THE EFFECT OF PYRETHROID IMPREGNATED MOSQUITO NETS ON FIELD MALARIA VECTOR POPULATIONS IN EXPERIMENTAL HUTS AND IN INDIVIDUAL LOCAL HOUSES

Malinee Prasittisuk[1], Chusak Prasittisuk[2], Veerapol Pothichiti[3], Boonserm Aum-aung[1] and Piti Mongklangkul[1]

[1]Malaria Division, Department of Communicable Disease Control, Ministry of Public Health, Nonthaburi, Thailand; [2]WHO/SEARO, New Delhi, India; [3]Malaria Center 1 Phrabuddhabat, Saraburi, Thailand

Abstract. Studies were carried out in Tak Province, northwest Thailand to determine repellency and killing effects of four commercially available pyrethroids etofenprox, deltamethrin, lambdacyhalothrin and permethrin treated mosquito nets on field malaria vector populations in experimental huts and local houses. The studies reveal that all four test pyrethroids have a highly repellency effect. Repellency ratio between lifted and torn nets also showed some different among the four pyrethroids. Mosquito net treated with 0.3 g/m^2 permethrin was most toxic to mosquito followed by 0.02 g/m^2 deltamathrin, etofenprox 0.3 g/m^2 and 0.02 g/m^2 lambdacyhalothrin. However, careful consideration for future use should also include problem of cross-resistance, persistence of chemicals and also type of mosquito net material.

INTRODUCTION

The goal of malaria control in most endemic countries is to prevent malaria mortality and reduce malaria morbidity (WHO, 1993a) and the socioeconomic losses provoked by the disease. At present, the problem of resistance to insecticide and evasive behavior of vectors is more widespread and have evolved more rapidly than expected. DDT has been used for malaria control for more than 40 years, and acceptance of DDT residual spraying has gradually declined. The reasons are being that no clear impact of DDT on malaria is observable and the public's becoming increasingly concerned with environmental contamination. DDT has already been, banned in many countries (WHO, 1993c).

Pyrethroids are now becoming more important in controlling mosquito vectors of human diseases (Elliot, 1978). Most pyrethroid insecticides are relatively non-toxic to birds, but highly toxic to fishes and other aquatic organisms. The efficacy of four pyrethroid compounds commonly available for public health use were tested their efficacy on bed nets in terms of personal protection (Bradley *et al*, 1986; Curtis *et al*, 1992). The specification for public health use and interim specifications for these compounds have been issued by the World Health Organization for Pesticide Evaluation Scheme (WHOPES), except etofenprox which has been tested in Phase II and III trials in Thailand in 1994 (Prasittisuk *et al*, 1994; Prasittisuk M, personal communication) and Sri Lanka (Wiggerama-singhe, personal communication). The aim of this experiment is to determine the killing and repellence effects of the four pyrethroid impregnated mosquito nets under field conditions in the experimental huts and in local houses.

MATERIALS AND METHODS

Chemicals

Four pyrethroids used in this study are *lambda-cyhalothrin* [(±) - cyano-3-phenoxybenzyl (=) cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate], *deltamethrin* [(S)-α-cyano-m-phenoxybenzyl(1R 3R) -3-(2, 2 dibromovinyl)-2, 2-dimethylcyclopropane carboxylate], *permethrin* [3-phenoxy benzyl(+) cis, trans 3-(2, 2, -dichlorovinyl) -2, 2-dimethyl cyclopropane carboxylate] and *etofenprox* [2-(4-ethyl-phenyl)-2-methylpropyl 3-phenoxybenzyl ether]. These insecticides are commercially available for agricultural and public health uses.

Mosquito nets

Mosquito nets employed in this study were produced by Siam Dutch Company, Bangkok, Thai-

land. The characteristic for the net type is a new stronger thread, denier 100, suitable to withstand rough handling, made from polyester which well resistant against UV beams. Mosquito nets of size: width × length × height were 130 × 180 × 150 (cm) impregnated with the above four chemicals and were tested in the experimental huts to determine for mass killing and repellency effects to mosquitos.

