J Vector Borne Dis 45, June 2008, pp. 143–150

Effect of washing on the bioefficacy of insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs) against main malaria vector *Anopheles stephensi* by three bioassay methods

J. Rafinejad[a], H. Vatandoost[a], F. Nikpoor[a], M.R. Abai[a], M. Shaeghi[a], S. Duchen[b] & F. Rafi[a]

[a]*School of Public Health & Institute of Health Research, Medical Sciences/University of Teheran, Iran;* [b]*Institut de Recherche pour le Development, Montpellier Cedex 1, France*

Abstract

Background & objectives: The use of pyrethoid impregnated bednets is one of the main malaria vector control strategies worldwide. The objective of the present study was to evaluate the bioefficacy of bednets impregnated with various pyrethroids after repeated washings.

Methods: The effectiveness of bednets impregnated with permethrin, deltamethrin, bifenthrin, etofenprox and long-lasting bednets like OlysetNet® and PermaNet® which were provided by WHOPES was evaluated. The tests were carried out according to the WHO-recommended methods. Malaria vector, *Anopheles stephensi* was exposed to impregnated bednets for 3 min and the mortality was measured after 24 h recovery period. Knockdown was measured as well.

Results: Results of three methods of bioassay tests showed that between two LLINs, PermaNet® was more efficient than OlysetNet®. Results of ITNs exhibited that deltamethrin and permethrin were more effective than etofenprox and bifenthrin as impregnants.

Interpretation & conclusion: Findings of this study will be useful for WHO, local authorities and people who wish to use different pyrethroid-impregnated bednets for malaria vector control.

Key words *Anopheles stephensi* – bednet – Iran – malaria – pyrethroids

Introduction

Malaria is considered as a main vector-borne diseases worldwide. Different methods for mosquito control have been proposed by the investigators. An important innovation during the past decade is the widespread introduction of insecticide-treated mosquito nets (ITNs and LLINs) for protection against malaria transmission. Pyrethroids are today the only class of insecticides recommended for the impregnation of mosquito nets due to their rapid knock-down effects and high insecticidal potency at low dosages combined with relative safety for human contact, domestic handling and their low mammalian toxicity[1]. Pyrethroid-impregnated nets have an impact on reducing mortality and morbidity due to malaria[2,3]. If the coverage is good they also provide community protection by significantly reducing the vector populations[4,5]. For the treatment of net, WHOPES recommended alphacypermethrin, cyfluthrin, deltamethrin, permethrin, etofenprox and bifenthrin at recommended dosage[6,7]. The concentration which is recommended depends on texture of net. Advantages of ITNs are: improved personal protection and rational use of in-

secticide. Advantages of LLINs can be categorized as: ready to use, dirt-repellent net, long-lasting efficacy, and high durability to washing and wide mesh size to provide good airflow. Applied objectives of current research are comparison between efficacy of ITNs and LLINs and to find sensitivity and calibration of different bioassay tests for ITNs and LLINs.

Material & Methods

Deltamethrin, permethrin, bifenthrin and etofenprox; PermaNets and OlysetNets were provided by WHO Collaborating Centre, Montpellier, France. Out of 6 polyester mosquito nets four nets were 75 denier (ITNs: permethrin, deltamethrin, bifenthrin and etofenprox) and two nets were long-lasting nets (PermaNet with 100 denier and OlysetNet with 150 denier). Mosquito nets were conventionally treated with permethrin EC (500 mg a.i./m^2), deltamethrin SC (25 mg a.i./m^2), etofenprox EW (200 mg a.i./m^2) and bifenthrin SC (25 mg a.i/m^2) by the WHO Collaborating Centre, Montpellier, France. Ten pieces (25 × 25 cm) of the net were cut (two on each side-by-side samples collected for bioassays) and were subjected to chemical assay. A seventh net was left as untreated (negative control). Eight pieces from the same net (40 × 40 cm) were used for cone and tube bioassays and tunnel test before and after every washing (e.g. 0x, 1x, 2x, 3x, 4x), following WHO standard washing procedure[8]. The nets were washed and dried once a week. Cone and tube bioassays and tunnel test were performed on one randomly selected net for each insecticide before next washing. Each selected 40 × 40 cm net was cut into the following pieces (Fig. 1) and used as specified below**:** (i) a 25 × 25 cm net was used in cone bioassay, followed by tunnel test; (ii) a 25 × 15 cm net was used for tube bioassay; and (iii) a 15 × 15 cm for chemical analysis. Detergent (Le chat) was provided by WHO Collaborating Centre, Montpellier, France via WHOPES.

