

**REPORT OF THE FIFTH
WHOPES WORKING GROUP MEETING**

**WHO/HQ, GENEVA
30-31 OCTOBER 2001**

**Review of:
OLYSET NET
BIFENTHRIN 10% WP**

WORLD HEALTH ORGANIZATION
COMMUNICABLE DISEASE CONTROL,
PREVENTION AND ERADICATION
WHO PESTICIDE EVALUATION SCHEME

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1. INTRODUCTION

The fifth WHOPES Working Group Meeting, an advisory group to the WHO Pesticide Evaluation Scheme (WHOPES), was convened at WHO Headquarters, Geneva, 30-31 October 2001. The objective of the meeting was to review the reports of the testing and evaluation of Olyset Net[®] (Sumitomo Chemical Co., Ltd, Japan) and bifenthrin 10% Wettable Powder (FMC, USA), for malaria vector control.

The meeting was opened by Dr M. Neira, Director, Communicable Disease Control, Prevention and Eradication. In her opening remarks she referred to vector control being an essential component of vector-borne disease control, especially in view of increasing drug resistance and the absence of vaccines. She stated that vector control is part of the preventive measures to reduce or interrupt transmission and plays an essential role in epidemic prevention and containment.

Dr Neira noted the importance of chemical control in the integrated approach to vector control and therefore the essential role of WHOPES in supporting the technical requirements of the vector-borne disease control programmes at WHO and country level, notably the Roll Back Malaria (RBM) partnership. Dr Neira referred to the recent WHO meeting with Regional Advisors for the development of a global strategic framework for vector control and expressed the hope that it would lead to the strengthening of WHO's activities in this field.

Dr L. Savioli, Coordinator, Strategy Development and Monitoring for Parasitic Diseases and Vector Control, also noted the need for increasing guidance and support to national vector-borne disease control programmes.

He also referred to the recent WHO initiative in support of dengue prevention and control among the Member States, as well as activities in support of the global elimination of lymphatic filariasis and the potential role of vector control.

Dr Morteza Zaim, Scientist in charge of WHOPES, presented the objectives and an overview of the scheme to the participants, and noted that the recommendations of WHOPES on the use of pesticides in public health are to expedite the registration of such products by the Member States. He also indicated that the reports of the WHOPES Working Group Meetings are well received by national registration authorities and control programmes, as an excellent source of information and consolidation of available information on pesticides evaluated by WHOPES and that every effort will be made to make the reports more useful and widely distributed.

Dr Zaim also briefed the participants on the memorandum of understanding which has been signed between WHO and FAO in 2001, by which the two organizations have agreed to cooperate in a joint programme to develop specifications for pesticides. This unified procedure will enhance the development of high-quality standards for pesticides; their acceptability by governments, industry and traders, and make the development of such specifications more efficient and cost-effective to industry, to WHO and FAO. He noted that the procedure for the establishment of WHO specifications has, therefore, been revised and harmonized with the new procedure of FAO, which started in 1999, as described in the "Manual on the Development and Use of FAO Specifications for Plant Protection Products, fifth edition", published as FAO Plant Production and Protection Paper 149, available at <http://www.fao.org/waicent/faoinfo/agricult/agp/agpp/pesticid/>. Dr Zaim also noted that the first Manual on Development and Use of FAO and WHO Specifications

for Pesticides is expected to become available in mid-2002. Under the "new" procedure the specifications do not necessarily apply to nominally similar products of other manufacturers, nor to those where the active ingredient is produced by other methods of synthesis. The scope of these new WHO specifications may be extended to apply to similar products when WHO has been satisfied that the additional products are equivalent to those which formed the basis of the reference specification.

The meeting was attended by 10 scientists (see list of participants, Annex 2). Dr B. Sharp was appointed as Chairman and Dr I. Vythilingam as Rapporteur. The meeting was convened in plenary sessions at WHO/HQ in Geneva, 30-31 October 2001, and the reports of the WHOPES supervised trials and relevant published literature, as well as the reports submitted by national disease and vector control programmes (see bibliography, Annex 1) were reviewed and discussed. Recommendations on the use of the above-mentioned products were made.

2. REVIEW OF OLYSET NET

2.1 Specifications

Olyset Net is a long lasting insecticide-treated mosquito net and does not require re-treatment after washing. The net material is 100% high-density polyethylene, blended with permethrin 2% (w/w) as active ingredient, corresponding to about 1000 mg of active ingredient/m². The insecticide moves through the threads of the net by diffusion.

Olyset Net is made of wide mesh (4mm x 4mm). The product has the following additional specifications:

Fiber thickness	>150 denier
Weave type	Raschel
Weight	50 g/m ²
Melting point	About 130 °C
Flashing point	341 °C
Ignition point	349 °C

2.2 Efficacy – background/supporting documents

Cambodia - Efficacy of Olyset Nets for malaria vector control was studied in comparison to the use of untreated mosquito nets by the National Malaria Centre, Phnom Penh (Chheang and Sandy, 1994). A block consisting of two hamlets (183 families) received Olyset Nets, where as a second block of three hamlets (208 families) received 500 untreated nets. *Anopheles dirus* and *An. minimus* were the main malaria vectors.

The entomological data recorded before and after the intervention indicated decrease of more than 70% of

indoor biting density of the main vectors, but with no significant impact on the outdoor biting density. Moreover, a sharp decline (about 60%) in parous rate (of sample caught inside, but not in outside collected samples) was reported for both vector species. The significant decrease observed in the number of malaria cases (94%) was attributed to the use of Olyset Nets but malaria incidence was also reduced (67%) where untreated nets were implemented.

Viet Nam - Persistence of permethrin in Olyset Nets was examined after seven months of practical use inside and outside houses (exposed to sunlight) in northern Viet Nam (Itoh and Okuno 1996), where the nets were hung down at openings of houses to control dengue vector mosquitoes. Permethrin remained 79% and 58% of the initial content in the nets used inside and outside the houses, respectively. When an unused net was washed with acetone to remove the active ingredient from the surface of thread, diffusion of the active ingredient from the inside of thread to the surface was accelerated by keeping the net at 60°C (5 hours) to 70°C (1-5 hours).

Viet Nam - Olyset Nets were used as screens against *Aedes aegypti* (pyrethroid susceptible) at all openings of 500 houses and their bioefficacy was tested at monthly intervals (Nguyen *et al.* 1996). The study revealed that almost all adult *Ae. aegypti* were knocked down after 9-16 minute exposure to the nets that were hung inside the houses or unused Olyset Nets stored indoors. The adulticidal activities remained 100% for eight months (the entire period of the study). The bioefficacy was however drastically reduced after two months, when the nets were hung outdoors: the percent mortality of *Ae. aegypti* dropped to 40% in five months and 20% in six months. The intervention resulted in a complete control of the *Ae. aegypti* population throughout the six months duration

of the trial. It was concluded that Olyset Net screen reduced dengue transmission in the experimental area, although larger scale studies are required to validate this conclusion.

Solomon Islands - Chemical and biological assays were carried out on Olyset Nets and nets conventionally treated with permethrin (target dose of 500 mg/m²), distributed to 64 villages in Honiara, from October 1993 to February 1996, to study their efficacy for the Pilot Malaria Control Program (Ikeshoji and Bakotee 1997). Olyset Nets lost 76% of the surface dose of permethrin by one washing with soap in ultrasonic washing machine for five minutes, but regained 82% by exposing to strong sunlight (50-60°C) in a polyethylene bag for two hours. Washing with no soap did not decrease the bioavailability of the insecticide. From various bioassays and chemical analysis made during this trial, it was expected that a bio-available concentration of 100 mg/m² permethrin would have an impact on the vector mosquito population when a 60 mg/m² concentration would provide personal protection in reducing man-vector contact.

Tanzania - In Kibaha district, coast region of Tanzania where high malaria transmission occurs three villages received Olyset Nets (1994) while two remained as control and comprehensive evaluation (entomological, parasitological and clinical) were carried out (Njunwa *et al.* 1996).

