WHO/CDS/NTD/WHOPES/2007.1

REPORT OF THE TENTH
WHOPES
WORKING GROUP MEETING

WHO/HQ, GENEVA
11—14 DECEMBER 2006

Review of:
SPINOSAD 0.5% GR AND 12% SC
LAMBDA-CYHALOTHRIN 10% CS
K-O TAB 1-2-3®
INTERCEPTOR®

World Health Organization

Control of Neglected Tropical Diseases
WHO Pesticide Evaluation Scheme

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1. INTRODUCTION

The tenth meeting of the WHOPES Working Group, an advisory group to the WHO Pesticide Evaluation Scheme (WHOPES), was convened at WHO headquarters in Geneva, Switzerland, from 11 to 14 December 2006. The objective of the meeting was to review the reports of testing and evaluation of the following four products: (i) spinosad 0.5% GR (granule) and 12% SC (suspension concentrate) of Dow AgroSciences, France, for mosquito larviciding; (ii) lambda-cyhalothrin 10% CS (slow-release capsule suspension) of Syngenta, Switzerland, for indoor residual spraying against malaria vectors; (iii) K-O TAB 1-2-3® (an insecticide treatment kit) of Bayer Environmental Science, France, for treatment of mosquito nets for malaria prevention and control; and (iv) Interceptor®, alpha-cypermethrin long-lasting (coated) insecticidal mosquito net of BASF, Germany, for malaria prevention and control.

The meeting was attended by 11 scientists (see Annex 1: List of participants). Dr Mir S. Mulla was appointed as Chairman and Dr Purushothaman Jambulingam as Rapporteur. The meeting was convened in plenary and group sessions, in which the reports of the WHOPES supervised trials and relevant published literature and unpublished reports were reviewed and discussed (see Annex 2: References). Recommendations on the use of the above-mentioned products were made.

The meeting also reviewed the results of laboratory studies on the deltamethrin long-lasting (coated) insecticidal mosquito nets of Netto Group (Thailand), Hiking Group Shandongtex Genfont (China) and Tianjin Yorkool (China), as part of the requirements for extension of WHO specifications for deltamethrin long-lasting (coated) insecticidal mosquito net (LN), as well as the reports of the WHOPES multi-centre study to develop simple and reliable method to determine the bioefficacy of pyrethroid-treated nettings, and made recommendations for further action.

2. REVIEW OF SPINOSAD 0.5% GR AND 12% SC

Spinosad is a natural product produced by fermentation technology that employs the bacterium *Saccharopolyspora spinosa* (Actinomycetales) from which it is obtained by extraction and purification of the whole broth. Spinosyns A and D are present in the isolated spinosad, in proportions of 65–95% and 5–35%, respectively,[1] together with traces of spinosyn-related compounds and other materials derived from the fermentation and purification process.

The two main spinosyns (A and D) are closely related structurally. They represent more than 85% of technical spinosad and are responsible for most of its insecticidal activity. They differ only in the presence of an additional methyl group attached to the bridging carbon of the indacene moiety in spinosyn D.

Spinosyns A and D have very low vapour pressures, making them essentially non-volatile. Spinosyns A and D are weak bases. Spinosyn A has rather low, and pH-dependent, water solubility, with that of D even lower. As may be expected for weak bases, the water solubility decreases with increasing pH in both cases. Both spinosyns are resistant to hydrolysis in sterile, buffered water, with no detectable hydrolysis at pH 5 and increasing but very slow hydrolysis at pH 7 and pH 9. Aqueous photolysis of A and D at pH 7 was rapid, with a half-life of less than one day.

Spinosad acts as a nicotinic agonist. It alters the function of nicotinic and GABA-gated ion channels, depolarizing insect neurons and resulting in neuron excitation. Spinosad has shown no cross-resistance with existing insecticides and can be rotated with all other classes of currently used mosquito larvicides.

[1] WHO specifications and evaluations for public health pesticides – Spinosad. Available at http://www.who.int/whopes/quality.

2.1 Safety assessment

The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) assessed the toxicity of spinosad in 2001 (JMPR, 2001).

Spinosyn A is rapidly absorbed from gastrointestinal tracts and is distributed relatively evenly in the organism. It is metabolized mainly via glutathione conjugation and excreted mainly in the faeces. After a single dose, about 90% is excreted within 24 hours. Limited information on spinosyn D is available, which indicates a similar pattern of disposal.

Spinosad has low acute toxicity by oral (LD_{50} >5000 mg/kg), dermal (LD_{50} >5000 mg/kg) and inhalation routes (LC_{50} >5 g/m^3), does not cause dermal sensitization in Buehler or maximization tests, is only slightly irritating to the eye and is non-irritating to the skin. In studies in mice and rats, spinosad did not induce tumours. Spinosad did not induce point mutations in bacteria or murine lymphoma cells, or chromosomal aberrations or unscheduled DNA synthesis in vitro or micronuclei in mice in vivo. Spinosad at low-to-moderate doses showed no teratogenecity, neurotoxicity or reproductive impairment.

Spinosad has the potential for bioaccumulation; it is photolabile, but resistant to hydrolysis. It is moderately toxic to fish, practically non-toxic to birds, and highly toxic to honeybees.

The WHO Programme on Chemical Safety is of the view that the use of spinosad as a mosquito larvicide poses no undue threat to the health of users or to the environment. However, it notes that this assessment concerns only spinosad with an equivalent impurity profile.[2]

[2] WHO specifications and evaluations for public health pesticides – Spinosad. Available at http://www.who.int/whopes/quality.

2.2 Efficacy – background and supporting documents

Tapachula, Mexico

Bond et al. (2004) carried out studies to determine the bioactivity of spinosad against larvae of *Aedes aegypti* and *Anopheles albimanus* in the laboratory and in the field. The studies used a formulation of spinosad (Tracer^{R} naturalyte® containing 480 g/L AI, Dow AgroSciences) intended for the control of crop pests.

Laboratory bioassays using cultured mosquitoes were carried out at 26 ±1 °C, photoperiod 12:12 light:dark and 75–85% relative humidity. A total of 25 third- and fourth-instar larvae were placed in 150 ml water in plastic cups and exposed to five concentrations of 1–100 ug/L AI of spinosad, replicated four times. The larvae were exposed for 1 hour and then transferred to 100 ml clean dechlorinated water to which a small amount of food was added. Each experiment was repeated three times on different dates. Mortality was assessed after 24 hours.

Two field tests were performed in circular brown 1.5 L capacity plastic containers with 1 L of dechlorinated water, set in open shade under the eaves of a laboratory building. The containers were enriched with grass infusion to attract gravid mosquitoes for oviposition. The containers were treated with 1 mg/L and 10 mg/L AI spinosad. In the first field trial, spinosad was applied at 1 mg/L and 10 mg/L and in the second trial at 5 mg/L AI. The containers were observed for all insects, and living and dead insects were counted and removed weekly. Immatures were classified to genus visually, while other insects were determined to family.

Regression analysis and quadratic linear analysis of bioassay data of the laboratory studies indicated LC_{50} values of 0.025 and 0.024 mg/L AI for larvae of *Ae. aegypti* and *An. albimanus* respectively (Table 1).

In the first field trial, wild *Ae. Aegypti* were noted in greater numbers than *Culex* mosquitoes. *Ae. aegypti* larvae were completely controlled for 22 weeks at 10 mg/L AI, but the level of control dropped to 80% or lower after 13 weeks at 1 mg/L AI.

Culex mosquitoes were almost completely suppressed for 15 to >22 weeks at 1 and 10 mg/L AI respectively (Table 2).

In the second field trial, spinosad at 5 mg/L AI yielded complete control of *Ae. aegypti* larvae for 13 weeks. The overall control for the period of 21 weeks was about 84%. Temephos at the recommended rate (100 g of 1% sand granules/m^3) yielded similar results. *Culex* mosquitoes were controlled for 17 weeks at the rate of 5 mg/L AI of spinosad (Table 2).

Although this research has provided some important information, documenting a high level of activity even with one-hour exposures, the data have to be carefully evaluated as the procedures and techniques used are different from the WHO standard procedure for testing and evaluation of mosquito larvicides (WHO, 2005).[3] This includes the exposure of mosquito larvae, in the laboratory, for one hour and then transferring them to clean water for assessment of mortality after 24 hours.

Antalya, Turkey

Cetin et al. (2005) conducted bioassays on field-collected third- and fourth-instar larvae of *Culex pipiens* in septic tank water using five concentrations (0.01–0.12 mg/L AI) of spinosad. The formulation Conserve® 12% SC (Dow AgroSciences) was premixed with water and used. For bioassays in the laboratory, 25 larvae from septic tanks were transferred to 1 L of septic tank water in plastic containers and treated with spinosad premixed dilutions to obtain the needed concentrations. Each treatment and control was replicated four times, and each experiment repeated three times. The containers were set up at 27 ± 2 °C, photoperiod 12:12 light:dark and 60 ± 10% RH.

