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REPORT OF THE SEVENTH WHOPES WORKING GROUP MEETING

WHO/HQ, GENEVA
2–4 DECEMBER 2003

Review of:
VECTOBAC WG
PERMANET
GOKILAHT-S 5EC

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

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1. INTRODUCTION

The seventh meeting of the WHOPES Working Group, an advisory group to the WHO Pesticide Evaluation Scheme (WHOPES) was convened at WHO headquarters, Geneva, 2–4 December 2003. The objective of the meeting was to review the reports of the testing and evaluation of VectoBac® WG (Valent BioSciences, USA) for mosquito larviciding, PermaNet® (Vestergaard Frandsen, Denmark) for malaria prevention and control, and Gokilaht®-S 5EC (Sumitomo Chemical Co., Japan) for space spraying against mosquitoes.

The meeting was opened by Dr L. Savioli, Coordinator, Strategy Development and Monitoring for Parasitic Diseases and Vector Control. In his remarks, Dr Savioli emphasized the need for the development of novel vector control strategies, as the number of available tools is rather limited at the present time. He further noted that WHOPES is playing a critical role in the development of vector control agents and their formulations.

Dr Morteza Zaim, Scientist in charge of WHOPES, presented the objectives of the meeting as well as an overview of the Scheme to the participants and noted that WHOPES recommendations are intended to expedite registration of public health pesticides by the Member States. He noted the close collaboration of WHOPES with the Programme on Chemical Safety (PCS) and mentioned that no public health pesticide is considered by the Scheme for field testing until the safety assessment has been carried out by PCS. Dr Zaim also noted that the reports of the WHOPES Working Group Meetings are a consolidation of the available information on pesticides evaluated by the Scheme and an excellent resource for pesticide registration authorities and national control programmes. He further emphasized that every effort is made to ensure that the reports are useful and widely available.

Dr Zaim briefed the participants on the new FAO/WHO procedure for developing pesticide specifications, by which the specifications are linked to the product of the manufacturer which provided the data package and the pesticide product for testing/evaluation. Details of the data package requirements and procedures for development of specifications are included in the *Manual for development and use of FAO and WHO specifications for pesticides*, 1st ed., published by the Food and Agriculture Organization of the United Nations (FAO) in 2002.

Dr H. Endo, Director, WHO Department of Communicable Disease Control, Prevention and Eradication, also attended the meeting and expressed his great interest in the proceedings and deliberations of the Working Group. Dr Endo made specific reference to the emergence and spread of vector-borne diseases, such as West Nile Virus, and reiterated, that in view of the occurrence of these types of epidemic, vector control research and the development of new strategies for vector control are attracting increasing attention. He emphasized the high priority that WHO is giving to vector biology and control as an important cross-cutting activity, despite the availability of only limited resources.

The meeting was attended by 11 scientists (see Annex, List of participants). Dr Mir S. Mulla was appointed as Chairman and Dr P. Jambulingam as Rapporteur. The reports of the WHOPES supervised trials and relevant published literature (see Section 6, References cited) were reviewed and discussed. Recommendations were made on the use of VectoBac® WG, PermaNet®, and Gokilaht-S® 5EC.

2. REVIEW OF VECTOBAC WG

VectoBac® is a bacterial larvicide. The active ingredient in VectoBac is composed of viable *Bacillus thuringiensis israelensis* (H-14) endospores and delta-endotoxin crystals.

Bacillus thuringiensis (*Bt*) is a facultative anaerobic, Gram-positive bacterium forming parasporal crystalline inclusions, which are toxic to certain invertebrates, especially species of insect larvae belonging to the insect orders of Coleoptera, Diptera and Lepidoptera. The parasporal inclusions consist of different insecticidal crystal proteins (ICP). A susceptible insect larva must ingest the ICP or spore–ICP complexes. The efficacy of the ICP depends on solubilization in the midgut, conversion of the protoxin to the biologically active toxin by proteolytic enzymes, specific membrane receptor binding by the C-terminal domain of the active toxin, and pore formation by the N-terminal domain, with subsequent lysis of the gut epithelial cells. Germination of spores and proliferation of vegetative cells into haemocoel may result in a septicaemia, contributing to the cause of death. Receptor binding by the ICP is the major determinant of host specificity. *Bacillus thuringiensis* has many subspecies that exhibit toxicity to a variety of insects. Over the past 50 years, most of the *Bt* products have been used for the control of agricultural pests. It was not until 1980 that a *Bt* subspecies, known as *israelensis* (*Bti*), was discovered and developed for use in mosquito and onchocerciasis control programmes.

2.1 Safety assessment

The human and environmental safety of *Bt,* including *Bti,* has been assessed by WHO (1999). The ICP spores and vegetative cells of the *Bti* subspecies, when administered by different routes, were found to be mostly non-pathogenic and non-toxic to various animal species. *Bti* has no adverse affect on birds, earthworms, fish, or numerous other non-target aquatic vertebrates in laboratory and field studies. A few species of aquatic invertebrates, however, are susceptible to *Bt. Bti* has little toxicity to non-target arthropods.

After the application of *Bti* to an ecosystem, the vegetative cells and spores may persist at gradually decreasing concentrations. The ICPs, however, are rendered biologically inactive within hours or days.

With the exception of case reports of ocular and dermal irritation, observations of occupational exposure have revealed no adverse health effects. Antibody titres to the vegetative cells, spores and spore–crystal complexes have been demonstrated in workers spraying *Bti* products; however, no adverse health effects were reported. *Bt* has no adverse effect on human health when present in drinking-water or food.

Owing to their specificity, *Bti* products are unlikely to pose any hazard to humans, other vertebrates, and non-target invertebrates, provided that they are free from non-*Bt* microorganisms and biologically active products other than the ICPs. They are safe for use in aquatic environments, including drinking-water and reservoirs, for the control of mosquitoes, blackflies, and larvae of nuisance insects.

The VectoBac WG formulation of *Bacillus thuringiensis* subsp. *israelensis* is a fermentation product, consisting of brownish fine-sized granules with loose appearance that disperse readily when mixed with water. VectoBac water-dispersible granule (WG) is non-toxic by ingestion, skin contact, or inhalation. The following are extracts from the Manufacturer's Material Data Safety Data Sheet and Label recommendations of the WG formulation.

Active ingredient	*Bti* 37.4%
Potency	3000 International Toxic Units
Acute oral LD_{50} (rat)	>5000 mg/kg
Acute dermal LD_{50} (rat)	>5000 mg/kg
Inhalation	No lethality was observed in rats after a 4-hour exposure at a highest obtainable inhalation exposure chamber concentration
Dermal irritation	Transient, slight or mild irritation was noted in a dermal toxicity study with this product.
Ocular irritation	Transient, redness and conjunctival irritation was observed in test animals in a study with this product. No positive ocular effects were observed. Was classified as a non-irritant
Dermal sensitization	In a study with this product, no skin sensitization was observed
Carcinogenicity	None of the components are classified as carcinogens
Corrosiveness	Not expected to have any corrosive properties

2.2 Efficacy – background/supporting documents

California – The efficacy and residual activity of WG formulations of *Bti* (4000 International Toxic Units (ITU)/mg) and *Bacillus sphaericus* (*Bs)* were assessed against *Culex* mosquitoes to determine the minimum effective dosages of these formulations in 250-ml fibreglass tubs (Su & Mulla, 1999). In the laboratory, the efficacy of VectoBac Technical Powder (7000 ITU/mg) was also studied. Bioassays were carried out against early fourth-instar larvae of colonized *Culex quinquefasciatus*. Suspensions at the final concentrations of 0.1, 0.01 and 0.001% (w/v) were added to 120-ml waxed paper cups containing 100 ml of tap water and 25 larvae. One

drop of 10% larval food was added to each test cup after treatment. A preliminary test was conducted for each material, with 3 replicates, using three or four concentrations from 0.005 to 0.010 ppm and control. The bioassay was carried out at 27-29 °C. Mortality was recorded 24 and 48 hours after treatment. If mortality in the control exceeded 5%, the test was discarded. Based on the preliminary observations, the final test was set up by using three or four concentrations, yielding 10-95% mortality, and nine replicates for each concentration and control. The dose–response data were subjected to probit regression analysis, and median lethal concentration (LC_{50}), 90% lethal concentration (LC_{90}), and their 95% confidential intervals were calculated.

Fibreglass tubs (1.0 x 1.0 x 0.4 m deep), placed in an open, sunlit area, were used for field-testing the material. Before flooding, the tubs were enriched with rabbit pellets (crude protein >17%) at 100 g per tub to provide continuous and sustained oviposition by the mosquitoes. The tubs were filled to a depth of 30 cm with 236 litres of water from an irrigation reservoir. The water level was kept constant by float valves. The tests were carried out in two sets, one using WG of *Bti* at 1.1 to 2.7 lb/acre (0.5 to 1.22 kg/ha) and the other using 0.27 to 0.53 lb/acre (0.122 to 0.240 kg/ha). Each of the treatments and the control were assigned at random using five replicates. The tubs were treated 7 days after flooding, when third and fourth instars and a few pupae were present. The WG formulation was made into a suspension in distilled water at 1% (w/v) and the required quantities were applied to the water surface using a 1-ml or 5-ml pipette.

Mosquito larvae and pupae were sampled on day 0 (pre-treatment) and at different intervals after treatment, taking five dip samples per tub, one from each corner, and an additional one from an area of aggregated mosquito larvae. Immature samples were categorized into early (first and second) instars, late (third and fourth) instars, and pupae. The average numbers in each category were compared with the control and treatments on each sampling by day, using one-factor analysis of variance. The species composition of the

mosquitoes was determined from the control tubs, identifying about 150 fourth instars on each sampling day, and compared by a chi-square test.

Bioassays against *Cx. quinquefasciatus* showed that the LC_{50} and LC_{90} values were 0.024 and 0.059 ppm respectively for *Bti* WG.

In the simulated field trial, the density was not statistically different before treatment in control and treatment plots, where the applied dosages were 1.1 and 2.7 lb/acre (0.5 and 1.22 kg/ha) . The formulation at the two dosages yielded almost 100% control of all the stages on day 2 post-treatment and a significant reduction of total immature and late instars and pupae on day 7 post-treatment ($p < 0.05$). The formulation was not effective against larvae and pupae on day 12-post treatment at either dosage, but there was significant reduction of late instars ($p < 0.05$). On days 19 and 25 post-treatment, the formulation was no longer effective at the low dosage on any of the mosquito stages. At the dosages of 0.27 and 0.53 lb/acre (0.122 and 0.240 kg/ha), a significant reduction in the number of total immature and late instars and pupae was achieved on days 3 (late instars and pupae >95%) and 7 (100% pupae) post-treatment, and the 2 dosages were equally effective ($p > 0.05$). On day 12 post-treatment, however, the low dosage lost its efficacy and the high dosage showed partial effect or no effect. In both sets of experiments, the proportions of *Cx. stigmatosoma* and *Cx. quinquefasciatus* in controls decreased from 53.3% to 6.5%, and from 34.8% to 6.9% respectively, whereas *Cx. tarsalis* increased from 11.9% to 86.5% on different days of sampling. The minimum and maximum water temperatures ranged from 20.0 °C to 24.4 °C, and from 32.2 °C to 36.1 °C respectively during the first test, and from 13.3 °C to 22.2 °C and 25.6 °C to 33.3 °C, respectively, during the second test. The pH values were 7.5 to 8.0. The study showed that the minimum effective dosage for the *Bti* WG formulation was 0.27 to 0.53 lb/acre (0.122 to 0.240 kg/ha), with a residual effect for 7–12 days.

Penang, Malaysia – The larvicidal efficacy of thermal fogging indoor application of VectoBac WG (3000 ITU/mg) was evaluated in comparison with that of VectoBac 12AS liquid (1200 ITU/mg of *Bti*) against *Aedes aegypti, Ae. albopictus, Cx. quinquefasciatus*, and *Anopheles dirus* (Yap, Lee & Zairi, 2002). The test site consisted of more than 20 lanes of residential terraced houses in an urban settlement in Bayan Baru, Penang Island. A minimum of 10 similar-sized, single-story concrete houses (each with five assessment cups) at alternate positions (with one house in between) from a single lane were chosen for spraying of each of the formulations. Different lanes, at least 50 m apart, were chosen for the spraying of each formulation. The formulations were tested at four dosages, WG: 2.91×10^9, 1.45×10^9, 0.71×10^9, and 0.36×10^9 ITU/ha (970, 480, 240 and 120 g/ha), and AS: at 2.87×10^9, 1.46×10^9, 0.71×10^9, and 0.36×10^9 ITU/ha (2390, 1210, 600, and 300 ml/ha). WG was diluted with seasoned water in the ratios of 1:20, 1:40, 1:80, and 1:160, and the liquid formulation at 1:8, 1:16, 1:32, and 1:64. The assessments for each of the species were carried out with 20 laboratory-cultured, late 3rd- or early 4th-stage larvae placed in a cylindrical paper cup (top diameter 8cm and height 10 cm) filled with 200 ml of seasoned tap water at two checkpoints, one in the living-room and the other in the kitchen. Each of the selected houses was sprayed for a period of 56.6 $\pm$ 2.5 seconds and an area of approximately 120 m^2 was covered. With the mean discharge rate of the machine at 245.0 $\pm$ 5.0 ml/min, the mean volume sprayed per house was 231.0 $\pm$ 3.0 ml. Spraying was conducted between 18:00 and 19:30 hours.

Larvae were brought back to the laboratory after 1 hour of exposure and kept in clean paper cups. The mortality of larvae was recorded 24 and 48 hours after treatment. For comparative efficacy, the percentage mortality was transformed into arcsine values, and the means were compared using the Duncan multiple range test.

The mean temperature (27 $\pm$ 1.2 °C) and relative humidity (90 $\pm$ 3.5%) of indoor premises recorded reflect the normal hot and humid indoor tropical environment. VectoBac WG yielded

more than 96% mortality at 48 hours after spraying against all four mosquito species at three of the dosages tested (2.91×10^9, 1.45×10^9, and 0.71×10^9 ITU/ha). VectoBac 12AS gave more than 92.5% mortality at 48 hours after spraying at 2 of the higher doses tested (2.87×10^9 and 1.46×10^9 ITU/ha). Larvae of *An. dirus* were significantly more susceptible than the other test species to both formulations ($p < 0.05$). Even with the lowest dosage, the two formulations were effective, causing >66% mortality at 48 hours after spraying. The results in the kitchen and living-room were similar ($p > 0.05$) for both the formulations against the four mosquito species being tested. Overall, VectoBac WG provided significantly better larvicidal activity ($p < 0.05$) compared with VectoBac 12AS at similar dosages.