Washing procedure: Net samples (40 × 40 cm) were individually introduced into 1–l beakers containing

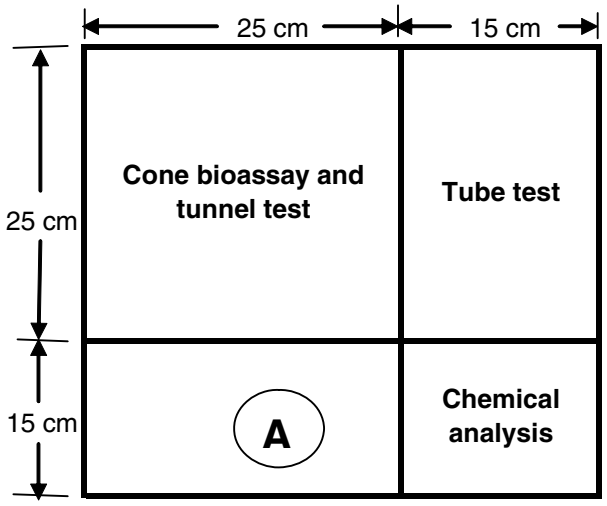

Fig. 1: Schematic presentation of cutting and using 40 × 40 cm nets for cone and tube bioassays and tunnel test. Nets were cut by a sharp scissor, without stretching the net. **A** is the section which was cut and used as the top of the WHO tube (cylinder)

0.5 liter deionized water, with 2 g/L soap "Le chat" (added and fully dissolved just before introduction of net samples). Beakers were immediately introduced into a water bath at 30°C and shaken for 10 min at 155 movements per min. The samples were then removed and rinsed twice for 10 min in clean, deionized water in the same shaking conditions as stated above. Nets were dried at room temperature and stored at 30°C in the dark between washes.

Bioassay tests: Three bioassay methods were used for the determination of biological efficacy of pyrethroids on treated mosquito nets. Non-blood fed, 2–3 days old susceptible female *Anopheles stephensi* (Diptera: Culicidae) mosquitoes (BEECH strain), susceptible to all pyrethroids reared in the insectary of School of Public Health & Institute of Health Research, Teheran University of Medical Sciences were used for all experiments.

Cone and tube tests: Mosquitoes were exposed to netting samples for 3 min after which they were held for 24 h with access to 10% sugar solution. Knockdown was measured in log time up to 64 min post-exposure and mortality after 24 h. In cone bioassays,

five mosquitoes were introduced into a cone at a time. At least 60 mosquitoes were tested on a netting sample (25 × 25 cm).

In tubes (cylinder) bioassays, 10 female mosquitoes were introduced into a cylinder at a time and at least 60 mosquitoes were tested (6 × 10) on a netting sample. The 25 × 15 cm nets were folded to 12.5 × 15 cm and introduced in the test tubes, lining the inside-surface of the tube. Three metallic clips were used instead of two to maintain the netting within test tubes properly. The top screen of the tube (see A in Fig. 1) was also made of the same fabric (same treatment and same number of washes). The tube was kept vertical during the 3 min exposure. The average mortality and knockdown time for mosquitoes in cone bioassay and mosquitoes in tube bioassays were calculated for each insecticide used for treatment of nets separately. Mosquitoes exposed to untreated nets were used as controls. Bioassays were carried out at 25± 2°C and 75 ± 10% relative humidity.

