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CYFLUTHRIN (EW 050)-IMPREGNATED BEDNETS IN A MALARIA CONTROL PROGRAM IN GHASSREGHAND (BALUCHISTAN, IRAN)

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ABSTRACT. In a study carried out in the Ghassreghand Division (Baluchistan, Iran) from March through November 1995, efficacy of cyfluthrin-impregnated bednets was compared to that of untreated nets, in relation to malaria control. Ten villages with a total population of 4,572 and 3 villages with a total population of 1,935 were used as treatment and control, respectively. The collection, impregnation (target dosage of 40 mg active ingredient [AI]/m²), and redistribution of the nets (9% nylon, 52% light cotton, 30% medium cotton, and 9% heavy cotton), carried out in mid-April, were done by local health workers, supervised by the senior research staff. *Anopheles culicifacies* was considered to be the main vector of malaria in the named area. This species is mainly zoophilic, endophilic, and exophagic. The initial uptake of the insecticide was lower than the target dosage, with high variation (nylon, 12.5 ± 5.4 mg AI/m²; light cotton, 33.3 ± 26.1 mg AI/m²; medium cotton, 25.9 ± 20 mg AI/m²; heavy cotton, 17.6 ± 12.5 mg AI/m²). The use of impregnated mosquito nets (used primarily outside) had no significant effect on the incidence of malaria. No difference was detected in the parasite density of patients with positive slides. No significant effect was observed in the parous rate, human blood index, and sporozoite rate of anopheline vectors. Only the indoor resting densities of *An. culicifacies* and other malaria vectors were drastically reduced after the introduction of the cyfluthrin-impregnated nets into the treatment villages. The residual activity of cyfluthrin was lower than expected. The mortality of anophelines brought in contact with the treated nets for 3 min in bioassays dropped to less than 55% in 3 months. The loss of chemical activity was greatest for the light cotton nets, followed by the medium cotton nets. Cyfluthrin-treated nets were mildly irritating to host-seeking female anophelines in the laboratory. The protective rate of impregnation (all fabric kinds included) in preventing female mosquitoes from biting through the impregnated nets was initially 5–6 times that of the nonimpregnated nets. The study did not detect any significant difference between the use of untreated versus impregnated bednets in the Ghassreghand area. In planning future medium-scale trials, comparison of new compounds and formulations to the more widely used pyrethroids such as permethrin and deltamethrin is highly recommended.

KEY WORDS: Malaria control, impregnated bednets, pyrethroids, cyfluthrin, Iran

INTRODUCTION

The Ghassreghand Division (composed of two subdistricts: Holonchekan and Sarbook), with a total population of about 40,000, is a malarious area in the heart of Baluchistan, Iran, where the annual incidence of malaria averages about 30–50 per thousand population. This is a rice-growing region and local people have long used bednets (mostly cotton fabric) for personal protection against mosquito bites. The average daily temperatures in the warmest and coolest months of the year are 34°C and 14.5°C, respectively. The average yearly rainfall is about 120 mm.

Two distinct peaks of malaria transmission occur in the Ghassreghand area: one in April–May and the other in September–October (Zaim et al. 1993). *Plasmodium vivax* and *Plasmodium falciparum* are the 2 malaria parasites present in this area. The proportion of *P. falciparum* among all malaria infections has cyclical changes, but it has been reported as 0.46, 0.75, and 0.37 for the years 1992, 1993, and 1994, respectively. About 50% of the malaria cases occur in people less than 15 years old.

Anopheles culicifacies Giles and *Anopheles pulcherrimus* Theobald are the most common anopheline species in the Ghassreghand area, where indoor residual spraying (2 rounds per year) was practiced for many years. *Anopheles dthali* Patton, *Anopheles stephensi* Liston, *Anopheles fluviatilis* James, and *Anopheles superpictus* Grassi, the other known malaria vectors in Iran, are also present in this area, but in very small numbers. However, *An. culicifacies,* identified as species A of the complex, is regarded as the main vector (Zaim and Javaherian 1991, Zaim et al. 1993). This is mostly an endophilic species, but a large population also rests outdoors (Zaim et al. 1995). The human blood index (HBI) for *An. culicifacies* was found to be related to the ratio of the human and cattle population in each village; however, the average HBI was found to be about 5%. Bloodfeeding occurs throughout the night, with 25% occurring before 2100 h and only 65% before midnight (Mowlaii et al. 1991).

Anopheles culicifacies is mostly exophagic because almost all inhabitants as well as animals rest outdoors during the night during the transmission season. Early-morning pyrethrum space spray catch of indoor-resting mosquitoes in animal and human shelters is considered to be the best sampling tool for studying the population of the species in the study area (Zaim et al. 1995).