Germany – The study describes the production of ice-pellets (named IcyPearls) from droplets of VectoBac WG suspensions, using a liquid nitrogen process and presents the results of a large-scale field test of these pellets against floodwater mosquitoes (Becker, 2003). Four plots of about 100 m^2, each for a different application dosage, were chosen for sampling. At each plot, 10 dips were taken with a standard dipper (350 cm^3). The number of larvae of each instar were counted and recorded before as well as 24 and 48 hours after treatments. The larval density in an untreated plot was used as a control. Data were statistically analysed using Student's *t*-test. The granules were applied by means of an insulated bucket equipped with a rotating device (seeder) and operated by the pilot of a helicopter. The grain size of the IcyPearls averaged 4 mm (33 mm^3). About 30 grains/m^2 were deposited when 10 kg of IcyPearls were applied.

The species composition in the test sites included *Ae. vexans* (85–90%), *Culiseta annulata* (15%), *Ochlerotatus sticticus* (4.7%), and *Ae. rossicus* (4.4%). The application of 100, 200, and 400 g of VectoBac WG in 10 kg of IcyPearls per hectare resulted in larval mortality rates of 91.4%, 97%, and 99.9%, respectively, at 48 hours after the application. The mortality rates achieved with the application of 100 and 200 g of WG in 10 kg of IcyPearls were significantly different ($p < 0.05$), but

there was no significant difference between the application of 200 and 400 g of WG/ha ($p > 0.05$). The application of 400 g of WG in 5 kg of IcyPearls per hectare resulted in 97.6% mortality. This was not significantly different from the mortality achieved with the same rate of the active ingredient in 10 kg of IcyPearls per hectare.

The study showed that the IcyPearls application method was very effective against mosquito larvae at application rates of 100–200 g of VectoBac WG per hectare, which are equivalent to application rates of 0.3–0.6 x 10^9 ITU/ha and are about one-third of the ITU dose normally used in corn-cob-based treatments for floodwater species. An effective control at these lower doses has been attributed to: a) the *Bti* ice pellets melting on the water surface and releasing the toxins in the feeding zone of the mosquito larvae; b) the toxins remaining inside the ice pellets and not being lost by friction in the spraying equipment, c) excellent penetration of the vegetation due to the pearl-like shape and specific weight of the granules; and d) excellent dispersion of WG in water and the low rates of settling of the active ingredients. In routine treatments, even with 7.5 kg of IcyPearls per hectare containing 250 g of VectoBac WG (0.75 x 10^9 ITU/ha), 48 pearls per m^2, there was almost 100% mortality at all sites. The ice formulation increases the swathe size to more than 30 cm, as against 15 cm used for other formulations, and is expected to reduce the costs of aerial application.

Western Kenya – The efficacy of WG formulation of *Bti* (2700 ITU/mg) was evaluated for the control of *An. gambiae sensu lato* in a malaria-endemic area of western Kenya. The laboratory bioassays were followed by efficiency and residual effect assessments in open-field experiments (Fillinger, Bart Knols & Becker, 2003). Preliminary tests were conducted in triplicates of three to six different concentrations (serial dilutions of a stock suspension prepared by suspending 100 mg of product in 1 litre of distilled water) and control. Based on the preliminary trials, five to six different concentrations (ranging from 0.01 to 2 ppm) were applied to 1 litre of lake water dispensed in 1.5-litre plastic containers containing 50 larvae

each along with controls in triplicate. Third-instar larvae of laboratory-reared *An. gambiae s.s.,* originally colonized from specimens from the south-east of the United Republic of Tanzania, were used. The larvae were not fed during the experiments and the mortality was scored after 24 hours. Moribund larvae were considered dead and included in the analyses. If mortality in the control treatment exceeded 10%, the test was discarded and repeated. The bioassays were repeated twice. All the tests were conducted at ambient temperature that ranged from 23 °C to 30 °C. Data from all replicates were pooled and analysed using probit-regression.

The simulated field trials were conducted with offspring of wild *An. gambiae s.l.* females that oviposited in the experimental tub. Plastic tubs (diameter 0.6 m) were buried in an open sunlit field, without vegetation, in two lines (1.5 m apart). Soil and mud from known *An. gambiae* breeding sites were added to each tub (one-third of its volume) to provide suitable biotic and abiotic conditions for mosquitoes. The tubs were subsequently filled with water from Lake Victoria to a depth of 0.2–0.3 m. Colonization of experimental tubs occurred within 2 days and sometimes included larvae of *Cx. quinquefasciatus* and *Cx. tigripes.* After colonization, completion of the larval life cycle was found to take no more than 10 days because of high water temperatures (25 $\pm$ 2 °C in the morning, 31 $\pm$ 2 °C in mid-afternoon). In order to ensure adequate numbers of third- and fourth-instar larvae, treatments were undertaken after 8 days after setting the tubs. By removing mature pupae from all tubs twice a day, care was taken not to allow adult mosquitoes to emerge.

Treatment concentrations were calculated on the basis of a standard water depth of 0.1 m and a fixed surface area and ranged from 0.2 to 1.6 mg/litre (equivalent to surface application of 0.2–1.6 kg/ha) for the *Bti* tests. Six tubs served as controls and six received a specific concentration of the test formulation. Tubs were matched on the basis of larval density so that control and test treatment tubs had similar densities at the start of the experiment. It was noted that the WG

formulations dispersed readily when mixed with water and remained dispersed for at least several minutes.

The *Bti* WG was applied uniformly over the water surface, at concentrations representing one, two, four, and eight times the $LC_{95,}$ with one hand-held sprayer at a fixed volume (250 ml) per tub. All tubs were examined daily and the average number of larvae per dipper (250 ml) was determined by taking five dips per tub, four from the periphery and one from the centre. Immature mosquitoes were classified in three categories: early (first and second) instars, late (third and fourth) instars, and pupae. All larvae were counted, classified to the genus and development stage, and then returned to their respective sites. The percentage reduction in larval mosquito densities was calculated, using the formula of Mulla. The average numbers of all larval instars, late instars, and pupae in the control and treatment tubs were compared daily by non-parametric Kruskal–Wallis one-way ANOVA ($\alpha = 0.05$) and Kruskal–Wallis multiple-comparison Z-value test ($\alpha = 0.05$).

Laboratory bioassays with *Bti* against third-instar larvae of *An. gambiae s.s.* showed that the average concentrations of 0.021 mg/litre (57 ITU/litre) and 0.21 mg/litre (567 ITU/litre) caused 50% and 95% mortality, respectively, after 24 hours of exposure.

In the simulated field trial, the efficacy of *Bti* WG formulation did not differ significantly between *Anopheles* and *Culex* larvae. *Culex* constituted up to 15% of the total larval population in the trial and results from both genera were therefore pooled. Pre-treatment larval densities were statistically similar between control and treated regimens. Treatments were made three times at weekly intervals and different concentrations (0.2-1.6 mg/litre) were tested. *Bti* WG provided 88–100% mortality within 24 hours at all application dosages. The number of larvae in the control during the first treatment period increased, but it declined naturally thereafter, which could have been caused by the reduction in oviposition attractancy often observed in ageing breeding sites and/or an increase in predator density. Considering the late instars only, a reduction

rate of 88–100% was observed up to the fourth day after treatment; nevertheless, a continuing recolonization of all treated sites by early instars was observed.

All concentrations tested were equally effective up to 2days post-treatment for the total number of larvae and up to 4 days when considering the late instars only. No significant difference was seen at any time between the different concentrations being tested. All the dosages were equally effective in lowering pupal populations and gave an overall reduction in mosquito emergence.

2.3 Efficacy – WHOPES supervised trials

Florida – The efficacy of VectoBac WG was compared with that of temephos 1% GR against larvae of three Florida species, *Ae. aegypti, Cx. quinquefasciatus*, and *An. quadrimaculatus*, in outdoor tubs (simulated field conditions) (Nayar & Ali, 2001). Laboratory reared late third- and early fourth-instar larvae of the three species of colonized mosquitoes were exposed to two dosages (0.125 and 0.5 kg/ha) of VectoBac WG and temephos (0.5 and 1.0 kg/ha). Polyethylene tubs (diameter 1 m) containing 100 litres of water (aged for 24 hours) were placed outdoors, under a rainproof shelter with 60% shade cloth. One hundred laboratory-reared late third- and early fourth-instar larvae of one of the mosquito species were introduced into each tub to be tested, and 0.5 g of larval food was added to each tub. Each tub was covered with a nylon mesh screen to prevent other insects from laying eggs in tubs and to exclude falling debris.

After 2–3 hours of the larval acclimation, the tubs were treated with VectoBac WG evenly over the water surface, in a completely randomized manner, using hand-spray bottles; temephos 1% GR was also scattered evenly over the water surface by hand. Four replicates of each dosage of each material and controls were used. All tubs were examined after 48 hours to score the post-treatment larval mortality, and all the live and dead larvae from each tub were collected, using one

net assigned to each tub. A fresh batch of 100 laboratory-reared late third- and early fourth-instar larvae of the same mosquito species, and mosquito food, were introduced into each tub at intervals of 3-4 days, and 48-hour post-treatment mortality/survivorship observations were continued. The test for each species was terminated when the larval mortality caused by the highest treatment rate of VectoBac WG was less than 50%. The percentage reduction data were analysed using one-way ANOVA, with Tukey multiple comparison test.

Against *Cx. quinquefasciatus*, both materials at both low and high dosages gave 87.2–98.7% mean larval reductions at 2 days post-treatment. However, VectoBac WG gave only 21.9% (0.125 kg/ha) and 40.8% (0.5 kg/ha) mean larval reductions at 5 days post-treatment, while temephos 1% GR gave 41.8% (0.5 kg/ha) and 81.6% (1.0 kg/ha) reductions. Activity of both the materials at the two rates declined to less than 23% (range: 5.9–22.9%) at 9 days post-treatment.

Against *Ae. aegypti*, both rates of both test materials produced 100% mortality at 2 days post-treatment. At 5 days post-treatment the higher dosages of each material caused 100% larval mortality while the lower rate of temephos 1% GR and that of VectoBac WG gave 85.6% and 95.4% larval reductions respectively. At 9 days post-treatment, the reductions were <25% at the lower dosages, but >70.7% at the higher dosage of temephos 1% GR and 50% at the higher dosage of VectoBac WG). At 12 days post-treatment, the high dosage of both materials gave <16% larval mortality.

Temephos 1% GR and VectoBac WG were effective against *An. quadrimaculatus*, causing 96.5–100% mortality at both dosages on day 2 post-treatment. The residual activity was low or negligible (2.3–17.1% mean larval mortalities) at 5 days post-treatment for both rates of temephos 1% GR and the lower rate of VectoBac WG. The higher dosage of VectoBac (0.5 kg/ha) gave significantly higher ($p < 0.05$) mean larval mortality of 57.7%. For all three species, no significant difference ($p > 0.05$) was observed in the percentage survival of controls between evaluation days. Thus both materials were effective against

Cx. quinquefasciatus and *An. quadrimaculatus* for 2–5 days and against *Ae. aegypti* for 5–9 days (Table 1). The higher dosage of VectoBac was significantly ($p < 0.05$) more effective than the lower dosages against *An. quadrimaculatus.*

Peru – The efficacy and residual activity of VectoBac WG and temephos 1% GR were evaluated and compared on *An. pseudopunctipennis* (the main malaria vector along the coast of Peru) and *Cx. quinquefasciatus* in artificial ponds in Lima, and on *An. albimanus* (one of the main malaria vectors along the northern coast of Peru and resistant to pyrethroids) in rice fields in Querecotillo, Piura (Ventosilla et al., 2002). The lethal concentrations of the products for each species were determined by the standard bioassays. Eggs or first-instar larvae were collected from natural breeding sites, reared to third- and early fourth-instar larvae, and used in the assay. The temephos concentrations used for *Cx. quinquefasciatus* ranged from 0.06 to 0.4 mg/litre of formulated product, and for VectoBac WG the concentration ranged from 0.004 to 0.02 mg/litre of formulated product. The concentration range of temephos for *An. pseudopunctipennis* was 0.6–6 mg/litre of formulated product and for VectoBac WG 0.06–0.6 mg/litre of formulated product. Three replicates of 25 larvae placed in 150 ml of distilled water for each concentration were used in each bioassay, with their corresponding controls. Solutions were prepared using distilled water (pH 5.5–6.5). Larvae were fed before each bioassay.

Three replicated bioassays were carried out, with an interval of one week between successive tests to correct for possible variation in results caused by larval manipulation and rearing techniques. Larval mortality was measured 24 hours after exposure. Both LC_{50} and LC_{95} were determined for each formulation and compared using a probit regression model.

Residual larvicidal activity was studied in six artificial breeding sites made of cement (2 x 1 x 0.5 m). Fifty second and third instars of *An. pseudopunctipennis* larvae were put into a metal-framed screened cage, with a mesh size of 0.2–0.5 mm^2, 150 larvae of *Cx. quinquefasciatus* in another one. Each pond

had four cages (two with *An. pseudopunctipennis* and two with *Cx. quinquefasciatus* larvae). Two artificial ponds were selected at random for application of VectoBac WG at 50 mg/m^2 (0.5 kg/ha), two for temephos at 2 g/m^2 (20 kg/ha), and two for the controls. Each pond was covered with a metallic mesh (mesh size 1 x 1 cm). The test period was 15 days; a cohort of larvae every 48 hours was exposed and the mortality rates (dead larvae/total larvae) were scored every 24 hours. The variation of residual effect of temephos and VectoBac WG was analysed using single-classification ANOVA after arcsine transformation of data.

The persistence of VectoBac and temephos was evaluated against *An. albimanus* in rice fields in Querecotillo district, Sullana Province, Piura Department. The average size of the rice fields was 278 m^2. Five replicates of each treatment – VectoBac WG, temephos GR, and control – were selected using two parameters: (i) location in relationship to irrigation flow, and (ii) the presence of larvae. The larval density was recorded 24 and 72 hours before application and 24, 48, and 96 hours and 10 and 15 days post-application. A proportion of the late third- and fourth-instar larvae were collected and identified before treatment. A minimum of 60 larval samples were collected at random using the standard dipper (0.5 litre). The number of non-target fauna was recorded, and species were identified. VectoBac WG and temephos GR were applied, using a backpack sprayer. Temephos GR was washed with the same rice-field water, and this liquid was then sprayed.[1] The remaining sand was packed in satin and placed in each corner of each rice field. The study was conducted in the winter season, when the mosquito population was low in Lima and Piura.