Tunnel test: Efficacy (mortality and blood feeding inhibition) of netting samples was studied in the laboratory by releasing non-blood fed female *An. stephensi* mosquitoes, aged 5–8 days, in a tunnel (square section 25 × 25 cm) made of glass, 60 cm length. At each end of the tunnel, a 25 cm^2 cage was fitted (extension) and covered with polyester netting. At one-third of the length (20 cm within the tunnel), a disposable cardboard frame was placed with the treated netting sample. The surface of netting 'available' to mosquitoes was 400 cm^2 (20 × 20 cm) with nine holes, each 1 cm in diameter: one whole was located at the centre of the square and the other eight were equidistant and located at 5 cm from the border. In the shorter section of the tunnel, a bait (guinea pig) was placed, unable to move. In the cage at the end of the longer section of the tunnel, 100 females were introduced at 1800 hrs. Females were free to fly in the tunnel but had to make contact with the piece of netting and locate the holes in it before passing through to reach the bait. The following morning, at 0900 hrs the mosquitoes were removed by hand using a suction glass tube and counted separately from the two sections of the tunnel and the immediate mortality was recorded. Live females were placed in plastic cups with honey solution; delayed mortality was recorded after 24 h. During tests, cages were maintained at 27 ± 2°C and 80 ± 10% relative humidity under subdued light. One tunnel with untreated netting was always used as a negative control. Blood feeding inhibition was assessed by comparing the proportion of blood-fed females (alive or dead) in treated and control tunnels. Overall mortality was measured by pooling the immediate and delayed (24 h) mortalities of mosquitoes from the two sections of the tunnel. The proportion of mosquitoes able to pass through the netting was also recorded. By comparison with control, an eventual reduction in penetration was calculated. This reduction provided an indication on the repellent effect of the insecticide and to which extent this effect was correlated with mortality[8]. If the control mortality was between 5 and 20%, the percentage mortality was corrected using Abbott's formula[9]. For calculating KD_{50} the probit analysis of Finney[10] and mortality analysis with SPSS (univariate analysis) was used.

Results

ITNs and cone test: The mortality of *An. stephensi* exposed to permethrin-impregnated nets by using conical test decreased from 90% in unwashed net to 78% after six washes. There was no significant difference in KD_{50} and 1 h knockdown between unwashed and 6x washed nets (Table 1). The mortality against deltamethrin-treated nets was 99.2% on unwashed and 70% on 6x washed nets. KD_{50} increased significantly from 4.16 to 37.2 min ($p<0.05$). After six washes the 1 h knockdown was 62.7%, which is significantly lower than that of unwashed net (Table 1). Against bifenthrin-treated nets the mortality was 59% on unwashed and 27.6% on 6x washed nets. KD_{50} was not measurable, but 1 h knockdown reduced by 50% (Table 1). The mortality against

etofenprox-treated nets was 74.5% and 25.5% on unwashed and 6x washed nets, respectively. KD_{50} after six washes was 48.38 min which was 9-fold more than the unwashed one. Knockdown after 1 h decreased 3.6 fold after six washes (Table 1). Mortalities on unwashed nets treated with different insecticides were in the order of deltamethrin > permethrin > etofenprox > bifenthrin. However, after six washes the mortalities were in the order of permethrin > deltamethrin > bifenthrin > etofenprox.

LLINs and conical test: The mortality against OlysetNet was 97% on unwashed net and decreased to 9% on 20x washed one. Percent knockdown in 1 h declined from 100% to 5% after 20 washes (Table 2), whereas against PermaNet mortality was 94.9% and 90% on unwashed and 20x washed nets, respectively. There was no significant difference between 1 h knockdown time between the unwashed and 20x washed ones (Table 2). PermaNet showed higher efficacy than the OlysetNet.

ITNs and tube test: The mortality of *An. stephensi* exposed to permethrin-treated nets in tube test decreased from 100% on unwashed net to 73.3% on 6x washed net. KD_{50} values for unwashed and 6x washed nets were 0.72 and 5.44 min respectively. There was no change in 1 h knockdown. The mortality against deltamethrin-treated net was 100 and 94.1% respectively on unwashed and 6x washed nets. KD_{50} slightly increased when the nets were washed six times. On bifenthrin-treated nets, the mortality reduced from 67.6% on unwashed net to 25% in 6x washed one and 1 h knockdown reduced from 100% to 28.3%. In case of etofenprox-treated nets the mortality was 100% on both unwashed and 6x washed nets (Table 1). The mortality after 6x washes was in the order of etofenprox > deltamethrin > permethrin >bifenthrin.

