of the 21st century. DMP treated wristbands were evaluated for their repellent efficiency against three major predominant vector mosquitoes, *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* at 1.5 and 2 mg/cm^2 under laboratory conditions. As shown in Table I, II and Figure 1, it is clear that the DMP treated wristband has strong repellent action against all three major vector mosquitoes.

Anopheles stephensi was sensitive to the DMP treated wristbands and the present result is consistent with a study conducted in Iran, we have found that two repellents includes Iranian and Merck dimethyl phthalate (DMP) were evaluated against *Anopheles stephensi* and *Culex pipiens* and found that there were significant differences in repellent sensitivity between *Anopheles stephensi* and *Culex pipiens* at the ED_{95} level. *Anopheles stephensi* was highly sensitive to the DMP[18].

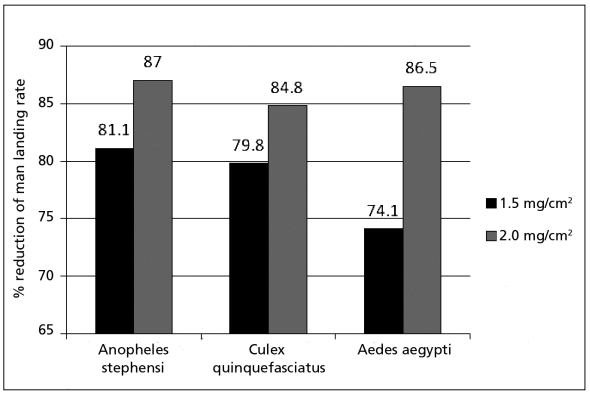

Figure 1. Percentage reduction of man landing rate with DMP treated wristbands at concentrations of 1.5 and 2.0 mg/cm^2 against mosquito vectors of disease under the laboratory conditions.

The DMP repellency effectiveness against all tested mosquito species particularly against *Aedes aegypti* provided 74.4% of reduction of man landing rate at 1.5 mg/cm^2 and its offered maximum of 86.5% at a concentration of 2.0 mg/cm^2. Similar result was observed by a earlier study when the fabric strips (anklets, wristbands, shoulder and pocket strips) impregnated with DEET (*N,N*-diethyl-*m*-toluamide) were tested against mosquito vectors of disease under the field conditions. The DEET impregnated anklets, wristbands, shoulder and pocket fabric strips at a concentration of 2 mg/cm^2 provided 5 h complete protection against mosquitoes bites and the reduction of man-landing rate varied between 65.85-100%. However, DEET impregnated fabric strips at a concentration of 1.5 mg/cm^2 provided 4 h complete protection against mosquito bites and the reduction of man-landing rate varied between 51.21-100%[14]. Previous study of Lee et al.[17] (2004) tested Neem oil against mosquito vectors and reported similar results of the efficacy according to the different dose.

In the present investigation, the results suggested that DMP treated wristbands proved to be a means of effective personal protection measure against three mosquito species tested. However few earlier studies findings showed that DMP has a poor repellency against various insects. Twenty-five per cent of N-diethyl phenyl acetamide (DEPA) incorporated macrogol ointment and tal-

cum base formulation offered more than 8 h and 6 h protection, as compared to DMP for 3 h and 1 h 30 min, respectively[19]. Another study reports the results showing effectiveness of an insect repellent N,N-diethylphenylacetamide (DEPA) against stable fly, *Stomoxys calcitrans,* and compared to N,N-diethyl-m-toluamide (DEET) and dimethyl-phthalate (DMP). DEPA gave maximum protection time of more than 6 h at 3% concentration followed by DEET and DMP[20]. This may possibly due to disparate mode of application.

DMP treated wrist bands are extremely useful when and where other kinds of personal protection measures are impossible and impractical. 84.8% of protection achieved against *Culex quinquefasciatus* at a concentration of 2.0 mg/cm^2. In one other study in Pakistan, the mean protection percent of permethrin treated uniforms against different species of mosquitoes has been estimated 57%[21]. Another investigation, in the Vietnamese forest, showed that mosquito bites on DEET users were reduced by more than threefold when sitting next to an unprotected partner who used a solvent control in comparison with the condition when treated subjects sat alone[22].