Field studies were carried out in 20 septic tanks. The surface area of each tank was measured and the amount of premixed SC in water calculated for a given dosage and applied with a

[3] *Guidelines for laboratory and field testing of mosquito larvicides*. Geneva, World Health Organization, 2005 (WHO/CDS/WHOPES/GCDPP/2005.13; available at: http://www.who.int/whopes/guidelines/en/).

compression sprayer. Larval density was assessed by taking five (500 ml) dips per tank at intervals after treatment (1, 7 and 14 days), and the percentage reduction in mosquito larvae was calculated.

From laboratory bioassays, the LC_{50} and LC_{90} values for 24 hours were calculated to be 0.027 and 0.111 mg/L AI for *Cx. pipiens.* Mortality in controls was less than 4% (Table 1).

In field trials in septic tanks, the magnitude of reduction was 100% on days 1 and 7 post-treatment at 100 and 200 g/ha AI, but the level of control declined to 79% and 83%, 14 days post-treatment respectively. At the two low dosages (25 and 50 g/ha AI), the level of control was 74% or lower on days 1 and 7 post-treatment. It is evident that 100 and 200 g/ha AI of spinosad are efficacious in septic tanks, but the longevity of control is only 7 to 10 days, thus requiring frequent treatments with the SC formulation (Table 2).

Rome, Italy

Romi et al. (2006) evaluated Laser^R 4.8% EC (Dow AgroSciences) in the laboratory against three species of mosquitoes. All tests were carried out at 27 ± 1 ºC, photoperiod 16:8 light:dark and 70% RH. Up to five concentrations (0.001–0.1 mg/L AI) and controls with three replicates each were used. Plastic containers with 200 ml dechlorinated tap water were inoculated with 20 third-instar larvae and mortality recorded 24 and 48 hours post-treatment.

In these studies, spinosad proved highly effective against *Cx. pipiens* (24 hour LC_{50}–LC_{90} 0.0064–0.018 mg/L AI), followed by *Ae. aegypti* (0.0096–0.015) and *An. stephensi* (0.039–0.101). The latter species was more tolerant (Table 1).

Spinosad persistence was monitored in plastic trays containing 1 L of dechlorinated tap water. Tests were run in duplicate and food was given to the larvae. The trays were stocked with 30 second- and third-instar larvae and mortality was recorded daily. Dead larvae were removed and 50 fresh larvae were added daily. At the concentration of 0.04 mg/L AI, spinosad persisted for 6 weeks, killing more than 50% of *An. stephensi* larvae. The persistence

was 8 weeks for *Aedes* and *Culex* larvae at the concentration of 0.01 mg/L AI.

2.3 Efficacy – WHOPES supervised trials

Montpellier, France

Darriet et al. (2005) carried out bioassays with technical material of spinosad (dissolved in ethanol) against *Ae. aegypti, An. gambiae* and *Cx. quinquefasciatus* larvae. Each bioassay was triplicated using late third- and early fourth-instar larvae. Batches of 20 larvae were tested in 99 ml of distilled water, adding 1 ml of insecticide solution at the required concentration. Five lots per concentration and five to eight concentrations per replicate, providing 0–100% mortality, were used for each replicate. Temperature was maintained at 27 °C throughout the test, and larval mortality was recorded after 24 hours of exposure. Mortality was corrected by Abbott formula and the results analysed by log-probit method.

Spinosad was found to be more active against larvae of *An. gambiae*, followed by *Cx. quinquefasciatus* and *Ae. Aegypti*. The LC_{50} and LC_{90} values were as follows: *An. gambiae* (0.01–0.032 mg/L AI) *Cx. quinquefasciatus* (0.093–0.49 mg/L AI), *Ae. aegypti* (0.35– 0.92 mg/L AI) respectively. Interestingly, the strains with multiple resistance were equally susceptible as the susceptible strains of each species (Table 1).

With sublethal concentrations (24-hour LC_5, LC_{20}, LC_{40} and LC_{60} values), additional mortality occurred in larvae, pupae and adults. Even at LC_{40}, there was almost 100% cumulative delayed mortality in larvae and pupae.

Riverside, California and Nonthaburi, Thailand

Mulla (2006) bioassayed technical material of spinosad (90.4%) and the 12% SC formulation in the laboratory against second- and fourth-instar larvae of *Cx. quinquefasciatus* and *Ae. Aegypti*. Various concentrations within the activity range were tested. Technical material was dissolved in acetone as 1% stock solution from which serial dilutions were prepared. The SC formulation was prepared as 1% suspension in distilled water. Aliquots of the

appropriate acetonic and aqueous dilutions were added to 100 ml of water in disposable paper cups containing 20 larvae. Each concentration was replicated three times and the tests run on two or three different days. The tests were run at 25 ± 1 °C, 14:10 dark:light photoperiod and 40% RH. Mortality was read 24 and 48 hours after treatment.

The spinosad technical material showed good activity against larvae of *Ae. aegypti* and *Cx. quinquefasciatus.* The LC_{50}–LC_{90} values (fourth instars) were 0.084 and 0.155 mg/L AI, for *Ae. aegypti* (24 hours). For *Cx. quinquefasciatus*, these values were 0.094 and 0.281 mg/L AI. The SC formulation was more active than the technical material against both species. The LC_{50}–LC_{90} values (24 hours) for *Ae. aegypti* (fourth instars) were 0.030–0.061, while those for *Cx. quinquefasciatus* were 0.013–0.038 respectively (Table 1). The second instars were generally slightly more susceptible than the fourth instars. Also, the mortality was higher after 48 hours of exposure than after 24 hours.

Mulla (2006) also carried out field studies against *Ae. aegypti* in earthen jars filled with 200 L water from a domestic water line, in Bang Bua Thong District of Nonthaburi Province. The jars were set in rows on a concrete slab under a roof and covered with celocrete covers except during assessment and treatment. The jars were not exposed to sunlight but were located in open shade at all times.

The jars were filled from a domestic water supply and 0.5 g of ground-up larval food was added initially and at weekly intervals. The jars were treated with various concentrations of spinosad using two formulations (12% SC and 0.5% GR). The SC formulation was premixed with water, and aliquots of appropriate strength suspensions were added to each jar for a given concentration. The SC formulation was used at 8.4, 3.9, 2.23, 0.89 and 0.0 mg/L AI, while the GR 0.5% was used at 1.0, 0.5, 0.25, 0.10 and 0.0 mg/L AI. Two water regimens were used: full jars at all times (water level replenished weekly or biweekly) and full jars with half of the water removed and replenished weekly.

For assessment, the treated and untreated jars were each challenged with 25 third-instar larvae of laboratory-cultured *Ae.*

aegypti every week. For 3–4 weeks, the larval mortality was assessed 48 hours after the addition of each cohort of larvae. All pupal skins (indicating successful emergence) were removed and counted one week after the introduction of each cohort of larvae. Pupal skins were easy to count as the exuviae all floated on water on the meniscus layer along the edge of the jars and could be easily removed by a syringe and transferred to white trays for counting. The data were recorded in charts and the percentage inhibition of emergence was calculated from the pupal skins on the basis of the original number of larvae (25).

For the first 4 weeks, both formulations produced essentially 100% larval mortality at all concentrations. This was true for both the full and the full–half-full removed and refilled jars. After these initial evaluations, knowing that larvae are killed in 48 hours, larval counting was skipped (because it was difficult to count) and pupal skins were counted weekly, in succeeding 17 cohorts, up to the end of the experiment 139 days after treatment (Table 2).

From cohort 5 (5 weeks post-treatment) to cohort 21 (21 weeks post-treatment), pupal skins were assessed (emergence). 100% inhibition of emergence was obtained up to 11 cohorts (69 days post-treatment) in all dosages of 12% SC and 0.5% GR in both water regimens. The longevity of 12% SC at the practical concentration of 0.89 was for 97 and 104 days (inhibition of emergence 90% and 88%), but the highest dosage yielded much longer persistence for up to 132 days post-treatment (inhibition of emergence 96%).

The 0.5% GR was applied at dosages of 0.1–1.0 mg/L AI. It produced almost 100% inhibition of emergence at all four dosages (1.0, 0.5, 0.25 and 0.1 mg/L AI) up to 76 days, while the highest dosage (1.0 mg/L AI) gave >90% inhibition of emergence for up to 111 days. The lower dosages showed <90% inhibition of emergence after 91 days. The level of control was somewhat longer in full–half-full jars. Removal and refilling probably resulted in the release of adsorbed spinosad (Table 2).

Sistan and Baluchestan, Islamic Republic of Iran
Laddoni (2006) carried out studies in the laboratory and under simulated field situations on two formulations of spinosad (12% SC and 0.5% GR) against *Anopheles* and *Culex* mosquitoes.