LLINs and tube test: For OlysetNet the mortality rate decreased from 100% on unwashed net to 92.6% on 20x washed net. KD_{50} increased 3.6-fold after 20x washes. But 1 h knockdown time did not change at all. The mortality rate and 1 h knockdown of *An. stephensi* exposed to PermaNet showed no signifi-

Table 1. Mortality and knockdown of *An. stephensi* exposed to different types of ITNs in different bioassays

Method of test	Unwashed net			6x washed net		
	Mortality (%)	KD_{50} (min)	% Knock-down (1 h)	Mortality (%)	KD_{50} (min)	% Knock-down (1 h)
Permethrin-treated net						
Cone	90 ± 3.9	2.01	100	78 ± 6.5	2.31	100
Tube	100	0.72	100	73.3 ± 8.8	5.44	100
Tunnel	100	–	44 ± 4.96	7 ± 2.6	42.05	74 ± 4.4
Deltamethrin-treated net						
Cone	99.2 ± 0.11	4.16	100	70 ± 6.4	37.22	62.7 ± 7.71
Tube	100	3.13	100	94.1 ± 3.2	5.79	100
Tunnel	93.8 ± 1.7	–	44 ± 5	50 ± 5	–	24 ± 4.27
Bifenthrin-treated net						
Cone	59 ± 6.3	–	25 ± 5.6	27.6 ± 5.6	–	12.3 ± 3.9
Tube	67.6 ± 5.9	3.85	100	25 ± 5.6	–	28.3 ± 5.8
Tunnel	71.6 ± 4.5	–	9 ± 2.7	43.3 ± 5.4	–	3 ± 1.7
Etofenprox-treated net						
Cone	74.5 ± 5.4	5.23	100	25.5 ± 5.7	48.38	27.6 ± 5.9
Tube	100	1.81	100	100	12.24	98.2 ± 1.7
Tunnel	87.6 ± 3.3	–	5 ± 2.2	65.5 ± 4.8	–	5 ± 2.2

Table 2. Mortality and knockdown of *An. stephensi* exposed to OlysetNets and PermaNets in different bioassay tests

Method of test	Unwashed net			20x washed net		
	Mortality (%)	KD_{50} (min)	% Knock-down (1 h)	Mortality (%)	KD_{50} (min)	% Knock-down (1 h)
OlysetNet						
Cone	97 ± 2.2	0.61	100	9 ± 3.3	–	5 ± 2.8
Tube	100	1.36	100	92.6 ± 3.2	4.80	100
Tunnel	100	90.65	37 ± 4.8	96 ± 2	53.35	56 ± 5
PermaNet						
Cone	94.9 ± 3.3	5.54	100	90 ± 4.04	3.97	100
Tube	100	2.10	100	100	1.28	100
Tunnel	100	59.28	57 ± 5	92 ± 2.7	53.15	56 ± 5

cant difference between unwashed and 20x washed nets (Table 2). PermaNet showed higher efficacy than the OlysetNet.

ITNs and tunnel test: The mortality of *An. stephensi* exposed to permethrin-treated nets in tunnel test decreased from 100% on unwashed net to 7% on 6x washed one. The KD_{50} of 6x washed net was 42.05 min. The 1 h knockdown increased to 74% on 6x washed net which is 1.7-fold more than the unwashed one. The mortality against deltamethrin-treated net was 93.8% on unwashed net and 50% on 6x washed net. KD_{50} was not measurable. Unwashed bifenthrin-treated net produced a mortality of 71.6% whereas 6x washed net produced 43.3%. Etofenprox-treated unwashed net produced a mortality of 87.6% whereas 6x washed net produced 65.5% mortality. There was no significant difference in terms of 1 h knockdown time between unwashed and 6x washed nets (Table 1). In the tunnel test the order of efficacy of 6x washed nets was: etofenprox > deltamethrin > bifenthrin > permethrin.

LLINs and tunnel test: For OlysetNet the mortality rate decreased from 100% on unwashed net to 96% on 20x washed nets and KD_{50} value reached to 53.35 min after 20 washes. The mortality rate of *An. stephensi* exposed to PermaNet decreased from 100% on unwashed net to 92% after 20 washes. KD_{50} also decreased after progressive washes (Table 2).