Impregnated mosquito nets have shown to be effective in reducing exposure to biting mosquitoes and other pest and nuisance insects (Curtis 1992, Sexton 1994, Lines 1996). Use of impregnated

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Table 1. Population, history of malaria cases, and the annual parasite incidence (API) per 1,000 population for the group of villages under the trial for the field evaluation of bednets impregnated with cyfluthrin EW 050 in Ghassreghand, Baluchistan, Iran (1992–1994).

	1992			1993			1994		
Study zone	Population	No. cases	API	Population	No. cases	API	Population	No. cases	API
Treatment	4,317	210	48.6	4,378	127	29.0	4,322	125	28.9
Control	1,761	105	59.6	1,916	34	17.7	1,844	36	19.5

mosquito nets substantially reduces the number of infective mosquito bites received by a person in a malarious area (Rozendaal 1989). Whether this reduction in the inoculation rate has an effect on malaria prevalence rates and malaria morbidity will depend on a variety of local factors among which are the level of endemicity and seasonal fluctuations in malaria, and the immune status of the population. In areas with low transmission of malaria, impregnated bednets seemed to be effective in substantially reducing parasite rates (Li Zuzi et al. 1989). In highly endemic areas, a reduction in the sporozoite inoculation rate resulted in a reduction in the number of fever cases and in a reduction of the parasite densities in positive slides (Carnevale et al. 1988).

Relatively little attention has been given to comparative trials to determine which insecticides, doses, and types of fabric are most effective (Curtis et al. 1996). In most cases the insecticides used for large-scale trials have been permethrin, deltamethrin, and lambdacyhalothrin, but more recently other pyrethroids such as cyfluthrin have been used successfully (Yadav and Sharma 1994).

Curtis et al. (1992) reported that 30 mg active ingredient [AI]/m² of cyfluthrin EW 050 on polyester caused the same mortality rates in *Anopheles gambiae* Giles as did 50 mg AI/m² in bioassays, and this mortality was not significantly different from that of permethrin at 200 mg AI/m². The same authors also reported that nets with holes, treated with 50 mg AI/m² of cyfluthrin, performed well after 15 months of domestic use.

Impregnated bednets have important operational advantages over residual spraying: less insecticide is needed to cover the relevant surfaces, control programs can be organized locally and hence operational costs are reduced, and foreign exchange costs are reduced because spray equipment is not needed and locally available materials may be used to manufacture the bednets (Miller and Gibson 1994).

The main objective of this study was to assess the potential of using cyfluthrin (EW 050)-impregnated bednets, as compared to untreated nets, for malaria control in Ghassreghand. Objectives included the study of the impact of impregnation on the indices of the vector potential as well as the persistence of the insecticide on fabrics in the local conditions of Baluchistan.

MATERIALS AND METHODS

Our investigation was carried out in the Ghassreghand area between March and November 1995. The preintervention phase lasted for 2 months and coincided with the beginning of the mosquito activity in the region. The impregnation of the bednets was carried out in mid-April 1995.

Study area

Ten villages with a total population of 4,572 inhabitants were selected randomly from the list of ecoepidemiologically homogenous villages in the Ghassreghand area. The village inhabitants were allocated bednets impregnated with cyfluthrin EW 050 (OMS 2012, Bayer AG, Leverkusen, Germany) at the target dose of 40 mg AI/m². Three villages with a total of 1,935 inhabitants were also selected randomly as controls. The history of malaria in the treatment and control villages, during the years 1992 and 1994, is presented in Table 1. It is noteworthy that all villages in the Ghassreghand area have primary health care (PHC) services. Also, almost all households in the Ghassreghand area have bednets, and all family members use the nets regularly.

All mosquito control activities had been stopped in the study villages and their 1.5-km vicinity beginning in the autumn of 1994. The last indoor residual spraying was carried out in the area in late August 1994, using propoxur at 2 g AI/m².

Impregnation of the nets

All inhabitants in the treatment as well as the control villages used bednets, mostly made of cotton (91%), but with great variation in size, thickness, weight, structure, and number of fibers per square centimeter, and hence of different absorptive capacities. Single treatment of the nets by application of enough emulsion just to wet the netting was not considered operationally feasible and dipping in bulk mix and wringing out the surplus was chosen as the method of choice for impregnation of the nets. However, based on our experience from a pilot study that was carried out in 1994 (Zaim, unpublished data), and in order to reduce the variance of insecticide deposits on the nets, they were grouped into fabric type, either nylon or cotton. The latter nets were further classified into light (52%), medium (30%), and heavy fabrics (9%). Different concentrations of insecticide, based on the absorptive capacities of the fabrics were prepared. Individual nets (at least 10 nets of each type of fabric) were soaked in a known volume of water, wrung out, and allowed to drip so that the excess water fell back into the bucket. The final volume of water in the bucket was measured, and the difference between this and the original volume was the amount of water retained by the net. This was divided by the surface area of the net to obtain the retention capacity in milliliters per square meter. The average absorptive capacities, determined on 42 bednets, were found to be 82.2, 132.1, and 144.6 ml/m^2 for light, medium, and heavy cottons, respectively, and 36.7 ml/m^2 for nylon fabrics. After impregnation, the nets were laid on plastic sheets in the shade to partially dry. They were then hung on a wire and allowed to dry completely before they were returned to the people. A total of 877 bednets (4 single, 8 double, and 865 family size) were impregnated in this trial.