The available one year report indicated a 55% reduction of parasite rate (*P. falciparum*) and a reduction of up to 81% of malaria parasite density in children (< 9 years) in treated villages and a great reduction (50-70%) in anaemic status.

Bioassays with *An. gambiae* were done with two protocols: three minute exposure under WHO cones or time to obtain 80% knock down. Three minute exposure induced # 70-80% mortality after six months as well as after 12 months and washed Olyset Nets appeared slightly better than unwashed. Times for 80% KD were about 5-6 minutes, even after 12 months of use. At six months the washed nets performed better (4 minute instead of 5-6 minutes) than unwashed. Exposure to sun during drying did not enhance efficacy.

Malaysia - Efficacy and wash resistance of Olyset Nets were studied in laboratory and compared to nylon multifilament and polyethylene monofilament nets conventionally treated with permethrin, at target dosage of 500 mg/m² (Vythilingam *et al.* 1996). The percentage mortality of mosquitoes exposed for 10 minutes to Olyset Net, nylon and polyethylene nets, after 15 washes with water only, was 95%, 83% and 26%, respectively for *An. maculatus* and 100%, 92% and 82% for *Ae. aegypti*. Exposed to nets washed four times with soap and water, the mortality figures were about 87%, 80% and 3%, respectively for *An. maculatus* and 90%, 50% and 5% for *Ae. aegypti*.

Ability of *Ae. aegypti* and *An. maculatus* to pass through treated netting was also studied in laboratory, by constructing a small cage covered with Olyset Net, which was placed inside a larger cage covered with fine nylon mesh and a guinea pig was placed inside the small cage as bait. For control, an untreated set of identical cages were made. Mosquitoes (100) were released in the outer cage and left for about two hours, after which they were collected separately (still outside or inside the small cage and numbered unfed or blood fed, dead or alive) and transferred to a cup for 12-hour post treatment mortality recordings. Using Olyset Nets 95% of *Ae. aegypti* and

82.5% of *An. maculatus* were recovered from the outer cage, while in control nets these percentages were respectively of 58% and 89.5%. However, none of the Olyset Net penetrating mosquitoes had fed and all died within 12 hours. In the control experiment, 45% of *Ae. aegypti* and 19% of *An. maculatus* were well blood fed, both species were able to penetrate large mesh (fish net 3x3 mm) untreated nets and to go out again.

Senegal - Efficacy of Olyset Nets was studied during a rainy season in the hyperendemic malaria villages of Wassadou in south-east Senegal (Faye *et al.* 1998), where *An. gambiae s.l.* (mainly *An. gambiae* during rainy season and *An. arabiensis* during dry season) has been the main vector. A total of 520 mosquito nets were distributed to the whole population of the village and entomological and malariometric indices were compared to a control village where mosquito nets were not distributed. The study revealed that the number of bites by *An. gambiae s.l.* (no significant pyrethroid resistance) decreased by 69%; sporozoite index decreased by 76%; inoculation rate decreased by 93%; and parity rate decreased by 8%. The study also showed that the density of residual resting fauna decreased by 90% (85% for blood fed specimen and 94% for gravid females) with increased exophilic behaviour of *An. gambiae* after Olyset Net implementation, indicating a strong repellent effect of these ITNs. Olyset Nets did not stop malaria transmission, but strongly reduced it (over 90%) with about 50% reduction in malaria cases.

Côte d'Ivoire - The efficacy of Olyset Nets on malaria transmission and morbidity was studied in Kafine (Doannio *et al.* 1999), where the nets were distributed first in the southern half of the village and then in the whole village. The biting and parity rates of *An. gambiae* (strongly pyrethroid-resistant, 96% *kdr* allelic frequency), as well as

sporozoite index and entomological inoculation rates were studied before and after the intervention. Comparative analysis of data showed that use of Olyset Nets had no effect on the biting or the entomological inoculation rate, and therefore malaria transmission. This absence of effect was attributed to the high prevalence of the *kdr* gene in this pyrethroid-resistant population of *An. gambiae*.

A cohort study of 163 children under five years of age who did or did not use Olyset Nets, as well as the study of patients seeking treatment for malaria attacks, revealed no difference between the user and non-user groups, with respect to children exhibiting *P. falciparum* trophozoites (*Plasmodic index* # 85%) or gametocytes (# 20%) or to the mean parasite load (Henry *et al.* 1999). But the rate of malaria attacks (high *P. falciparum* parasitaemia (> 5000 par./mm³) associated with fever) was reduced by half in children sleeping regularly under Olyset Nets compared to non-users (3.4% versus 8.7%). These studies confirmed experimental hut observations that pyrethroid-treated mosquito nets could significantly reduce man/vector contact even against pyrethroid-resistant *An. gambiae*.

Côte d'Ivoire - Four Olyset Nets were compared with a standard untreated polyester mosquito net, as control, in verandah-trap huts at Yaokoffikro village, near Bouaké, by standard WHOPES phase II procedures (N'Guessan *et al.* 2001a). The study comprised three examples of Olyset Nets previously used in a village for over three years (one washed, one dirty, one very dirty), as well as a previously unused Olyset Net, newly unwrapped from the same original batch. Bioassays, using susceptible female *An. gambiae*, 3-minute exposure, gave more than 99% mortality on "new" Olyset Net. The mortality rates on the used Olyset Nets averaged 83, 85 and 55% for the washed, dirty and very dirty nets, respectively. Olyset Nets

were found to remain remarkably effective for at least three years, under field conditions.

Olyset Nets showed some deterrence against wild pyrethroid-resistant (96% *kdr*) *An. gambiae* (44 and about 20% reduction in entry rate of *An. gambiae* by the new and unwashed dirty nets, respectively), but not against pyrethroid-resistant *Cx. quinquefasciatus*. The insecticidal repellency of Olyset Nets increased exophily of pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus*, by 19 and 14%, respectively, as compared to untreated control nets (exophily rate of 30-40%). Blood feeding rates in the hut with a sleeper under the untreated net were 16 and 35% for pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus*, respectively, and 22-26% of both species in huts with washed or dirty used Olyset Nets.

Mortality rates of pyrethroid-resistant *An. gambiae* and *Cx. quinquefasciatus* from the huts were, respectively, 27.5% and 17% with the new Olyset Net, 15% and 17.5% with the washed Olyset Net, 16-25% and 17-20% with the dirty old Olyset Net, and 3% and 8% with the untreated polyester net.

2.3 WHOPEs supervised trials

2.3.1 Laboratory study

Wash resistance and dynamics of the insecticide in Olyset Nets were studied in the laboratory (Hougard *et al.* 2000), using soap, "savon de Marseille" (2 g/L) and a 24 hour interval between washing (30°C, agitation 155 mv/min for 20 minutes in demineralised water) and standard bioassays of three minutes exposure (pyrethroid susceptible *An. gambiae*), as well as 24 hours between two successive washes. It was noted that:

- Only a fraction of permethrin is bio-available;

- the biological activity of Olyset Nets (24 hours after washing) is drastically reduced in laboratory after only two washes (29% knock down in 60 minutes and 75% mortality in 24 hours post exposure);
- reactivation by heat is very effective, even at a relatively low temperature (60°C) for a relatively short period of time (60 minutes) - 100% knock down in 60 minutes and 90% mortality in 24 hours post exposure was reported for 24 times washed Olyset Net; and
- After washing up to 10 times, spontaneous reactivation occurs in less than 15 days when nets are kept under "tropical" conditions (30°C & 80% RH).

2.3.2 Field studies

Experimental hut trials are used to study the impact of insecticide treated mosquito nets on: detergency (= fewer mosquitoes enter the room), excito-repellency (= increase in exophilic behaviour), feeding inhibition (= those that do enter have a lower probability of feeding), and killing (= fraction of blood-seeking females killed, before or after they have succeeded in feeding). The first three of these are indicators of personal protection, and benefit individual users. The fact that some blood-seeking females are killed is primarily important because community-wide use of treated nets can, in some circumstances, produce a "mass effect", i.e. a reduction in the density of infective mosquitoes in the area and, by the way, protect the whole community, including those not using ITNs.