Discussion

The present study assessed the efficacy and wash-resistance of four pyrethroids namely deltamethrin, permethrin, etofenprox and bifenthrin as impregnants; and OlysetNet and PermaNet the two long-lasting insecticidal nets using different bioassay methods. Results of different bioassay methods on permethrin-impregnated nets revealed 100% mortality on unwashed nets in both tube and tunnel tests, but in cone bioassay only 90% mortality was recorded. In contrary, after six washes the mortality was 7% in tunnel test. In comparison to tunnel test higher mortality was observed in cone and tube tests on 6x washed nets. Percentage of 1 h knockdown on unwashed and 6x washed nets was 100% in both cone and tube tests, but in tunnel test 44 and 74% mortality was recorded on unwashed and 6x washed nets, respectively. These results are attributed to the large space of tunnel, hence mosquitoes are not obliged to contact the nets impregnated with insecticide.

Military shirt fabric impregnated with permethrin against *An. farauti* caused 94–100% mortality in unwashed and decreased to 28% after two washes fabric in Australia[11]. They found <20% knockdown mortality after three washes. Using bifenthrin, the authors found 100 and 55% mortality on unwashed and 3x washed nets, respectively[11]. After two washes they reported only 25% of knockdown. The effect of cold

water washing on the persistence of both chemicals in fabric by chemical assays showed that between 58 and 66% of both chemicals was lost from the test fabric after a single wash. The authors did not mention the method for determination of amount of chemical loss in impregnated bednets.

Mortality rate of *An. stephensi* exposed to deltamethrin impregnated nets revealed differences in methods of tests. Only tube test showed 100% mortality with unwashed nets. Results showed that the tube test is more appropriate than the other two tests. There was no change of 1 h knockdown in unwashed and six washed nets using tube test. However, the results for cone and tunnel test on these parameters were significantly different ($p<0.001$)[12]. In the same study the field trials with deltamethrin treated nets (KO-Tab) at 25 mg/m^2 against *An .stephensi* and *An. culicifacies* in India showed considerably reduced insecticidal action (65–78%) after two washes[12].

Nylon bednets impregnated with permethrin, cypermethrin, deltamethrin, lambdacyhalothrin and pirimiphos-methyl were evaluated in 1988 against wild adult mosquito populations, mostly *Mansonia africana* Theobald and *An. gambiae* Giles *sensu lato*, entering experimental verandah-trap huts in The Gambia. Washing three times in the traditional manner with local cow-fat soap reduced the initial dosages by about 85% of cypermethrin and lambdacyhalothrin, 99.8% of pirimiphos-methyl and left no detectable residues of deltamethrin or permethrin. The unwashed permethrin-treated bednet reduced the number of mosquitoes entering a hut by 60% of *An.gambiae s.l.* and 68% of *Mansonia* spp. Washing completely removed the efficacy of deltamethrin and permethrin treated bednets, whereas nets treated with cypermethrin, lambdacyhalothrin or pirimiphos-methyl remained significantly effective after washing[13].

In a study by Corbel *et al*[14], mixture of bifenthrin and carbosulfan was sprayed on mosquito net samples and their efficacy was tested against a susceptible strain of *An. gambiae* and the observed mortality was significantly more than expected in the absence of any interaction. They recommended that one strategy for resistance management would be to treat mosquito nets with a mixture comprising two insecticides having different modes of action. In the present study, mortality of *An. stephensi* exposed to etofenprox was more pronounced when tube test was used. Cone test had least efficacy on mortality with unwashed nets. Prasittisuk *et al*[15] conducted a study on repellency and killing effect of etofenprox, deltamethrin, lambdacyhalothrin and permethrin-treated mosquito nets on field malaria vector populations in experimental huts and local houses in westnorth of Thailand and found permethrin was most toxic to mosquitoes than others.

In the present study, 100% mortality was observed using tube and tunnel tests with OlysetNet. Cone test only yielded 97% mortality on unwashed nets. After 20 washes using cone test the mortality reached to the level of 9%, whereas in tube and tunnel tests the mortalities were 92.6 and 96% respectively. OlysetNet was evaluated in a hut trial using verandah-trap in Ivory Coast[16]. Bioassays with 3 min exposure of susceptible *An. gambiae* resulted in > 99% mortality. They concluded that OlysetNets remain remarkably effective against susceptible *An. gambiae* for at least three years under the field conditions. Similarly, OlysetNet trials in India against *An. culicifacies* and *An. fluviatilis* using cone test also reported high efficacy and wash resistance of OlysetNets even after 20 washes[17,18].

