LABORATORY EVALUATION ON THE TOXIC EFFECTS OF BEDNETS IMPREGNATED WITH PERMETHRIN AGAINST MALARIA MOSQUITOS

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Abstract. In the laboratory, bednets impregnated with 250mg ai/m^2 and 500mg ai/m^2 permethrin caused respectively the mean mortalities of 86.6% within 13 months and 87.2% within 17 months on laboratory-bred *An. sinensis*, while they caused average mortalities of 58.3% within 4 months and 73.8% within 10 months on *An. dirus* respectively. The bioassay results of KT50 and LT50 on the two species showed that KT50 is shorter than LT50 after exposure to the treated bednets. The ratio is 1:2.16 - 1:3.05. It was observed *Anopheles* had obviously secondary knocked down after exposure to the treated bednets and there is obvious resurgent after *Anopheles* have been knocked down. When the temperature goes up the resurgence gets shorter, the resurgence rate gets higher and the mortality gets lower. It showed that permethrin has stronger knocking down effect than killing effect.

INTRODUCTION

Anopheles sinensis and *Anopheles dirus* are the important malaria vectors in China. Due to their exophilism, the conventional DDT residual spray is unsatisfactory. Attention has been paid on the practice of using permethrin-impregnated bednets for the control of mosquito vectors transmitting malaria (Curtis *et al*, 1990; Rozendaal *et al*, 1989; WHO, 1989). Because the different doses were used for bednets impregnated with permethrin in various parts of the world, the results in terms of the toxic and residual effect on the mosquitos were greatly different. The objective of the study is to determine the toxic and residual effect of bednets impregnated with different dosages of imported and domestically available permethrin on *Anopheles dirus* and *Anopheles sinensis*, the important malaria vectors in China.

MATERIAL AND METHODS

Insecticide

10% oil-emulsified permethrin was produced in Jiangsu Provincial Pesticide Institute, PR China and the 26% oil-emulsified permethrin was produced by International Chemical Industry (ICI) UK.

Species

Laboratory bred *An. sinensis* and *An. dirus* were used in the bioassay. The mosquitos were at Sella stages III-IV when tested.

Bednets impregnated with permethrin

The 32 count cotton yarn cloth was used for tests and impregnated with permethrin at dosages of 250mg ai/m^2 and 500mg ai/m^2, respectively. Finally the treated cotton yarn cloth and bednets were air-dried for long-lasting use.

Bioassay of the mortality

During the tests of mortality the plastic funnels were provided by WHO for bioassay. Each test was conducted in 5 different sites on the treated nets. On each site 20 laboratory-bred *An. sinensis* or *An. dirus* were confined in bioassay funnels and exposed for 30 minutes. Then the mosquitos were kept in cultivating cages and fed a sugar solution for observing 24 hour survival rate. Tests were performed once per month and if the mortality was found to be less than 50%, the test was discarded. Besides, blank controls were carried out in each test.

Half knockdown time (KT50)

Plastic contact containers were provided by WHO, which were used for resistance test. A plastic funnel was attached to one end of the containers for promptly separating and taking out the mosquitos being knocked down. In the test the containers were lined inside with the impregnated cotton yarn cloth and 25 mosquitos were put into each of the containers. Meanwhile the number of mosquitos knocked down in different lengths of time was recorded and the tests were all repeated for the sake of accuracy.

Half lethal time (LT50)

Plastic containers were provided by WHO, which were used as in the resistance test. In the tests the containers were lined inside with permethrin-impregnated cotton yarn cloth and 25 mosquitos were put into each of the containers. After the mosquitos contacted the impregnated cotton yarn cloth at five different lengths of time, they were transferred into double cages for observing their mortality in 24 hours.

All bioassays were repeated for obtaining accurate results. The above mentioned tests were completed at the room temperature of 27 ± 1°C in laboratory and the results of the KT50 and LT50 were analysed by computer.

RESULTS

Residual bioassay

In the laboratory, the bednet cloths impregnated with 250mg ai/m² and 500mg ai/m² caused average mortality of 58.3% within 30 ~ 154 days and 73.8% within 30 ~ 310 days on laboratory-bred *An. dirus* after treatment, respectively (Table 1).

The bednets impregnated with 250mg ai/m² domestic and imported permethrin in caused 50 ~ 100% and 48.7 ~ 100% mortalities of *An. sinensis*, with average mortalities of 85.7% and 87.4% 17 ~ 410 days after impregnation, respectively.