Tanzania - Two verandah-trap huts in a Tanzanian village were used to assess the effectiveness of Olyset Nets against *An. gambiae s.l.* and *Cx. quinquefasciatus* (Curtis

et al., 2001). Two successive trials were performed and in each trial six nets were tested in rotation in the huts over a six week period.

The first trial compared two Olyset Nets versus two polyester nets, conventionally treated with permethrin (PTN) at 200 mg a.i./m² (one with broad 4 mm mesh such as Olyset Net and one 1.5 mm mesh but with six holes of 4 x 4 cm) and two untreated nets (in the same conditions as PTN).

The second trial compared three conditions of Olyset Net: unwashed, "washed" (hand washed five times in cold soapy water) and "washed and heated" (= washed five times as other one but placed for 15 minutes in hot water at 80°C after each wash) versus untreated 4 mm size mesh nets.

In both trials, there was no evidence for any deterrence to hut entry of anopheline or culicine mosquitoes due to Olyset Nets or nets conventionally treated with permethrin. This absence of deterrence may be attributed to the design of the experimental huts and not to the insecticide used on the nets.

The first trial showed that unwashed Olyset Net caused significant mortality among anophelines (84% versus 7% with the untreated net), and the effect was especially marked among those remaining in the huts and not escaping from them.

The 4 x 4 mm mesh net, conventionally treated with permethrin, reduced blood feeding of *An. gambiae* significantly better than the unwashed Olyset Net (respectively 2.5% and 13% well blood fed specimens). However, the 1.5 x 1.5 mm mesh net conventionally treated with permethrin produced significantly less

An. gambiae mortality than the Olyset Net, (respectively # 30% and 56%). Among malaria vectors not escaping from the hut, mortality was high with both permethrin treated nets as with the Olyset Net and far higher than with the untreated nets.

Against *Cx. quinquefasciatus* the Olyset Net significantly reduced blood feeding compared with the untreated nets (respectively # 18% and 46% of well blood fed specimens). As in other studies previously carried out in this area, mortality of this species due to pyrethroids was low, but it was significant compared to untreated nets, (respectively 8.5% and 0.5%) especially among those which remained in the huts. Olyset Nets and conventionally treated nets had the same lethal effect.

The nets conventionally treated with permethrin gave similar results to the Olyset Nets in terms of blood feeding inhibition and lethal effect. Overall the results with 4 x 4 mm mesh intact nets were similar to those with holed 1.5 x 1.5 mm nets.

The second trial showed that compared to untreated 4 mm mesh net, Olyset Nets washed and "washed + heated" were effective in reducing feeding (3% versus 16%) and in killing 60-70% versus 25%) of *An. gambiae*. Heating procedure did not improve their performance. Unwashed, washed or washed and heated Olyset Nets were as effective in preventing blood feeding and in killing mosquitoes (washed nets being even more effective in reducing blood feeding).

Against *Cx. quinquefasciatus* the three models of Olyset Nets similarly reduced blood feeding (and significantly when compared to an untreated net, respectively # 10% versus 40% specimens blood fed) and had the same lethal influence as untreated nets (< 10%).

Côte d'Ivoire - Efficacy of Olyset Nets against almost susceptible *An. gambiae* (84% mortality following one hour exposure to permethrin 1%) and *An. funestus* was studied in verandah-trap huts in M'bé village, by standard WHOPES phase II procedures (N'Guessan *et al.* 2001b). Comparisons were made to nets (poly-filament, 100 denier) conventionally treated with permethrin (25/75 and 40/60 *cis/trans*) at target concentration of 500 mg/m², and to an untreated polyester net as control.

Olyset Nets performed as well or better than nets conventionally treated with permethrin in reducing man/vector contact as they significantly reduced entry and blood feeding rates, and significantly increased the exit and mortality rates of the two vector species. For Olyset Nets, or nets conventionally treated, the reduction in deterency of *An. gambiae* and *An. funestus*, was 60% and 90%, respectively, and for blood feeding rates the reductions were 65-70% and 60-80%. The excito-repellency of Olyset and nets conventionally treated were increased by 70-80% over the untreated nets.

Olyset Nets performed significantly better than nets conventionally treated in inducing overall mortality of *An. gambiae* (40% vs. 26%) and *An. funestus* (60% vs. 30%).

No significant difference was noted in efficacy of the two *cis/trans* isomer ratios of permethrin products used in this study.

2.4 Conclusions

Olyset Net is a new concept of long-lasting insecticide treated net (ITN) where the insecticide is incorporated into the polyethylene polymer before yarn extrusion. The

insecticide, permethrin (*cis-trans* 40/60), 2% w/w, is slowly released from the polymer, allowing bio-availability of the insecticide at the surface of the fibre. Through this process, the residual efficacy is longer than that of conventionally treated nets. Since only a small fraction of permethrin is bio-available at the surface of the fibre at any time, and there is no need for treatment at peripheral level, the use of Olyset Net is even safer than that of conventionally treated nets.

There is evidence that Olyset Nets are as effective as nets conventionally treated with permethrin in killing vector mosquitoes, reducing blood feeding, as well as having an excito-repellent effect. They also have significantly longer residual efficacy. In two trials from West Africa, it was shown that about 50% of the original insecticide content is still in the fibre after four years of continuous use at village level, providing good impact on mosquitoes during bioassays and in experimental huts. Field tests at village scale level in high transmission area, showed that the use of Olyset Nets has reduced high density parasitaemia and malaria attacks by about 50% in spite of pyrethroid resistance (*kdr*) in the vector.

Washing Olyset Nets with soap under standard laboratory conditions has reduced efficacy as with conventionally treated nets. However, a major advantage of Olyset Net is that its biological efficacy is resumed by diffusion of the insecticide from the inside of the yarn to the surface. This "regeneration" occurs naturally but is accelerated when nets are exposed to heat. However, under tropical conditions, Olyset Net washed 10 times recovered its efficacy in less than 15 days. In experimental huts, after five washes with local soap, Olyset Net without heating remained as effective as non-washed net.

Although there were concerns of users about the small size of the nets and the large mesh size, Olyset Nets have been generally well accepted in communities who appreciated its efficacy and strength.

2.5 Recommendations

1. Considering safety, efficacy and evidence of long lasting effect, Olyset Nets are recommended for prevention of malaria. Nevertheless, long-term efficacy of Olyset Nets, including the dynamics of insecticide release, should be further investigated under different eco-epidemiological settings.
2. The concept of long-lasting nets should be promoted.
3. Based on experience acquired in testing Olyset Nets, standardised criteria and test procedures should be developed for testing and evaluation of long-lasting insecticide treated mosquito nets.
4. Investigations on potential use of long-lasting insecticide treated materials for prevention and control of vector borne diseases other than malaria should also be promoted.
5. Instructions for use of Olyset Nets and other long-lasting insecticide treated materials should be developed.

3. REVIEW OF BIFENTHRIN 10% WP

3.1 Safety assessment

Bifenthrin¹ is a synthetic pyrethroid insecticide and acaricide. It has a very low vapour pressure (1.81×10^{-7} mm Hg), a low water solubility (less than 1 µg/l) and a good stability to hydrolysis and photolysis (2 years at 50°C under natural daylight).

It has moderate acute toxicity and the technical material is classified as “moderately hazardous (Class II)” by the WHO Programme on Chemical Safety (WHO 1998). The toxicity of bifenthrin has been assessed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) (FAO 1992) and the following conclusions have been noted:

When bifenthrin was orally administered to rats, the compound was absorbed and eliminated mainly via faeces (70-80% within 48 hr). Hydrolysis and hydroxylation were the major steps in the biotransformation. Tremors and clonic convulsions were the most consistent clinical signs of intoxication noted. Bifenthrin is less toxic by the dermal route ($LD_{50} > 2000$ mg/kg body weight rabbit) than the oral route ($LD_{50} > 56$ mg/kg body weight rat). It is a mild irritant to the rabbit eye and no irritation was observed after dermal application on abraded and intact skin. It is not a skin sensitizer in the guinea pig. In rats, dermal absorption is low.