Experimental huts

Four experimental louvre trap huts made of plywood that were modified from Smith *et al* (1972) were constructed in village No. 5, Ban Pade, Phradhart Pha Dang Subdistrict, Mae Sot District, Tak Province. This area is 120 km from Tak Province. The dimensions of the huts were 1.5 × 2.0 × 2.0 (width × length × height) meters. The floors were raised half meter from the ground and the roof made of local thatch, which was available in the area. A detachable window trap was installed opposite the side of the louvres. The louvres were modified as approximately 50 slatts made of plywood, which was 2 cm apart and set at 30° to the vertical. These were arranged to facilitate the entry of mosquitos into the huts in order to direct as many mosquitos as possible to fly to the window trap if they became excited or irritated. The door of the hut was situated at one corner beside the window trap and kept closed all the time. Surrounding the hut poles, grease was applied to deter scavengers such as ants. The distance from hut to hut was approximately 500-800 meters.

Local house

Five local houses were chosen with the similar distribution manner as for experimental hut in the same village. These houses had been sprayed with DDT for 5-10 years, but it had been withdrawn three years before commencing the study. Mosquito nets were rotated for observation each night for mosquito collections.

Impregnation method

The method of impregnation by Shreck and Self (1985) was carried out in this study. Individual mosquito net was soaking in a polythene bag. Sizes and type of fabrics were measured and identified prior to impregnation. Surface areas of mosquito nets were measured. The total surface area of mosquito nets and the amount of water and insecticide required were calculated.

An appropriate amount of water (280 ml) was poured into a polythene bag and the amount of calculated pyrethroids added into the bag, and mixed thoroughly until the solution became milky. A new folded mosquito net was placed in the bag, the air was released out of the bag and the opening was wrapped with a rubber band. The net was soaked, squeezed until all material became wet, left in the polythene bag about 1-2 hours for uniform distribution of chemical.

Mosquito collection

From experimental huts: In each hut one man served as a human bait and men were rotated among the four huts every day. The first hut a man slept

$$\text{Amount required} = \frac{\text{Total surface area} \times \text{target dosage}}{\text{\% active ingredient in 100}}$$

Mosquito nets of large size were used in this experiment, types and dosages of pyrethroids for impregnated mosquito nets are described below:

Pyrethroids	Active ingredient	Amount in mg/area	Amount in g/area	Chemical company
1. Lambdacyhalothrin	2.5% EC	20 mg/m^2	(0.02 g/m^2)	ICI Company Ltd UK
2. Deltamethrin	2.5% EC	20 mg/m^2	(0.02 g/m^2)	Hoechst Schering AgrEvo
3. Permethrin	10% EC	300 mg/m^2	(0.3 g/m^2)	(Thai) Ltd
4. Etofenprox	20% EC	200 mg/m^2	(0.2 g/m^2)	Mitsui Toatsu Co Ltd Japan

with no net. In the second hut, one man slept in a mosquito net into which were cut 6-8 holes of 15 cm in diameter at each side, but no chemical treatment; these two huts served as controls. In the other two huts one man each slept in a net cut into 6-8 15 cm diameter holes at each side and the nets were treated with pyrethroids. This study was carried out during the peak of mosquito density from September to November 1992 and from June to October 1993. Four consecutive night studies were carried out for each pyrethroid insecticide. The men were assigned to sleep one night in the experimental huts prior to the test, and every study night, they entered at 1800 hours and left at 0600 hours the next morning. Collection of mosquitos from window traps was performed by two insect collectors on an hourly basis from 1900 to 0100 and then at 0600 hours in the morning. The abdominal condition of each individual specimen was recorded. The doors of the huts were kept closed throughout the night until morning. The insect collectors entered the hut in the morning to search for mosquitos resting or dropping on the floor. Numbers and species of mosquitos knocked down or dead were recorded at the time of collection and living mosquitos were kept in a cool box and mortality recorded after 24 hours.

Landing catches from mosquito nets provided in local houses: (1) Two nets were used for comparison, one net served as control with no treatment, the other served as a pyrethroid treated mosquito net. In each house with each net one man sat inside the net, one side of the net was lifted to allow mosquitos to enter the net. The other man sat outside the net approximately 3-5 meters away.

(2) Three nets were used for this study. All nets were cut with holes as mentioned for the net tested in the experimental huts. This observation was made in order to imitate the nature and normal practice of local used, since the nets might be utilized for certain periods and for a period of time the nets would tear off. One net served as control with no treatment. The other two nets were treated with pyrethroid insecticides at the dosages previously mentioned. One man sat inside each net which was well tucked under the mattress, and one man sat outside the nets. Collection methods were as above.

Collected mosquitos were identified by genus and species, their abdominal conditions were scored as unfed or fed, they were kept at 28°C in a moisture cooler box and mortality was recorded after 24 hours.