The variation in the mean densities of *An. albimanus* larvae was analysed, comparing pre-treatment values with each day post-treatment, using ANOVA for single-factor repeated measures design, in treated and control rice fields. The data were adjusted using logarithmic transformation ($\log x + 1$) before

[1] Not a standard application procedure.

analysis. The reduction of larval densities was compared using the formula of Mulla.

Against *An. pseudopunctipennis*, the LC_{50} and LC_{95} of VectoBac WG were 0.143 and 0.491 mg/litre respectively. For temephos, the LC_{50} and LC_{95} against *An. pseudopunctipennis* were 0.691 and 3.128 mg/litre of granules. Against *Cx. quinquefasciatus*, the LC_{50} and LC_{95} of temephos were 0.092 and 0.245 mg/litre. The LC_{50} and LC_{95} of VectoBac WG against *Cx. quinquefasciatus* were 0.008 and 0.018 mg/litre, indicating that *Culex* was more susceptible to VectoBac WG.

The residual activity of VectoBac WG against *An. pseudopunctipennis* in the artificial ponds was 4.5 days, yielding a 21.1–54.1% reduction in larval population. Thereafter, the activity decreased. The larvicidal activity of temephos against this species was higher, yielding a reduction of 88-99.1% up to 15 days (Table 1). The mean percentage mortality of *Cx. quinquefasciatus* with VectoBac WG treatment was from 98.5–99.5% up to day 6.5. Thereafter, the efficacy decreased gradually. The larvicidal activity of temephos was high, producing 94.1–99.5% of control up to 15 days.

The ponds were evaluated for 15 days, but only data for 10 days were analysed by ANOVA, because three ponds were dry on the 15th day. The larval densities (number larvae/5 dips) from ponds that did contain water on the 15th day were estimated as geometric means.

In the rice fields, larval density showed a decrease after application and a gradual increase from the second day of application. The reduction of the larval population was 99.9% one day after treatment, decreasing to 75.0% at the end of the test in the rice fields treated with VectoBac WG. Temephos reduced the larval population by 97.5% during the same period.

Salatiga, Central Java, Indonesia – The optimum application rate and residual activity of VectoBac WG against *An. maculatus* were determined in stream pools and against *An. sundaicus* in brackish water ponds and were compared with

values for temephos 1% sand granules (GR) (Damar, Widyastuti & Blondine, 2002).

The trial against *An. maculatus* was carried out in ground pools along streams in the Menoreh hills. The upper parts of streams were used as control and the lower parts for treatment. Different sides of the stream were used for VectoBac WG and temephos 1% GR. For a small-scale trial, 15 pools in three categories (small, medium, and large, with a size ranging from 3 to 30 m^2) were selected, three for each dosage and three for control. Three dosages of VectoBac WG (250, 500, 750 g/ha) and one dosage of temephos 1% GR (10 ppm, the standard dosage used in the control programme) were evaluated. Temperature and pH were recorded during the trial. The larval density was determined through 5 dips in small and 10 dips in medium and large pools one day before and 1, 2, 4, 7, 14, and 21 days after treatment. Based on the small-scale trial, VectoBac at 500 and 750 g/ha and temephos 1% GR at 10 ppm were tested on a larger scale following a similar procedure. Twenty-four ground pools in three categories (small, medium, and large) were randomly allocated, six pools for each treatment and control.

In a small-scale field trial against *An. sundaicus*, six ponds (size ranging from 250 to 800 m^2) were used for treatment with VectoBac WG (three each for 500 and 750 g/ha dosages) and three each for temephos (10 ppm) and untreated control. The pH, salinity, and temperature were recorded during the trial. Application of VectoBac WG and temephos 1% GR was done using a backpack sprayer mist blower with a solid stream nozzle. Larval collections (20 dips for medium ponds and 25 for large ponds) were made 1 day pretreatment and 1, 2, 4, 7, 14, and 21 days post-treatment. For the medium-scale evaluation, six ponds each were selected for treatment and control. VectoBac WG at 500 and 750 g/ha and temephos 1% at 10 ppm were evaluated following a similar procedure. Percentage reduction of larval densities was calculated using Mulla's formula.

In pools treated with temephos (small-scale trial), the reduction of *An. maculatus* larval densities was 100% for 21 days. At all three dosages of VectoBac WG, the reduction of *An. maculatus* larval densities was 100% for 4 days. At 250 g/ha, the reduction was 70% at day 7 and <70% at day 14 post-treatment. At 500 g/ha and 750 g/ha the effect lasted for 14 days with >70% reduction in larval densities. In the medium-scale trial, temephos at 10 ppm and VectoBac WG at 500 and 750 g/ha gave similar results (>70% reduction for 7–14 days).

In the ponds treated with temephos in the small-scale trial, a 92.1–100% reduction of *An. sundaicus* larval densities was evident for 21 days. With VectoBac at 500 g/ha and 750 g/ha, the reduction was 70–100% for 14 days after treatment. In the medium-scale trial, temephos gave similar results (95.2–100% reduction for 21 days). In ponds treated with VectoBac at 500 g/ha, the reduction ranged from 95.1% to 100% for 7 days and was 51.68% 14 days post-treatment. The reduction at 750 g/ha was 73.8 –100% for 14 days, except in large ponds where it was 21.8% on post-treatment day 14.

VectoBac WG was significantly more effective at dosages of 750 and 500 g/ha than it was at 250 g/ha. Temephos was effective for 21 days, causing 90–100% mortality, and was better than VectoBac at 500 and 750 g/ha, which was effective for 14 days, causing 70–100% mortality (Table 2). There was no significant difference between the application rates of 500 and 750 g/ha of VectoBac WG, and the mortality rates did not differ significantly with the size of the pools at any of the tested dosages. The physical characteristic of water in the stream pools before and during the trial did not fluctuate significantly; the average water temperature was 25–26 °C, and pH was 7.

Penang, Malaysia – The bioefficacy and residual activity of VectoBac WG against the dengue vectors *Ae. aegypti* and *Ae. albopictus* were compared with those of temephos 1% GR (Zairi, 2003a). In the laboratory study, three dosages of VectoBac WG (1, 2 and 5 mg/litre) and one of temephos (0.11 g/litre) were used, along with untreated water as a control. The study was conducted in two different types and sizes of

containers, i.e. large glass jars (3000 ml) and earthenware jars (45 litres), containing 2000 ml and 40 litres of water respectively. Both types of jars had their water level marked at 10 cm from the top of the jar. The water was allowed to season for at least 48 hours before the experiment. Twenty laboratory-cultured late third- or early fourth-instar larvae of *Ae. aegypti* or *Ae. albopictus* were introduced into the jars at 24 hours, 3 days, 7 days, 2 weeks, 3 weeks, and 4 weeks post-treatment until the efficacy was less than 50%. VectoBac WG was weighed and sprinkled on the water surface of the containers. The larval mortality was recorded at 24 and 48 hours post-treatment. Two treatment regimes were adopted: one set of jars (three per dosage and three controls), with no replenishment of water; the other set (also three per dosage plus three controls) were replenished daily (300 ml for glass jars and 6 litres for earthenware jars) to simulate daily water usage. Three sets of experiments were carried out.

A total of 100–150 houses in the suburbs of Penang Island were surveyed, using ovitraps to determine the presence of *Ae. aegypti* and *Ae. albopictus*. The number of mosquito larvae found in household water containers was recorded. Except for 10 houses with a relatively high mosquito population, being used as untreated controls, positive containers in all other houses were treated with VectoBac WG at 2 mg/litre.

In addition, 30 houses were chosen in the Teluk Kumbar Township, with a relatively higher *Aedes* breeding population; 10 houses were used for each formulation (VectoBac WG and temephos GR) and the remaining 10 houses for control. Three large glass jars (3000 ml) and three earthenware jars (45 litres) were placed in and around each selected house and treated with VectoBac WG at 2 mg/litre and temephos at 0.11 g/litre. The water volume used per jar and the regime for water replenishment were the same as in the simulated study in the laboratory. In both field trials, the number of larvae found in the jars was recorded at 24 and 72 hours after treatment and thereafter every fifth day post-treatment for 2 months. From the larvae and pupae collected in the jars at 5-day interval until the

end of the trial, *Ae. albopictus* (58%), *Ae. aegypti* (41.5%), and *Cx. quinquefasciatus* (0.5%) were identified.

In the laboratory, VectoBac WG was effective against *Ae. aegypti* and *Ae. albopictus* released in earthenware and glass jars with and without replenishment of water, causing >70% larval mortality for 5–8 weeks at 1 and 2 mg/litre and for 7–9 weeks at 5 mg/litre (Table 2). Temephos at 0.11 g/litre was effective in both test designs, giving more than 90% larval mortality throughout the 11-week experimental period.

In the trial against the natural mosquito population in household containers, there were no *Aedes* larvae breeding after the treatment (3–50 days) (Table 2). With the containers introduced into the houses and treated with temephos GR, 100% of larval reduction was recorded up to 65 days in all the jars. VectoBac WG was effective against *Ae. albopictus* and *Ae. aegypti* mosquitoes in the earthenware jars with daily water replenishment for 65 days, giving 78.4% reduction of larvae, compared with 80% of reduction for 40 days in jars that were not replenished. In the large glass jars, with water replenishment, VectoBac WG provided good larvicidal effect up to day 55, with 85.8% reduction; when water was not replenished, VectoBac WG gave a 66.7% reduction on post-treatment day 45.

Table 1. Residual effect of VectoBac WG, as mean percentage reduction of larval density, in standing/stagnant mosquito breeding sites or simulated field conditions

Place	Habitat	Species	Dosage (g/ha)	Duration of effect (days)		
				>90%	>80%	>70%
California	Fibreglass tubs	*Cx. stigmatosoma*	300	3	3	7
		Cx. tarsalis and	590	3	3	7
		Cx. incidens	1200	2	2	2
			3000	2	2	7
Florida	Polyethylene tubs	*Cx. quinquefasciatus*	125	0	2	2
			500	2	2	2
		Ae. Aegypti	125	5	5	5
			500	5	5	5
		An. Quadrimaculatus	125	2	2	2
			500	2	2	2
Peru	Artificial ponds	*An. Pseudopunctipennis*	500	0	0	0
		Cx. quinquefasciatus	500	6.5	6.5	6.5
Western Kenya	Plastic tubs	*An. Gambiae*	200	3	3	3
			400	4	4	4
			800	4	4	4
			1600	4	4	4

Table 1 (continued)

Place	Habitat	Species	Dosage (g/ha)	Duration of effect (days)		
				>90%	>80%	>70%
Indonesia						
small-scale	Stream pools	*An. Maculatus*	250	4	7	7
			500	7–14	14	14
			750	7	7	14
	Brackish ponds	*An. Sundaicus*	500	7	7	14
			750	7	7	14
medium-scale	Stream pools	*An. maculatus*	500	4	7	14
			750	4–7	4–7	7–14
	Brackish ponds	*An. Sundaicus*	500	7	7	7
			750	7	7	7–14
Peru	Rice fields	*An. Albimanus*	500	4	7	10

Table 2. Residual effect of VectoBac WG, as mean percentage reduction of larval density against container-breeding mosquitoes in Penang, Malaysia (laboratory and field studies)

NR = no replenishment of water; R = replenishment.

Habitat	Species	Dosage (mg/litre)	Duration of effect (weeks)		
			>90%	>80%	>70%
Earthenware jars (NR)	*Ae. Aegypti*	1	6	6	6
		2	4	5	6
		5	5	7	7
Earthenware jars (R)	*Ae. Aegypti*	1	6	6	6
		2	5	6	6
		5	8	8	9
Glass jars (NR)	*Ae. Aegypti*	1	7	7	8
		2	5	8	8
		5	8	8	8

Table 2 (continued)

Habitat	Species	Dosage (mg/litre)	Duration of effect in weeks		
			>90%	>80%	>70%
Glass jars (R)	*Ae. Aegypti*	1	6	6	8
		2	7	8	8
		5	8	9	9
Earthenware jars (NR)	*Ae. Albopictus*	1	6	6	7
		2	6	7	7
		5	8	8	8
Earthenware jars (R)	*Ae. Albopictus*	1	6	6	6
		2	6	6	6
		5	8	8	8
Containers	*Ae. Aegypti* and *Ae. Albopictus*	2	1-7	1-7	1-7

Table 2 (continued)

Habitat	Species	Dosage mg/L	Duration of effect in weeks		
			>90%	>80%	>70%
Earthenware jars (R)	*Ae. Aegypti* and	2	7	7	9
Earthenware jars (NR)	*Ae. Albopictus*		5	6	6
Glass jars (R)	*Ae. Aegypti* and	2	6	8	8
Glass jars (NR)	*Ae. Albopictus*		5	5	5

2.4 Conclusions

The microbial larvicide *Bacillus thuringiensis* subsp. *israelensis* produces insecticidal crystal proteins (ICP) on fermentation. These proteins are highly specific with toxicity only to mosquito and blackfly larvae. The ICP, spores, and vegetative cells of *Bti* administered by different routes were found to be mostly non-pathogenic and non-toxic to various organisms. *Bti* has no adverse effects on humans, birds, earthworms, fish, and many aquatic invertebrates other than mosquitoes and blackflies. A few species of aquatic invertebrates are susceptible to high dosages of *Bti*. Thus, *Bti* products are unlikely to pose any hazard to humans, other vertebrates, or invertebrates provided that they are free from non-*Bti* microorganisms and from biologically active products other than the ICPs. *Bti* free of microbial and chemical contaminants is safe for use in aquatic environments, including drinking-water sources where mosquitoes breed.

The water-dispersible granular formulation (WG) of *Bacillus thuringiensis,* subsp*. israelensis* has several advantages over the conventional formulations currently in use, including improved stability, ease of handling, low bulk density, and ease of shipment. Additionally, the WG formulation disperses and mixes well with water, requiring little agitation.

In laboratory tests, VectoBac WG has shown good activity against mosquito species of the genera *Aedes, Anopheles*, and *Culex*. The WG formulation showed a high level of activity against *Culex* mosquitoes with LC_{50} in the range 0.008-0.024 mg/litre and LC_{90} 0.018–0.059 mg/litre. The product is more effective against *Ae. aegypti,* a container-breeding mosquito, than against *Anopheles* mosquitoes (LC_{50} = 0.021– 0.143 mg/litre, LC_{90} = 0.21–0.491 mg/litre).