In the present study, PermaNet caused 100% mortality in both tube or tunnel tests. Similar to OlysetNet cone test only yielded 94.9% mortality. The mortality in 20x washed nets was reduced in cone and tunnel tests. However, tube test exhibited 100% mortality even after 20 washes. Both tube and cone tests were appropriate to perform the 1 h knockdown in unwashed and 20x washed nets. The mortality after

both washes was 100%.

In contrast, in a trial in Colombia and Bolivia[19] using cone test for evaluation of PermaNet, lambdacyhalothrin, deltamethrin and alphacypermethrin impregnated polyester nets against *Anopheles* spp reported 100% mortality after three washes. The procedure of their washing was competently different from that in our study. In this study the nets were washed with soap powder and tap water by local women. They found that the mortality after four washes declined considerably for lambdacyhalothrin, deltamethrin and alphacypermethrin. The wash resistance offered by PermaNet was much better and long-lasting. Graham *et al*[20] showed 97% mortality against *Anopheles* in Pakistan, Iran and Tanzania trials using PermaNet after 21 washes. However, local washing regimes gradually reduced the insecticidal efficacy of conventionally treated nets with deltametrhrin and alphacypermethrin. In the present study, the results of three methods of bioassay tests showed that deltamethrin and permethrin were more efficient than etofenprox and bifenthrin. For LLINs results of three methods of bioassay tests showed that PermaNet® is more effective than OlysetNet®. Bioefficacy of PermaNet® was evaluated in both laboratory and field conditions against *An. culicifacies* and *An. stephensi*, in India[21] and reported high mortality (>80%) even after 20 washes.

Our observation revealed that in cone test the sample net is located only at the bottom of cone. Due to repellency effect of pyrethroids and natural behaviour of mosquitoes, they have no tendency to rest at the bottom of the device. On the other hand, the excitorepellency phenomenon of insecticides may have an influence on the results of this method and the mosquitoes may easily move upward and rest on the wall of the cone or cotton swab. It seems that the exposure time is not really three min. In tube test, the sample net covers all surfaces of the tube except its bottom where mosquitoes due to their natural behaviour do not rest. To fit the nets inside the tube is sometimes difficult. In tunnel test, it is assumed that the test situation is close to the natural circumstances but the mosquitoes may rest anywhere in the compartments as well as the sample net. Additionally, the age of mosquito used for tunnel test was older than other methods. Hougard *et al*[22] in their experiments on seven pyrethroid impregnated nets against susceptible and resistant strains of *An. gambiae* and *Cx. quinquefasciatus* using cone test found that the performance of irritancy and knockdown effect of pyrethroids depends mainly on strain and insecticide. In the present study also similar results were observed.

Although LLINs are recommended for malaria control purposes, their performance should be monitored in the field under various ecological settings to assess their durability and long-term effectiveness for malaria prevention and control in Iran. Strategic plan of each country should be carefully designed to preserve the effectiveness of ITNs. Considering the different methods of bioassay it seems that cone bioassay test is an easier to perform than the other methods.

Acknowledgement

The authors would like to appreciate very much the kind cooperation of the staff of Medical Entomology and Vector Control Department, School of Public Health & Institute of Health Research, Medical Sciences/Teheran University. The authors were provided useful comments from Dr M. Zaim and Dr P. Guillet, WHO (HQ), Geneva. This research is financially supported by WHO/EMRO.

References

1. Zaim M, Aitio A, Nakashima N. Safety of pyrethroid-treated mosquito nets. *Med Vet Entomol* 2000; *14:* 1–5.
2. Choi HW, Breman JG, Teutsch SM, Liu S, Hightower AW, Sexton JD. The effectiveness of insecticide-impregnated bednets in reducing cases of malaria infection: A meta-analysis of published results. *Am J Trop Med Hyg* 1995; *5* (5): 377–82.
3. Lengeler C, Cattani J, De Savigny D. Net gain: a new

method for preventing malaria deaths. Ottawa: International Development Research Center and Geneva: World Health Organization 1996; p. 260.