RESULTS

Repellency and killing effects

Experimental huts: There were very few mosquitos found dead inside the experimental huts. The data for analysis used only numbers collected from window traps. Table 1 shows the numbers of *An. dirus*, *An. minimus* and *Culex* spp collected from control experimental huts. 93.4% of *An. dirus*

Table 1

Numbers of *An. dirus*, *An. minimus* and *Culex* spp collected in the exit traps of experimental huts**.

Pyrethroids	Control huts						Huts with treated nets					
	An. dirus		*An. minimus*		*Culex* spp		*An. dirus*		*An. minimus*		*Culex* spp	
	Fed Alive (Dead)	Unfed Alive (Dead)	Fed Alive (Dead)	Unfed Alive (Dead)	Fed Alive (Dead)	Unfed Alive (Dead)	Fed Alive (Dead)	Unfed Alive (Dead)	Fed Alive (Dead)	Unfed Alive (Dead)	Fed Alive (Dead)	Unfed Alive (Dead)
Lambda-cyhalothrin	45 (1)	0 (0)	103(2)	0 (0)	1,277 (5)	126 (3)	1 (0)	17 (17)	0 (0)	49 (28)	0 (0)	18 (66)
Deltamethrin	33 (2)	2 (0)	101 (0)	10 (0)	278 (7)	241 (3)	0 (0)	6 (15)	0 (0)	19 (41)	2 (0)	6 (14)
Permethrin	6 (0)	6 (0)	46 (0)	16 (0)	290 (3)	144 (1)	0 (0)	0 (12)	0 (0)	1 (43)	6 (0)	0 (137)
Etofenprox	30 (0)	0 (0)	95 (0)	1 (0)	212 (3)	14 (0)	0 (0)	3 (13)	0 (0)	24 (32)	0 (0)	3 (9)

leaving untreated nets had succeeded in feeding as against 92.7% of *An. minimus* but only 66.8% of *Culex* spp. On the other hand, in the huts employing treated mosquito nets with all four pyrethroids, more than 95% of mosquitos collected from window traps were unfed. This indicates that all four tested pyrethroids have a high repellency effect.

Fig 1 shows the mortality from window trap collection of *An. dirus, An. minimus, Culex* spp and other anopheline mosquitos collected from window traps that were exposed to nets impregnated with the four pyrethroids. Permethrin consistently produced the highest mortalities, and chi-square tests for homogeneity showed that the mortalities of *An. dirus, An minimus* and *Culex* spp varied very significantly ($p < 0.01$) between the four pyrethroids.

In local houses: The raw data from which repellency and mortality were calculated from landing catches of mosquito nets with one side lifted up and from mosquito nets with holes or torn nets.

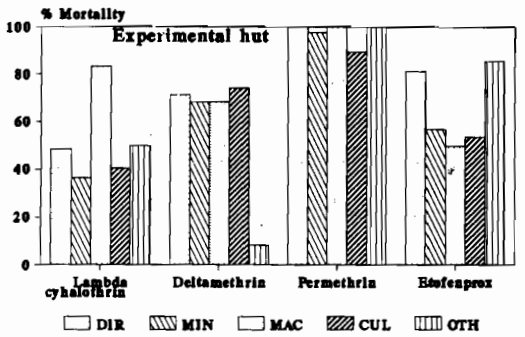

Fig 1–The efficacy of synthetic pyrethroids threated mosquito net in louvre trap hut.

A "repellency ratio" was calculated, according to the following formula:

$$\frac{\text{N outside treated net}}{\text{N outside treated net}} + \frac{\text{N outside control net}}{\text{N inside control net}}$$

$$= \frac{\text{N inside treated net}}{\text{N inside treated net}} \times \frac{\text{N inside control net}}{\text{N outside control net}}$$

where N = number of landing on the human bait.

After mosquito nets were treated with pyrethroids and introduced for practical use in local houses, the ratios of mosquitos collected between inside and outside untreated mosquito nets were almost the same. Repellency ratio between lifted nets and torn nets also show some difference among the 4 pyrethroids, *ie* lambdacyhalothrin, deltamethrin and etofenprox (Table 3). These findings confirm the finding from experimental huts as previously stated that all 4 chemicals tested have repellency effects. These findings from normal practical use of mosquito nets support the idea that when mosquitos contact nets impregnated with pyrethroids they have the possibility to be killed.

A ratio of 1 indicates no effect of impregnation, a ratio of > 1 an increased tendency for landing on the outside bait, a ratio of < 1 an increased tendency for landing on the inside bait. The chi-square test was used to test whether the ratio differed significantly from 1 when the numbers were too small.