The collection, impregnation, and redistribution of the nets was carried out in mid-April 1995, coinciding with the normal habit of using bednets by the local people. These activities were carried out by local health workers, supervised by the senior research staff. The nets received a code number at the time of collection. Also, a questionnaire recording the size (single, double, or family size), type of the fabric, and frequency of use (throughout the season or occasionally) was completed. A small piece of netting of a similar type was then sewn to the net for bioassay and chemical residue analysis.

Residual analysis and bioassays

The code numbers of the nets were used for random selection of bednets from which netting samples were taken 2 days after impregnation and every month thereafter. The netting samples were put in glass test tubes along with their relevant collection information, then brought to the laboratory where bioassays were performed on them. They were then sent to the World Health Organization (WHO) Collaborating Centre in Gembloux, Belgium (Sation de Phytopharmacie), for residue analysis, using gas–liquid chromatography (GLC).

The bioactivity of the insecticide was studied through bioassays using 12- to 24-h-old, unfed, laboratory-reared female *An. stephensi.* Female mosquitoes were exposed for 3 min in groups of 5, to each sample lining the interior surface of WHO adult mosquito susceptibility test tubes. For comparison, mosquitoes were exposed to untreated nylon netting. At the end of the exposure time, the mosquitoes were transferred to clean holding tubes where they were maintained on 10% sucrose solution. Percent knock down was recorded at 5-, 15-, 30-, and 60- min intervals, from which the median knock-down time (KDT_{50}) was calculated. Mortality was recorded after 24 h. It is noteworthy that a laboratory colony of *An. culicifacies* was not available and it was assumed that a susceptible laboratory colony of *An. stephensi* could be used for detecting changes in the bioactivity of the insecticide on the fabrics.

Also, unfed 24- to 48-h-old laboratory-reared female *An. stephensi* were exposed, in groups of 5, to treated (collected from the trial area) and untreated netting samples, while the holding tubes were kept in contact with human skin. The "protective rate" was calculated as the ratio of the percentage of mosquitoes that fed in the control to that in the treatment.

Entomological surveillance

Monthly indoor pyrethrum space spray catches (conducted every 30 days) were performed early in the morning, in a total of 60 fixed human and animal shelters (40 in the treatment and 20 in the control zone), from March (2 months before the impregnation of the nets and at the start of mosquito activity) through November, 1995. Almost all inhabitants used their bednets outside the houses and the possible excito-repellency effect of the insecticide on the indoor-resting population and hence the collection technique was assumed to be minimal. It is noteworthy that pyrethrum space spray catch has been shown to have good correlation with human biting collections of *An. culicifacies* in Baluchistan (Zaim et al. 1995).

Anopheline species collected were classified according to blood-digestion stages (abdominal conditions). Empty and freshly fed individuals were dissected and scored as nulliparous or parous on the basis of the tracheolar skeins of their ovaries (Detinova 1962). Blood smears were prepared on Whatman papers (Whatman International Ltd., Kent, United Kingdom) from the fed individuals and were used for the determination of the HBI, using an enzyme-linked immunosorbent assay (ELISA), as described by Edrissian and coworkers (Edrissian et al. 1985). The head and thorax of each individual were removed and kept in a small vial for the determination of the sporozoite rate using an ELISA, as described by Wirtz et al. (1991, 1992). The monoclonal antibodies were kindly provided by R. A. Wirtz of Walter Reed Army Institute of Research, Washington, DC.

The monthly mean indoor resting densities in the treatment and control areas were subjected to Student's t-test, after being transformed to $\ln(x + 1)$. The monthly parous rates, HBIs, and sporozoite rates of the 2 areas were subjected to a chi-square test.

Parasitologic surveillance

Blood slides (thick smears) were collected from one half of the population (randomly selected) of

Table 2. The monthly mean indoor resting density (m), human blood index (HBI), parous rate (p), and sporozoite rate (sp) of female *Anopheles culicifacies* collected in the villages under the trial of cyfluthrin-impregnated bednets, in Ghassreghand (Baluchistan), Iran, 1995. The total number tested is given in parentheses.