¹ (2-methylbiphenyl-3-ylmethyl (z)-(1*RS*, 3*RS*)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2, 2-dimethylcyclopropanecarboxylate)

Long-term studies in rats and mice indicated that bifenthrin is unlikely to pose a carcinogenic hazard to humans. No teratogenic, fetotoxic or embryotoxic effects were found. Bifenthrin was unlikely to present a genotoxic hazard. Reproduction was not impaired by treatment. However, a tumorigenic potential for bifenthrin in mice cannot be excluded.

The acceptable daily intake (ADI) for man has been established at 0-0.02 mg a.i./kg body weight on the basis of the no observed adverse effect level (NOAEL) of 1.5 mg a.i./kg body weight/day in the 12 month study in dogs using a 100-fold safety factor.

The following are extracts from the Toxicological Evaluation report of JMPR (FAO 1992).

Acute oral LD ₅₀ (rat)	>56 mg/kg
Acute dermal LD ₅₀ (rabbit)	>2,000 mg/kg
Skin irritation (rabbit)	No irritation. With guinea pigs no sensitization reaction was noted.
Eye irritation (rabbit)	May cause mild eye irritation

3.2 Efficacy – WHOPES supervised trials

3.2.1 Laboratory studies

Laboratory investigations on the activity of bifenthrin against mosquitoes - The objectives of the study (Finot *et al.* 1997) were to evaluate the potential of bifenthrin for mosquito control and suggest further testing on the basis of laboratory investigations such as larval tests, filter paper tests on adults, topical applications (adults) and irritability tests (adults). In addition, preliminary investigations were

carried out on possible cross-resistance with other insecticides.

The test species included susceptible strains of *An. gambiae* s.s., Kisumu (Kenya), *Ae. aegypti*, Bora Bora (French Polynesia), and *Cx. quinquefasciatus* S-Lab (California) and *An. gambiae* s.s. strain VK-PR (resistant to permethrin and dieldrin), *Ae. aegypti* strain LHP (resistant to DDT and pyrethroids) and *Cx. quinquefasciatus* strain BK-PR (resistant to DDT, organophosphate compounds and pyrethroids).

Larval test: Larval bioassay was carried out with five concentrations of alcoholic solutions of active ingredient and late third or young fifth instar larvae with 4 cups per concentration and three replicates. Mortality was recorded after 24, 48 and 72 hours and results were subjected to probit analysis.

Results showed that *An. gambiae* s.s. ($LC_{50} = 0.0087$) was about 10 times less susceptible than *Ae. aegypti* (0.00076) and *Cx. quinquefasciatus* (0.00067). This phenomenon was not commonly observed with other pyrethroids such as deltamethrin and permethrin.

Topical applications on adults: 0.1 ul active ingredient diluted in acetone was applied with a calibrated micro-capillary tube on the dorsal pronotum of three to five days old unfed female mosquitoes, which were maintained on a cold surface after a brief anaesthesia with CO₂. After treatment, females were maintained in batches of 25 in plastic cups at 28°C and 80% RH with sucrose added. Mortality was checked after one hour and after 24 hours. For each test 25 females were used. There were two replicates, each using five concentrations that provided between five and 100% mortality. The entire experiment was triplicated. Contrary to the results for

larval stages, the susceptibility of *An. gambiae* ($LD_{50} = 0.21$), *Ae. aegypti* (0.16) and *Cx. quinquefasciatus* (0.29) was similar and the inherent toxicity of bifenthrin was about 10 times more than permethrin.

Tarsal contact with insecticide deposits on filter paper:
A range finding test using the WHO filter paper method was carried out to determine the knock down effect and the concentration giving 95 - 100% mortality of adults after 24 h. Bifenthrin was found more active than permethrin 25/75 with 100% mortality at 0.125% instead of 0.5%. However, it has a significantly lower KD effect with only about half of individuals KD_{30} min. after exposure to a 100% lethal concentration and not even 100% after 60 min. KD_{50} and KD_{95} are more than double that of permethrin and deltamethrin at respective LC_{100} . The KD_{50} at 0.25% of bifenthrin for *An. gambiae* was 22 sec, for *Ae. aegypti*, 34 sec and *Cx. quinquefasciatus* was 34.8 sec as compared to 11.3, 11.8 and 14.17 sec respectively at 1.0% permethrin. The difference observed was stronger with *Ae. aegypti*. Bifenthrin is not as fast acting as permethrin and deltamethrin. Based on the susceptibility tests a diagnostic concentration of 0.25% to *An. gambiae* and *Ae. aegypti* and 1.0% to *Cx. quinquefasciatus* has been suggested.

Irritability tests: Irritability tests were carried out with susceptible strains of *An. gambiae* s.s., Kisumu (Kenya), *Ae. aegypti*, Bora Bora (French Polynesia), and *Culex quinquefasciatus* S-Lab (California). Using the standard cones, the time taken for the individual mosquitoes to first-take off after a 90 sec pre-observation time, the number of successive take-offs and the total time spent by mosquitoes on the treated paper (0.125%) was recorded. This was repeated 50 times for one test and tests were duplicated with different impregnated papers and insect batches. A control with non-impregnated paper

was done. Probit analysis was done to determine the time for 50% take-off and its confidence limits. Limited tests were also carried out in both cases with 1% permethrin (*cist-trans* 25/75) and deltamethrin 0.025% for comparison.

At 0.125%, bifenthrin had a comparable irritant effect on the three test species (same time spent on the treated surface, comparable time for 50 (18.2 - 29.4 sec) and 95% take-off (260.3 - 363.5 sec). However, this irritant effect was at least three times lower than that of permethrin (50% take off: 6 sec & 95% take off: 35.9 sec) and deltamethrin (50% take off: 7.1 sec & 95% take off: 34.0 sec). It was significantly prolonged (50% take off: 276.1 sec) with the pyrethroid resistant strain (VK-PR).

The study showed bifenthrin to be less irritating than permethrin and deltamethrin, even with *Cx. quinquefasciatus*, which is more susceptible to the irritant effect of pyrethroids and has reduced knock down.

Resistance to bifenthrin was observed in *kdr* resistant strains of *Anopheles*, *Aedes* and *Culex* species. In the tests on adults by topical applications and with insecticide papers, the concentration which gave 100% mortality on a susceptible strain gave only five to 10% mortality with a resistant strain with a complete loss of KD effect.

Establishment of diagnostic concentration - Using the standard WHO bioassay for adult mosquitoes, diagnostic concentration for bifenthrin has been established (Hemingway and Morgan 1998). The insecticide papers were prepared with serial dilutions of the insecticide (0.001 – 1% w/v) in Dow Corning 556 silicone fluid and an equal volume of acetone. Papers were allowed to dry for 12 hours at room temperature and stored at 4°C until used.

One-day old adults of *An. stephensi*, (Beech strain), *An. gambiae* (Kisumu strain), or *An. albimanus* (Panama strain) from susceptible colonies were exposed for 1 hour to a range of concentrations of insecticide impregnated papers using the standard WHO adult bioassay test kits. Twenty-five mosquitoes of mixed sex were exposed to each test and at least six replicates were taken at each concentration giving mortalities between 0-100% for each species. For each species a minimum of three data points between 0-100% mortality were obtained.

The slopes for log-dosage probit mortality lines were good, indicating homogeneity of response of all the three mosquito species across the range of insecticide concentrations tested. The LC_{50} for *An. stephensi*, *An. gambiae*, and *An. albimanus* was 0.031, 0.0167, and 0.012 and LC_{90} was 0.058, 0.0327 and 0.058 respectively. The diagnostic concentration for bifenthrin calculated at double the LC_{99} was 0.1%. This dosage when tested against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. arabiensis* and *An. sacharovi* caused 100% mortality.

Papers at this diagnostic concentration, when stored for 4-6 weeks at 4°C and then tested against *An. stephensi* and *An. gambiae* produced complete mortality, indicating that there is no adverse interaction between the silicone fluid and insecticide on storage.