In simulated field studies, VectoBac WG also exhibited good efficacy against mosquito larvae. *Culex* mosquitoes were controlled (>70% reduction) by application rates of 125 to 500 g/ha for 2–7 days. *Ae. aegypti* mosquitoes were controlled (>70% reduction) at 125 to 500 g/ha for 5 days. In some

studies, however, control was achieved for 5–9 weeks at application rates of 1–5 mg/litre. *Anopheles* species were controlled (>70% reduction) for 2–4 days at dosages of 125 to 800 g/ha. *Anopheles* spp. breeding in brackish water and rice fields were effectively controlled at 500 to 750 g/ha for 7-14 days, as were mosquitoes in stagnant areas of streams (Tables 1 and 2).

The WG formulation can be applied to mosquito larval habitats as aqueous sprays as well as thermal and cold fogs, and can be applied indoors and outdoors. *Bti* formulations, especially, the WG formulation, may offer a good option for sustainable mosquito control. Although the efficacy of *Bti* against stagnant water mosquitoes is short-lived, the conservation of natural enemies under a regimen of *Bti* treatments may eventually lead to regulation of mosquito larvae by the predators in some developmental sites. This aspect of *Bti* intervention measures should be determined in operational control programmes.

2.5 Recommendations

1. VectoBac WG possesses high efficacy against larvae of many mosquito species in a variety of habitats, and is safe to humans, wild life, and other non-target biota. It is recommended for mosquito larval control in open bodies of water at dosages of 125–750 g/ha. Higher dosages may be applied in habitats with dense vegetation and high in organic matter.

2. VectoBac WG is highly effective against container-breeding mosquitoes (*Ae. aegypti* and *Ae. albopictus*) and is recommended at target dosages of 1–5 mg/litre.

3. VectoBac WG should meet WHO specifications and should be free of chemical and microbial contaminants.

3. REVIEW OF PERMANET

3.1 Specifications

The PermaNet® is a deltamethrin-treated mosquito net manufactured by Vestergaard-Frandsen, Denmark, and proposed in 2000 as a long-lasting, wash-resistant insecticidal net. The net is made of polyester, multi-filament fibres and treated to a target concentration of 55 mg a.i./m^2 with deltamethrin. The deltamethrin is bound in a resin coating that reduces the amount of insecticide lost during routine washing. In 2002, the manufacturer released a "second generation" PermaNet 2.0 that is reported to have improved manufacturing quality control and wash resistance. PermaNet 2.0 is available in standard shapes and sizes and different colours. The following are extracts from the manufacturer's product standard for PermaNet® 2.0:

		Test standard
Yarn	75 or 100 denier Multi-filament: 36 filaments 100% polyester	ISO 2060 ISO 1833
Mesh size	156 holes/$inch^2$	
Weight	75 denier: 30 g/m^2 100 denier: 40 g/m^2	ISO 3801
Bursting strength	75 denier: 220 kPa 100 denier: 320 kPa	ISO 2960
Fabrication	Warp-knitted	ISO 8388
Dimensional stability	Shrinkage <5%	ISO 5077
Fire safety	16	CFR 1610

3.2 Efficacy – background/supporting data

Based on the information provided by the manufacturer to WHO, the difference between first-generation PermaNet and PermaNet 2.0 is improved manufacturing quality assurance.

Reports on the testing/evaluation of first-generation PermaNet have therefore been included in this assessment.

3.2.1 Laboratory studies

First-generation PermaNet

Colombia and Bolivia – The wash resistance of PermaNet was compared with that of conventionally treated nets under laboratory and semi-field conditions in Colombia and Bolivia (Gonzales et al., 2002). Test mosquitoes were wild, unfed *Anopheles* (*An. darlingi* and *An. albitarsis*) and *Culex* species. For the laboratory study, PermaNets were compared with nets treated with deltamethrin (K-O Tab® at 25 mg a.i./m^2), lambda-cyhalothrin micro-encapsulated suspension (15 mg a.i./m^2), and alpha-cypermethrin SC (40 mg a.i./m^2). Nets were washed gently for 5 minutes using bar soap and cold water. Each net was bioassayed after 3 washes while the PermaNet was bioassayed again after 4, 10, and 20 washes. The authors used a non-standard bioassay method for testing nets, as mortality was low in standard WHO cone bioassays with a 3-minute exposure. Nets were tested against 40 mosquitoes in cone bioassays with an exposure time of 30 minutes. Knockdown was recorded at 60 minutes and mortality at 24 hours after exposure. After 3 washes, *Anopheles* mortality was 100% on all nets except for the net treated with lambda-cyhalothrin where mortality was 97.6%. Conventionally treated nets were not tested after 3washes. Mortality on the PermaNet was 100% after 4 and 10 washes for both *Anopheles* and *Culex* species. After 20 washes, mortality was 81.3% and 87.5% respectively.

For the study of wash-resistance under local conditions, women washed the nets in their usual way. Nets were soaked for 30-60 minutes in tap water with washing powder and then rubbed over rocks for 3-5 minutes. Nets were then allowed to dry in a shady place. The insecticides and washing frequencies tested are listed in Table 3.

Table 3. Insecticides and washing frequencies tested (Gonzales et al., 2002)

Insecticide	Concentration (mg a.i./m^2)	Washing frequency
Deltamethrin tablets	25	2 days
Deltamethrin SC	25	2 days
Deltamethrin tablets	25	7 days
Alpha-cypermethrin SC	40	7 days
PermaNet	55	7 days

Table 4. Bioassay mortality (%) of *Anopheles* species on nets washed every 7 days (Gonzales et al., 2002)

Insecticide	1 wash	2 washes	3 washes	4 washes	10 washes	20 washes
Deltamethrin SC, 25 mg a.i./m^2	70.2	67.4	31.8	41.3		
Deltamethrin WT, 25 mg a.i./m^2	84.2	71.6	63.3	43.5		
PermaNet, 55 mg a.i./m^2				92.6	83.7	87.1

Bioassays were performed in the same manner as for the laboratory experiments. When nets were washed every 2 days, vector mortality after 4 washes declined to 43.5% for deltamethrin tablets and 41.3% for deltamethrin SC. When nets were washed every 7 days, mortality after 4 washes was 43.3% for nets treated with deltamethrin, 63.8% for nets treated with alpha-cypermethrin, and 92.6% for the PermaNet. Mortality for the PermaNet was 83.7% after 10 washes and 87.1% after 20 washes. Results for bioassays against *Anopheles* species are summarized in Table 4.

PermaNet was slightly less effective against *Culex* species, with 83.8% mortality after 10 washes and 71.2% mortality after 20 washes.

Iran (Islamic Republic of) – Bioassay tests were conducted against *An. stephensi* to measure the effects of washing, drying, dust, and smoke on the efficacy of PermaNet compared with a net treated with deltamethrin tablets at 25 mg a.i./m^2 (Kayedi et al., 2002). Nets were washed for 3 minutes under local conditions and then rinsed twice in cold water. They were dried in different positions, either in the sun or in the shade. Some nets were left outside to allow dust and dirt to accumulate and then smoked for 3 minutes to simulate the effect of cooking indoors. Bioassays were conducted using wire balls after 0, 5, and 15 washes with field-collected *An. stephensi*. Bioassays included 3-minute exposures followed by measurement of knockdown at 60 minutes and mortality at 24 hours. Additional bioassays were conducted in which mosquitoes were introduced into the wire ball and observed until all were knocked down, recording the time of knockdown for each mosquito. Washes were repeated every 3 days. Results of bioassay mortality following a 3-minute exposure are summarized in Table 5.

Table 5. Bioassay mortality (%) of *An. stephensi* 24 hours after a 3-minute exposure (Kayedi et al., 2002)

Insecticide	Unwashed	5 washes	15 washes
Deltamethrin WT	84.1	79.5	64.5
PermaNet	93.6	98.6	89.8

Time to 50% knockdown (KT_{50}) increased from 529 seconds on an unwashed K-O Tab-treated net to 873 seconds after 15 washes. For the PermaNet, KT_{50} was 462 seconds on an unwashed net and 534 seconds on a net washed 15 times. The authors reported that the insecticidal activity of the PermaNet was significantly greater than the K-O Tab-treated nets after 5 and 15 washes. In addition, the bioactivity of K-O Tab-treated nets was significantly lower on nets washed 15 times, compared with nets that had been washed 5 times or had never been washed. Dust, dirt, and smoke had no effect on either of the nets, but a significant degree of insecticidal activity was lost when nets were left to dry in the sun.

3.2.2 Experimental hut studies

First-generation PermaNet

Côte d'Ivoire – PermaNet was evaluated in experimental hut trials in Côte d'Ivoire to determine the effect of washed nets on the behaviour of *An. gambiae* and *Cx. quinquefasciatus* in field conditions (Mosquiera et al., 2002). There was a high prevalence of *kdr* resistance in *An. gambiae* in this area (frequency of resistance allele = 99%). Six experimental huts were built next to rice fields, with concrete bases and a moat to exclude ants and other scavengers. Each hut had four window traps that allowed unimpeded entry of mosquitoes but limited their exit. Exiting rates were measured using a veranda trap located in the back of each hut. One human subject slept in each hut every night from 20:00 to 05:00. Sleepers were rotated through different huts throughout the study. In the morning, sleepers collected live and dead mosquitoes from their huts,

noting the location (room or veranda trap) where each was collected. Live mosquitoes were held for 24 hours to check for delayed mortality. Outcome measures included deterrent effect, excito–repellent effect, blood-feeding inhibition, and mortality. Six types of nets were tested: a conventional deltamethrin net (25 mg a.i./m^2), an unwashed PermaNet, a PermaNet washed 10 times, a PermaNet washed 20 times, and 2 untreated control nets. Nets were washed with Marseille soap in clean water, rinsed 3 times in clean water and allowed to dry for 5hours. Washing was done once a day before nets were installed in the huts. Two nets of the same type were rotated each week within the same hut throughout the study.

All treated nets, regardless of washing, reduced the entry rate of *An. gambiae* by 28– 48% compared with huts with control nets. The rate of early exit of *An. gambiae* females averaged 26% in the control huts, but was between 49% and 56% in huts with treated nets. Again, washing of the PermaNet did not reduce the exit rate compared with the unwashed PermaNet. Blood-feeding success was 34% in the control huts but less than 10% for all treated nets. PermaNets washed 10 times were less effective in reducing blood-feeding success compared with the deltamethrin control net and an unwashed PermaNet. However, no differences in blood-feeding success were observed between the PermaNet washed 20 times and the unwashed PermaNet or the deltamethrin control net. The overall mortality was less than 10% in the control huts, while it exceeded 50% in huts with a treated net. Washing of the PermaNets did not reduce the mosquito mortality in the experimental huts. Compared with the deltamethrin control net, washing did not reduce the efficacy of the PermaNet. Reductions in entry rates, induced exophily, reduction in blood feeding, and induced mortality are summarized in Table 6.

Similar trends were noted with *Cx. quinquefasciatus*. Compared with the untreated controls, entry rates were reduced by 37% in the deltamethrin control hut and by 26–32% in huts with PermaNets. No effect of washing was observed. Exit rates were 22.4% in the control huts and ranged from 51% to 61% for the treated nets. Washing of the PermaNets had no significant

impact on the exit rates. The blood-feeding success of *Cx. quinquefasciatus* was 35.4% in the control huts and less than 10% for all treated nets except for the PermaNet washed 10 times, for which blood-feeding success was 11.5%. Overall mortality was 4.3% for the control nets and greater than 40% for all treated nets. Interestingly, mortality induced by the PermaNets rose from 44.7% in the unwashed nets to 58.1% in the PermaNet washed 20 times.

Standard cone bioassays with a 3-minute exposure were done using a susceptible reference strain of *An. gambiae* (Kisumu strain) and the local field strain (Yaokoffikro) with a high level of pyrethroid resistance. Outcomes measured included knockdown immediately after exposure and mortality at 24 hours (Table 7). Bioassays using the Kisumu strain resulted in 27–40% knockdown, with slightly lower knockdown for the unwashed PermaNet and the PermaNet washed 20 times. Mortality was high, with >90% mortality for all treated nets. Mortality decreased slightly with increasing numbers of washes: mortality was 100% for the unwashed PermaNet, 96% for the PermaNet washed 10 times, and 91% for the PermaNet washed 20 times. Against the Yaokoffikro strain, knockdown was <5% for all treatments. Mortality was also lower for all treatments relative to the Kisumu strain. The unwashed PermaNet caused 40% mortality, the PermaNet washed 10 times caused 33% mortality, and the PermaNet washed 20 times caused 17% mortality. The deltamethrin control caused 59% mortality. The authors concluded that, overall, the PermaNets washed 10 or 20 times performed as well as an unwashed PermaNet and as well as a standard net treated with deltamethrin at 25 mg a.i./m^2.

In a study conducted in the same experimental huts, similar results were obtained with unwashed PermaNets when compared with nets treated with combinations of pyrethroid and non-pyrethroid insecticides (Guillet et al., 2001).

Table 6. Reduced entry rate, induced exophily, blood-feeding reduction, and induced mortality relative to control huts (Mosquiera et al., 2002)

Average number of females collected from 2 control huts = 387.5

	PermaNet, Unwashed	PermaNet washed 10 times	PermaNet Washed 20 times	Deltamethrin SC, 25 mg a.i./m^2, unwashed
Total females	279	201	250	253
Entry rate (reduction)	–28%	–48%	–36%	–34%
Induced exophily	+105%	+89%	+117%	+96%
Blood feeding (reduction)	–86%	–73%	–82%	–90%
Mortality	53%	47%	59%	50%

Table 7. Bioassay mortality (%) in standard WHO cone tests with a 3-minute exposure (Mosquiera et al., 2002)

	Control	PermaNet, unwashed	PermaNet washed 10 times	PermaNet washed 20 times	Deltamethrin SC, 25 mg a.i./m^2, unwashed
Kisumu	6	100	96	91	100
Yaokoffikro	2	40	33	17	59

Table 8. Average mortality of *An. gambiae* exposed in a 3-minute cone bioassay (Gimnig et al., 2003)

Two samples were tested from each net except for nets tested during the 6-month follow-up, when only one sample per net was tested. Columns with similar letters were not significantly different at a = 0.01.

	PermaNet	Factory-treated	Field-treated
Baseline	97.3 [a]	99.9 [a]	97.8 [a]
6 months	72.6 [a]	77.0 [a]	46.1 [b]
12 months	81.9 [a]	62.7 [a,b]	49.4 [b]
18 months	70.8 [a]	39.1 [b]	
24 months	42.5		

3.2.3 Field studies

First-generation PermaNet

Burkina Faso – PermaNet was evaluated under field conditions for 18 months in Burkina Faso against wild caught *An. gambiae s.l.* (Muller, Ido & Traore, 2002). New PermaNets were washed twice and mean deltamethrin concentrations were measured after xylene extraction and gas chromatography.