Month (1995)	Control				Treatment			
	m	HBI (%)	p (%)	sp (%)	m	HBI (%)	p (%)	sp (%)
March[1]	3.25	0 (20)	75 (24)	3.17 (63)	3.45	0 (54)	77 (26)	0.8 (125)
April[1]	6.9	2.4 (82)	50 (69)	1.06 (188)	22.4	0.79 (507)	72.5 (138)	0.89 (778)
May	24.2	1.4 (278)	68.8 (41)	0 (350)	2.5	5.13 (78)	74.3 (35)	2.06 (97)
June	9.25	5.7 (122)	74.4 (90)	0 (167)	0.86	4.76 (42)	76.4 (17)	9.67 (31)
July	5.7	24 (80)	86.8 (38)	0.9 (111)	0.15	20 (5)	100 (1)	33.3 (6)
Aug.	5.2	22.2 (27)	39.6 (53)	2.08 (96)	1.7	14.29 (35)	38.7 (31)	1.41 (71)
Sept.	13.4	12.6 (159)	38.7 (93)	2.84 (211)	15.9	3.65 (384)	43.3 (254)	2.73 (622)
Oct.	6.6	4.11 (73)	75.5 (49)	2.38 (127)	11.07	2.5 (202)	91.5 (141)	0 (376)
Nov.	1.25	0 (7)	43 (7)	15.4 (26)	1.8	0 (30)	87.5 (24)	0 (65)

[1] Preintervention phase.

the 13 villages in late April 1995, when the distribution of the impregnated nets was completed (prevalence rate of 1.03 and 1.75 per 1,000 population in the control and treatment villages, respectively). Thereafter, passive case detections (PCDs) and intensive active case detections (ACDs) (every 10 days) were carried out during the study period. Standard malaria epidemiologic information, including age, sex, history of travel especially in the past 15 days, history of malaria, date of onset of symptoms, and type of parasite, was gathered for all positive cases (confirmed by blood slides).

All positive slides as well as 10% of the negative slides were double-checked by senior laboratory technicians at the central laboratory in Teheran, Iran, who also counted the number of parasites per microliter of blood. The parasite densities of the slides positive for *P. vivax* (the most common malaria parasite in the study area) were then classified according to Bruce-Chwatt (1985). The frequency of the number of parasites per microliter of blood for the patients in the treatment and control areas were compared using a chi-square test.

RESULTS

Entomological surveillance

From March to November 1995, a total of 11,847 female anopheline mosquitoes were collected indoors, using pyrethrum space spray catches. *Anopheles culicifacies* was the most common anopheline species (89%) captured in the treatment villages, followed by *An. pulcherrimus* (4.4%), *An. dthali* (2.7%), *An. stephensi* (2.4%), *An. fluviatilis* (0.6%), *An. superpictus* (0.1%), and others (0.8%). However, the composition of anophelines was quite different in the control zone. In the village of Zeineddini (control village, untreated bednets), where no mosquito control activity has been in practice for about 5 years, *An. stephensi*, after 20 years of appearance in only very small numbers, once again became the predominant species (90.5%), followed by *An. culicifacies* (7.7%), *An. pulcherrimus* (1.1%), *An. dthali* (0.6%), and others (0.1%). However, in the other control villages, *An. culicifacies* was the most common species (92.2%), followed by *An. dthali* (4%), *An. pulcherrimus* (3.2%), *An. stephensi* (0.5%), and others (0.1%), which was very much comparable to the species composition in the treatment villages.

The monthly indoor resting density, HBI, parous rate, and sporozoite rate for *An. culicifacies* and the total anopheline vectors, excluding *An. culicifacies*, are presented in Tables 2 and 3, respectively. The monthly mean indoor resting densities in the control and treatment villages were compared, using Student's *t*-test. The monthly HBIs, parous rates, and sporozoite rates in the control and treatment villages were compared, using a chi-square test.

Density of *An. culicifacies* in the control villages continued to rise until the end of May, whereas in the treatment villages it decreased sharply following the introduction of the impregnated bednets into the area in mid-April, and stayed less than 2.5 per shelter for 4 months. The mean indoor resting density of female *An. culicifacies* was significantly higher ($P < 0.001$) in the treatment villages in April (before the introduction of the treated nets;

Table 3. The monthly mean indoor resting density (m), human blood index (HBI), parous rate (p), and sporozoite rate (sp) of the total anopheline vectors, except *Anopheles culicifacies,* collected in the villages under the trial of cyfluthrin-impregnated bednets, in Ghassreghand (Baluchistan), Iran, 1995. The total number tested is given in parentheses.