Comparative testing of bifenthrin 10% and 20% WDP on adult mosquitoes for residual effect - This study (Hougard *et al.* 1999) tested, under laboratory simulated conditions, the efficacy and residual effect of two wettable powder formulations of bifenthrin 10 WP and 20 WP on three substrates, wood (non-resinous hardwood), plaster of Paris and mud (from Africa). Plaster and mud were laid in Petri dishes (12 cm x 12 cm, 1.5 cm high), the size fitting

with standard WHO test cones. Treatment was carried out at 12.5, 25, 50 and 100 mg/m² with a Potter Tower on the basis of 46.7 ml/m² of insecticide suspension. Ten samples were sprayed for each substrate and each concentration. For each test series one sample was selected at random and all samples were used only once for bioassays.

Two to three-day old non-blood fed female *An. gambiae* s.s. (Kisumu strain) were exposed in triplicate for 30 minutes in WHO bioassay cones. Tests were carried out one day after treatment then after 1, 2, 3, 4, 5 and 6 months. KD rate was noted after 30 and 60 minutes. Percentage mortality was noted after 24 hours. Mosquitoes able to fly were counted as alive. Surviving mosquitoes with three or less legs were counted as dead.

In the non-resinous hard wood, a full efficacy (100%) was observed after three months with the 10 WP (whatever the dosage) and six months with 20 WP (except 12.5 mg/m²). Results with mud and plaster showed approximately the same trends, with an obvious dose-effect. With 10 WP formulation, regular decline of efficacy was observed from the first month and no effect was recorded after three to four months. The trend was the same with 20 WP formulation but the residual effect was longer with plaster (full efficacy after three months at 100 mg/m²) in comparison with mud (about one month). No effect was observed with plaster and mud after the fifth month.

The differences observed on various substrates and the two formulations were attributed to the availability of active ingredient at the surface and the particle size of the formulations.

Comparative testing of bifenthrin EW with six other pyrethroids for treatment of mosquito nets - A comparative laboratory study has been carried out under standardized conditions to test efficacy of bifenthrin EW against susceptible and pyrethroid-resistant *An. gambiae* and *Cx quinquefasciatus*, in comparison with alpha-cypermethrin SC, cyfluthrin EW, deltamethrin SC, etofenprox EW, lambda-cyhalothrin CS and permethrin EC (Hougard *et al.* 2001). Mortality, knock down and irritancy were measured using WHO cones, with three minutes exposure to treated netting materials. Mortality and blood feeding were also assessed in a feeding chamber ("tunnels"), simulating field conditions. In the latter studies, free flying, unfed females had to pass through a uniformly holed piece of treated netting material to reach the live bait and eventually take a blood meal. The following target dosages were used: alpha-cypermethrin 10% SC (40 and 10 mg/m²); bifenthrin 0.5% EW (25 and 6.25 mg/m²); cyfluthrin 5% EW (50 and 12.5 mg/m²); deltamethrin 10% SC (25 and 6.25 mg/m²); etofenprox 10% EW (200 and 50 mg/m²); lambda-cyhalothrin 2.5% CS (20 and 5 mg/m²); and permethrin 10% EC (500 and 125 mg/m²).

All insecticides performed very well in tunnel tests against susceptible *An. gambiae* at both dosages. Against resistant strain at 25% of the recommended concentration, however, permethrin, bifenthrin and deltamethrin performed the best. Efficacy against susceptible *Culex* has been confirmed in tunnel tests, bifenthrin being the most active in killing mosquitoes, especially at reduced insecticide dosage. However, none of the seven insecticides tested induced any significant mortality in tunnel tests against resistant *Culex*. Bifenthrin was very active in inhibiting blood feeding in susceptible and resistant *Anopheles*, with the highest inhibition rate at reduced dosages with the resistant strain. With susceptible *Culex* all insecticides performed very well,

including at reduced dosage. Against resistant *Culex*, bifenthrin was very effective, far more than the other tested pyrethroids.

The main attributes of bifenthrin, from another laboratory study (Finot *et al.* 1997), were confirmed in this study, i.e. low KD, low irritancy, high intrinsic activity and good impact on *Culex*. Excellent blood feeding inhibition, especially against resistant *Culex*, was noted. These attributes are of interest not only for treatment of mosquito nets but also for indoor residual spraying.

When ranked in comparison with the other tested pyrethroids based on mortality and blood feeding inhibition in tunnel tests at reduced dosage using both *Anopheles* and *Culex*, bifenthrin performed best in experiments on susceptibility strains as well as on resistant strains.

3.2.2 Field studies

Thailand - The residual effect of Bifenthrin, on surfaces of wood, bamboo inner-side and bamboo outer-side, at two dosages 25 mg/m² and 50 mg/m² against *An. minimus* was determined in Maetaeug District of Chaingmai Province, North Thailand (Prajakwong 1998).

Bioassays were carried out both in lab and field on similar sprayed surfaces. A total of 40 houses was selected from a village and randomly allocated into four groups, each group representing three types of surface. The surfaces from each group were treated with bifenthrin 10 WP at 25 mg/m² or 50 mg/m² or deltamethrin 20 mg/m² following a standard technique. One group was left untreated. For laboratory testing, sets of similar wall constructive material each 10 cm x 10 cm, were placed on the walls prior to spray and after spray these sets were taken to the laboratory.

Bioassay was carried out with 20 females of *An. minimus* exposed to 30 min. and observed for mortality after 24 hours. Tests were conducted weekly for one month and monthly for a year. Mortality below 70% was used as the cut off level to define satisfactory residual activity. Laboratory tests showed almost 100% mortality throughout the year of observation. In the field, the efficacy lasted for 48 weeks on bamboo outer surfaces, 40 - 44 weeks on bamboo inner surfaces and 28 - 30 weeks on wood surfaces. There was no difference in the effectiveness between bifenthrin WP and deltamethrin or between the two dosages of bifenthrin.

India - Persistence of bifenthrin 10% WP on different local surfaces and the efficacy of the insecticide on entry rate, indoor resting density, exito-repellency, immediate and delayed mortality and blood feeding rate were studied in a small-scale field trial in Gujarat (Yadav *et al.* 2000). The study also included determination of the best dose for larger scale trials and the perception of spraymen and sleepers in the sprayed huts.

The trial was carried out in Kheda district, Central Gujarat, where *An. culicifacies* is the major vector. Sibling species A, B and C are sympatric, the vectors A and C formed 26% of the total. *Anopheles culicifacies* is resistant to DDT, HCH and malathion. It is mainly endophilic and zoophilic.

Six comparable houses were selected from each of five villages and were randomised to six arms of treatment (based on baseline entomological collections during July and August 2000): Bifenthrin 10% WP was applied at 25, 50, 100 and 200 mg/m² (one round), malathion 25% WP at 2 gm/m² (three rounds) at six-week intervals and there was an unsprayed control. Most houses selected were made of mud, or brick walls, with or without mud

plastering. Roofs were made of earthen tiles or galvanized iron sheets supported by a wooden frame. Houses generally had 30 cm wide eaves. Cattle sheds were adjacent enclosures. In each house one dwelling room wall was selected and its eaves were covered from inside with black cloth. Two exit traps were fitted, as far as possible facing the rising sun.

To compare persistence of insecticide, various surfaces in different houses, such as mud-plastered walls, unpainted wood panels, brick walls and galvanised metal sheets were sprayed using standard methods. The target doses sprayed indoors were subjected to chemical residue analysis. Wide variation, however, was noticed. Contact bioassays were carried out on the surfaces following WHO procedure at the frequency of one day and 2, 4, 8, 12, 16, 20 and 24 weeks after spray. Five replicates of 20 females of *An. culicifacies* sibling species C, 48-72 hours old and sugar-fed were exposed for 30 minutes and mortality was recorded immediately after exposure as well as after 24 hours.

The results indicate that 25 mg/m² produced 100% mortality up to week 16 on galvanised metal sheets, 8 weeks on mud surface, 4 on brick and wood surfaces respectively. The dose of 50 mg/m² produced 100% mortality on galvanised metal sheets (up to week 24), mud (up to week 8), brick (up to week 4) and wood surfaces (up to week 8). On mud walls, which are the most common surfaces, more than 80% mortality was observed until 24 weeks.