Mean deltamethrin concentration was 47.1 mg/m^2 on unwashed nets (n = 6), 38.6 mg/m^2 on nets washed once (n = 3), and 20.1 mg/m^2 on nets washed twice (n = 3).

Randomly selected nets that had been distributed to villagers for use in an area of north-western Burkina Faso were collected after 6months, 12 months, and 18 months for bioassay and determination of deltamethrin concentrations. At 6 months, the average deltamethrin concentration on nets (n = 8) was 24.1 mg/m^2. Average deltamethrin concentration fell to 3.7 mg/m^2 at 12 months (n = 11) and to 1.6 mg/m^2 at 18 months (n = 5).

In standard 3-minute bioassays, the mortality of *An. gambiae s.l.* was 84% after 6 months of use in the field (n = 7), 54% after 12 months (n = 9), and 7% after 18 months (n = 2).

Malawi – PermaNet was evaluated against *An. gambiae* (Kisumu strain) under field conditions over 2 years in Malawi (Gimnig et al., 2003). PermaNets were compared with standard deltamethrin-treated nets, using K-O Tabs for a target dose of 25 mg a.i./m^2, and against factory-treated nets at a target dose of 50 mg a.i./m^2. Nets were distributed in July 2001 to 514 households in 15 villages. Baseline bioassays were conducted on two patches (25 cm x 25 cm) taken from 12 unused nets from each treatment arm. At 6-month intervals, 25 nets were sampled from each treatment group. Two net patches were taken from each net sampled, except during the 6-month follow-up when only one patch per net was taken. Net patches were exposed to a standard 3-minute bioassay in a

cone test, and mortality was recorded 24 hours post-exposure. The strain of mosquitoes tested was *An. gambiae* Kisumu. At least 40 mosquitoes were tested on each net patch. Repeated measures Poisson regression was used to compare mosquito mortality rates between treatment arms at each time period.

At baseline, the mosquito mortality was >95% for all treatment arms, and no significant differences were observed between the different nets (Table 8). After 6 months, the average bioassay mortality for the field-treated nets was 46.1%. This was significantly lower than the average bioassay mortality for factory-treated nets (77.0%) and PermaNets (72.6%). There was no significant difference between the factory-treated nets and the PermaNets. After 12 months of use in the field, the average bioassay mortality of the field-treated nets was again the lowest at 49.4%, which was significantly lower than the mortality caused by the PermaNets (81.9%). Factory-treated nets caused intermediate mortality (62.7%), and there were no significant differences between these nets and either the PermaNets or the field-treated nets. Since bioassay mortality was consistently below 50% for the field-treated nets, these nets were re-treated and dropped from further consideration. After 18 months in the field, PermaNets caused 70.8% mortality in the bioassays, which was significantly higher than the 39.1% mortality caused by the factory-treated nets. After 24 months in the field, however, the average bioassay mortality for the PermaNets had fallen to 42.5%.

Using standard criteria for minimal effectiveness of the nets (knockdown at 60 minutes $\geq$75%, or mortality at 24 hours $\geq$50%), 100% of all nets were minimally effective at the baseline. The factory-treated nets rapidly lost effectiveness, with 60% being minimally effective at 6 months and 40% minimally effective at 12 months. For the factory-treated nets, 88% were minimally effective after 6 months, 64% after 12 months, and 44% after 18 months. More than 80% of the PermaNets retained minimal effectiveness up to the 24-month follow-up, when only 44% of nets were minimally effective.

Kenya – PermaNets (55 mg a.i./m^2) were evaluated under field conditions against *An. gambiae* (pink-eye strain) in comparison with a conventional deltamethrin-treated net (K-O Tab, 25 mg a.i./m^2) and four additional long-lasting net candidates (Lindblade et al., 2003). Three nets were treated with deltamethrin and three with permethrin at varying concentrations. All nets were made of polyester except the Olyset net which was made of high-density polyethylene.

Nets were distributed in July 2002 to compounds with three houses. Each compound received either deltamethrin- or permethrin-treated nets, which were randomly assigned to each house on the compound. Each net was permanently marked with a unique identifier. At intervals of 3–4 months, all nets were visited and three cone bioassays with a 3-minute exposure were conducted on each net. Ten *An. gambiae* (pink-eye strain) were tested in each cone and mortality was recorded at 24 hours post-exposure. If bioassay mortality exceeded 50%, that net was revisited within 1 month. If a net had two consecutive visits where bioassay mortality was <50%, that net was considered to have "failed".

After 1 year, the PermaNet performed best, with only five failures. Statistical analysis indicated that PermaNets were significantly less likely to fail, compared with a standard deltamethrin-treated net.

Uganda – PermaNets were evaluated under field conditions in western Uganda against *An. gambiae* (Kisumu strain) and *Cx. quinquefasciatus* (S-lab, a susceptible reference strain); their retention of insecticidal efficacy was also compared with that of standard deltamethrin-treated nets (Killian et al., 2003). To assess changes in net efficacy over time, 450 PermaNets and 140 conventional deltamethrin-treated nets (25 mg a.i./m^2) were distributed in December 2000 to 295 households in western Uganda. Regular surveys were conducted to assess frequency of adverse reactions, proper use of nets, the condition of the nets, the frequency of washing, and the owner's perception of the nets.

At approximately 6-month intervals, 40 nets from each study arm were randomly sampled and two pieces were removed from each net sampled. These pieces were used for WHO cone bioassays. Two additional pieces were taken for chemical residue analysis. After the 6-month survey, only one sample was taken from each net for bioassay and one sample for chemical residue analysis. Bioassays were conducted with *An. gambiae* (Kisumu strain) and *Cx. quinquefasciatus* (S-lab strain). Bioassays were a standard 3-minute exposure in a WHO cone test, with knockdown recorded at 60 minutes and mortality recorded at 24 hours post-exposure. Deltamethrin residues were measured by gas chromatography after xylene extraction.

Adverse reactions observed among net users included nose, eye, and skin irritation, objections to the smell, and feeling hot. There was no association between net type and the frequency of adverse reactions, although the PermaNets seemed to cause fewer adverse reactions than conventional nets. Most nets were in use at the first survey, with only 2.2% of the PermaNets and 2.0% of the conventional nets not hanging in the households. Washing frequency was lower for the PermaNets early in the study but increased in the latter half of the study. There were no significant differences, however, in washing frequency between the net types at any survey.

Chemical residue analysis and bioassays were conducted on the conventional nets at baseline and after 6 months, and on the PermaNets at baseline, 6 months, 12 months, and 20 months. Bioassay mortality of *An. gambiae* was >95% at baseline. By 6 months, the bioassay mortality for conventional nets had fallen to 12.4% and for PermaNets to 31.7%. PermaNets were followed for an additional 14 months, while the conventional nets were re-treated after the 6-month follow-up. Bioassay mortality continued to decline, to 23.9% at 12 months and to 15.5% at 20 months. Both nets caused higher rates of knockdown but the trends were the same. For *Cx. quinquefasciatus*, baseline mortality was low: 75.5% for PermaNets and 85.8% for the conventional nets. At 6 months, bioassay mortality fell to 8.1% for the PermaNets and to 2.4%

for the conventional nets. Using criteria for minimal net effectiveness of ≥75% knockdown or ≥50% mortality of *An. gambiae* in bioassays, all nets were minimally effective at the baseline. At 6 months, only 25% of conventional nets were minimally effective while 70% of the PermaNets were minimally effective. By 20 months, only 30% of PermaNets were minimally effective. The bioassay mortality results are summarized in Table 9.

Table 9. Bioassay mortality (%) of *An. gambiae* after a 3-minute exposure in WHO cone tests (Killian et al., 2003)

	Baseline	6 months	12 months	20 months
Deltamethrin WT 25 mg/m^2	99	12		
PermaNet	96	32	24	16

Chemical residue analyses correlated well with bioassay results. Initial deltamethrin concentrations were 47.5 mg/m^2 on PermaNets and 16.9 mg/m^2 on conventional nets. After 6 months, deltamethrin concentrations had declined to 2.5 mg/m^2 and 0.7 mg/m^2 respectively. After 6 months, deltamethrin concentrations on the PermaNets continued to decline, although the rate of decline slowed considerably and there was still 1.6 mg/m^2 on nets after 27 months.

Given the poor performance of both nets, samples were re-treated 14 months after the study began and these were followed up 6 months after re-treatment. Deltamethrin concentrations at this time were 3.8 mg/m^2 on the PermaNets and 3.3 mg/m^2 on the conventional nets. Bioassay mortality was 65.1% for re-treated PermaNets and 44.4% for re-treated conventional nets. These results indicate that the PermaNet can be re-treated in the same way as a conventional net, if necessary.

Statistical analysis of the deltamethrin concentrations and the bioassay results indicated that the decline in deltamethrin concentrations and bioassay performance differed between the PermaNets and the conventional nets. Additional statistical tests suggested that the PermaNets comprised of two groups of nets: one performed well over 20–27 months, while the second performed poorly within the first 6 months after distribution.

Second-generation PermaNet

Uganda – In October 2002, second-generation PermaNets (PermaNet) 2.0 were introduced into the studies reported above by Killian et al. (2003). Nets were tested at baseline and after 6 months in the field. These were compared with conventional nets re-treated at 21 months.

At baseline, the chemical residue analysis indicated 69.2 mg/m^2 of deltamethrin on the PermaNet. This declined only slightly to 65.5 mg a.i./m^2 after 6 months. The decline in deltamethrin concentration was much lower compared with the first-generation PermaNet on which deltamethrin concentration fell from 47.5 mg a.i./m^2 at baseline to 2.5 mg a.i./m^2 after 6 months. Bioassay results against *An. gambiae* correlated well with the deltamethrin concentrations. Mortality of mosquitoes exposed to PermaNets 2.0 was 100% at baseline and after 6 months. These results suggest that the PermaNet 2.0 is an improved product.

3.3 Efficacy – WHOPES supervised trials

3.3.1 Laboratory studies

First-generation PermaNet

Montpellier, France – PermaNets were tested against *An. gambiae* (Kisumu strain) under different washing and reactivation regimens to determine whether the reactivation time affected their efficacy and performance in a laboratory setting (Duchon, Finot & Hougard, 2002). Fifty samples (25 cm x 25 cm) from a single PermaNet were used for the washing study, while an additional five samples were kept for chemical analysis. Nets were divided into two groups; one group was washed every other day while the other group was washed every 7 days. Washing was done according to a standard WHO washing protocol. Net samples were placed in a beaker with 0.5 litre of deionized water and 2 g/litre of soap; they were shaken at 155 movements per minute for 10 minutes. The nets were rinsed twice for 10 minutes in clean water, using the same shaking conditions. They were then tested using a standard WHO cone test and in a tunnel test. In the cone test, 5 mosquitoes were tested at a time, with a total of 50 mosquitoes tested per sample. Mosquitoes were exposed for 3 minutes; knockdown was recorded after 60 minutes and mortality after 24 hours. Cone test bioassays were conducted on five net samples after 0, 1, 5, 10, 15, and 20 washes. Net samples were bioassayed only once and then set aside for chemical assays. After 20 washes, each net was reactivated at 60 °C for 6 hours.

With a 1-day interval between washes, the mortality remained above 80% after 15 washes then dropped to 12% after 20 washes. With a 7-day interval between washes, the mortality rapidly dropped to 32% after only 5washes. Heat reactivation of PermaNets washed 20 times restored some activity: nets washed at 1-day intervals (mortality = 77%) regained more activity than nets washed at 7-day intervals (mortality = 31%).

Montpellier, France – The PermaNet was evaluated against *An. gambiae* (Kisumu strain) under laboratory conditions to test its wash resistance compared with nets treated with deltamethrin SC at 25 mg a.i./m^2 using conventional treatment procedures (Hougard & Duchon, 2001). Four samples of the PermaNet and four of the conventional deltamethrin-treated net were washed up to 20 times; WHO cone bioassays with a 3-minute exposure were performed after 0, 5, 10, 15, and 20 washes. There were 4 days between washing and bioassay to allow for reactivation of the net. For each bioassay, the outcomes measured were knockdown after 60 minutes and mortality after 24 hours. Chemical analysis of unwashed PermaNets indicated an average deltamethrin concentration of 43.6 mg/m^2. At the baseline, both the PermaNet and the conventional nets caused 100% knockdown and 100% mortality.

After 5 washes, average knockdown was 54% and mortality was 8% for the conventional net. For the PermaNet, these figures were 100% and 91% respectively. Closer examination of the conventional net, with bioassays after each wash, indicated that bioactivity declines after just 2 washes and mortality falls below 10% after 5 washes. Conventional nets were not tested beyond 5 washes because mortality was low. After 10 washes, the PermaNet retained high knockdown activity (99%) but mortality declined to 26%. After 15 washes, the PermaNet had lost significant bioactivity as measured by both knockdown (52%) and mortality (6%).

To determine whether additional time was needed for reactivation after 15 washes, the PermaNets were divided into two lots. One lot was bioassayed 4 days after the last wash and the other lot was bioassayed 2 and 18 days after the last wash. Four days after the last wash, the average knockdown of the first lot was 52% and average mortality 6%. For the other lot, knockdown and mortality after 2 days were 67% and 7% respectively; however, 18 days after the last wash, knockdown had risen to 100% and mortality to 52%.

Two net samples were washed an additional 10 times with 4 days between washing and bioassay. After 25 washes, knockdown was 20% and mortality 15%. After 30 washes, knockdown was 9% and mortality 10%. Nets were tested again 16 and 33 days after the final wash. Knockdown rose to 63% after 16 days but fell to 32% after 33 days; mortality actually declined with longer reactivation periods. The two samples that had been washed 30 times were then retreated with deltamethrin SC at 25 mg/m^2. Both knockdown and mortality returned to 100%.

In tests of mosquito irritability, the median time for a mosquito to take off from a net was 9.7 seconds for an unwashed PermaNet, 10.8 seconds for a net washed 10 times, 29.7 seconds for a net washed 20 times, and 50.3 seconds for a net washed 30 times.

The efficacy of washed nets was also tested against *Cx. quinquefasciatus*. At baseline, both PermaNets and conventional nets caused 100% knockdown and 100% mortality. Conventional nets were not tested beyond 5 washes because mortality was low. After 10 washes, PermaNets caused 44% knockdown and 11% mortality. After 20 washes, these figures were 0% and 3% respectively and after 30 washes, there was 0% knockdown and 0% mortality of *Cx. quinquefasciatus*. Irritability tests demonstrated a similar decline in bioactivity, with *Cx. quinquefasciatus* taking off after 17.5 seconds from an unwashed PermaNet and after 146 seconds from a PermaNet washed 30 times. Re-treatment with deltamethrin SC 25 mg/m2 restored bioactivity, with 100% knockdown and 100% mortality.