	Control				Treatment			
Month (1995)	m	HBI (%)	p (%)	sp (%)	m	HBI (%)	p (%)	sp (%)
March[1]	87.6	1.7 (291)	68.4 (152)	0.26 (755)	2.4	0 (16)	75 (20)	0 (67)
April[1]	144.15	1.1 (705)	54.9 (233)	0 (1,691)	0.52	37.6 (16)	92 (12)	3.8 (26)
May	38.05	0.6 (331)	60.2 (176)	0.34 (587)	0.17	0 (6)	75 (3)	0 (6)
June	2.65	0 (32)	70 (20)	2.85 (70)	0.2	50 (2)	0 (6)	0 (10)
July	0.7	12.5 (8)	100 (4)	0 (9)	0.075	— (0)	— (0)	0 (2)
Aug.	1.95	3.4 (29)	33.3 (18)	3.1 (32)	0.15	0 (3)	100 (1)	0 (6)
Sept.	9.8	1 (94)	41.3 (92)	2.8 (284)	2.82	4 (74)	44.8 (67)	2.1 (97)
Oct.	66.25	0.7 (2.67)	55.4 (130)	0.25 (1,175)	1.27	0 (43)	86.2 (29)	63.1 (32)
Nov.	30.3	1.6 (124)	57.6 (158)	0.16 (641)	0.37	0 (7)	71.4 (7)	0 (16)

[1] Preintervention phase.

22.4 vs. 6.9 female *An. culicifacies* per shelter in the treatment and control villages, respectively) and in October and November (6 and 7 months after the introduction of impregnated nets).

The total anopheline vector density, excluding *An. culicifacies,* also decreased in the treatment villages after the introduction of the impregnated nets, and stayed less than 0.2 per shelter for 4 months. The total anopheline vector density, excluding *An. culicifacies,* was also significantly lower than the density of total anophelines in the control villages in September through November, when the density in the control villages increased to 66.25 per shelter in October. The high density of total anophelines in the control area was due to the high number of *An. stephensi* collected in the village of Zeineddini, especially between March and May.

No significant difference in the parity of *An. culicifacies,* the main endophilic species in the study site, could be detected between the treatment and control villages in the months of May, June, August, and September, 1–5 months after the introduction of impregnated nets (Tables 2). No statistical analysis was performed on the data of July, because not enough empty or newly bloodfed mosquitoes could be found for dissection in the treatment zone. However, the parity of *An. culicifacies* was significantly higher ($P < 0.05$) in the treatment zone in the months of April (before impregnation), October, and November (6 and 7 months after impregnation). Also, except in September and October ($P < 0.0001$), no significant difference was found in the HBI of *An. culicifacies* between the treatment and control villages (Table 2). No statistical analysis was performed on the parous rate and HBI of other anophelines because of the relatively small number collected in the treatment zone during April through August.

A total of 9,016 anophelines (56.5% *An. stephensi,* 38.9% *An. culicifacies,* 2.5% *An. pulcherrimus,* 1.7% *An. dthali,* 0.37% *An. fluviatilis,* and 0.03% *An. superpictus*) were subjected to an ELISA test. Seventy-six individuals (0.84%) were found to be positive (38.2% *P. falciparum* 2A10, 47.2% *P. vivax* NVS 3, and 14.4% *P. vivax* NK 247), 53 of which were *An. culicifacies.* No significant difference was found between the sporozoite rate of *An. culicifacies* in the treatment and control villages throughout the study period.

Parasitologic surveillance

The proportion of blood slides taken and the proportion of malaria cases found by PCD, ACD, survey, and follow-up; the proportion of infection with *P. faciparum*; malaria incidence according to age groups and sex; as well as the proportion of indigenous and imported cases and the proportion of relapses, are presented in Table 4. These data were subjected to the chi-square Mantel–Haenzel test for any significant difference.

No significant difference was found between the total malaria incidence; proportion of positive females; proportion of the indigenous and imported cases and the relapses; and the proportion of infection with *P. falciparum* between the treatment and control villages. However, the proportion of malaria cases found by ACD in the treatment zone ($P =$

Table 4. Malariometric indices in the villages under the trial of cyfluthrin-impregnated bednets in Ghassreghand (Baluchistan), Iran, 1995.

Activity[1]	Control	Treatment
Proportion of blood slides taken/population		
PCD	0.105 (306/2,900)	0.112 (903/8,025)
ACD	0.398 (1,155/2,900)	0.328 (2,632/8,025)
Survey and follow up	0.496 (1,493/2,900)	0.559 (4,490/8,025)
Proportion of infection with *Plasmodium falciparum*	0.158 (13/82)	0.171 (39/227)
Proportion of positive females	0.304 (25/82)	0.334 (75/227)
Malaria incidence in		
<5 years	0.034 (15/482)	0.047 (29/611)
6–10 years	0.033 (20/390)	0.057 (45/782)
11–15 years	0.049 (15/340)	0.076 (51/665)
>16 years	0.033 (32/956)	0.044 (152/2,290)
Total	0.042	0.049
Proportion of malaria cases found by		
PCD	0.646 (53/82)	0.495 (106/227)
ACD	0.304 (25/82)	0.462 (105/227)
Survey and follow up	0.048 (4/82)	0.070 (16/227)
Proportion of indigenous cases/malaria cases	0.500 (41/82)	0.041 (94/227)
Proportion of relapses/malaria cases	0.341 (28/82)	0.365 (94/227)
Proportion of imported cases/malaria cases	0.158 (13/82)	0.220 (50/227)

[1] PCD, passive case detection; ACD, active case detection.