Susceptibility test of the local population of *An. culicifacies* to bifenthrin (0.1%) at one-hour exposure and 24 hour holding showed 100% knockdown/mortality. Mortality with malathion (5%) impregnated test papers was 57%.

Airborne bioassays were carried out (at four days interval) by exposing sugar-fed female mosquitoes in cubical cages, hung 50 cm below the ceiling and 50 cm from the wall for one hour. Knockdown after one hour exposure and mortality after 24 hours was recorded. Airborne effect of 25 mg and 50 mg of bifenthrin and malathion on day one was between 89 and 100%, which declined significantly by the second week. At 100 and 200 mg dosage, the effect remained for a longer period.

Floor sheet, exit trap and indoor resting collections were made at weekly intervals in the houses selected. Before spraying (July & August), the densities were comparable. After spraying (September-March), the densities declined in houses treated with bifenthrin (one round) as compared with the control. In malathion-sprayed houses (three rounds) densities declined initially but re-appeared intermittently. Generally, densities in rooms with bifenthrin 100 and 200 mg dosage were relatively low. The impact of 25 and 50 mg dosage on indoor resting densities of *An. culicifacies* and other anophelines was almost comparable.

Since the houses were not made ant-proof, a very low number of mosquitoes were found dead in the morning on the floor sheets. Compared with malathion-treated and control rooms the entry rate in bifenthrin-sprayed rooms was lower.

The excito-repellency rate of *An. culicifacies* (proportion of mosquitoes collected in exit traps versus total entry) in bifenthrin-sprayed houses ranged from 0.31 to 0.50 as compared to 0.12 in malathion and 0.13 in control rooms. The difference was significantly higher ($P < 0.001$). However, no significant difference was seen between malathion and control ($P > 0.05$). Similar results were also observed with other mosquitoes.

The proportion of fed to total entry of *An. culicifacies* in rooms with bifenthrin 25, 50, 100 and 200 mg dosage was 0.40, 0.30, 0.33 and 0.38 respectively compared with 0.40 in rooms with malathion and 0.31 in control rooms. The difference was not statistically significant ($P > 0.05$). The difference between the feeding rates of other mosquitoes among bifenthrin, malathion and control huts was statistically significant ($P < 0.01$). At higher dosage, the proportion of fed among all entered mosquitoes declined significantly ($P < 0.01$).

Delayed mortality among *An. culicifacies* and other mosquitoes caught from insecticide-sprayed rooms was higher than for controls but the difference was not significant ($P > 0.05$). The overall mortality was comparable among bifenthrin 25 mg, malathion and controls but was higher with bifenthrin 50, 100 and 200 mg dosages.

Semi-structured interviews showed that none of the five spraymen had any side effects after spraying bifenthrin. They observed that bifenthrin did not have an odour or leave stains on surfaces. The average number of persons sleeping in sprayed rooms was 2.9 (ranging from one to five persons per room). Almost all (except one) appreciated the efficacy of this insecticide, even after one month.

Since the mud-plastered walls were more common in the rooms, persistence of residual action on mud walls was considered as the basis for selecting a dose for the phase III trial. On mud walls 25 mg bifenthrin produced 100% mortality up to eight weeks, >80% until 20 weeks and 66.7% by 24 weeks. The dose of 50 mg/m² was found to have a marginally better residual action. The airborne effect, the effect on indoor resting density and the

excito-repellent effect were also comparable. Considering the risk of faster development of resistance with a higher dose of 50 mg, two rounds of 25 mg, three months apart were preferred to one round of 50 mg to cover six months transmission season in the area.

Thailand - Efficacy of bifenthrin 10% WP for indoor residual spraying against *An. minimus s.l.* was studied and compared to deltamethrin, in Mae Hong Son Province (Prajakwong *et al.* 2001). The study was carried out from January 1999 to April 2001, before intervention, and May 2000 to April 2001, after intervention. Nine index villages, all located in the forest or forest fringes, in Muang district, Mae Hong Son province, were selected for the study, based on high endemicity of malaria. Three villages were sprayed with 25 mg/m² bifenthrin 10% WP, three villages were sprayed with 20 mg/m² deltamethrin 10% WP, and three villages were left untreated.

Indoor and outdoor human bait collections (three nights per village), animal bait collections and light trap collections were carried out monthly. Age grading, malaria sporozoite rates and susceptibility status were determined. Surface contact bioassay tests were carried out at monthly intervals using a *An. minimus s.l.* laboratory strain. Mosquitoes dead or knock down were counted at the end of the exposure periods and after 24 hrs. Susceptibility to the 0.05% deltamethrin and 0.1% bifenthrin was determined.

Eight experimental huts (3m x 4m x 2 m) were constructed, four in bifenthrin area (three sprayed with bifenthrin and one left unsprayed). Four huts were constructed in deltamethrin-sprayed areas, three huts sprayed, one left unsprayed. Floor sheet, indoor resting and exit trap collections were made.

The malaria cases reported from active case detection (ACD) and passive case detection (PCD) were collected monthly from Vector Borne District Control units. Mass blood surveys were performed in nine index villages every six months. The spray operations were conducted twice, during April for the first round and August for the second round. The average coverage of house spraying was 88% and 92% in deltamethrin and bifenthrin-treated areas, respectively. Since *An. dirus s.l.* densities were low further analysis was on *An. minimus* only.

Analysis of variance showed a significant difference in mean density of *An. minimus s.l.* from outdoor and indoor human bait collections in bifenthrin, deltamethrin treated and control areas ($P < 0.05$), both before and after intervention. Therefore, further comparisons were made between pre- and post-application results in each treatment area.

The result indicated that there was a significant difference in the *An. minimus s.l.* area from indoor and outdoor human bait collections between pre and post-application in bifenthrin-sprayed areas ($P < 0.001$), while no significance was found in deltamethrin areas ($P > 0.05$).

Parous rates, determined from at least 50 females of *An. minimus* dissected at each survey declined significantly after intervention in bifenthrin and deltamethrin-sprayed areas ($P < 0.001$); there was no significant difference in the control area ($P > 0.05$).

Wild caught *An. minimus s.l.* was susceptible to both deltamethrin and bifenthrin, resulting in 98-100% mortality at the diagnostic dose.

Surface contact bioassay tests showed that the mortality rate in bifenthrin 25 mg/m² gradually reduced but the

persistence remained for six months since more than 70% mortality rate was observed. Deltamethrin 20 mg/m² gave large fluctuation in the mortality rate after the second month after application.

The average number of *An. minimus s.l.* entering the exit trap from three sprayed experimental huts in the bifenthrin area was lower than with deltamethrin. The resting mosquitoes on the wall was higher in the bifenthrin huts, which indicate a low excito-repellent effect. No dead mosquitoes were observed after 24 hour observation from both exit trap and resting mosquito collections.

Mass blood survey coverage ranged from 65.0 to 98.7%. Approximately 75 – 83% of the patients recorded from mass blood survey were indigenous malaria cases. Analysis of indigenous cases before and after intervention indicated that parasite rate was reduced from 3.3% to 0.67 in bifenthrin-treated villages, which was significant ($t = 2.941$, $df = 10$, $p > 0.05$). There was no significance reduction in those of deltamethrin (8.8% to 5.17%) and control areas (4.17 to 3.0%) ($P > 0.05$).

Mexico - The objectives of the trial (Arredondo-Jiménez *et al.* 2001) were to determine the susceptibility of *An. albimanus* and *An. pseudopunctipennis*, the vectors of southern Mexico, against bifenthrin, to select optimum dosage from a small-scale trial and to establish the effect of bifenthrin on biting, resting behaviour, parity and malaria infection rates of vectors.