The fact is remaining that several personal protection devices need electricity for their operation and therefore it may not be useful in the remote rural and forest areas, where there is a lack of electricity. This type of circumstance repellent treated wristbands is extremely supportive and valuable. In addition, DMP treated wristbands offered 86.5% of reduction of man landing rate against *Aedes aegypti* at a concentration of 2.0 mg/cm^2. While studying the effectiveness of net jacket treated with DEET in field against biting midges viz., *Culicoides furens, Culicoides hollensis* and *Culicoides mississipiensis* has shown that it provided 98-99% protection, but against *Culicoides barbosai* it provided only 59% protection[23].

Human volunteers wearing fabrics, in the form of armbands, anklets, headbands, collar, and shoulder & pocket strips impregnated with *Vitex negundo* leaves extract were used, to test their repellent efficacy at two concentrations viz., 1.5 and 2.0 mg/cm^2 under the field conditions. At 1.5 mg/cm^2 concentration was found to be more efficacies and it provided 6 h complete protections against mosquito bites. Complete protections were for 8 h found at 2.0 mg/cm^2 against mosquitoes bites[24]. Mosquito control and personal protection from mosquito bites are currently the most important measures to control vector borne diseases. The use of repellents is a practical and economical means of preventing the transmission of the diseases to humans[6].

Vector-borne diseases are the major public health problem and it does contribute substantial socio-economic impediment in the tropical world. Indeed, developing countries are the foremost sufferer and major victims for several vector-borne diseases as a result of lack of affordability to acquire indispensable personal protection devices. On the other hand, repellent treated wristbands are incredibly economical, reusable by means of reimpregnation with repellents and it does not require any special expertise to use. In addition, it's having long lasting repellent activity than direct application on the exposed parts of skin and one can avoid considerable level of adverse effects like allergic reactions too. Therefore, repellent treated wristbands could serve as a mean of effective as well efficient personal protection device against both day and night-biting mosquitoes in order to reduce the man-vector contact and eventually vector-borne diseases.

References

1) World Health Organization. Malaria vector control and personal protection. Report of a WHO study group. WHO Tech. Rep. Ser. No. 936, World Health Organization, 2006.

2) Georghiou G, Lagunes-Tejeda A. The occurrence of resistance to pesticides in Arthropods. FAO, Rome, 1991, p. 318.

3) World Health Organization. Vector resistance to pesticides. Fifteenth Report of the WHO Expert Committee on Vector Biology and Control. WHO Technical Report Series. 818:1-62. World Health Organization, Geneva, Switzerland, 1992.

4) Hotez PJ, Remme JHF, Buss P, Alleyne G, Morel C, Breman JG. Combating tropical infectious diseases: report of the disease control priorities in developing countries project. Clin Infect Dis 2004; 38: 871-878.

5) Curtis CF. Personal protection methods against vectors of disease. Rev Med Vet Entomol 1992; 80: 543-553.

6) Gupta RK, Rutledge LC. Laboratory evaluation of controlled-release repellent formulations on human volunteers under three climatic regimens. J Am Mosquito Control Assoc 1989; 5: 52-55.

7) Coleman RE, Robert LL, Roberts LW, Glass JA, Seeley DC, Laughinghouse A, Perkins PV, Wirtz RA. Laboratory evaluation of repellents against four anopheline mosquitoes (Diptera: Culicidae) and two phlebotomine sand flies (Diptera: Psychodidae). J Med Entomol 1993; 30: 499-502.

8) Thavara U, Malainual Y, Chansang C, Phan-Urai P. Evaluation on the use of repellent soap. Bull Dept Med Sci 1990; 32: 203-207.