Second-generation PermaNet

Montpellier, France – PermaNets 2.0 were evaluated against *An. gambiae* (Kisumu strain) under laboratory conditions to determine their efficacy after a series of washes compared with nets conventionally treated with deltamethrin (Duchon, Herve & Hougard, 2003a). Five PermaNets 2.0 and five conventionally treated nets (deltamethrin 25 mg a.i./m^2) were evaluated

against non-blood-fed female *An. gambiae s.s.* (Kisumu strain) that were 2–5 days old. Seven pieces of netting (25 cm x 25 cm) were cut from each net and one piece from each net was washed 0, 2, 4, 6, 10, 15, or 20 times (five samples for each number of washings). After washing the appropriate number of times, the netting samples were exposed to WHO cone bioassays and the netting sample was then stored at 4 °C before being chemically analysed for its deltamethrin concentrations. Washing was done by placing the netting samples in a beaker with 500 ml of water and 2 g/litre of soap (savon de Marseille) and shaking in a water-bath at 30 °C for 10 minutes at 155 movements per minute. Washing was done at 7-day intervals. Bioassays involved 3-minute exposures of 5 mosquitoes in a cone test. The test was repeated 10 times so that 50 mosquitoes were tested against each sample. After exposure, mosquitoes were held in paper cups for 24 hours. Knockdown was recorded after 60 minutes and mortality was recorded after 24 hours. Tunnel tests were done after 0, 10, and 20 washes. A guinea-pig was held in one end of a 75-cm tunnel (25 cm high and 25 cm wide), separated from the rest of the tunnel by a sample of the netting material that was being tested. Nine holes were cut in the netting material which was positioned so that mosquitoes had to enter through the netting to feed on the guinea-pig. Female mosquitoes were introduced into the tunnel and were collected approximately 15 hours later. Immediate and delayed mortality were recorded, as was with blood-feeding inhibition.

For the cone tests, there was no decline in knockdown caused by PermaNet 2.0 even after 20 washes, while a significant reduction was observed after just 4 washes of conventional nets. Mortality decreased from 100% to 26% after just 2 washes of the conventional nets and fell below 10% after 6 washes. For the PermaNet 2.0, a similar though much less marked trend was observed. Mortality remained stable at 30–50% after 10 washes.

Deltamethrin concentrations on the nets were measured after the bioassays by a WHO Collaborating Centre in Belgium. For the conventional net, the initial concentration was 24.7 mg/m^2. This declined to 5.7 mg/m^2 after 2 washes, 2.5 mg/m^2 after 4 washes, 0.7 mg/m^2 after 6 washes, and 0.3 mg/m^2 after 10 washes. Deltamethrin was essentially undetectable at less than 0.1 mg/m^2 after 15 and 20 washes . For the PermaNet 2.0, the initial concentration of deltamethrin was 47.5 mg/m^2. This declined gradually to 21.5 mg/m^2 after 20 washes. A summary of these findings is given in Table 10.

For the tunnel tests, with conventional nets washed 10 or 20 times, mortality was very low (less than 30%) and more than 50% of mosquitoes had succeeded in obtaining a blood meal. For the PermaNets 2.0, mortality was greater than 95% and feeding inhibition was 100%, regardless of how many times the nets had been washed.

3.3.2 Experimental hut studies

First-generation PermaNet

Côte d'Ivoire – The effect of washing of PermaNets on mosquito behaviour was assessed in experimental huts in Côte d'Ivoire in an area where *An. gambiae* remains susceptible to pyrethroids (Traore-Lamizana et al., 2003). Hut construction and test methods were similar to those used by Guillet et al. (2001) and Mosquiera et al. (2002). Nets tested in the huts included an unwashed PermaNet, a PermaNet washed 10 times, a PermaNet washed 20 times, a deltamethrin control net, and an untreated control net. The PermaNets were washed for 10 minutes using bar soap in clean water. Nets were rinsed three times in clean water and allowed to air dry before the next wash. Nets were washed once per day. There were three replicates for each arm of the study, with one replicate per arm tested each night and replicates rotated each night. The same type of net was used in the same hut throughout the study.

Table 10. Deltamethrin concentrations (mg/m^2) ± 95% C.I. of PermaNets 2.0 and conventional deltamethrin-treated nets after repeated washing (Duchon, Herve & Hougard, 2003a)

	Unwashed	2 washes	4 washes	6 washes	10 washes	15 washes	20 washes
Conventional net	24.7 ± 1.1	5.7 ± 0.7	2.5 ± 0.5	0.7 ± 0.2	0.3 ± 0.2	<0.1	<0.1
PermaNet 2.0	47.5 ± 10.1	43.4 ± 11.9	46.1 ± 11.3	39.0 ± 12.1	33.5 ± 6.3	29.4 ± 10.3	21.5 ± 10.1

Table 11. Reduced entry rate, induced exophily, blood-feeding reduction, and induced mortality relative to control huts (Traore-Lamizana et al., 2003)

Total number of females collected from control huts = 1357

	PermaNet, unwashed	PermaNet washed 10 times	PermaNet washed 20 times	Deltamethrin SC, 25 mg/m^2, unwashed
Total females	297	862	631	269
Entry rate (reduction)	–78%	–36%	–61%	–80%
Induced exophily	+76%	+75%	+69%	+76%
Blood feeding (reduction)	–48%	–27%	0%	–66%
Mortality	48%	27%	17%	44%

Compared with an untreated control, deterrence was highest with the deltamethrin control (80%) and the unwashed PermaNet (78%), and lowest for the PermaNet washed 10 times (36%). Compared with the control hut, there were significantly fewer *An. gambiae* in all huts with a treated net except for the hut with the PermaNet washed 10 times. Significantly more *An. gambiae* exited into the veranda trap in huts with treated nets compared with the hut with an untreated net. The exit rate was 35.2% in the control hut and ranged from 69.1% in the hut with the PermaNet washed 20 times to 75.8% in the huts with either an unwashed PermaNet or a deltamethrin control net. Compared with the control net, the deltamethrin control, the unwashed PermaNet, and the PermaNet washed 10 times significantly reduced the blood-feeding success. However, there was no difference in blood-feeding success between the control net (17.5%) and the PermaNet washed 20 times (17.7%). The overall mortality rate was 6.2% for the control nets but significantly higher for the unwashed PermaNet (47.8%) and the deltamethrin control nets (43.5%). Mortality declined significantly in nets washed 10 times (26.5%) and 20 times (17.3%). Results for entry rate and blood feeding, as well as induced exophily and mortality, are summarized in Table 11.

Standard cone tests were conducted on all nets when they were installed in the huts and again 3 months later. Fifty mosquitoes (*An. gambiae*, Kisumu strain) were tested per net in a standard 3-minute exposure, with mortality recorded at 24 hours. Mortality did not change over the course of the study but differences were noted between the nets. The control net caused very little mortality (<5%). The unwashed PermaNet and the deltamethrin control nets caused 100% mortality while the PermaNets washed 10 and 20 times caused 64.9% and 40% mortality respectively.

Second-generation PermaNet

Benin – Experimental hut trials of the PermaNet 2.0 were carried out in Benin in an area where *An. gambiae* is susceptible to pyrethroids (Akogbeto et al., 2003). Experimental huts were constructed near rice fields at the Malanville field station. Hut construction and methods were similar to those used by Guillet et al. (2001) and Mosquiera et al. (2002). Collections were done 3 days a week over 6 months.

Nets tested included an unwashed PermaNet 2.0, a PermaNet 2.0 washed 10 times, a PermaNet 2.0 washed 20 times, a conventional deltamethrin-treated net, and an untreated polyester net. Nets were washed using bar soap in clear water at weekly intervals. Washing lasted 10 minutes and was followed by three rinses in clear water. Two nets were prepared for each arm of the study, except the untreated control arm. One net was used for the experimental hut study, while another was set aside for chemical residue analysis.

Washed and unwashed PermaNets 2.0 reduced hut entry rates of *An. gambiae s.l.* by 25–28%; these rates were significantly lower than those for the conventional deltamethrin-treated net (54%). Exophily of *An. gambiae* was 21% for the untreated control net and significantly higher for all the deltamethrin-treated nets except for the PermaNet 2.0 washed 20 times. Mortality was high (>60%) for all insecticide-treated nets and 23% for the untreated net. The differences were all statistically significant. The mortality induced by the PermaNet 2.0, however, declined with increasing numbers of washes. Results are summarized in Table 12.

Standard cone bioassays with a 3-minute exposure were conducted on each net used in the hut trial at the beginning and the end of the trial. Knockdown was recorded at 60 minutes and mortality at 24 hours post-exposure. Mosquitoes used in the bioassays were *An. gambiae* (Kisumu strain) and a local susceptible strain of *An. gambiae s.s.*

Bioassays conducted at the start of the trial indicated high efficacy of all insecticide-treated nets. Mortality and knockdown of Kisumu strain mosquitoes were 100% for all treatments except the PermaNet 2.0 washed 20 times, which caused 87% mortality and 90% knockdown. A similar trend was observed for the local strain, although the overall mortality and knockdown of these mosquitoes was lower for all nets tested except the unwashed PermaNet 2.0. At the end of the trial, the nets lost some efficacy. For the Kisumu strain, bioassay mortality was highest for the unwashed PermaNet 2.0 (97%) and lowest for the PermaNet 2.0 washed 20 times (63%). For the local strain, the trend was similar but lower overall. Bioassay mortality was highest for the unwashed PermaNet 2.0 (77%) and lowest for the PermaNet washed 20 times (56%). Knockdown rates at the end of the hut trial were all $\geq$90% for both mosquito strains tested against all net treatments.

Pakistan – PermaNets 2.0 were evaluated in laboratory and field conditions in Pakistan (Graham & Rowland, 2003). Pieces of netting (25 cm x 25 cm) were washed at weekly intervals in 500 ml of 2 g/litre soap solution ("Sufi"). Nets were washed in a shaker bath with 155 movements per minute for 10 minutes. This was followed by two rinses in clean water for 10 minutes, again with shaking at 155 movements per minute. PermaNets 2.0 were compared with polyester nets conventionally treated with deltamethrin. Bioassays were done after 0, 3, 5, and 10 washes using *An. stephensi* in a standard 3-minute exposure in a WHO cone test. Washing and bioassays were performed on four replicates per treatment.

All nets caused high mortality (>95%) at the baseline. Mortality for the conventionally treated nets dropped and after 10 and 15 washes nets was 63% and 27% respectively. For the PermaNet 2.0, mortality was 99.5% after 5 washes, 99.0% after 10 washes, and 93% after 15 washes.

Washed and unwashed PermaNets were also evaluated in outdoor, overnight platform trials. Two men slept under nets on elevated, ant-proof platforms 6 m x 5 m. Six holes (4 cm x 4 cm) were cut in each test net to simulate a torn net. The entire platform was covered with an untreated net. For the first half of the night, mosquitoes attracted to the sleepers and to a cow tethered nearby were captured and placed inside the untreated net. At dawn, live and dead mosquitoes were collected and blood-feeding success was scored. Live mosquitoes were held for 24 hours to assess delayed mortality. Nets tested were untreated nets as controls, PermaNets, and conventional deltamethrin-treated nets. Three nets were rotated for each treatment type and a total of 21 replicate nights were run for treatment type. The PermaNets and the deltamethrin-treated nets were washed 0, 10, or 20 times. Washing was done by soaking the nets for 60 minutes in water with 2 g/litre of soap before washing with soap for 30 minutes in a 2-way spin washing-machine. Finally, nets were rinsed for 15 minutes in the washing machine before being dried horizontally out of direct sunlight.

Outcome measures in the platform bioassay included the number of mosquitoes that successfully blood-fed, the number of mosquitoes dead at dawn, and the number dead after 24 hours. All treated nets caused significantly higher mortality among *Culex* spp. mosquitoes than an untreated net. Mortality of *Culex* spp. mosquitoes was higher in washed and unwashed PermaNets compared with washed and unwashed conventional deltamethrin-treated nets. There was no significant difference in *Culex* spp. mortality among the PermaNets washed 0, 10, or 20 times. For anopheline mosquitoes, washed and unwashed PermaNet caused significantly higher mortality than an untreated net or a conventional deltamethrin-treated net that was washed 20 times. There was no significant difference in anopheline mortality between conventional nets that were unwashed or washed 10 times and any of the PermaNet treatments.

Table 12. Reduced entry rate, induced exophily, blood-feeding reduction and induced mortality relative to control huts (Akogbeto et al., 2003)

PermaNets 2.0 were washed once per week
Total number of females collected from control huts = 644

	PermaNet, Unwashed	PermaNet Washed 10 times	PermaNet washed 20 times	Deltamethrin SC, 25 mg/m^2, unwashed
Total females	461	468	482	297
Entry rate (reduction)	–28%	–27%	–25%	–54%
Induced exophily	+57%	+30%	+9%	+37%
Blood feeding (reduction)[a]	NS	NS	NS	NS
Mortality	88%	73%	61%	87%

[a] Blood feeding was very low in all treatments, including the control, since there were no holes in the nets.

Table 13. Percentage mortality and percentage blood-fed *Anopheles* spp. in overnight platform bioassays (Graham & Rowland, 2003)

Nets were holed and washed at weekly intervals

	Mortality (%)	Blood-fed (%)
Deltamethrin, unwashed	18	12
Deltamethrin, washed 10 times	21	8
Deltamethrin, washed 20 times	14	12
PermaNet 2.0, unwashed	21	13
PermaNet 2.0, washed 10 times	22	11
PermaNet 2.0, washed 20 times	21	16
Untreated control	16	9

Blood-feeding inhibition of *Culex* spp. was greatest for conventional nets washed 10 times. Washed and unwashed PermaNets caused significantly greater feeding inhibition in *Culex* spp. compared with an untreated net, an unwashed conventional net, or a conventional net washed 20 times. For anophelines, there was no evidence in blood-feeding inhibition for the PermaNets, with >10% of anophelines successfully feeding in all PermaNet treatments (Table 13). Feeding success was lowest for the conventional net washed 10 times (7.8%), followed by the untreated net (9.0%).

The low mortality relative to the control and low blood-feeding rates in all treatments were probably the result of the zoophilic nature of mosquitoes tested in this region.