In laboratory bioassays, spinosad technical material (90.4%) was dissolved in acetone giving 1% stock solution, diluted serially in acetone, and 1 ml of the appropriate dilution added to 99 ml of chlorine free water to achieve the desired dosages. Each treatment and control was replicated four times, using 20–25 early third-instar larvae per container. Tests were run at 25 ºC, photoperiod 12:12 light:dark, and mortality read after 24 hours.

Larvae of both *An. stephensi* and *Cx. quinquefasciatus* were susceptible. The 24-hour LC_{50} and LC_{90} of technical material were: 0.01 and 0.04 mg/L AI against *Cx. quinquefasciatus*, and 0.004 and 0.02 for *An. stephensi*. Delayed mortality beyond 24 hours increased significantly in *An. stephensi* (Table 1).

Simulated field studies were run in constructed ponds (1 x 1 m and 50 cm deep), lined with plastic sheeting covered with soil or mud to a depth of 10 cm. The ponds were filled with local water to a depth of 25–35 cm and allowed to stand for 2 weeks before treatment. The ponds supported natural populations of mosquitoes. Each treatment and control was replicated four times.

The 12% SC and 0.5% GR formulations were administered at four rates (20, 30, 40 and 50 g/ha AI) equalling 2, 3, 4 and 5 mg/m^2. The SC was applied as aqueous spray using compression sprayers, the GR was scattered over the surface area and larvae and pupae were sampled by the dipper technique taking five dips per pond before and every other day after treatment until larval recovery reached 90% of pretreatment level. The immatures were categorized into first- and second (early) and third- and fourth (late)-instar larvae, and pupae. Density of immatures was reported as number/10 dips per pond/day. Percentage reduction was calculated for early- and late-instar larvae and pupae daily.

A total of 56 ponds (28 for *Anopheles* and 28 for *Culex*) inhabited by wild populations were employed. The plots were treated twice.

Reductions for both larvae (early and late) and pupae were calculated. The percentage reduction based on late instars and pupae was more accurate than that using the early instars.

During the course of these tests, *An. culicifacies* (55.6%) and *An. stephensi* (21.6%) predominated other anophelines, while in the *Culex* group the predominant species were *Cx. tritaeniorhynchus* (50.4%), *Cx. pipiens* (34.6%) and *Cx. pseudovishnui* (11.4%).

Both formulations at the application rates of 2, 3, 4 and 5 mg/m^2 AI of spinosad yielded similar results, giving >80% (close to 100% at higher dosages) reduction in both *Anopheles* and *Culex* species for 7–11 days. At 4 and 5 mg/m^2 AI application rates, there were some adverse effects on non-target biota, recovering in 1–2 weeks. The lower and practical dosage of 1, 2 and 3 mg/m^2 AI of both formulations yielded quite variable results, with no tangible trends. The 4 mg/m^2 AI of both formulations produced 80–100% reduction in all mosquitoes for 9–11 days, while the 5 mg/m^2 also produced >80% reduction for 9–11 days (Table 2).

Laddoni (2006) also carried out large-scale field studies in rice paddies, developmental sites for natural populations of mosquitoes, in Sistan and Baluchestan. The 12% SC and 0.5% GR formulations were administered at four rates (20, 30, 40 and 50 g/ha AI), equalling 2, 3, 4 and 5 mg/m^2. The sections of rice field used were 650–900 m^2, water depth about 25 cm and water temperature 27 ± 3 °C. Each treatment and control was replicated four times. Mosquito immatures were sampled by taking 20 dips per plot before and daily after treatment, and recorded as 1–2, 3–4 and pupae per 10 days. The percentage reduction in larvae and pupae in the treated plots was recorded.

The 12% SC formulation was applied as an aqueous spray using a compression sprayer. The granules were mixed with fine sand to increase bulk[4] and were scattered by hand. Species composition was determined by bringing fourth-instar larvae from the control at various intervals.

[4] It is not desirable to mix granules with other inert materials.

From these studies, it was concluded that the practical dosage for *Culex* and *Anopheles* in open and vegetated natural breeding grounds will be 40–50 g/ha AI (4–5 mg/m^2 AI). No significant difference in efficacy was noted between the two formulations at 40 or 50 g/ha AI, using two-way analysis of variance. At 40 g/ha AI, the SC and GR formulations provided more than 80% reduction of *Anopheles* and *Culex* larvae, up to 9 days post-treatment (Table 2).

Pondicherry, India

Sadanandane et al. (2006) determined the efficacy of spinosad technical material against *Cx. quinquefasciatus* larvae in the laboratory.

Technical material spinosad was dissolved in acetone to achieve a 1% stock solution. This solution was serially diluted and aliquots of the appropriate dilutions were used in bioassays against field-collected fourth-instar larvae. Eight concentrations (0.025–0.200 mg/L AI) were employed using 250 ml paper cups filled with 200 ml of dechlorinated water containing 25 fourth-instar larvae. Each concentration and control was replicated three times, and mortality assessed at 24 hours.

The 24-hour LC_{50} and LC_{90} values of technical spinosad for *Cx. quinquefasciatus* larvae were 0.085 and 0.189 mg/L AI respectively (Table 1).

Small- and medium-scale evaluations of 12% SC and 0.5% GR formulations of spinosad were also carried out against *Cx. quinquefasciatus*, a domestic polluted-water breeder, in cesspits, drains and abandoned wells, all polluted with sullage, debris and garbage (Sadanandane et al., 2006).

In the *small-scale studies*, the GR and SC formulations were tested in the habitats at 50, 100, 150 and 200 mg/m^2 AI. Three to five replicates of cesspits, drains and wells were selected for each dosage and control.

Sampling was done by dipping twice a week for 1–2 weeks before treatment and then every 2 or 3 days after treatment. Three dips were taken in each replicate, and larvae and pupae were counted.

Spinosad 12% SC was premixed with water and applied with a compression sprayer, while 0.5% GR was scattered over the surface manually. After treatment, larvae and pupas were dipped every 2 or 3 days and continued until density in treated sites reached that of the control.

In cesspits, as expected, there was little reduction in pupal number on day 1 post-treatment at all three dosages (50, 100 and 150 mg/m^2 AI) – pupae are not susceptible to spinosad – but the reduction reached >90% subsequently, with 100% reduction observed on day 7 post-treatment at all dosages. On days 14 and 21, the reduction in pupae was 66–77% and 40–50% respectively at all dosages, indicating low efficacy from day 21. Similar trends in late larval reductions were noted. The trends in efficacy of GR 0.5 in cesspits were essentially the same as with SC120. The pupal reduction was 80–100% on day 7 post-treatment at 50, 100 and 150 mg/m^2. On day 21 the reduction was below 80%. The larval reduction trends were essentially the same, indicating that the dosages used here can provide control for 1–2 weeks in cesspits (Table 2).

In drains, the reduction in pupae was dose-dependent. As expected, the low doses (50 and 100 mg/m² AI of 12% SC) had little reduction in pupal numbers on day 1, but yielded 100% at day 4 and 80% at day 7 post-treatment, declining to 38% and 56% on day 14. At the higher doses (150 and 200 mg/m² AI), the effect lasted 17 days, with reductions >90%. Thereafter, pupal control was mediocre even at the highest dosage. Larval reductions followed the same trend as above, with 99–100% reduction on day 4 and 83–96% on day 7 at 50 and 100 mg/m² AI, the effect declining to below 50% on day 14. At 150 and 200 mg/m² AI, 12% SC produced 100% reduction at day 10, and >90% at days 14–17, declining to 43–64% at 21 days. The GR formulation yielded greater reduction of pupae at the two low dosages and was >80 reduction at day 14. At the 150 and 200 mg/m² AI, 95–100% pupal reduction was noted on days 21 and 31. Thereafter, the effect declined to 20%. The 0.5% GR showed greater persistence in the drains compared with 12% SC, but this may not be the case in every situation (Table 2).

In disused wells, the SC formulation at 50, 100 and 150 mg/m² AI showed considerable longevity, reducing pupal density by 100% for 31, 35 and 41 days post-treatment respectively. The level of reduction >80% was for 41, 58 and 69 days respectively. Beyond these durations, the extent of pupal reduction was lower. Similar longevity was shown against late-instar larvae, although at every dosage the duration of persistence was somewhat shorter. The GR formulation possessed shorter persistence than the SC. A greater than 95% reduction was noted on days 21, 28 and 37 at 50, 100 and 150 mg/m² AI. The persistence against late-instar larvae was similar to that against pupae. The duration of persistence against early instar was shorter at the low dosages, but similar to late instars (Table 2).