0.008) and the proportion of malaria cases found by PCD in the control zone ($P = 0.019$) were significantly higher.

The frequency distribution of *P. vivax* malaria cases according to parasite density per microliter of blood in the 2 malaria transmission seasons (April–July and August–November) in the control and treatment villages are presented in Table 5. No significant difference was found in the parasite density in the study villages, in each transmission season, using a chi-square test.

Residue analysis and bioassays

Table 6 presents the results of the residue analysis of the netting samples taken randomly from the impregnated bednets every month. Despite the careful and highly supervised impregnation procedure, great variation was observed in the residue analysis of bednet samples. The average initial uptake of the insecticide by nylon was unexpectedly low, at only 12.5 mg AI/m^2. The average initial uptake for light cotton and medium cotton was 33.3 and 26 mg AI/m^2, respectively. The latter 2 types of fabric constituted 82% of the nets in the trial area. The rate of loss of chemical activity was greatest for light cotton, followed by medium cotton. The reported amount of residue of cyfluthrin 4 months after impregnation of the nylon nets was unexpected. Also, great variation was reported in the residue analysis of heavy cotton.

Table 7 presents the results of the KDT_{50} and 24-h mortality of 12- to 24-h-old, unfed laboratory-reared female *An. stephensi* when exposed to different kinds of fabrics impregnated with cyfluthrin for 3 min. Mortality rates were considered more stable and relevant than KDT_{50} values. Loss of biological activity was fastest for the light cotton nets, followed by the medium cotton nets. Mortality rates and KDT_{50} values, obtained in bioassays using different fabrics, showed a good correlation (ranging between 0.61 and 0.87), except for that of nylon nets. The KDT_{50} and 24-h mortality values at time

Table 5. Frequency distribution (%) of *Plasmodium vivax* malaria cases according to parasite density per microliter of blood, in the 2 malaria transmission seasons, in the villages under the trial of cyfluthrin-impregnated bednets in Ghassreghand (Baluchistan), Iran, 1995.

	Control		Treatment	
Parasite density per microliter of blood	1st transmission season	2nd transmission season	1st transmission season	2nd transmission season
<100		7 (23.3)		15 (27.7)
101–200			2 (3.8)	
201–400	1 (3.6)		1 (1.9)	
401–800	2 (7.1)		3 (5.8)	
801–1,600	2 (7.1)		5 (9.6)	
1,601–3,200	7 (25)	5 (16.7)	10 (19.2)	6 (11.1)
3,201–6,400	4 (14.4)	6 (20)	14 (26.9)	10 (18.5)
6,401–12,800	7 (25)	7 (23.3)	10 (19.2)	9 (16.7)
12,801–25,600	3 (10.7)	2 (6.7)	3 (5.8)	9 (16.7)
>25,601	2 (7.1)	3 (10)	4 (7.8)	5 (9.3)

zero for the heavy cotton nets were unexpectedly poor.

The protective rates of the impregnated fabrics to mosquito bites at 0, 1, 2, 3, and 4 months after impregnation were 4.75, 3.64, 8.43, 2.98, and 2, respectively. Regression analysis revealed a low correlation coefficient of −0.39, which was mainly due to the results of the biting experiments conducted on the fabrics collected 2 months after impregnation. When these data were removed, the correlation coefficient increased to −0.97. The regression coefficient did not change ($b = -0.62$), and the a coefficient decreased from 5.59 to 4.57. Cyfluthrin-treated fabrics were mildly irritating to host-seeking females. The initial protection rate of the impregnated fabrics was 5–6 times that of the untreated nets.

DISCUSSION

Mosquito nets are a familiar household item in the Ghassreghand area. More than 98% of the households own bednets, mostly made of cotton. They are mainly used against mosquito bites, but they are also used for privacy purposes (Zaim et al., unpublished data).

Baluchistan has long been under indoor residual spraying for malaria control. However, because of the wall staining and odor properties of the insecticides used in the past, the compliance of the residents with the program has drastically decreased. In a survey conducted in 1994 (Zaim et al., unpublished data) only about 40% of the residents had their premises completely sprayed. The living and sleeping rooms as well as storage rooms were among the ones the residents most frequently did not allow to be sprayed. This has led health authorities to give high priority to research on the feasibility of using impregnated bednets as a substitute for indoor residual spraying for malaria control in Baluchistan, where the PHC infrastructure in certain areas, especially Ghassreghand, is reasonably good and any such program can be well planned and implemented, using the PHC services.