3.4 Conclusions

PermaNet 2.0 is a long-lasting insecticidal mosquito net, treated with deltamethrin (55 mg a.i./m^2) mixed in a resin that coats the netting fibres and releases the insecticide progressively, so that the net retains efficacy after repeated washings. Although PermaNet is treated at a higher concentration than nets conventionally treated with deltamethrin (target concentration 25 mg a.i./m^2), the transient side-effects reported for first generation PermaNet were no more frequent .

The first-generation PermaNet gave inconsistent results in terms of initial deltamethrin concentration and wash resistance Some batches indicated wash resistance up to 20 WHO standard laboratory washes, while others were no more wash-resistant than nets conventionally treated with deltamethrin. Re-treatment of exhausted PermaNet by conventional dipping restored insecticidal activity. PermaNet 2.0 gave consistent results for initial deltamethrin concentration and wash resistance.

Unwashed PermaNet 2.0 was just as effective as conventional deltamethrin-treated nets against both susceptible and pyrethroid-resistant mosquitoes. Laboratory washing studies and experimental hut studies comparing washed and unwashed PermaNet 2.0 confirm that it retains insecticidal activity for up to 20 washes.

In response to the urgent needs of control programmes, when a WHO-recommended insecticide has been used in the manufacture of long-lasting insecticidal mosquito nets, interim recommendations may be given after specific requirements of laboratory and small-scale field studies (experimental huts) have been met. Interim recommendations will be reviewed and full recommendations may be given, based on data from large-scale studies in different settings.

3.5 Recommendations

1. Considering the safety, efficacy and wash-resistance of PermaNet 2.0, *interim recommendation* is given for its use in the prevention and control of malaria.

2. WHO should support and facilitate large-scale field studies of PermaNet 2.0 to confirm its long-lasting efficacy in the prevention and control of malaria and other vector-borne diseases in different settings.

4. REVIEW OF GOKILAHT-S 5EC

4.1 Safety assessment[1]

Gokilaht®-S 5EC is an emulsifiable concentrate formulation consisting of *d,d-trans*-cyphenothrin, a synthetic pyrethroid insecticide.

Information on the safety and adverse effects of *d,d-trans*-cyphenothrin comes almost exclusively from studies performed on "cyphenothrin". Cyphenothrin is nominally a racemic mixture of (RS, 1RS-*cis-trans*-cyphenothrin); however, the product that has usually been called cyphenothrin is actually enantio-enriched, =95%R, =75% trans. *d,d-trans*-Cyphenothrin is a further enriched enantiomer mixture, essentially a single stereoisomer (1R-*trans*-chrysanthemic acid esterified with enantiopure (s)-a-cyano-3-phenoxybenzylalcohol). As the product does not contain any new impurities either, it is practically excluded that the toxicity of *d,d-trans*-cyphenothrin is qualitatively different from that of "cyphenothrin". Structure–activity data from other pyrethroids would indicate that, quantitatively, *d,d-trans*-cyphenothrin could be somewhat more toxic than the "racemic" cyphenothrin; the limited available information on acute toxicity of cyphenothrin and *d,d-trans*-cyphenothrin seems to confirm this prediction: acute oral LD_{50} values of cyphenothrin and *d,d-trans*-cyphenothrin in male rats have been reported as 318 and 188 mg/kg body weight respectively, and in females as 419 and 220 mg/kg. On the other hand, similar differences in the toxicity to *Daphnia magna* and rainbow trout were not observed – in fact, the LC_{50} values of cyphenothrin for these aquatic species were somewhat lower than those of *d,d-trans*-cyphenothrin.

[1] This assessment is based on the confidential summary of toxicity studies on *d,d-trans*-cyphenothrin, provided by Sumitomo Co., Japan, and on a summary of toxicology data on cyphenothrin from the California Environmental Protection Agency, and was performed by International Programme on Chemical Safety (IPCS) Secretariat.

It is considered that the safety assessment presented below, although based on information on cyphenothrin, is valid also for *d,d-trans*-cyphenothrin.

Cyphenothrin has moderate acute oral toxicity, and the International Programme on Chemical Safety (IPCS) has classified it as "moderately hazardous". Structurally it is a type II pyrethroid, and the clinical signs of toxicity include tremor, salivation, and convulsions. Neither the human nor the environmental safety of cyphenothrin has been evaluated by WHO or by the Joint Expert Committees of WHO and FAO (JECFA/JMPR).

Cyphenothrin did not induce gene mutations in bacteria, or sister chromatid exchanges in Chinese hamster ovary cells in vitro, or micronuclei in bone marrow erythrocytes in mice. It gave no evidence of carcinogenicity in acceptable long-term studies in rats or mice. No effects on reproductive capacity was observed in two-generation studies in rats, and no embryotoxicity or teratogenicity was observed in rats or rabbits at the highest tested dose levels (which were toxic to the dams).

Cyphenothrin was negative in eye and skin irritation studies, and did not indicate sensitizing potential in a Buehler test.

In a 52-week toxicity study in dogs, tremors, salivation, convulsions, and one death were observed at the dose of 60 mg/kg per day. Tremors and reduced spontaneous movements were observed at a dose of 30 mg/kg, and at 10 mg/kg the animals exhibited vomiting. The no observed effect level (NOEL) was given as 3 mg/kg body weight per day.

d,d-trans-Cyphenothrin is very toxic to *Daphnia* (48-hour LC_{50} = 1.2 µg/litre) and to rainbow trout (96-hour LC_{50} = 0.38 µg/litre), but toxicity to birds is low (bobwhite acute dietary toxicity LC_{50} > 5620 mg/kg). Cyphenothrin is not mobile in the environment. With the proposed conditions of use, environmental exposure is expected to be low.

The following are extracts from the Material Safety Data Sheet (MSDS) of the manufacturer, Sumitomo, for cyphenothrin:

Acute oral LD_{50} rat	318 mg/kg male, 419 mg/kg, female
Acute dermal LD_{50}, rat	>5000 mg/kg
Acute inhalation LD_{50}	1850 mg/m^3
Skin irritation, rabbit	Not an irritant
Eye irritation, rabbit	Not an irritant
Sensitization	Not a sensitizer

4.2 Efficacy – background/supporting documents

Manufacturer (Sumitomo) – The biological efficacy of *d,d-trans*-cyphenothrin has been studied and compared with that of cyphenothrin and permethrin. The primary lethal activity of *d,d-trans*-cyphenothrin (Gokilaht-S), cyphenothrin (Gokilaht) and permethrin against the housefly (*Musca domestica*), *Cx. pipiens pallens*, and the German cockroach (*Blatella germanica*) by topical application is almost 2 times and 2.2 times higher than that of cyphenothrin and permethrin, respectively. The mean knockdown activity (KD_{50}) of *d,d-trans*-cyphenothrin against the housefly is 2.2 times and 7.3 times higher than that of cyphenothrin and permethrin, respectively. Knockdown activity of *d,d-trans*-cyphenothrin against *Cx. pipiens pallens* is 2.5 times and 6.9 times that of cyphenothrin and permethrin, respectively. Against the housefly, the lethal activity of *d,d-trans*-cyphenothrin is 2.32 and 1.90 times higher than cyphenothrin and permethrin, respectively. In tests against crawling insects (German cockroaches) using the CSMA (Chemical Specialties Manufacturers Association) spray method, *d,d-trans*-cyphenothrin exhibited 4.2 times and 57 times higher knockdown activity than cyphenothrin and permethrin. *d,d-trans*-Cyphenothrin is 3.4 times and 4.4 times as lethal as cyphenothrin and permethrin to German cockroaches. The relative flushing activity of *d,d-trans*-cyphenothrin against German cockroaches is 3.0 and 14.9 times greater than that of cyphenothrin and permethrin.

India – The biological efficacy of cyphenothrin 5% EC has been studied (IIBAT, 2003) in Peet–Grady chambers against the housefly and mosquitoes (*Ae. aegypti* and *An. stephensi*) and on wood and cement against American cockroaches (*Periplaneta americana*) and German cockroaches. In tests against the housefly, a concentration of 15 mg/chamber gave a mean KT_{50} of 9.9 minutes in two replicates. By increasing the concentration to 25 mg/chamber, the mean KT_{50} was reduced to 6.4 minutes. A similar trend was observed in tests against mosquitoes. Against *Ae. aegypti*, at a concentration of 2.5 mg/chamber, mean KT_{50} was 9.6 minutes, while at 7.5 mg/chamber, the mean KT_{50} was 5.6 minutes. Similar results were obtained against *An. stephensi*. In residual tests against American and German cockroaches, cyphenothrin performed more effectively on wood than on cement at concentrations of 100, 125, and 150 mg a.i./m^2.

4.3 Efficacy-WHOPES supervised trials

4.3.1 Laboratory studies

France – The biological activity of *d,d-trans*-cyphenothrin against mosquitoes has been studied (Duchon, Herve & Hougard, 2003b) by methods including larval bioassays, filter-paper tarsal contact tests, adult irritability test, topical application (adult), and surface residual effect. The test mosquito species were susceptible and resistant strains of *An. gambiae* and *Cx. quinquefasciatus*. The reference susceptible strains of *An. gambiae* originated from Kenya and those of *Cx. quinquefasciatus* originated from California. The resistant strains were field collected (*Cx. quinquefasciatus* from Côte d'Ivoire and *An. gambiae* from Burkina Faso) and maintained under continuous selection with permethrin. Both were homozygous for the *kdr* gene. In the resistant *Cx. quinquefasciatus* strain, monooxygenase resistance mechanisms were also involved.

Larval tests

Larval bioassays were conducted with 5-8 concentrations of the technical material of *d,d-trans*-cyphenothrin and late third- or early fourth-instar larvae in triplicates. Larval mortality was recorded after 24 hours and the results were subjected to probit analysis. Results showed that *d,d-trans*-cyphenothrin was about 7-10 times more toxic against susceptible *Cx. quinquefasciatus* than against susceptible *An. gambiae*. For the resistant strains, the ratio was approximately 40. *d,d-trans*-Cyphenothrin was less effective against the *kdr* resistant strains than against susceptible strains of both mosquito species.

Adult tests

Tarsal contact tests were carried out using filter-papers treated with technical grade *d,d-trans*-cyphenothrin. The tests followed the WHO filter paper method: 25 females were exposed to the filter-paper in WHO susceptibility-testing tubes for 1 hour. Each concentration was replicated four times and each test was replicated three times. Mortality was recorded 24 hours after exposure.

In contrast to the larval test results, cyphenothrin was much more active against susceptible *An. gambiae* than against susceptible *Cx. quinquefasciatus*; 0.5% of *d,d-trans*-cyphenothrin induced 100% mortality of *An. gambiae* compared with 8% for *Cx. quinquefasciatus*. Based on direct mortality observations, the tentative diagnostic dosage was determined to be 1% and 16%, respectively, for the two species.

In the resistant strain of *Cx. quinquefasciatus*, 8% *d,d-trans*-cyphenothrin induced only 15% mortality, but at 4% it induced 100% mortality in *An. gambiae*. Again, these results are based on direct observation.

d,d-trans-Cyphenothrin also induced high knockdown effects in both susceptible and resistant strains. For *An. gambiae*, 100% knockdown was induced in the susceptible strain by a 0.1% concentration of the technical grade and in the resistant strain

by a 5% concentration. For *Cx. quinquefasciatus*, the corresponding values were 1% and 2%.

Irritability tests

Female mosquitoes were released into plastic cones affixed to treated filter-paper containing 0.5% a.i. The time elapsed between the first landing and the next take-off of the mosquito was recorded as the "time for first take-off". The times for 50% and 95% of the mosquitoes to take off (FT_{50} and FT_{95}) were calculated from cumulative frequencies.

The results showed that *An. gambiae* was three times more irritated by *d,d-trans*-cyphenothrin than *Cx. quinquefasciatus*, irrespective of resistance status. The FT_{50} was 6.5 seconds for susceptible *An. gambiae* and 6.8 seconds for the resistant strain, compared with 21.3 seconds and 17.9 seconds for susceptible and resistant strains of *Cx. quinquefasciatus*, respectively.

Topical application

A calibrated microcapillary tube was used to apply 0.1 µl active ingredient diluted in acetone to the dorsal pronotum of 2–5-day-old unfed female mosquitoes, which were maintained on a cold surface after brief anaesthesia with CO_2. After treatment, females were maintained at 27 ± 2 °C and 80 ± 10% relative humidity and allowed to feed on honey. Mortality was recorded after 24 hours. Fifty females were used for each concentration. Three replicates were tested. The data were subjected to computerized probit analysis.

The results showed that, based on the LC_{50} values, topically applied *d,d-trans*-cyphenothrin was 7.3 times more toxic to the susceptible strain of *An. gambiae* than to the susceptible strain of *Cx. quinquefasciatus*. Similarly, the resistant strain of *An. gambiae* was 10 times more susceptible than the resistant strain of *Cx. quinquefasciatus*.

Efficacy and residual effect on solid surfaces

This study was conducted in the laboratory to test the efficacy and residual effect of *d,d-trans*-cyphenothrin on three substrates: hardwood (non-resinous), plaster of Paris, and mud (from Africa). The EC formulation of Gokilaht-S was sprayed manually onto the substrate (10 samples each) which was then allowed to dry in a horizontal position. After drying, samples were stored in a vertical position at 28 °C and 80% relative humidity. For each test series, one sample was selected randomly and was tested only once. After spraying, efficacy was measured every month for 6 months. Standard WHO cone tests were conducted in which 15 non-fed 2–5-day-old females were introduced per cone. Outcome was measured as knockdown (KD) after 60 minutes and 24-hour mortality. Only the susceptible strain of *An. gambiae* was tested.

No activity was observed on mud or plaster surfaces, even at the highest dosage of 500 mg/m^2 immediately after spraying. On wood, however,100% mortality was observed at 500 mg/m^2. The observed differences in performance may be due to rapid absorption by the porous mud and plaster surfaces. On the non-resinous wood surface, the chemical retained its efficacy. In the residual testing on wood, *d,d-trans*-cyphenothrin was still effective after 6 months at 200 mg/m^2 (87% mortality) and at 500 mg/m^2 (96% mortality).

Malaysia – The biological efficacy of Gokilaht-S 5EC as space spray was studied against *Ae. aegypti, Cx. quinquefasciatus*, and *An. sinensis*, using a modified glass chamber and Peet–Grady chambers (Zairi, 2002).