In the *medium-scale trials*, the SC and GR formulations were applied at the dose of 150 mg/m² AI to cesspits and drains and at the dose of 50 mg/m² AI to wells. A total of 72 cesspits (168.5 m²) 13 street drains (1690 m long, surface area 902 m²) and 41 disused wells (86.7 m²) were selected, but some of these sites were excluded due to other interventions. From the cesspits, 22 were assigned to 12% SC, and 20 to 0.5% GR treatments. Of the 39 wells, 12 were assigned to each 12% SC, and 0.5% GR; 6 were assigned as controls. Among the 13 drains, 5 were assigned to 12% SC and 4 to 0.5% GR, with proper controls. Pre-treatment larval and pupal densities were determined twice, and then assessed by dipping twice-weekly post-treatment. Immatures were categorized as early (first and second) and late (third and fourth) instars and pupae. The percentage reduction in treated habitats was based on population of late instars and pupae. Duration of efficacy was based on the level of reduction >80% (Table 2).

In cesspits, pupal populations were reduced by 96–100% for 2 weeks. Beyond that period, the reduction was 80% or lower. Against late-instar larvae, this dose produced 100% reduction of late instars for 7 days, declining to 73–90% 14 days post-treatment. Similar trends in the reduction of early-instar larvae were noted (Table 2).

In drains, both formulations at 150 mg/m² AI produced pupal reduction of 89–100% for 18 days, the reduction declining to 71–

74% on day 21 post-treatment, decreasing further in time. Late-instar larvae experienced >90% reduction up to 14 days, decreasing to 45–53% on day 21. Reductions in early instars were not as high as in pupae and late instars, and this trend is to be expected because of the short duration of exposure of early-instar larvae to the active agent (Table 2).

From these studies, it is evident that that the duration of control is dependent upon formulation, dosage, species and the type of habitat.

2.4 Conclusions and recommendations

Spinosad is a natural product produced by fermentation technology employing the Actinomycetales bacterium *Saccharopolyspora spinosa*. Spinosyns A and D, the two major active principles, are present in the isolated spinosad in proportions of 65–95% and 5–35%, respectively. Spinosad offers a new mode of action as a nicotinic agonist. It has shown no cross-resistance with existing insecticides and can be rotated with other classes of currently used mosquito larvicides for resistance prevention and management.

Spinosad has a favourable toxicological profile. It has relatively low acute toxicity by the oral, dermal and inhalation routes. It has been found to cause no tumours, teratogenicity, neurotoxicity or reproductive impairment at low-to-moderate doses. It causes slight eye irritation but is non-irritating to the skin. The WHO Programme on Chemical Safety considers spinosad to be a mosquito larvicide that poses no undue threat to the health of users or to the environment. However, it notes that this assessment relates to spinosad, with the equivalent impurity profile of that used for development of WHO specifications.[5]

The bioactivity of technical and formulated spinosad was studied against larvae of several important species of mosquitoes in the laboratory. The LC_{50} (24-hour mortality) of technical material

[5] WHO specifications and evaluations for public health pesticides – Spinosad. Available at http://www.who.int/whopes/quality.

against *Ae. aegypti* larvae ranged from 0.155 to 0.35 mg/L AI in various studies, while the LC_{90} values were in the range of 0.185 to 0.92 mg/L AI. For *Cx. quinquefaciatus*, the LC_{50} values were 0.01 to 0.094 mg/L AI while the LC_{90} values were 0.04 to 0.49 mg/L AI in the various tests. *Anopheles* species *(An. albimanus*, *An. gambiae* and *An. stephensi)* were more susceptible, with the LC_{50} being 0.004 to 0.01 mg/L AI; the LC_{90} values were 0.02 to 0.032 mg/L AI. The activity of formulated material was generally three- to five-fold greater than that of the technical material.

Suspension concentrate (12% SC) and granule formulation (0.5% GR) were employed in field studies. These formulations yielded persistent control (>90% mortality) of *Ae. aegypti* in water storage containers for 76–111 days at the dosages of 0.1, 0.25, 0.50 and 1.0 mg/L AI. Shorter residual activity was shown against *Culex* in disused wells, ranging from 31 to 41 days (>80% reduction) at the dosages of 50 to 150 mg/m^2 AI. However, in highly polluted bodies of water such as septic tanks, cesspits and street drains, the residual activity was much shorter at the dosages of 50 to 150 mg/m2 AI applied to the various habitats. In ponds and rice fields, the dosages of 20–50 g/ha AI yielded control >80% against *Anopheles* and *Culex* species for 9–11 days. In all of these studies, the two formulations (12% SC and 0.5% GR) essentially produced the same level and duration of control. It is clear from these studies that the duration of control is greatly influenced by the target species and by the habitat parameters where the target species propagates (Table 2).

Noting the safety and efficacy of the two formulations of spinosad (12% SC and 0.5% GR), the meeting recommended:

- the use of spinosad 12% SC and 0.5% GR formulations in open bodies of water such as ponds, impoundments and rice fields, at the rate of 20–50 g/ha AI, with an expected duration of efficacy of 9–11 days;

- the use of spinosad 12% SC and 0.5% GR at dosages of 0.1–0.5 mg/L AI to provide control of container breeding mosquitoes, with an expected duration of efficacy of 10–12 weeks;

- the use of 12% SC and 0.5% GR formulations in highly polluted habitats and where larviciding is indicated, at the rate of 25–100 mg/m^2 AI, with an expected duration of efficacy of 1–2 weeks;

- that WHO should carry out further risk assessment for the use of spinosad in potable water as a mosquito larvicide, noting the relative safety profile of the product;

- that WHOPES should explore with the manufacturer the possibility of developing a tablet or other sinkable formulation for the control of mosquitoes in water storage containers, noting the floating of the 0.5% GR formulation on the water surface, a characteristic that is undesirable for many water storage container habitats.

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.[6]

[6] WHO specifications for public health pesticides are available on the WHO homepage on the Internet at http://www.who.int/whopes/quality/en/.

Table 1. Bioactivity of spinosad against third- and fourth-instar mosquito larvae in the laboratory – mortality assessed 24 hours post-treatment

Country and location	Product	Activity (mg/L active ingredient) against					
		Ae. aegypti		*Anopheles*		*Cx. quinquefasciatus*	
		LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Mexico, Tapachula	Tracer® SC	0.025	-	0.024[a]	–	–	–
Turkey, Antalya	Conserve® SC	–	–	–	–	0.027	0.111
France, Montpellier	Technical	0.35	0.92	0.01[b]	0.032[b]	0.093	0.49
Italy, Rome	Laser® EC 4.8	0.0096	0.015	0.039[c]	0.101[c]	0.0064	0.018
USA, Riverside	Technical	0.084	0.155	–	–	0.094	0.281
	12% SC	0.030	0.061	–	–	0.013	0.038
Iran (Islamic Republic of), Tehran	Technical	–	–	0.004[c]	0.02[c]	0.01	0.04
India, Pondicherry	Technical	–	–	–	–	0.085	0.189

[a]*An. albimanus*, [b]*An. gambiae*, [c]*An. stephensi*

Table 2. Efficacy of spinosad formulation as tested against mosquito larvae in simulated field and field trials against various species in various habitats

Country and location	Product	Species	Habitat	Dose (active ingredient)	Residual activity	% reduction
Mexico, Tapachula	Tracer® SC	*Ae. aegypti*	Plastic containers	1.0 mg/L	8 wks	50
				5.0 mg/L	13 wks	100
		Culex spp.		10.0 mg/L	>22 wks	100
				1.0 mg/L	15 wks	50
				5.0 mg/L	17 wks	100
				10 mg/L	>22 wks	100
Turkey, Antalya	Conserve® SC	*Culex pipiens*	Septic tanks	25 g/ha	7 d	40
				50 g/ha	7 d	74
				100 g/ha	7-14 d	80-100
				200 g/ha	7-14 d	80-100
Thailand, Nonthaburi	12% SC	*Ae. aegypti*	Jars (200 L)	0.89 mg/L	104 d	>90
				2.23 mg/L	111 d	>90
				3.9 mg/L	118 d	>90
				8.4 mg/L	132 d	>90
	0.5% GR	*Ae. aegypti*	Jars (200 L)	0.1 mg/L	76 d	>90
				0.25 mg/L	83 d	>90
				0.50 mg/L	83 d	>90
				1.0 mg/L	111 d	>90

Table 2 (continued). Efficacy of spinosad formulation as tested against mosquito larvae in simulated field and field trials against various species in various habitats

Country and location	Product	Species	Habitat	Dose (active ingredient)	Longevity	% reduction
Iran (Islamic Republic of), Sistan & Baluchestan	12% SC & 0.5% GR	*Anopheles* and *Culex*	Ponds (1 m²)	2.0 mg/m²	7-9 d	>80
				3.0 mg/m²	7-9 d	>80
				4.0 mg/m²	9-11 d	80–100
				5.0 mg/m²	9-11 d	80–100
	12% SC & 0.5% GR		Rice fields	3.0 mg/m²	Variable	
				4.0 mg/m²	7–11 d	>80
				5.0 mg/m²	9–11 d	>80
India, Pondicherry	12% SC & 0.5% GR	*Cx. quinque-fasciatus*	Cesspits	50 mg/m²	7 d	80–100
				100 mg/m²	7 d	80–100
				150 mg/m²	7–14 d	80–100

Table 2 (continued). Efficacy of spinosad formulation as tested against mosquito larvae in simulated field and field trials against various species in various habitats

Country and location	Product	Species	Habitat	Dose (active ingredient)	Longe-vity	% reduction
India, Pondicherry	12% SC and 0.5% GR	*Cx. quinque-fasciatus*	Street drains	50 mg/m²	4-7 d	80–100
				100 mg/m²	7-10 d	80–100
				150 mg/m²	17–21 d	80–100
			Disused wells	50 mg/m²	21–31 d	80–100
				100 mg/m²	28–35 d	80–100
				150 mg/m²	37–41 d	80–100

3. REVIEW OF LAMBDA-CYHALOTHRIN 10% CS

The lambda-cyhalothrin slow-release 10% capsule suspension (CS) of Syngenta (Basel, Switzerland) was evaluated for indoor residual spraying against malaria vectors. WHOPES has already tested and evaluated lambda-cyhalothrin WP for indoor residual spraying. The evaluation of the new formulation (CS) was carried out in comparison with the WP formulation, using a *fast track* procedure.