To determine the diagnostic dose, susceptibility tests were carried out in the lab. at 0.01, 0.03, 0.05, 0.1 and 0.2%. Eight replicates of 25 females were tested for each dose. Log-probit analysis was used to estimate LD₅₀ and LD₉₉. The LD₉₉ for *An. albimanus* was 0.05 and for

An. pseudopunctipennis was 0.6%. The discriminative dose was set at $2 \times LD_{99} = 0.1\%$

Susceptibility of field-collected F1 mosquitoes was determined before and after intervention to diagnostic dosages of bifenthrin. The mortality rate was 100% in *An. albimanus* and 98 – 99% in *An. pseudopunctipennis*, indicating that both anopheline species were susceptible to bifenthrin at 0.1% before and after the trials.

The field dosage was assessed by surface bioassay test on different surfaces. Three dosages 25, 50 and 100 mg (a.i.)/m² were tested. Eight houses were selected as representative of different materials: cement, plaster, bricks or bamboo walls and wood or palm thatched roofs. Each concentration was applied in two houses and two houses were left untreated. Standard wall bioassays were conducted every second week with an exposure of one hour of *An. albimanus* and *An. pseudopunctipennis* (Tapachula strains).

Based on the residual effect with mortality of $\geq 75\%$ and a target duration of 20 weeks, the target dosage of 37.5 mg (a.i.)/m² was selected for larger scale evaluation. At this dosage a sustained residual effect of at least 22 weeks against both these species was observed, with mortalities of $\geq 75\%$ on all surfaces tested (cement, wood, bamboo pole and palm thatch).

The village scale study was conducted in southern Chiapas, Mexico, around Tapachala city (17° 25'N and 92° 20' W), along the coastal plain where *An. albimanus* is the vector and a foothill area where *An. pseudopunctipennis* is the predominant vector. The area is endemo-epidemic with periodic outbreaks. In the *An. albimanus* area nine villages with approximately 200 houses were selected and three of each were

randomly allocated for bifenthrin, deltamethrin and no treatment.

In the *An. pseudopunctipennis* area, three villages were treated with bifenthrin and three were untreated. Assessment of mosquito populations was carried out one month prior to spraying and nine months post treatment follow-up in *An. albimanus* areas (May 1999 to February 2000) and six months in *An. psuedopunctipennis* areas (February to July 2000). A split plot design was followed. Two rounds of bifenthrin and deltamethrin spraying were carried out in the *An. albimanus* area while in the *An. pseudopunctipennis* area one round of spraying was done. The period between applications was decided when the mortality rate from wall bioassays dropped below 75%.

Human landing rate and indoor/outdoor resting densities were determined twice a week using standard procedures. Wall bioassays were carried out every two weeks. Mosquitoes were also released into huts covered with netting curtains and those found resting on the curtain and found dead were collected and classified into blood fed and unfed. The live ones were observed for 24 hrs mortality.

Analysis of variance for split plot design indicated that mosquito abundance was significantly different with respect to treatments. However there was no difference between the bifenthrin and deltamethrin with respect to mortalities. Human-vector contact was not reduced in the *An. albimanus* area, but it was reduced in the *An. pseudopunctipennis* area.

Parous rates were significantly reduced in both areas, indicating an impact on the age structure of the mosquito population. Resting collections showed that the feeding

success was reduced (from 50% to 30%) and mortality increased (from 0 to 67%) in bifenthrin-treated villages from *An. albimanus* area.

The house curtain experiments indicated no significant repellent effect of bifenthrin. Mark, release and recapture results showed that mosquito mean resting time was not significantly different between treatments while mortality was statistically different in bifenthrin-treated huts.

The assessment of infection rate was done only in the *An. albimanus* area. Less *P. vivax* infected mosquitoes were observed in the bifenthrin-treated area as compared to the control. The infection rate was higher in control (1.08) compared to bifenthrin (0.36).

A structured questionnaire survey showed that acceptability by residents and spray men to bifenthrin and deltamethrin was similar, although more residents referred to itching (50%) and were reluctant (25%) to accept further sprays with deltamethrin. In contrast, only 20% of residents complained about itching following bifenthrin spray and more than 90% would accept further treatments with bifenthrin.

Mexico - A single village control trial was conducted against domestic Triatominae, using three synthetic pyrethroids: bifenthrin (10 WP), deltamethrin (2.5% SC) and cyfluthrin (10 WP) in Chalcatzingo, Morelos (Ramsey-Willoquet 2000). Spray intervention was preceded by a pre-intervention entomological evaluation of 100% of houses followed by four post-intervention (PI) evaluations at 1, 3, 6 and 12 months, carried out by timed manual collections. Two *triatomine* species, *Triatoma pallidipennis* and *T. barberi* infest this community, with an overall pre-intervention adjusted infestation index of 38%, of which 17% represents intradomiciliary infestation.

HPLC analysis of filter papers attached to the walls just before spraying showed that bifenthrin and cyfluthrin applications were close to their mean target doses, whereas deltamethrin was less than half the desired dose. For this reason, deltamethrin results are not considered further in this evaluation. Wall bioassays indicated a steady decrease in mortality, reaching zero before one year. Mortality of individual nymph stages varied widely, with highest mortality in second-stage nymphs. Stage 3 nymphs had greatly reduced mortality, declining to only 10-40% mortality at one month post-spray.

There was a six- to 13- fold decrease in intradomiciliary infestation rates for cyfluthrin and bifenthrin-treated areas, resulting at one month post-intervention in 0% and 0.6% infestation rates, respectively. Intradomiciliary infestation steadily recovered for bifenthrin in the treated area after one month, but more slowly after three months for the cyfluthrin-treated area. Peridomestic infestation recovered to pre-intervention levels in the bifenthrin area within three months. Studies of the peridomestic infestations suggest these were the main factor for repopulation of domestic areas. Morphometric analysis of intradomiciliary, peridomiciliary and sylvatic *T. pallidipennis* populations indicated that gene flow occurs between the three. It was suggested that future control of this species may involve integrated control of small rodent populations within the community, followed by domestic and peridomestic spray application.

3.3 Conclusions

Bifenthrin, a non-alpha-cyano pyrethroid, has moderate acute toxicity and the technical material is classified, as are other public health pyrethroids (except for etofenprox), as moderately hazardous by the WHO. Bifenthrin is less

toxic by the dermal route than the oral route. It is a mild irritant to the eye and is not a skin sensitizer.

Based on the standard WHO bioassay conducted in three laboratories, against adult species of *An. stephensi*, (Beech strain), *An. gambiae* (Kisumu strain), or *An. albimanus* (Panama strain) and *An. albimanus* and *An. psuedopunctipennis*, and *Ae. aegypti* diagnostic concentration of between 0.1% and 0.25% was determined. One study concluded that the diagnostic concentration for *Cx. quinquefasciatus* was 1.0%. The group concluded that for malaria vectors the tentative diagnostic concentration should be 0.2%

Forced tarsal contacts with insecticide deposits on filter paper and irritability tests have shown that bifenthrin has a relatively low irritant and knock down effect compared with permethrin and deltamethrin. Field studies comparing bifenthrin to deltamethrin confirmed this excito-repellency effect. Bifenthrin consistently provided a high kill by allowing mosquitoes to rest on treated surfaces for longer periods. The airborne effect was similar to that of other pyrethroids. Relative to other public health pyrethroids, laboratory studies of bifenthrin indicated good efficacy against *Cx. quinquefasciatus*.

Bifenthrin resistance was observed in pyrethroid resistant strains (*kdr*) of *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* in the tests on adults by topical applications and with forced tarsal contact with insecticide deposits on filter papers. The concentration which gave 100% mortality with a susceptible strain gave only five to 10% mortality with a resistant strain with a complete loss of KD effect.

Field studies were carried out in India, Thailand and Mexico, using bifenthrin 10WP at the concentrations of

25-200mg ai/m², on wood, bamboo, palm, mud, galvanized iron and cement blocks. The dosage of 25-50 mg ai/m² provided residual effectiveness of between three and six months (> 80% mortality in surface bioassay tests).

Following indoor residual application, acceptability of bifenthrin by residents and spray men was good.