Bednets have been used in different parts of the world with varying results. Considerable reduction in mortality (e.g., Gambia) and morbidity (e.g., Burkina Faso, Gambia, Kenya, and Tanzania) have been reported from the use of impregnated mosquito nets (Lines 1996). The location of these trials span a wide range of transmission intensities, from 2 infective bites per person per year in the Gambia to 300 in Burkina Faso (Sexton 1994). In areas of less intense transmission, impregnated bednets appeared to lead to a reduction in the prevalence of infection as well as in the incidence of the disease.

Table 6. Results of the chemical residue analysis of impregnated bednet samples (mg/m²) taken randomly from the bednets impregnated with cyfluthrin EW 050 in Ghassreghand (Baluchistan), Iran. The tests were conducted at the World Health Organization Collaborating Centre in Belgium, by gas–liquid chromatography.

	Type of fabric											
Months after treatment	Nylon			Light cotton			Medium cotton			Heavy cotton		
	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD
0	8	12.5	5.4	18	33.3	26.1	11	25.9	20	5	17.6	12.5
1	8	5.7	4.5	13	28.1	17.8	6	22.9	13	5	27.9	30.1
2	5	4.5	2.6	10	16.8	17.9	9	16.0	10	7	26.7	17.9
3	7	2.6	1.8	10	12.0	9.4	8	14.6	9	6	30.6	29.8
4	12	9.3	9.0	16	10.6	13.1	17	8.4	9	11	14.0	17.0

Table 7. Median knock down time (KDT_{50}) and the 24-h percent mortality (M) of 12- to 24-h-old laboratory-reared female *Anopheles stephensi* exposed to cyfluthrin-impregnated fabrics for 3 min at monthly intervals after impregnation.

Months after treatment	Type of fabric							
	Nylon		Light cotton		Medium cotton		Heavy cotton	
	KDT_{50}	M	KDT_{50}	M	KDT_{50}	M	KDT_{50}	M
0	2.09	95.0	12.70	100	18.95	93.0	32.38	40.0
1	12.86	88.5	19.40	92.3	28.57	84.7	27.88	92.3
2	11.76	75.0	25.18	57.0	14.13	80.0	11.67	93.3
3	21.82	53.3	28.13	40.0	67.34	15.0	16.76	40.0
4	24.20	48.9	147.4	26.0	83.00	16.6	31.60	38.0

However, in this study, no significant effect was found on the incidence of the disease, resulting from the use of cyfluthrin-impregnated mosquito nets. Also, no significant difference could be detected in the parasite density of patients with positive slides. No significant effect was observed in the parous rate, HBI, and sporozoite rate of the anopheline vectors, resulting from the use of cyfluthrin-impregnated mosquito nets. Only the densities of the vectors were significantly reduced when the impregnated nets were introduced into the treatment villages.

Reduction in the density of the local mosquito population, as well as reductions in the parous rate and the sporozoite rate, a "mass effect," have been reported in village-scale trials of treated nets in Burkina Faso, Tanzania, Kenya, and Zaire (Magesa et al. 1991, Robert and Carnevale 1991, Beach et al. 1993, Karch et al. 1993). Outside Africa, clear evidence for a mass effect has been reported with *Anopheles minimus* Theobald in Assam, India (Jana-Kara et al. 1995), but not with *An. minimus* in Thailand (Somboon 1993). The difference between the latter 2 cases appears to be related to the behavior of the mosquito. *Anopheles minimus* in Assam is still endophilic and anthropophilic, whereas in Thailand it is now zoophilic and exophilic (Lines 1996).

All the anopheline vectors in the Ghassreghand area, especially *An. culicifacies,* the main vector, are zoophilic. The HBI for *An. culicifacies* has been found to be about 5% (Mowlaii et al. 1991). *Anopheles culicifacies* is mainly endophilic, but a large population also rests outdoors (Zaim et al. 1995). It is noteworthy, however, that almost all inhabitants as well as animals rest outdoors during the night (bednets are used outside) during the transmission season, and *An. culicifacies* has been found to be mainly exophagic during this period. A mass effect on the population of the vectors in the Ghassreghand area, resulting from the use of impregnated mosquito nets, has not been observed. This is in agreement with the statements made by Lines (1996) and Carnevale and Coosemans (1995) that a mass effect is improbable with zoophilic species.

Synthetic pyrethroids combine several advantages for use on nets. As well as having excito-repellent properties, they are quick-acting, and are effective in the small quantities that can be made to adhere to fabrics. However, the insecticide–fabric interactions are complex and difficult to predict. Little is known of the external factors determining the residual activity of the insecticide deposits on the net.