3.1 Safety assessment

Lambda-cyhalothrin (alpha-cyano-3 phenoxybenzyle 3 – (2-cholro-3, 3, 3-trifluroprop-1-enyl)-2, 2-dimethylcyclo propene carboxylate, a 1:1 mixture of the (2) – (1 R, 3R), S-ester and the (2)-(1S,3S), R-ester)) is a pyrethroid insecticide consisting of one of the enantiomer pairs of cyhalothrin.

WHO has assessed the toxicity of lambda-cyhalothrin technical material and classified it as “moderately hazardous” (Class II), on the basis of acute oral toxicity data (WHO, 1990). The hazards and risks were summarized as follows: harmful; irritating to eyes, skin and upper respiratory system; ingestion could lead to neurological symptoms such as tremours and convulsions; a hazard of ingested liquid formulations is aspiration of the solvent into the lungs (chemical pneumonitis); very toxic to fish and honey-bees. Exposure of the general population to lambda-cyhalothrin is expected to be very low and not likely to represent a hazard under normal conditions of use. With good work practices, hygiene measures and safety precautions, lambda-cyhalothrin is unlikely to present an occupational exposure hazard.

The following are extracts from the material safety data sheet of the manufacturer for lambda-cyhalothrin 10% CS:

Acute oral LD_{50} (rat)	>5000 mg/kg
Acute inhalation LC_{50} (rat)	>4.62 mg/L; 4 h
Acute dermal LD_{50} (rat)	>4000 mg/kg
Skin irritation (rabbit)	Slightly irritating May cause temporary itching, tingling, burning or numbness of exposed skin (paraesthesia)
Eye irritation (rabbit)	Mild eye irritation
Sensitization (man)	Likely to cause skin sensitization

3.2 Efficacy – background and supporting documents

United Republic of Tanzania

Curtis et al. (1998) carried out a trial in 12 villages in an intense malarious area of north-eastern United Republic of Tanzania in 1995–1996. The village populations ranged between 971 and 2445, with an average of 18% below the age of 6 years. The malaria vectors *An. gambiae* and *An. funestus* were present in all villages. *An. arabiensis* was present in six villages. The vector population and malaria cases occurred perennially, peaking in the rainy season (April–June).

Villages were randomly assigned to the four arms of the study. Lambda-cyhalothrin 10% CS was used at the dosage of 30 mg/m^2 AI for indoor residual spraying in four villages. The same insecticide was used for treatment of polyester bednets in four villages at 10 mg/m^2 AI and in another four villages at 20 mg/m^2 AI. Another four villages were monitored as a control. This report describes only the results of indoor residual spraying.

The four villages were sprayed in December 1995 and re-sprayed after 7–8 months. Bioassays were carried out in a small sprayed hut in an unsprayed village on mud walls and palm-thatch doors. WHO cones were used for the bioassays. *An. gambiae* were exposed for 10 minutes to the sprayed surface[7] and for 3 minutes

[7] WHO guidelines require 30-minute exposure.

to the nets. The mosquito populations were monitored using monthly light trapping in each village in the bedrooms of five unsprayed sentinel houses which were provided with untreated bednets. Sporozoite proteins were detected in heads and thoraxes of anophelines by enzyme-linked immunosorbent assay (ELISA).

The mortality rate was 100% up to 7 months after spraying. Light trap and ELISA testing showed a significant reduction of malaria vector populations and of sporozoite rates. Window exit trap and pyrethrum spray catches were carried out in treated rooms. There was an overall reduction of 25% in the number of blood-fed females. However, analysis of blood-meals showed no diversion from biting humans to biting animals. Incidence of reinfection was measured by weekly monitoring of cohorts of 60 children per village, after clearing pre-existing infection with chloproguanil–dapsone. There was a reduction in probability of reinfection per child per week by 54%. Monthly cross-sectional surveys in each village among 50 randomly selected children aged 1–6 years for fever, parasitaemia and haemoglobin level showed significant reductions. However, passive surveillance by resident health assistants showed no evidence for reduced prevalence of fever or parasitaemia.

Viet Nam

The efficacy of residual spraying of lambda-cyhalothrin 10% CS at 30 mg/m^2 AI was evaluated in three trial sites, one each in north, central and south Viet Nam (Le Khanh Thuan, 2006). Before the trial, the insecticide formulation was tested and its quality confirmed in a national laboratory. Susceptibility of the vectors *An. dirus* and *An. minimus* in the north, *An. aconitus* and *An. dirus* in the centre and *An. sundaicus* and *An. dirus* in the south were tested. Tests were conducted with wild-caught mosquitoes using 0.05% lambda-cyhalothrin impregnated papers, following WHO standard procedure. In all the tests, 100% mortality was observed, indicating that, with the exception of *An. sundaicus*, the vectors were susceptible to lambda-cyhalothrin. *An. sundaicus* was resistant (mortality rate 53–57%). In the trial sites, the targeted households for spraying ranged from 303 to 372, with populations ranging from 1488 to 1675, and a parallel control site with the households ranging from 301 to 401 and population from 1507 to

2083. All the targeted (100%) households were sprayed at 30 mg/m^2 AI following WHO standard procedure.

Contact bioassays were carried out to determine the residual effect of the insecticide formulation on the sprayed surfaces of mud, wooden, bamboo, brick and leaf walls. Mosquitoes were exposed for 30 minutes to the sprayed surface, and the 24-hour mortality was recorded. In the north, >80% mortality of *An. minimus* was recorded up to 3, 4 and 2 months in the wooden, bamboo and brick walls respectively. In the centre, the residual effect (80%) against *An. aconitus* lasted for 4 months on the wooden as well as the bamboo walls. On the brick wall, only 63% mortality rate was recorded. With *An. dirus*, the residual effect was found to last for 6 months on wooden and bamboo walls. In the south, >80% mortality of *An. dirus* was recorded up to 4, 5 and 3 months on wood, bamboo and brick walls respectively. The mortality rate of *An. sundaicus* was >80% only on bamboo and leaf walls for 1 month. On other surfaces, the mortality rate was <77% from the beginning. Overall, the residual effect was relatively longer on the wooden walls and against *An. dirus.*

Entomological collections were carried out before spraying in fixed houses in both experimental and control villages. All indoor, outdoor and night human landing collections (18:00–06:00) were carried out for 3 nights, daytime indoor resting catches (07:00–22:00) for 2 days and light trap catches (18:00–06:00) in two fixed houses for 3 nights. Similar collections were done at monthly intervals after the spraying to determine the impact of spraying on the anopheles population.

There were no major differences in the composition of anopheles species between the treatment and control sites in the north and south during the post-treatment period. In the centre, however, the number of species was reduced during the post-treatment period compared with the pretreatment and control sites. The human landing rates, density of daytime resting mosquitoes and light trap catches of *An. minimus* and *An. dirus* were greatly reduced in all the treated sites compared with the unsprayed sites. The density of other anophelines in the north and south and daytime resting

density of *An. sundaicus* in the south, however, did not show significant reductions (P>0.05).

Three surveys (24 hours, one week and one month after spraying) were conducted among the spray operators as well as villagers to assess the perceived side-effects of the spraying. Questionnaires were used for interviewing the spray operators and 150 villagers in each survey. Of 30 spray operators engaged, only 6.6% in the centre reported side-effects such as headache, itching, smell and 3.3% sneezing. In the other two sites, the spray operators reported no side-effects. The villagers in the north reported no side-effects, while those from the centre and south reported headache (2–4%), itching (1.3–4%), smell (1.3%), eye disturbances (0.6%), dizziness, face skin allergy (4%) and fever and sneezing (0.66%). Only headache, dizziness, itching and sneezing remained one week after the spraying, but these side-effects also disappeared within a month.