4. *A global strategy for malaria control*. Geneva: World Health Organization 1993; p. 40.
5. Takken W. Do insecticide-treated bednets have an effect on malaria? *Trop Med Health* 2002; *7:* 1022–30.
6. Chavasse DC, Yap HH. *Chemical methods for the control of vectors and pests of public health importance.* Geneva: World Health Organization 1997. WHO/CTD/WHOPES/97.2.
7. Najera JA, Zaim M. *Malaria vector control, decision making criteria and procedures for judicious use of insecticides.* Geneva: World Health Organization 2002. WHO/CDS/WHOPES/2002.5.
8. *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets* 2005. Geneva: World Health Organization 2005. WHO/CDS/WHOPES/GCDPP.
9. Abbott WS. A method for computing the effectiveness of insecticides. *J Econ Entomol* 1925; *18:* 265–7.
10. Finney DJ. Probit analysis. III edn. Cambridge: Cambridge University Press 1971; p. 227.
11. Frances SP, Watson K, Constable BG. Comparative toxicity of permethrin and bifenthrin-treated cloth fabric for *Anopheles farauti* and *Aedes aegypti*. *J Am Mosq Control Assoc* 2003; *9*(3): 275–8.
12. Bhatt RM, Yadav RS, Adak T, Babu CJ. Persistence and wash-resistance of insecticidal efficacy of nettings treated with deltamethrin tablet formulation (K-O Tab) against malaria vectors. *J Am Mosq Control Assoc* 2005; *21:* 54–8.
13. Miller JE, Lindsay SW, Armstrong JR. Experimental hut trials of bednets impregnated with synthetic pyrethroid or organophosphate inseciticide for mosquito control in The Gambia. *Med Vet Entomol* 1991; *5*(4): 465–76.
14. Corbel V, Darriet F, Chandre F, Hougard JM. Insecticide mixture for mosquito net impregnation against malaria vectors. *Parasite* 2002; *9*(3): 255–9.
15. Prasittisuk M, Prasittisuk C, Pothichiti V, Aum_aung B, Mongklangkul P. The effect of pyrethroid impregnated mosquito nets on field malaria vector populations in experimental huts and individual local houses. *Southeast Asian J Trop Med Public Health* 1996; *27*(30): 610–6.
16. N' Guessan R, Darriet F, Doannio JMc, Chandre F, Carnevale P. Olyset net efficacy against pyrethroid-resistant of *An. gambiae* and *Cx. quinquefasciatus* after 3 years field use in Cote d Ivoir. *J Med Vet Entomol* 2001; *15:* 97–104.
17. Sharma SK, Upadhyay AK, Haque MA, Padhan K, Tyagi PK, Ansari MA, Dash AP. Wash resistance and bioefficacy of Olyset net : a long-lasting insecticide-treated mosquito net against malaria vectors and non-target household pests. *J Med Entomol* 2006; *43*(5): 884–8.
18. Ansari MA, Sreehari U, Razdan RK, Mittal PK. Bio-efficacy of Olyset nets against mosquitoes in India. *J Am Mosq Control Assoc* 2006; *22*(1): 102–6.
19. Ordonez Gonzalez J, Kroeger A, Avina AI, Pabon E. Wash resistance of insecticide-treated materials. *Trans R Soc Trop Med Hyg* 2002; *96:* 70–5.
20. Graham K, Kayedi MH, Maxwell C, Kaur H, Rehman H, Malima R, Curtis CF, Lines JD, Rowland MW. Multicountry field trials comparing wash-resistance of PermaNet and conventional insecticide-treated nets against anopheline and culicine mosquitoes. *Med Vet Entomol* 2005 ; *19*(1): 72–83.
21. Sreehari U, Mittal PK, Razdan RK, Ansari MA, Rizvi MMA, Dash AP. Efficacy of Permanet® 2.0 against *Anopheles culicifacies* and *Anopheles stephensi*, malaria vectors in India. *J Am Mosq Control Assoc* 2007; *23*(2): 220–3.
22. Hougard JM, Duehon S, Darrirt F, Zaim M, Rogier C, Guillet P. Comparative performances under laboratory conditions, of seven pyrethroid insecticide used for impregnation of mosquito nets. *Bull WHO* 2003; *81*(5): 324–33.

Corresponding author: Dr H. Vatandoost, School of Public Health & Institute of Health Research, Medical Sciences/ University of Teheran, Teheran, Iran.
E-mail: hvatandoost@yahoo.com

Received: 27 September 2007 *Accepted in revised form:* 3 February 2008