9) Schreck CE. Techniques for the evaluation of insect repellents: a critical review. Ann Rev Entomol 1977; 22: 101-119.

10) Debboun M, RE Coleman, PK. Gupta, D Strickman. Soldier acceptability of a camouflage face paint combined with DEET insect repellent. Mil Med 2001; 166: 777-782.

11) Kalyanasundaram M, Srinivasan R, Subramanian S, Panicker KN. Relative potency of DEPA as a repellent against the sandfly Phlebotomus papatasi. Med Vet Entomol 1994; 8: 68-70.

12) Paul JR, Sabin AB. Dimethyl phthalate as a repellent in control of Phlebotomus papatasi or sandfly fever. War Med 1994; 6: 27-33.

13) Maxwell CA, Wakibara J, Tho S, Curtis CF. Malaria infective biting at different hours of the night. Med Vet Entomol 1998; 12: 325-327.

14) Karunamoorthi K, Sabesan S. Field trials on the efficacy of DEET impregnated anklets, wristbands, shoulder and pocket strips against mosquito vectors of disease. Parasitol Res 2009; 105: 641-645.

15) Sharma VP, Ansari MA. Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in kerosene. Ind J Med Entomol 1994; 31: 505-507.

16) Yap HH, Jahangir K, Chong AS, Adanan CR, Chong NL, Malik YA, Rohaizat B. Field efficacy of a new repellent, KBR 3023, against Aedes albopictus (Skuse) and Culex quinquefasciatus (Say) in a tropical environment. J Vector Ecol 1998; 23: 62-68.

17) Lee WJ, Lee HS, Ahn YJ, Lee DK. Laboratory evaluation of controlled-release repellent treated pulp fabric on human volunteers against mosquito vectors. Entomol Res 2004; 34: 37-42.

18) Khoobdel M, Oshaghi MA, Jonaidi N, Shayaeghi M, Abaei MR, Panahi Y, Vatandoosi H, Vatani H, Tajik A, Saeedfar H. Laboratory evaluation of Dimethyl Phthalate against Anopheles stephensi and Culex pipiens. Pakistan J Biol Sci 2007; 10: 745-750.

19) Prakash S, Kumar S, Suryanarayana MVS, Sharma RK, Rao KM. Effectiveness of macrogol and talcum base formulations of a new insect repellent N, N-diethyl phenyl acetamide. Int J Cosmetic Sci 2007; 10: 23-28.

20) Parashar BD, Gupta GP, Sikder N, Rao KM. Diethylphenylacetamide: a new insect repellent against stable fly, Stomoxys calcitrans. Med Vet Entomol 1993; 7: 259-262.

21) Sholdt LL, Schreck CE, Qureshi A, Iqball M. Field bioassay of permethrin – treated uniforms and extended duration repellent against mosquitoes in Pakistan. J Am Mosquito Control Assoc 1988; 4: 233-236.

22) Nguyen SH, Nguyen TV, Phan CD, Marchand RP. Repellent cream with DEET: an effective and cheap method to reduce bites of Anopheles dirus species A in the forest. First Asian Congress on Parasitology and Tropical Medicine, Kuala Lumpur, 2004.

23) Schreck CE, Kline D, Smith N. Protection offered by the insect repellents jacket against four species of biting midges (Diptera:Culicoides). Mosq News 1979; 39: 139-142.

24) Karunamoorthi K, Ramanujam S, Rathinasamy R. Evaluation of leaf extracts of Vitex negundo L. (Family: Verbenaceae) against larvae of Culex tritaeniorhynchus and repellent activity on adult vector mosquitoes. Parasitol Res 2008; 103: 545-550.

Acknowledgements

The authors sincerely thank Dr. Vijai Dhande, the then Director, Vector Control Research Centre, Pondicherry, India for approving this research work and the facilities provided to carry out this study. In addition, we are greatly indebted to Dr. M. Kalyanasundarm, Deputy Director, Vector Control Research Centre, Pondicherry, India for his constant encouragement throughout the studies.