Glass-chamber testing

A glass chamber measuring 70 x 70 x 70 cm was used. Twenty laboratory-reared sucrose-fed adult females of susceptible *Ae. aegypti* or *Cx. quinquefasciatus* or *An. sinensis* strains were released into the chamber. Gokilaht-S was sprayed into the chamber with a hand-sprayer and the dosage was determined. The test dosages were 5.80–5.90 mg/m^3, 2.82–2.95 mg/m^3,

1.45–1.50 mg/m^3, and 0.74–0.75 mg/m^3. The number of mosquitoes knocked down was recorded at regular intervals up to 20 minutes. After this, all mosquitoes were transferred into plastic containers and fed with a 10% sucrose pad. Mortality was recorded after 24 hours. Controls were set up similarly in another chamber without spraying. All tests were at least triplicated at 26–28 °C and 65–85% relative humidity.

Peet–Grady chamber testing

A glass chamber measuring 180 x 180 x 180 cm was used. The test procedures and the test mosquitoes (50 each for each test) were similar to those used in the glass-chamber tests. The dosages tested were: 0.63–0.69 mg/m^3, 0.34–0.35 mg/m^3, and 0.17 mg/m^3.

Twenty-four-hour mortality in glass-chamber and Peet–Grady chamber tests ranged from 81% to 100% for all species; the least susceptible was *An. sinensis*. In addition, the calculated time to knockdown was inversely related to dosage, but there were wide confidence intervals, which extended greatly over time.

4.3.2 Field studies

Malaysia – The field efficacy of Gokilaht-S 5EC against *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* was compared with that of cyphenothrin EC using thermal fogging and cold fogging machines indoors and outdoors (Zairi, 2003b). The test site for outdoor spraying was an open football field measuring 100 x 200 m (2 ha), while the indoor spraying was conducted in single-storey terraced houses in an urban settlement on Penang Island.[1]

Outdoor test

The spray equipment consisted of vehicle-mounted cold foggers and thermal foggers for outdoor testing. The application

[1] Thermal and cold fog applications used diesel oil and water as carriers, respectively.

dosages were 0.5, 1.0, and 3.5 g a.i./ha with a spray volume of 0.5 litre/ha. The adulticidal effect of Gokilaht-S 5EC and cyphenothrin EC was assessed by introducing 20 laboratory-reared, sucrose-fed adult females aged 2–5 days into cylindrical cages, made of fine mesh netting with wire frame supports, placed at 10, 25, 50, 75, and 100 m downwind and 1.5 m above ground. The knockdown rate of the test mosquitoes was recorded at 0,10, 20, 30, 40, and 60 minutes post-spraying. After field exposure, the mosquitoes were transferred into clean plastic cups and fed with 10% sucrose pads. Mortality was measured after 24 hours. Larvicidal activity was evaluated by placing paper cups each containing 20 larvae (late third and/or early fourth instars) of a test species below each test cage. After field exposure, the larval cups were returned to the laboratory and mortality was recorded after 24 hours. The temperature, relative humidity, and wind velocity during cold fogging trials were 25.2–27.0 °C, 79.0–88.0%, and 0.60-1.30 m/s.

In the study using the vehicle-mounted cold fogger, Gokilaht-S 5EC at 3.5 g a.i./ha induced KD rates of 93% and 94% after 20 minutes and 24-hour mortality rates of 96% and 92% against *Ae. aegypti* and *Ae. albopictus* respectively. By comparison, cyphenothrin at the same dosage induced lower 20-minute KD rates (93% and 85%) and lower 24-hour mortality (80% and 70%) against both species. Dosages of 1 and 0.5 g a.i./ha of each formulation induced lower but significant knockdown and mortality in both mosquito species. At all dosages, both Gokilaht-S 5EC and cyphenothrin EC showed low adulticidal activity against *Cx. quinquefasciatus*, with less than 50% mortality at 24 hours post-spraying. However, Gokilaht-S 5EC at 3.5 g a.i./ha induced 79% knockdown at 60 minutes post-treatment. No larvicidal activity of either formulation was observed in any of the test mosquitoes.

For the outdoor thermal fogging using a vehicle-mounted thermal fogger, the temperature was 24.1–29.5 °C, the relative humidity 79–90%, and the wind velocity 0.40–1.80 m/s. As with cold fogging, both tested products exhibited high adulticidal activity. Gokilaht-S 5EC at a dosage of 3.5 g a.i./ha induced

mortality of 98% and 94% against *Ae. aegypti* and *Ae. albopictus*, respectively. KD_{20} rates of 100% for *Ae. aegypti* and 99% for *Ae. albopictus* were observed. Similar high mortality and knockdown were observed with cyphenothrin EC at 3.5 g a.i./ha. At lower dosages, both formulations induced moderate (>60%) KD after 60 minutes and 24-hour mortality. No larvicidal activity of either formulation against either species was observed. At 3.5 g a.i./ha, adulticidal activity of both products was lower against *Cx. quinquefasciatus* than against other species (24-hour mortality 49% for Gokilaht-S 5EC and 68% for cyphenothrin EC). However, both products at this dosage induced >85% KD after 20 minutes. Gokilaht-S 5EC also showed larvicidal activity against *Cx. quinquefasciatus* at 3.5 g a.i./ha, with 67% mortality at 24-hours post-treatment. Lower dosages showed no larvicidal activity. No larvicidal activity was observed for cyphenothrin EC.

Indoor tests

Portable thermal and cold foggers were used for indoor tests. The dosages tested were 1.0, 2.5, and 5.0 g a.i./ha with a spray volume of 10 litres/ha or 120 ml per house area of 120 m^2. The living-room and kitchen in each of five selected houses were sprayed. In each room, adult female *Ae. aegypti, Ae. albopictus*, and *Cx. quinquefasciatus* were exposed in separate wire-framed cages placed at a height of 1.5 m. Control cages were installed in houses in another street situated >50 m away. The knockdown rate was determined at 0, 10, 20, 30, 40, and 60 minutes post-fogging. After exposure, the mosquitoes were brought back to the laboratory and transferred to clean plastic cups with 10% sucrose pads. Mortality was recorded after 24 hours. Larvicidal activity was evaluated by placing paper cups containing 20 larvae (late third and/or early fourth instars) of each test species below each test cage. After field exposure, the cups were returned to the laboratory and larval mortality was recorded after 24 hours.

Table 14. Summary of the results of bioefficacy testing of indoor and outdoor space spraying with Gokilaht-S 5EC (TC) and cyphenothrin EC (C), in percentage mortality, against *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus,* Penang, Malaysia (Zairi, 2003b)

Application		Dosage (g a.i./ha)	*Ae. Aegypti*		*Ae. albopictus*		*Cx. quinquefasciatus*	
			TC	C	TC	C	TC	C
Outdoor[a]	Cold	3.5	100	75	97	82	53	45
		1	66.7	52	43	57	22	3
		0.5	60	70	40	58	7	28
	Thermal	3.5	97	100	97	100	67	73
		1	82	83	68	37	13	8
		0.5	23	53	40	60	7	13
Indoors	Cold	5	100	100	100	100	100	100
		2.5	100	100	100	100	98	98
		1	97	89	90	88	74	48
	Thermal	5	100	100	100	95	97	95
		2.5	99	93	97	79	87	75
		1	90	92	81	56	72	54

[a] Mortality at 50-m observation post

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In indoor cold fogging tests, both products induced >89% and >88% mortality of *Ae. aegypti* and *Ae. albopictus* at all three dosages. KD rates of =99% and =97% after 10 minutes were observed for both products at dosages of 5.0 and 2.5 g a.i./ha; at a dosage of 1.0. g a.i./ha, 10-minute KD values were 47% and 36% respectively. Gokilaht-S 5EC induced 24-hour larval mortalities of 54–60% against *Ae. aegypti* and *Ae. albopictus* at 5.0 and 2.5 g a.i./ha. Cyphenothrin EC at 5.0 g a.i./ha induced 84% and 91% larval mortality against *Ae. aegypti* and *Ae. albopictus* respectively. At 5.0 and 2.5 g a.i./ha against *Cx. quinquefasciatus*, both Gokilaht-S 5EC and cyphenothrin induced mortality >98% and 10-minute KD >9 5%. Only cyphenothrin at 5.0 g/ha exhibited significant larvicidal activity (86.5%).

For indoor thermal fogging at all dosages, both products induced >90% mortality against *Ae. aegypti* and >80% mortality against *Ae. albopictus*. Against *Ae. albopictus*, Gokilaht-S 5EC induced at least 82% mortality at all three dosages, whereas cyphenothrin induced significant mortality only at the highest dosage. Both Gokilaht-S and cyphenothrin also induced >87% adult mortality against *Cx. quinquefasciatus* at 5.0 and 2.5 g a.i./ha.

Only Gokilaht-S exhibited significant larvicidal activity at the highest dosage (46% and 52% for *Ae. aegypti* and *Ae. albopictus*, respectively, and 22% for *Cx. quinquefasciatus*). Cyphenothrin, however, produced 39%mortality at the highest dosage against *Cx. quinquefasciatus.*

Adulticidal effects of both products applied with cold and thermal fogging equipment are summarized in Table 14. For comparability, outdoor mortality data are presented for cages placed 50 m from the delivery point. Indoor data are summarized as the average of mortality results from living-room and kitchen.

For outdoor application, using both cold and thermal fogging equipment, Gokilaht-S outperformed cyphenothrin at 3.5 and 1.0 g a.i./ha against all three mosquito species. For indoor applications Gokilaht-S also performed as well as or better than cyphenothrin.

USA – Field bioefficacy of Gokilaht-S 5EC applied as an aerosol from a vehicle-mounted cold fogger was evaluated in an open field at various dosages (0.5, 1.0, 2.0, and 4.0 g a.i./ha) against *Ae. albopictus* and *Cx. quinquefasciatus* in sentinel cages at distances of 10, 25, 50, and 100 m downwind from the fogger. Bioefficacy was compared with untreated controls and a treated control of resmethrin/piperonyl butoxide (PBO) at 3.9 g a.i./ha (Perich, 2003). The meteorological parameters during the tests were: temperature 16–24 °C and relative humidity 61–92%. In two trials, the wind speed ranged from 1.2 to 3.7 m/s and in the third trial from 0.0 to 0.8 m/s.

In tests against *Ae. albopictus*, Gokilaht-S induced >80% 24-hour mortality at 10 m and 25 m at dosages of 0.5, 1.0, and 2.0 g a.i./ha. At 4 g a.i./ha, mortality was 60–70% at distances of 10 m and 25 m. Resmethrin/PBO at these distances induced <60% mortality. At distances of 50 m and 100 m, Gokilaht-S induced > 0% mortality, except at 2.0 g a.i./ha for which >98% mortality was observed. The mortality induced at 50 m and 100 m by resmethrin/PBO was <30%. In *Cx. quinquefasciatus*, a dose–mortality relationship was observed. At distances of 10 m and 25 m, a dosage of 0.5 g a.i./ha induced <50% mortality while a dosage of 2 g a.i./ha induced >90% mortality. At 50 m and 100 m, only dosages of 2 and 4 g a.i./ha gave mortalities of >80%. Resmethrin/PBO induced <20% mortality in *Cx. quinquefasciatus* at all distances. The overall median diameter of spray droplets at all distances was 13–25 µm.

4.4 Conclusions

d,d-trans-Cyphenothrin, a synthetic pyrethroid, has moderate acute toxicity to mammals. Technical material is classified, like are most other public health pyrethroids, as moderately hazardous by the WHO. This compound is less toxic by the dermal route than by the oral route. It is a mild eye irritant and does not cause dermal sensitization.

Based on the standard WHO filter-paper bioassay, conducted in the laboratory against susceptible strains of *An. gambiae* and *Cx. quinquefasciatus*, the tentative diagnostic concentration of *d,d-trans*-cyphenothrin was 1% and 16% against *An. gambiae* and *Cx. quinquefasciatus*, respectively.

Based on forced tarsal contacts with insecticide deposits on filter-paper and irritability tests, *d,d-trans*-cyphenothrin has a relatively low irritant effect but a high knockdown effect on both susceptible and resistant strains of test mosquitoes. In glass-chamber studies, the knockdown and mortality effects were high against *Ae. aegypti* and *Cx. quinquefasciatus* but somewhat lower against *An. sinensis*.

The concentration of *d,d-trans*-cyphenothrin required to induce 100% mortality of a pyrethroid-resistant strain of *An. gambiae* (*kdr*) was 8times higher than for a susceptible strain. The concentration of *d,d-trans*-cyphenothrin that caused 100% mortality of susceptible *Cx. quinquefasciatus* caused only 15% mortality in the pyrethroid-resistant (*kdr* and monoxygenases) strain.

In indoor and outdoor field applications of cold and thermal fogs against *Ae. aegypti, Ae. albopictus* and *Cx. quinquefasciatus,* Gokilaht-S proved to be more effective than cyphenothrin and resmethrin/PBO at dosages of 2–5 g a.i./ha.

While targeting adult mosquitoes, Gokilaht-S also demonstrated a larvicidal effect, especially in indoor applications. Whereas this effect may be desirable for emergency control of container-breeding mosquitoes, outdoor applications require careful

consideration of the potential impact on non-target aquatic organisms.

4.5 Recommendations

1. Gokilaht-S 5EC (*d,d-trans*-cyphenothrin) is effective for space-spraying applications. For outdoor thermal or cold fog application, a dosage of 3.5–4.0 g a.i./ha is recommended, while 5.0 g a.i./ha is recommended for indoor thermal application and 2.5–5.0 g a.i./ha for indoor cold fog application.

2. For detection of resistance to *d,d-trans*-cyphenothrin in malaria vectors, a tentative diagnostic concentration of 1% is recommended. Establishment of the diagnostic concentration through multi-centre studies is recommended, especially for *Ae. aegypti*, which is of particular concern given the emergence and spread of pyrethroid resistance in this species.

3. Noting the limitations of standard procedures for testing space sprays using caged mosquitoes, WHO-recommended dosages may be subject to revision once additional studies have been carried out in different field settings and using assessments of natural target mosquito populations.

5. GENERAL RECOMMENDATIONS

1. Noting the specificity of pesticide products in terms of their safety and efficacy, the trade name of products should be used in the reports of the WHOPES working group meetings. Specifications for quality control, however, shall be developed and published using the name of the compound, to ensure the possibility of other manufacturers developing the product following established WHO procedures.[1]

2. Given the urgent need for new tools and improved technologies for vector and public health pest prevention and control, and particularly for long-lasting insecticide treated materials, WHO should stimulate and coordinate further research in this area.

3. Noting the urgent need for establishing standard procedures and criteria for testing and evaluating pesticides for vector and public health pest prevention and control, WHO should convene a consultation to review and update the existing guidelines.

[1] *Manual on development and use of FAO and WHO specifications for pesticides*, 1st ed. Rome, Food and Agriculture Organization of the United Nations, 2002.

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