3.4 Recommendations

1. Noting its safety and efficacy, bifenthrin 10 WP is recommended for indoor residual spraying against anophelines at 25-50 mg ai/m². The expected residual efficacy at the recommended dosage is three to six months.
2. For detection of resistance to bifenthrin in malaria vectors, a tentative diagnostic concentration of 0.2% is recommended
3. Further field testing and evaluation of bifenthrin for indoor residual spraying against Chagas vectors is recommended.

ANNEX 1. REFERENCES CITED

Arredondo-Jiménez, N.E. Rivero, I.R. Malo, L. Gonzalez-Ceron and M.H. Rodriguez (2001) Phase III trials of bifenthrin to control *Anopheles* in southern Mexico. Unpublished report to the WHO Pesticide Evaluation Scheme.

Chheang Y. and L. Sandy (1994) Final report on a field trial of Olyset Net for the control of malaria transmitted by *Anopheles dirus* and *Anopheles minimus* in Rattanak Kiri Province, Cambodia. Unpublished Report to Sumitomo Co Ltd, Osaka, Japan.

Curtis C.F., J. Myamba and C.A. Maxwell (2001) Report to WHOPES on trials in Tanzania of Olyset bednets. Unpublished report to the WHO Pesticide Evaluation Scheme.

Doannio, J.M.C., J. Dossou-Yovo, S. Diarrassouba, G. Chauvancy, F. Darriet, F. Chandre, M.C. Henry, I. Nzeyimana, P. Guillet and P. Carnevale (1999) Efficacité des moustiquaires pre-impregnées de perméthrine Olyset Net en zone de résistance des vecteurs aux pyréthrinoides. I – Evaluation entomologique. *Médecine Tropicale*, 59, 349-354.

Faye O. (1996) Field evaluation of preimpregnated mosquito nets "Olyset Net" produced by Sumitomo Chemical Co. Ltd on reduction of malaria transmission in a Sudanese Savannah Village of Senegal. Unpublished Report to Sumitomo Co Ltd, Osaka, Japan.

Faye O., L. Konate, O. Gaye, D. Fontenille, N. Sy, A. Diop, M. Diagne and J.F. Molez (1998) Impact de l'utilisation des moustiquaires pre-impregnees de perméthrine sur la

transmission du paludisme dans un village hyperendémique du Sénégal. *Médecine tropicale*, 58, 355-360.

Finot L., S., Duchon, F. Chandre and P. Guillet (1997) Laboratory investigation on the activity of bifenthrin against mosquitoes. Unpublished report to the WHO Pesticide Evaluation Scheme.

Food and Agriculture Organization of the United Nations (1992) Pesticide residues in food. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the Core Group on Pesticide Residues, Rome, FAO Plant Production and Protection, Paper 116.

Hemingway J. and J. Morgan (1998) Report for the World Health Organization on establishing a discriminating dosage of bifenthrin. Unpublished report to the WHO Pesticide Evaluation Scheme.

Henry M.C., J.M.C. Doannio, F. Darriet, I. Nzeyimana and P. Carnevale (1999) Efficacité des moustiquaires pré-imprégnées de perméthrine Olyset Net en zone de résistance des vecteurs aux pyréthrinoides. II. Evaluation parasito-clinique. *Médecine Tropicale*, 59, 355-357.

Hougard, J.M., S. Duchon, M. Malivert, L. Finot, J. Tiahing, D. Boucharine and P. Guillet (1999) Comparative testing of bifenthrin 10% and 20% WDP on adult mosquitoes. Unpublished report to the WHO Pesticide Evaluation Scheme.

Hougard J.M., L. Finot, M. Malivert, S. Duchon and D. Boucharinc (2000) Dynamics of perméthrin on Olyset Nets under laboratory conditions. Unpublished report to the WHO Pesticide Evaluation Scheme.

Hougard, J.M., S. Duchon, F. Darriet, M. Zaim and P. Guillet (2001). Comparative performances under laboratory conditions of seven pyrethroid insecticides for treatment of mosquito nets. Unpublished report to the WHO Pesticide Evaluation Scheme.

Ikeshoji T. and B. Bakotee (1997) Dynamics of permethrin on mosquito nets used in the malaria control program in Honiara, Solomon Islands. *Med. Entomol. Zool.* 48, 25-31.

Itoh T. and T. Okuno (1996) Evaluation of the polyethelene net incorporated with permethrin during manufacture of thread on efficacy against *Aedes aegypti* (Linnaeus). *Med. Entomol. Zool.* 47, 171-174.

N'Guessan R., F. Darriet, J.M. Doannio, F. Chandre and P. Carnevale (2001a) Olyset Net efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* after 3 years' field use in Côte d'Ivoire. *Med. Vet. Entomol.*, 15, 97-104.

N'Guessan R., M. Traore-Lamizana, F. Chandre and P. Carnevale (2001b) Field evaluation of Olyset Net against *Anopheles gambiae* and *Anopheles funestus*, main vectors of malaria in West Africa. Unpublished report to the WHO Pesticide Evaluation Scheme.

Nguyen H.T., T.V. Tien, N.C. Tien, T.U. Ninh and N.T. Hoa (1996) The effect of Olyset Net screen to control the vector of dengue fever in Viet Nam. *Dengue Bulletin* 20, 87-92.

Njunwa K.T., V.A.E.B. Kilimali, S.M. Marero, F.H.M. Msuya, R. Pilyimo and D. Kamuzora (1996) Permethrin incorporated bednets, "Olyset Net", reduce malaria transmission after twelve months of their use in

three villages of Kibaha District, coast region, Tanzania. Report Submitted to Sumitomo Chemical Company, Japan.

Prajakwong S. (1998) Dose response trial of bifenthrin for use as a residual mosquito adulticide in Thailand. Unpublished report to the WHO Pesticide Evaluation Scheme.

Prajakwong S., T. Banchong-aksorn, K. Sombatwatnang-gura, W. Suwonkerd, P. Panart, S. Chawprom, W. Tidthian (2001) Phase III trials – Effect of bifenthrin as an indoor residual spray for control of malaria vectors in Mae Hong Son Province, Thailand. Unpublished report to the WHO Pesticide Evaluation Scheme.

Ramsey Willoquet J.M. (2000) Field trial of pyrethroid insecticides against domestic and peridomestic populations of *Triatoma Pallidipennis* and *Triatoma barberi* in Morelos, Mexico. Unpublished report to the WHO Pesticide Evaluation Scheme.

Vythilingam I, B.P. Pascua and S. Mahadevan (1996) Assessment of a new type of permethrin impregnated mosquito net. *J. Bioscience*, 7.

Yadav, R.S., T. Adak, N. Nanda, H.C. Srivastava, S. Subbarao, B.R. Thapar (2000) Phase II evaluation of bifenthrin 10% WP indoor residual spraying for malaria vector control in India. Unpublished report to the WHO Pesticide Evaluation Scheme.

ANNEX 2. LIST OF PARTICIPANTS

- Dr P. Carnevale, Institut de Recherche pour le Développement, Montpellier, France.
- Dr M. K. Cham, Communicable Disease Control, Roll Back Malaria, World Health Organization, Geneva, Switzerland.
- Dr C. Frederickson, Pan American Health Organization, Brasilia, Brazil.
- Dr P. Guillet, Strategy Development and Monitoring for Parasitic Diseases and Vector Control, Communicable Disease Control, Prevention and Eradication, World Health Organization, Geneva, Switzerland.
- Dr P. Jambuligam, Vector Control Research Centre, Pondicherry, India.
- Dr L. Manga, WHO Regional Office for Africa, Harare, Zimbabwe.
- Dr M. Nathan, Strategy Development and Monitoring for Parasitic Diseases and Vector Control, Communicable Disease Control, Prevention and Eradication, World Health Organization, Geneva, Switzerland.
- Dr B. L. Sharp, Medical Research Council, Durban, South Africa.

- Dr I. Vythilingam, Institute for Medical Research, Kuala Lumpur, Malaysia.
- Dr M. Zaim, WHO Pesticide Evaluation Scheme, Strategy Development and Monitoring for Parasitic Diseases and Vector Control, Communicable Disease Control, Prevention and Eradication, World Health Organization, Geneva, Switzerland.