Zimbabwe

The residual efficacy of indoor residual spraying with lambda-cyhalothrin 10% CS was evaluated in comparison with lambda-cyhalothrin 10% WP and pirimiphos-methyl 50% EC against *An. gambiae s.l*. in Uzumba Maramba Pfungwa, Zimbabwe. A total of 21 huts, which had not been sprayed for the previous 12 months, were selected in a ward for insecticide spraying. Pirimiphos-methyl 50% EC was sprayed at 1g/m^2 AI and 2g/m^2 AI in two sets of three huts each respectively. In another three huts, lambda-cyhalothrin 10% WP was sprayed at 30 mg/m^2 AI. Lambda-cyhalothrin 10% CS at 20, 25 and 30 mg/m^2 AI was sprayed in three sets of three huts each respectively. Three huts were used as a control.

The knock-down (KD) and killing effects of the insecticide formulations were determined through bioassays conducted 24 hours post-treatment. A total of 10 non-blood fed females of *An. gambiae s.l* were exposed to the sprayed surfaces in a WHO bioassay cone for an hour,[8] and KD was recorded. The mosquitoes were held in paper cups, fed with 20% sugar solution and their mortality recorded 24 hours post-exposure. Five replicates were

[8] WHO procedure requires 30-minute exposure.

observed for each formulation and dosage. 100% KD was recorded after 45 minutes and 35 minutes in huts treated with pirimiphos-methyl 50% EC at 1 and 2 g/m^2 AI, respectively and after 30 minutes post exposure at all dosages of the two formulations of lambda-cyhalothrin.

The fumigant effect was measured 24 hours after spraying by exposing 20 *An. gambiae s.l* in a wire cage (square frame of steel rods covered with untreated mosquito netting) suspended 1 m above the ground and 1 m from the sprayed surface. KD mosquitoes were recorded after 4 hours and mortality after 24 hours. Five replicates were used for each formulation and for each dosage. The KD rate ranged from 75% to 85%, and mortality was 100% in the cages placed in treated huts. There was no significant difference between the formulations and dosages ($P>0.05$). On day 2 post-treatment, KD and mortality rates were significantly reduced to 25–30% and 10–15% respectively in huts treated with lambda-cyhalothrin formulations, whereas the same level of effect was maintained in huts treated with pirimiphos-methyl ($P<0.05$).

The exit rate of *An. gambiae* in the sprayed huts was assessed 24 hours after application of insecticides by releasing 50 blood-fed females in each hut at 18:00 and recording the number of mosquitoes found at 22:00 the following day in the exit window traps. Three replicates were done using three huts for each formulation and for each dosage. The exit rate was 3.3% in huts sprayed with lambda-cyhalothrin WP, while the exit rate was 6–8% in the huts sprayed with 10% CS formulation. There was no significant difference between exit rates in huts treated with the two lambda-cyhalothrin formulations ($P>0.05$). The exit rate ranged from 13% to 18% in huts treated with pirimiphos-methyl 50% EC.

The immediate mortality rate as measured from the number of dead mosquitoes collected from the white sheets spread on the floor of each hut was between 92% and 96.7%. There was no significant difference between the formulations of lambda-cyhalothrin as well as the dosages. The mortality rates in the huts treated with pirimiphos-methyl 50% EC were 82–87%.

Additionally, pirimiphos-methyl 50% EC was sprayed at 1 g/m^2 AI in 10 villages with a population of 2023 and at 2 g/m^2 AI in four villages with a population of 1081. The lambda-cyhalothrin 10% CS was sprayed in four villages (population: 1986) at 20 mg/m^2 AI, six villages (population: 1678) at 25 mg/m^2 AI and four villages (population: 1228) at 30 mg/m^2 AI. The spray coverage for pirimiphos-methyl 50% EC applied at 1 and 2 g/m^2 AI was 80.2% and 85.1% respectively and for lambda-cyhalothrin 10% CS applied at 20, 25, 30 mg/m^2 AI was 84.4%, 86.4% and 92% respectively.

Contact bioassays were conducted by exposing 10 field-collected/laboratory-reared *An. gambiae s.l* each in six WHO bioassay cones, three cones placed on the roof and three on the sprayed walls for an hour. Mortality rates were scored after 24 hours. A total of five rooms were used for each formulation and for each dosage, tests were conducted every month. The mortality rate of mosquitoes exposed to the roof was >80% up to 7 months in the huts treated at 30 mg/m^2 AI 10% CS or 10% WP; the same was found for 5 and 6 months respectively in the huts treated at 20 and 25 mg/m^2 AI 10% CS. On sprayed walls, the mortality rate was 100% for 6 months at 30 mg/m^2 AI for lambda-cyhalothrin 10% CS and 10% WP formulations, 4 months at 20 mg/m^2 AI of 10% CS and 5 months at 25 mg/m^2 AI. At all dosages after 5 months, the mortality rate was below 80%. At equal dosages, there was no significant difference between the two formulations ($P>0.05$). Irrespective of the type of formulation, 30 mg/m^2 AI produced higher mortality rates than 20 and 25 mg/m^2 AI. Between the two lower dosages, there was no significant difference ($P>0.05$).

Out of 10 spray operators involved, one commented that it was difficult to mix pirimiphos-methyl 50% EC formulation and two said the same for lambda-cyhalothrin 10% WP. Only 2 spray operators (20%) experienced the problem of itching during spray of lambda-cyhalothrin formulation; 2–3 people experienced choking, smelling and itching with pirimiphos-methyl 50% EC.

Structured questionnaires were administered to about 100 respondents for each formulation or dosage to evaluate the acceptability of the spraying of the insecticides. Most (81.4–87.5%)

of the respondents believed that the majority of pests died after spraying of lambda-cyhalothrin 10% CS formulation. About 10–30% experienced side-effects: skin irritation and cough were cited as the major side-effects. The majority (91.4–94.2%) were of the opinion that the insecticide formulation was effective (90.4–92.4%). The side-effects perceived by the respondents were 11.7% and 14.5% at 1 g and 2 g/m^2 AI of pirimiphos-methyl 50% EC respectively. Smell was an additional side-effect perceived by the respondents in the villages sprayed with pirimiphos-methyl 50% EC.

Application of both the formulations of lambda-cyhalothrin reduced malaria incidence significantly compared with the control, and there was no significant difference between the impact of the formulations or of the dosages. Given its relatively longer residual efficacy, 10% CS sprayed at 25 and 30 mg/m^2 AI was recommended.

3.3 Efficacy – WHOPES supervised trials

Benin

Hougard et al. (2005) assessed the efficacy of indoor residual spraying of lambda-cyhalothrin 10% CS in comparison with lambda-cyhalothrin 10% WP against *An. gambiae* in experimental huts. The evaluation was carried out in north Benin in a soudanian Savannah area. *An. gambiae s.l* is the main malaria vector, with 95% M form and 5% *An. arabiensis.* Both are susceptible to permethrin, deltamethrin and lambda-cyhalothrin. Five experimental huts (four treated and one non-treated) were used. The walls were made of cement-plastered breezeblocks and a roof of corrugated iron with plastic cover stretched underneath. Five local adult volunteers slept in the huts from 20:00 to 05:00, five times a week and were rotated between huts on successive study nights. Preliminary catches were carried out to ensure that there was no significant difference between the huts in attracting mosquitoes. Each treatment (lambda-cyhalothrin 10% CS: 20 and 30 mg/m^2 AI; lambda-cyhalothrin 10% WP: 20 and 30 mg/m^2 AI; and a control) was randomly allocated to one of the huts. Insecticide was applied once using a hand-operated compression

sprayer. The spray had been left to dry for one week before the start of the evaluation. Mosquito collections were made from the rooms and veranda five times a week for 6 months and were recorded as alive or dead and unfed or blood-fed. Surviving mosquitoes were provided with 10% honey solution and held for 24 hours before scoring delayed mortality.

Pre-treatment catches were not significantly different between the huts ($P>0.05$). In the control huts, a total of 123 catches were made during the post–treatment period. The mosquito entry rate (average number of mosquitoes entered per hut per night) was 8.95. Natural mortality remained very low or nil. The exit rate was between 40% and 51%, blood-feeding rate ranged from 80% in July–August to 100% in November–December.

The entry rate was significantly reduced ($P<0.05$) in the huts sprayed with both formulations except in huts treated with lambda-cyhalothrin 10% WP at 20 mg/m^2 AI. Fortnightly data show that the deterrence to the level of 40–60% was maintained up to 6 months at the two dosages of CS formulation and at 30 mg/m^2 AI of WP formulation. The CS formulation at 30 mg/m^2 AI and WP formulation (at both dosages) significantly induced exophily compared with the unsprayed hut, only after the first fortnight and lasted for only 3 months. Similarly, except with the WP formulation at 20 mg/m^2 AI, all dosages inhibited blood-feeding significantly compared with the unsprayed huts. However, the inhibition was very low (8%) and was observed only for 2 months post-treatment. The reduction of entry rate, induced exophily and inhibition of blood-feeding were independent of the formulations and dosages applied. The overall mortality rate ranged from 3% to 12% at 30 mg/m^2 AI CS formulation, significantly higher than the three other dosages ($P<0.05$), for 2 months.