The mortalities of 52.8 ~ 100% and 52.6 ~ 100% on *An. sinensis* were obtained within 17 ~ 520 days after the bednets impregnated with 500mg ai/m² domestic and imported permethrin, with an average mortalities of 87.2% and 87.0%, respectively (Table 2).

The results show that the residual effect of the permethrin-treated bednets on *An. dirus* and *An. sinensis* at a dose of 500mg ai/m² could last 10 and 17 months, respectively. The residual efficacy of permethrin-impregnated benets with the above-mentioned, two different doses on *An. sinensis* was longer than that on *An. dirus*. It was also observed that there were no obvious differences in terms of residual efficacy between the domestic and im-

Table 1

The residual effect of permethrin-impregnated bednets on *Anopheles dirus*.

Days after impregnation	No. of mosquitos tested	Corrected mortality (%)	
		250mg ai/m²	500mg ai/m²
30	75	81.0	100.0
68	75	55.0	92.0
91	75	40.0	81.0
124	75	59.0	71.0
154	75	35.0	57.0
221	50		59.0
240	52		78.0
276	60		70.0
310	50		58.0
Average		58.3	73.8

Table 2

Comparison of the residual effect of permethrin-impregnated bednets with two different does on *Anopheles sinensis*.

Days after impregnation	Corrected mortality (%)			
	Domestic permethrin		Imported permethrin	
	250mg ai/m²	500mg ai/m²	250mg ai/m²	500mg ai/m²
17	93.8	94.9	86.6	94.9
56	-	-	96.4	98.9
99	100.0	100.0	98.3	100.0
122	98.4	100.0	97.0	97.0
143	100.0	100.0	100.0	100.0
165	85.0	100.0	88.9	94.3
200	100.0	100.0	100.0	100.0
228	77.0	97.0	94.9	97.7
255	90.0	98.8	93.3	94.6
285	79.5	92.5	92.5	94.1
325	74.6	85.7	75.0	84.6
360	80.0	74.1	64.1	64.3
410	50.0	60.0	48.7	-
430	25.0	78.5	18.5	58.8
490		73.9		72.7
520		52.8		52.6
Average	85.7	87.2	87.4	87.0

ported permethrin used with the two doses ($p > 0.05$).

Relation between KT50 and LT50

The KT50 for *An. dirus* was 16.22-31.44 minutes averagely 18.95 minutes as determined 68-350 days after the permethrin-impregnated bednets at the dose of 500mg ai/m², while the LT50 was 8.71-47.7 minutes averagely 24.76 minutes. The ratio of KT50 to LT50 was 1:1.3 (Table 3).

When the bednets were impregnated with permethrin at dosages of 12.5mg ai/m², 50mg ai/m² and 100mg ai/m², KT50 were 19 minutes, 10.8 minutes and 7.64 minutes, respectively, and LT50 were 52.0 minutes, 24.0 minutes and 16.5 minutes, respectively for *An. sinensis*. The ratio of KT50 to LT50 were 1:3.0, 1:2.2 and 1:2.16 (Table 4).

Since revival could occur of the Anopheline mosquitos after being knocked down by permethrin-impregnated bednets, as revealed in the results, then the time required for lethal contact is longer than for knockdown. The knockdown efficacy of permethrin was greater than the lethal efficacy of the insecticide.

Knockdown rate and mortality of the mosquitos during the recovery period

An. dirus exposed for 5 and 10 minutes with the bednets impregnated with 500mg ai/m² permethrin were transferred into the recovery cages for observing the knockdown and lethal results. It was found that in the group exposed for 5 minutes there were no knockdown mosquitos, but all the mosquitos separated from the treated bednet were knocked down during the recovery period within 15-35 minutes with a KT50 of 20.87 minutes and a mortality of 4.2% only. 13.6% of the mosquitos exposed for 10 minutes were knocked down and all the mosquitos separated from the treated bednet were knocked down during the recovery period between 15-35

Table 3
The KT50 and LT50 for *Anopheles dirus* after exposure to bednets impregnated with 500mg ai/m² permethrin.

No. of days after impregnation	Knockdown time (min)		Lethal time (min)	
	KT50	KT95	LT50	LT95
68	16.22	27.54	-	-
91	-	-	8.71	25.30
124	-	-	21.38	47.86
154	-	-	34.67	123.03
191	16.67	32.28	20.99	55.46
221	16.60	28.46	18.07	52.36
249	16.46	26.16	22.28	80.72
276	16.23	29.18	22.36	75.68
310	18.72	30.47	26.65	134.25
350	31.44	60.77	47.70	227.67

Table 4

KT50 and LT50 of *An. sinensis* after exposure to permethrin-impregnated bednets with different dosages.

mg ai/m²	KT50 (min)	LT50 (min)	The ratio of KT50 to LT50
12.5	19.00	58.0	1:3.05
50.0	10.80	24.0	1:2.20
100.0	7.64	16.5	1:2.16

Table 5

The knockdown and lethal results for *An. dirus* during the recovery period after being exposed to 500mg ai/m² permethrin-impregnated bednets.