Permethrin was one of the first synthetic pyrethroids to be developed with stable residual activity (World Health Organization [WHO] 1990a), and the first to be specifically recommended by the WHO for use on mosquito nets. Deltamethrin, lambdacyhalothrin, and other alphacyano compounds tend to be rather more toxic than permethrin to mammals; however, this is compensated for by their very much greater toxicity to insects (WHO 1990b). For this reason, remarkably small quantities are needed on a net. Deltamethrin and lambdacyhalothrin are reported to persist on treated nets for 1 year, even with one or 2 washings (Njunwa et al. 1991, Miller 1994). Curtis and coworkers (1992) reported that polyester nets with holes, treated with cyfluthrin at 50 mg AI/m^2, performed well after 15 months of domestic use.

As already stated, in the Ghassreghand area, most of the bednets are made of cotton, but with extreme variation in size, thickness, weight, structure, and number of fibers/cm^2, and hence of different absorptive capacities. However, grouping of the cotton nets to 3 groups (light, medium, and heavy), for which different concentrations of insecticides were made, decreased the variation in the uptake of the insecticide to some extent, but still left high variance, as shown in the results of the monthly residue analysis performed by GLC. The rate of loss of chemical and biological activity of the insecticide was greatest for the light and medium cotton fabrics, constituting 82% of the total nets impregnated in this study. The mortality of anophelines brought in contact with the treated nets for 3 min in bioassays dropped to less than 70% in 2 months for light cotton nets and in 3 months for medium cotton nets. Such a remarkably low residual activity is believed to be mainly due to dust and dirt in the area, as well as the washing habits of the residents, which affects the residual activity of the insecticide. It is noteworthy that in the pilot project that was carried out in 1994 in the same area, the chemical activity of the insecticide dropped to one-tenth of the original activity in 5 months after impregnation of the nets (Zaim et al., unpublished data).

Washing habits of the study villagers were questioned at the end of the project. In the treatment zone, on average, the heavy, medium, and light cotton, and nylon bednets were washed 0.12, 0.83, 0.61, and 0.094 times, respectively, in the transmission season. However, in the control villages the nets were washed, on average, 1.86, 1.4, 2.2, and 0.57 times, respectively. The same study revealed that more than 82% of the villagers in both treatment and control villages sleep inside bednets throughout the transmission season. However, less than 10% used bednets when they traveled.

Quantifying the insecticide deposit as a quality control in fabric treatment is of prime importance. Presently, this can only be obtained by costly and sophisticated technique of GLC, and the results tend to be slow in return, making modifications of current procedures almost impossible. Hence, the development of a test suitable for use in field laboratories is clearly needed (Lines 1996). In this study, the initial uptake of the insecticide, despite the careful and highly supervised impregnation procedure, was lower than the targeted dosage for all 4 kinds of fabrics, especially for nylon nets, where the uptake was only 12.5 mg AI/m^2, as determined by GLC. This might be due to the binding properties of cyfluthrin to the fabrics. Probably less wringing out of the surplus emulsion would improve the uptake of the insecticide.

Bioassays are the only test we have that is simple and quick enough to allow routine testing of large numbers of treated nets. Establishment of a pattern of KDT_{50} or mortality of a test species toward different concentrations of the insecticide would allow these values to be used as a simple measure for the amount of the deposit of the insecticide on the fabric in the field. Such a study is now underway in our laboratory. In the present study, however, a reasonably good correlation was found between KDT_{50} and 24-h mortality, 24-h mortality and dosage, and KDT_{50} and dosage, in bioassays using light and medium cotton treated fabrics.

Miller and Gibson (1994) studied the behavioral responses of host-seeking *An. gambiae* and *Culex quinquefasciatus* Say to permethrin- and lambda-cyhalothrin-treated mosquito nets. Cotton netting significantly reduced the toxicity and irritancy of the permethrin treatments (at concentrations of 50, 400, and 1,000 mg AI/m^2), as compared to nylon netting. Lambdacyhalothrin at concentrations of 2, 5, 6, and 25 mg AI/m^2 was less irritating than permethrin. *Culex quinquefasciatus* was less irritated by permethrin but more by lambdacyhalothrin, than was *An. gambiae*. The authors concluded that "the responses were so characteristic that a signature behavior pattern could be attributed to each insecticide." In our study, cyfluthrin had mild irritancy to blood-seeking female *An. stephensi*. The initial protection rate of the impregnated nets in preventing the females from biting through the nets was 5–6 times that of untreated nets. Further behavioral studies are needed to define the nature of the response of different vector species to cyfluthrin-impregnated nets.

In the present investigation, many different features of cyfluthrin for the impregnation of bednets were studied and reported. However, the study did not detect any significant difference in malaria incidence between the use of untreated vs. impregnated bednets in the Ghassreghand area. In planning future medium-scale trials, comparison of new compounds and formulations to the more widely used pyrethroids such as permethrin and deltamethrin is highly recommended.

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