Killing effect

The observed mortality rates are given in Fig 2 and 3. With the control nets, there was no difference of mortality between those landing inside the

Table 2

Repellency effect of impregnating lifted and torn nets in local houses.

Pyrethroids	Lifted nets						Torn nets					
	An. dirus		*An. minimus*		*Culex* spp		*An. dirus*		*An. minimus*		*Culex* spp	
	Repellency ratio	X^2	Repellency ratio	X^2	Repellency ratio	X^2	Repellency ratio	X^2	Repellency ratio	X^2	Repellency ratio	X^2
Lambda-cyhalothrin	0.4	4.5*	1.2	1.1	1.5	2.9	1.7	1.9	2.1	17.5*	1.8	28.0**
Deltamethrin	1.2	0.14	0.9	0.3	1.2	3.5	1.0	0.0	1.1	0.3	1.8	57.4**
Permethrin	0.5	1.7	0.7	1.6	5.6	80.2**	3.0	4.7*	1.2	0.7	2.4	63.8**
Etofenprox	2.6	3.6*	1.0	0.1	1.1	0.7	1.1	0.1	1.2	1.1	0.5	29.4**

Table 3

Killing effect of impregnating lifted and torn nets in local houses.

Pyrethroids	Lifted nets						Torn nets					
	An. dirus		*An. minimus*		*Culex* spp		*An. dirus*		*An. minimus*		*Culex* spp	
	Control	In/out	Control	In/out	Control	In/out	Control	In/out	Control	In/out	Control	In/out
Lambda-cyhalothrin	3.4	27.6/0	0.8	29.5/4.2	0.8	35.9/5.4	0	33.3/30.4	1.3	30.6/10.2	0.2	21.1/4.5
Deltamethrin	1.8	52.4/21.1	0.4	56.8/8.3	0.4	44.5/69.9	1.5	4.1/18.2	0.5	58.6/30.8	0.7	76.9/46.8
Permethrin	0	83.3/14.3	1.0	75.0/2.4	0	52.5/4.9	0	100/24.0	1.2	95.3/12.4	0.3	85.7/5.4
Etofenprox	0	50.0/5.9	0.7	47.5/5.6	0.4	52.1/2.9	0	66.7/11.8	0	37.7/5.2	0.9	31.7/5.6

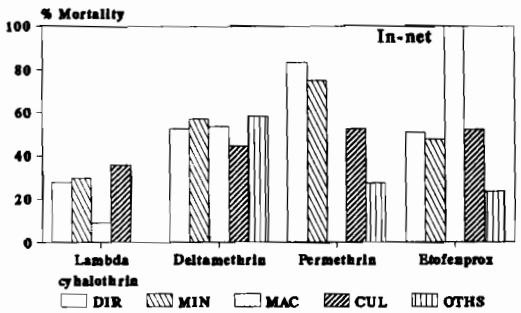

Fig 2A–The efficacy of synthetic pyrethroids treated mosquito net, mosquito collection inside one side raised up net.

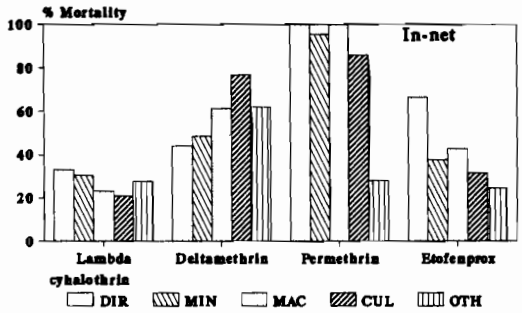

Fig 3A–The efficacy of synthetic pyrethroids treated mosquito net, mosquito collection inside hole (torn) net.

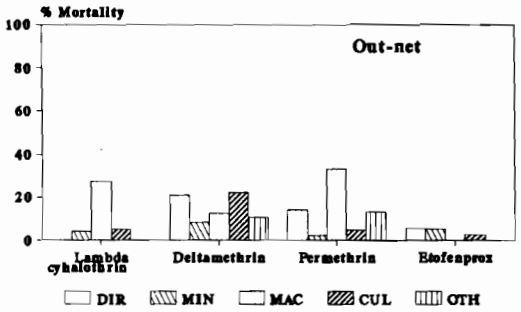

Fig 2B–The efficacy of synthetic pyrethroids threated mosquito net, mosquito collection from outside the net.