Day after impreg	No. knocked down in exposure			No. secondary knockdown after exposure (min)						KT50 (min)	mortality after 24 hours (%)
	No. tested	Exposed time	No. knocked down	10	15	20	25	30	35		
140	44	10	6		12	16	34	44		19.85	11.4
142	48	5	0	0	4	16	32	44	48	20.87	4.2

minutes with a KT50 of 19.85 minutes and a mortality of 11.4%. It shows that the secondary knockdown rate during the recovery period is very high, being 86.4 ~ 100%. But the mortalities were very low for the mosquitos exposed for 5 minutes and 10 minutes to the bednets (Table 5).

Revival of *An. dirus* knocked down under different temperatures

An. dirus were exposed for 50 minutes to the bednets 240 days after they were impregnated with 500mg ai/m² permethrin at a temperature of 27°C

Table 6

The revival of *An. dirus* knocked down after exposed to bednets impregnated with 500 mg ai/m² permethrin under different temperature.

Days after impreg	Temperature	No. of knock down	Mean recovery time (h)	Recovery time required after knocked down (h)									Recovery rate (%)
				4	6	8	10	12	20	24	28	32	
240	27°C	71	19.6	0	1	3	10	11	15	16	18		25.3
240	20°C	63	30.0	0	0	0	0	0	0	0	7	10	15.8

and of 20°C. After all the mosquitos were knocked down, the recovery time at 27°C was 6-28 hours, averagely 19.6 hours with a recovery rate of 25.3%. The recovery time at 20°C was 24-32 hours, average 30.0 hours with a recovery rate of 15.8%. The results demonstrate that the higher the temperature, the shorter of the time required for revival and the higher recovery rate, the lower the mortality will be (Table 6).

DISCUSSION

There are a number of papers concerning the toxic and residual effect of bednets impregnated with permethrin on mosquitos. However, due to the different dosages used, various exposure times and differences of the vectors involved, the results varied greatly and comparison could not be made. For example, Darriet *et al* (1984) reported that the residual effect of impregnated cotton bednets on *Aedes egypti* 60 minutes after exposure to bednets impregnated with 80mg ai/m² permethrin could last 4-5 months, but Lines *et al* (1987) reported that with bednets impregnated with the same dosage the mortality rates of *An. gambiae* were 0 and 40% after an exposure of 1 minute and 15 minutes to the bednets one month after impregnation. Loong *et al* (1985) found that the mortality of *An. maculatus* 10 minutes after being exposed to bednets or 30 minutes after being put into bednets reached 100% one year after impregnation with low dose permethrin (20mg ai/m²). It was thought that bednets impregnated with permethrin had an exciting repellent effect on the mosquitos and the mosquitos would often fly away several minutes or even several seconds after contact with the bednets. Therefore long exposure of the mosquito to the impregnated bednets was not recommended when conducting experiments. However, the results of bioassay obtained in our laboratory demonstrate that the average mortality of *An. sinensis* 30 minutes after exposure to the bednets one year after impregnation with 500mg ai/m² permethrin was 78.9%. Taking the results obtained in the experimental hut into consideration the average mortality was 87% one year after impregnation with the same dose of permethrin. The authors' experience is that an exposure of 30 minutes would be reasonable for laboratory determination. It also shows that although the mosquitos will become fly away after contact with the impregnated bednets, for sucking blood they have to repeatedly come into contact with the bednet again, thus along with an increase of opportunities of contact by the mosquitos with the bednets the mortality of the mosquitos will be increased.

The knockdown and lethal effect of permethrin on the mosquitos showed that the revival phenomenon apparently occurred to the mosquitos knocked down after exposure to the permethrin-impregnated bednets. For *An. sinensis* the ratio of KT50 to LT50 was from 1:1.26 to 1:1.30. It shows that the knockdown effect is stronger than the lethal effect and there is an additional secondary knockdown phenomenon. The recovery of the mosquitos after being knocked down was associated with the temperature, that is the higher the temperature is, the greater the recovery rate it is. It also indicated that there is a negative correlation between the temperature and the death of the mosquitos. Therefore these factors relating to the evaluation of the toxic effect of permethrin on mosquitos and its applicationin to epidemiological studies should be comprehensively considered in study design.

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