Bioassays were carried out every month using laboratory-reared, susceptible females of *An. gambiae* (Kisumu strain). Lots of 15 unfed mosquitoes, 3–5 days old were released into a WHO cone and exposed to the sprayed surface for 30 minutes on the four walls of each hut. After 60 minutes, KD mosquitoes were counted and kept for 24 hours in the laboratory and their mortality recorded.

The KD effect was around 70% and mortality nearly 100% on day 1 post-treatment. The KD effect declined rapidly on day 10 post-treatment (12%) and remained very low for 4 months (8–12%). Mortality also declined rapidly to around 20–34% for 4 months. There was no difference between the formulations, and the high dosage of 30 mg/m^2 AI was relatively more effective for 2 months.

Both bioassay and experimental hut results show that the residual efficacy of both formulations of lambda-cyhalothrin lasted for only 2 months.

Orissa, India

Jambulingam et al. (2005) carried out an experimental hut trial of indoor residual spraying with lambda-cyhalothrin 10% CS in comparison with lambda-cyhalothrin 10% WP against the malaria vector *An. fluviatilis (*sibling species S) in Malkangiri District of Orissa State, India. The district is endemic for falciparum malaria. *An. fluviatilis* is the main vector from October to March, predominantly endophilic and anthropophilic, and resistant only to HCH. *An. Culicifacies*, the secondary vector, is predominantly endophilic, abundant from June to August and resistant to DDT and HCH. The objective of the trial was to study and compare the impact of the residual spraying of the formulations at two application rates (20 mg/m^2 AI and 30 mg/m^2 AI) on mosquito behaviour such as house entry, induced exophily, blood-feeding success, and immediate and delayed mortality rates.

The study was conducted during September 2004–April 2005, when *An. fluviatilis* was abundant. A total of 20 experimental huts were built, the design resembling those of local tribes. The huts were made of mud walls and thatched roof (bamboo and straw) and consisted of a single room containing four windows with entry slots and screened veranda at the backside. The sprayable surface area in each hut was about 35 m^2. Four huts were randomly allocated for treatment with each formulation and four for control. Lambda-cyhalothrin formulations were sprayed at 20 mg/m^2 AI and 30 mg/m^2 AI in the huts using hand-held compression sprayers.

Mosquitoes were collected twice a week in each hut, 8 times pretreatment and 59 times post-treatment. Each collection included

live and dead mosquitoes, in rooms and veranda traps. Mosquitoes collected were identified, classified by abdominal conditions and the live ones held for 24 hours to record any delayed mortality.

An. fluviatilis was the predominant species, forming about 65% of the total collection (6109). *An. culicifacies* constituted only 17% of the collection. The numbers of *An. fluviatilis* collected in untreated huts were 117 and 2502 pre and post-treatment respectively. In the huts treated with 20 mg/m^2 AI and 30 mg/m^2 AI of lambda-cyhalothirn 10% CS and lambda-cyhalothrin 10% WP, the respective numbers (pre and post-treatment) were 148 and 357, 127 and 89, 186 and 158, 126 and 164. The entry of *An. fluviatilis* was reduced by 89–97% and of *An. culicifacies* by 61–78% in huts treated with WP and CS formulation. The reduction in the entry rates was not statistically different between the huts treated with the two dosages of WP and CS ($P>0.05$). In all the treatments, the reduction *An. fluviatilis* entry was >80% up to 7 months post-spraying. The relative increase in exit rates of both the species in the treatments was not significantly different from that recorded in untreated huts, indicating minimal induced exophily. Overall, the reduction in the proportion of blood-fed females of *An. fluviatilis* was significantly lower in the treated huts ($P<0.05$). The reduction in the feeding success was about 20%, and the difference between the dosages and formulations were not statistically significant ($P>0.05$). There was no impact on the blood-feeding success of *An. culicifacies*.

The immediate mortalities of *An. fluviatilis* were 4.5% and 3.4% in huts treated with 20 mg/m^2 AI and 30 mg/m^2 AI of WP, 10.1% and 17.7% in huts treated with CS, compared with 0.4% in untreated huts. The immediate mortality of *An. fluviatilis* was significantly higher in the huts treated with CS formulations, and increased with dosage of CS formulation ($P<0.05$). No immediate mortality was observed in *An. culicifacies*. The delayed mortalities of *An. fluviatilis* ranged from 33.1% to 43.8% and that of *An. culicifacies* from 14.5% to 22.4% in the four groups of treatments. There was no significant difference between the two formulations and dosages ($P>0.05$). The immediate mortalities were recorded only during the first 3 months following the treatment. The delayed mortalities were

relatively higher during the first 3 months post-treatment. Contact bioassays were carried out every week on the sprayed surfaces of the hut to assess persistence of the formulation. Wild-caught *An. fluviatilis* (9–13 blood-fed females) were exposed to the sprayed walls in WHO bioassay cones for 30 minutes and mortality was recorded after 24 hours. There was 100% mortality one week post-treatment, 60–79% during weeks 2–11 post-treatment and then >80% up to the end of the study period, i.e. 7 months. There was no significant difference in the residual activity of the two formulations at both the dosages (P>0.05).

Thus, lambda-cyhalothrin CS formulation was comparable with WP formulation in its residual efficacy, inhibition of blood-feeding and killing effect. However, at 30 mg/m^2 AI, CS was better in producing an immediate killing effect. Spray operators experienced no side-effects except for slight headaches that lasted for about 30 minutes. There was no complaining of any side-effect from the volunteers who slept in the experimental huts, who observed that lambda-cyhalothrin formulation had no odour or stain on the sprayed surfaces and could perceive the benefit of reduction in mosquito nuisance.

Gujarat, India

Yadav et al. (2006) carried out a village-scale trial of lambda-cyhalothrin 10% CS in comparison with lambda-cyhalothrin 10% WP at 20 and 30 mg/m^2 AI in Gujarat State, India, during 2004–2006 against the malaria vector *An. culicifacies*. The study villages were selected based on a preliminary study in several villages at risk of malaria. *An. culicifacies*, the main malaria vector, is predominantly endophilic and zoophagic. Susceptibility tests were conducted in the hot and wet season, which showed that *An. culicifacies* was highly susceptible to lambda-cyhalothrin. After a one-year baseline survey (June 2004–May 2005), a single round of spraying lambda-cyhalothrin formulations was carried out in June 2005 in comparable groups of three villages randomly allocated for each dosage; three villages were left unsprayed as a control. The number of houses in each group ranged from 1310 to 1989 and the population from 7168 to 9935. The impact of spraying on vector density, survival rate and entomological inoculation rate was evaluated. Hand catches, pyrethrum space spray collections and

light trap catches were carried out in five rooms in each village. Human landing collection was conducted in one village for each dosage and control. Two exit traps were fitted in each of the five rooms in each village for monitoring the mosquito exit rate. The entry rate, exit rate and proportion of human-blood fed mosquitoes and mortality rates were monitored every month.

Contact bioassays were carried out to determine the persistence of the formulation on sprayed surfaces, mud plastered walls and unpainted wood surfaces at monthly intervals up to 8 months post-spraying. Five replicates of 20 laboratory-reared 2–3-day-old sugar-fed females of *An. culicifacies* sibling species C were used for each dosage and type of surface in five houses. The mosquitoes were exposed for 30 minutes in WHO cones, KD mosquitoes were recorded and mortality was recorded 24 hours post-exposure.

On mud plastered walls, the residual activity (>80% mortality rate) of the two formulations at 30 mg/m^2 AI lasted for 4 months and at 20 mg/m^2 AI for 3 months. On wooden surfaces, the high dosage was effective for 5 months, while the low dosage was effective for 4 months. The residual effect was significantly longer on wooden surfaces than on the mud walls and significantly longer at 30 mg/m^2 AI than at 20 mg/m^2 AI. There was, however, no significant difference between the formulations.

The indoor resting density of *An. culicifacies* markedly declined in the sprayed villages compared with the unsprayed villages for 4 months post-treatment. The reduction was significantly greater in the villages sprayed with CS formulations than in the villages sprayed with WP formulation ($P<0.05$). The reduction was greater at CS 30 mg/m^2 AI, followed by CS 20 mg/m^2 AI, WP 30 mg/m^2 AI and WP 20 mg/m^2 AI. During the initial 4 months after spraying, the parous rate significantly reduced in sprayed villages, with greater reduction in villages sprayed at 30 mg/m^2 AI with the two formulations. There was no evidence of any deviation from human blood-feeding of the vector in sprayed villages compared with control villages. The EIR fell to zero in villages sprayed at 30 mg/m^2 AI of either formulation. At the lower dosage, the impact was not clear.