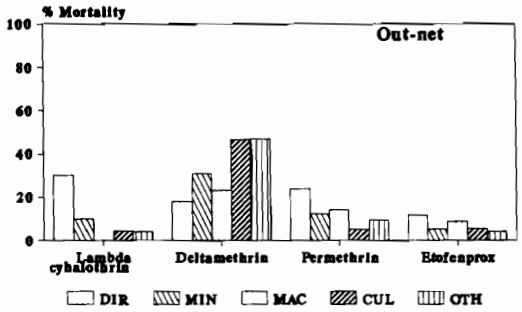

Fig 3B–The efficacy of synthetic pyrethroids treated mosquito net, mosquito collection outside hole (torn) net.

net and those landing outside, and the data from control nets have been pooled.

Observation was made on mosquitos as collected from one side lifted up net treated with lambdacyhalothrin and then recorded mortality after 24 hours. 27-36% of mosquitos collected inside the net died after being kept for 24 hours. On the other hand, mosquito nets treated with deltamethrin gave mortality rates of 44-77% of mosquitos collected inside the net while 8-70% were collected from outside. It was found that mosquito nets

treated with permethrin gave higher killing effects. Mortality rate of mosquitos collected from inside the net ranged from 52.5 to 83.3% while for those collected outside the net the rate was 2-33%. The net treated with etofenprox also gave a high killing effect, especially for mosquito collection from inside the net. These observations suggest that permethrin at the dosage of 0.3 g/m^2 was most toxic to mosquitos, followed by deltamethrin, etofenprox and lambdacyhalothrin.

Results from the nets with holes (torn nets) were similar to those for the lifted net. The results confirmed that permethrin at a dosage of 0.3 g/m^2 gave the highest killing effect, followed by 0.02 g/m^2 of deltamethrin, 0.3 g/m^2 etofenprox and 0.02 g/m^2 lambdacyhalothrin.

In order to determine whether they preferred to bite inside or outside the nets, data from all experiments were pooled from individual species and comparison was made between in-net and out-net ratios from the mosquito landing collection from lifted and torn nets. The biting ratios of the four major mosquito species indicate that mosquito species prefer to bite outside mosquito nets slightly more than inside the nets, but there was no statistical difference. Moreover, there were no statistical differences between the killing effects of *An. dirus* and *An. minimus* when they entered either to lifted nets or torn nets. This revealed that torn nets impregnated with pyrethroids could kill mosquitos and can prevent mosquitos biting. However, it was found that torn nets impregnated with deltamethrin, permethrin and etofenprox could kill *Culex* spp significantly more effectively than lifted nets treated with pyrethroids. Calculation was further made to compare killing effects of pyrethroids inside and outside mosquito nets with the control. Mosquitos collected from inside mosquito nets showed significantly higher mortality than those from outside the net (Table 3).

DISCUSSION

The experimental hut and landing catches from impregnated mosquito nets in local houses were investigated. Results obtained from experimental huts indicated that four commonly used pyrethroids, *eg* lambdacyhalothrin, deltamethrin, permethrin and etofenprox have highly repellency effects. All four pyrethroids gave partially killing effect. Permethrin at a dosage of 0.3 g/m^2 gave the highest killing effect, followed by 0.02 g/m^2 deltamethrin, 0.2 g/m^2 etofenprox and 0.02 g/m^2 lambdacyhalothrin. Results obtained from landing catches of impregnated and unimpregnated mosquito nets also confirmed the finding from experimental huts.

Permethrin is considered as one of the most safest pyrethroids. However, the problem of cross-resistance needs to be carefully considered for future use (Malcom, 1988). Ree and Loong (1989) noted that *An. farauti* and *Culex quinquefasciatus* showed a high degree of irritability to permethrin. In addition under field conditions, although people in most malaria endemic areas do not frequently wash their mosquito nets, permethrin may easily lose its persistence on nets even with only a few washes. Lambdacyhalothrin gives longer persistence after washing. With three minutes exposure periods, residual effect of 0.02 g/m^2 of lambdacyhalothrin lasts more than 12 months (Prasittisuk, personal communication). The advantage is that in an area where malaria transmission lasts 7-8 months, only one treatment is required in one year. Chemical analysis shows that following hand washing in cold water with soap, about half the initial content of pyrethroids is lost (Snow *et al*, 1988; Linsay *et al*, 1991). The loss of each compound tested after one wash was around 50%; there was a reduction in killing due to permethrin reduction to virtually zero after four washes, but with cypermethrin and lambdacyhalothrin mortality remained at 100% (Linsay *et al*, 1991). In Thailand there are 3 main types of material used for making mosquito nets, *eg*, cotton, nylon and polyester. Studies in China stated that permethrin is considered more effective on polyester than on cotton and there is little difference between the fabrics when deltamethrin is applied. With cotton, deltamethrin is much more effective than permethrin. Moreover, both permethrin and deltamethrin gave longer effects on cotton than polyester (Wu *et al*, 1987, cited by Curtis *et al*, 1991). However, assays of permethrin impregnated fabrics indicated that polyester is a better substrate for permethrin than cotton, and nylon is similar to polyester (Hossain and Curtis, 1989).

The experimental hut studies indicated that the four pyrethroids, lambdacyhalothrin, deltamethrin, permethrin and etofenprox, have a repellency effect and also a killing effect. Permethrin at a dosage of 0.3 g/m^2 was the most toxic to mosquitos, followed by deltamethrin, etofenprox and lambdacyhalothrin. The observation made by landing catch of

mosquitos inside and outside mosquito nets under natural conditions revealed that torn nets or nets with holes and impregnated with insecticide can kill mosquitos quite effectively.

ACKNOWLEDGEMENTS

This work was success with kindly assistance of Mr Porn Sawadwongporn, retired Entomologist, Malaria Center 2 Chiang Mai to whom I am deeply indepted. I am greatful to staff of Applied Research Branch, Malaria Division for their patient assistance. I am greatly indepted to Dr L Moleneaux for his kindly adviced and suggested to this work.

REFERENCES

Bradley AK, Greenwood BM, Greenwood AM, *et al.* Bednets (Mosquito nets) and morbidity from malaria. *Lancet* 1986; 2 : 204-7.

Curtis CF, Lines JD, Carnevale P, *et al.* Impregnated bed nets and curtains against malaria mosquitoes. In: Curtis CF, ed. Appropriate Technologies for Vector Control, Boca Raton, Florida: CRC Press 1990.

Curtis CF. Personal protection methods against vectors of disease. *Rev Med Vet Entomol* 1992; 80 : 543-53.

Elliot M, Janes NF, Potter C. The future of pyrethroids in insect control. *Ann Rev Entomol* 1978; 23 : 443-69.

Hossain MI, Curtis CF. Behavioural and killing effects of permethrin impregnated bednets-a laboratory evaluation. *Med Vet Entomol* 1989; 1 : 319-27.

Leahey JP. The Pyrethroid Insecticides. London: Taylor and Francis, 1985, pp. 427.

Linsay SW, Hossain MI, Bennet S, Curtis CF. Preliminary studies of the insecticidal activity and wash fastness of 12 synthetic pyrethroid treatments impregnated into bednetting assayed against mosquitoes. *Pest Sci*1991; 32 : 297-341.

Malcom CM. Current status of pyrethroid resistance in anopheline mosquitoes. *Parasitol Today* 1988; 4 : S13.

Prasittisuk C, Aumaung B, Prasittisuk M, Ketrungsee S. An experimental hut study of etofenprox against malaria vectors. The Third Conference on Malaria in Thailand, 8-10 February 1994 : 65 (Abstr).

Ree HI, Loong KP. Irritability of *An. farauti, An. maculatus* and *Cx. quinquefasciatus* to permethrin. *Jpn J Sanit Zool* 1989; 40 : 47-51.

Shreck CE, Self LS. Treating mosquito nets for better protection from bites and mosquito-borne diseases. *WHO/VBC*/85.914, 1985.

Smith A, Hudson JE, Obudho WO. Preliminary louvre-trap hut studies on the egress of *Anopheles gambiae* Giles, *Mansonia uniformis* Theobald, and *Culex pipiens fatigans* Wied from untreated huts. *Bull Entomol Res* 1972; 61 : 415-9.

Snow RW, Rowan KM, Lindsay SW, Greenwood BM. A trial of bednets (mosquito nets) as a malaria control strategy in a rural area of the Gambia, West Africa. *Trans R Soc Trop Med Hyg* 1988; 82 : 212-5.

WHO. A global strategy for malaria control. WHO Geneva 1993a.

WHO. WHO Ministerial Conference on Malaria, Amsterdam Netherland, October; 1993b.

WHO. Report of a study group on vector control for malaria and other mosquito borne diseases, Geneva. 1993c.

Wu N, Xiao Y, Huang F. Studies on the toxic effects of mosquito nets impregnated with deltamethrin and permethrin to mosquitoes (in Chinese, unpublished data)