In the rooms fitted with exit traps, the proportions of blood-fed females among the total entering the trap did not differ significantly (P>0.05) between the sprayed and unsprayed rooms. The exit rate varied from 8% to 20% with the dosages and formulations tested, and was lowest at 20 mg/m^2 AI of CS formulation. The delayed mortality of *An. culicifacies* for 4 months after spraying ranged from 9.3% to 21.8% at different dosages, formulations and control.

The perceived side-effects of the formulation were assessed among five spray operators and 30 householders (15 male and 15 female) in each group of villages through a semi-structured questionnaire. The side-effects perceived by the spray operators included facial burning, sneezing, dermal and eye irritation and headache which lasted for a day. The majority of the householders interviewed stated that they experienced itching and facial burning the day following spraying. On day 8 post-spraying, there were no reported side-effects. Almost all of them found that the formulations were effective in killing mosquitoes and other insects.

3.4 Conclusions and recommendations

Lambda-cyhalothrin, a pyrethroid insecticide, has a moderate acute oral toxicity, and the technical material has been classified by WHO as “moderately hazardous”. It is biodegradable and not mobile in the environment. It is a mild irritant to the skin and eye of a rabbit. Lambda-cyhalothrin is highly toxic for fish, aquatic invertebrates and honeybees. There is no evidence of genotoxicity. Lambda-cyhalothrin may cause transient itching and/or burning sensations in exposed human skin. No long-term adverse effects have been reported.

WHOPES has already tested and evaluated lambda-cyhalothrin WP for indoor residual spraying, and the evaluation of the slow-release 10% CS was carried out with the WP formulation, using a *fast track* procedure.

The indoor residual application of lambda-cyhalothrin CS in experimental huts reduced the entry and blood-feeding rates and

increased mortality rates in different vector species. Mortality rates were higher at 30 mg/m^2 AI than at 20 mg/m^2 AI.

The indoor application of the 10% CS formulation in a village-scale trial in India was effective in reducing vector densities, survival rates and sporozoite rates. In general, the higher dosage (30 mg/m^2 AI) showed a greater effect.

Overall, the residual activity (threshold 80% mortality) of lambda-cyhalothrin 10% CS applied at dosages of 20–30 mg/m^2 AI varied from 3 to 7 months on a range of sprayable surfaces and vector species. In all respects, the CS formulation showed comparable or better efficacy to the WP formulation, with greater ease of handling. On brick walls and cement-plastered surfaces, the residual activity was very short (<2 months).

Only transient, minor adverse effects (e.g. paraesthesia) were observed among people who slept in huts sprayed with lambda-cyhalothrin and among spray operators. The village-scale studies have shown that lambda-cyhalothrin 10% CS spray is acceptable to the community.

Noting the above, the meeting recommended:

- the use of lambda-cyhalothrin 10% CS for indoor residual spraying on a wide range of sprayable surfaces for malaria prevention and control at the dosage of 20 to 30 mg/m^2 AI, with expected residual activity of 3–6 months. It is not recommended for use on cement-plastered surfaces.

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.[9]

[9] WHO specifications for public health pesticides are available on the WHO homepage on the Internet at http://www.who.int/whopes/quality/en/.

4. REVIEW OF K-O TAB 1-2-3®

The K-O TAB 1-2-3® is a "dip-it-yourself" treatment kit manufactured by Bayer Environmental Science, France, for converting untreated *washed polyester* nets into long-lasting insecticide-treated nets. The kit is based on the water-dispersible tablet (WT) formulation of deltamethrin (K-O TAB®, Bayer Environmental Science) that has previously been evaluated by WHOPES and has been recommended for treatment of mosquito nets[10] (each 1.6 g tablet contains 0.4 deltamethrin, i.e. 250 g/kg). The K-O TAB 1-2-3 treatment kit includes the original deltamethrin tablet plus a binder. The company has disclosed the nature of the binder to WHOPES and this information will be treated as confidential. The company has certified that it will inform WHOPES of any change to the binder, as this may require the re-assessment of the safety and efficacy of K-O TAB 1-2-3 and therefore WHO's recommendation on the product.

4.1 Safety assessment

The human risk assessment for impregnation of mosquito nets with K-O TAB 1-2-3 and their subsequent use, provided by the manufacturer, were assessed by the Finnish Institute of Occupational Health (FIOH) on behalf of the WHO Programme on Chemical Safety. The WHO *Generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*[11] was used as the guiding document.

For all exposure scenarios during the preparation, maintenance and use of the K-O TAB 1-2-3 mosquito nets, the manufacturer concludes that the exposure is below the Acceptable Exposure

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[10] *Report of the third WHOPES Working Group meeting: review of deltamethrin 1% SC and 25% WT and etofenprox 10% EC and 10% EW, WHO/HQ, Geneva, 23–24 September 1999*. Geneva, World Health Organization, 1999 (CDS/CPE/WHOPES/99.4; available at: http://www.who.int/whopes/recommendations/wgm/en/).

[11] *A generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*. Geneva, World Health Organization, 2004 (WHO/CDS/WHOPES/GCDPP/2004.6 and WHO/PCS/04.1; available at http://whqlibdoc.who.int/hq/2004/WHO_PCS_04.1.pdf).

Limit, or AEL (0.0075 mg/kg bw/d). This conclusion is justified by the risk characterization presented.

Accidental ingestion of one K-O TAB 1-2-3 tablet by a child will lead to an exposure that is 520 times the JMPR acute reference dose for deltamethrin (0.05 mg/kg bw) and in the order of magnitude of the lowest reported LD_{50} values for deltamethrin administered in oil. In order to prevent accidental ingestion, the K-O TAB 1-2-3 tablets are sold in sachets which are difficult to open and which also contain a bittering agent. Considering the end use of the product, this formulation is probably the least unsafe.

The FIOH, on behalf of the WHO Programme on Chemical Safety, concluded that the assessment of health risks of the preparation, maintenance, and use of deltamethrin-treated mosquito nets by Bayer Environmental Science have been performed in compliance with the WHO Generic Model, and that the conclusions of their safety, when used as instructed, are justified.

4.2 Efficacy – Background and supporting documents

Cotonou, Benin and London, United Kingdom
Yates et al. (2005) tested three nets treated with different formulations of K-O TAB 1-2-3 by Bayer Environmental Science under laboratory conditions in Cotonou, Benin, and London, UK. One formulation was the K-O TAB 1-2-3, a second included a different binder, while the third had double the dose of binder and insecticide. A polyester net, treated with K-O TAB 1-2-3 at the London School of Hygiene and Tropical Medicine (LSHTM), and a PermNet® were also included. A total of 15 samples (120 x 30 cm) were removed from each net. The samples were kept in triplicate and washed 0.5, 10, 20 or 30 times. Washing was done according with WHOPES guidelines, with a single wash in a shaker bath at 155 movements per minute in 2 g/L of soap (Savon de Marseille) followed by two rinses in deionized water. To accommodate the larger size of the net samples, 750 ml of wash and rinse solutions were used. Once the nets had been washed a specified number of times, they were sectioned into three pieces, with one piece used for 3-minute exposure bioassays, one for median time-to-knock-down (KD) tests and one for chemical analysis.

Three-minute exposure tests were carried out by taping netting to the back of paper and inserting it into the exposure chambers of WHO susceptibility tests.[12] Batches of 10 female mosquitoes (*Anopheles gambiae*, Kisumu strain, 2–5 days old) were inserted into the recovery chamber, blown into the exposure chamber and then blown back into the recovery chamber after 3 minutes. Mosquito mortality was measured 24 hours after exposure. A total of 70 mosquitoes were tested for each net type and washing interval. All net types resulted in >95% mortality of mosquitoes up to 30 washes, except for the conventional K-O TAB. Mortality of mosquitoes exposed to this net dropped to 39.4% after 10 washes and to 16.1% after 30 washes.

Median time-to-KD tests were conducted using wire-ball frames at LSHTM. The net samples were wrapped around two circular pieces of wire, and 11 female mosquitoes (*Anopheles stephensi*, BEECH strain) were introduced into the netting and the time until the sixth mosquito was knocked down was recorded. Time to KD on conventional nets increased 2.4-fold, from 454 seconds on unwashed nets to 1089 seconds on nets washed 30 times. In contrast, time to KD of the K-O TAB 1-2-3 increased only 1.3 fold, while that of the PermaNet increased only 1.1 fold.

Chemical analysis of deltamethrin concentrations indicated that conventional nets had an initial target dose of just over 25 mg/m^2 AI. After five washes, 90% of the deltamethrin had been removed from these nets. K-O TAB 1-2-3 treated nets had initial doses of approximately 25 mg/m^2 AI. After five washes, 73% of the initial dose remained on the nets, while 62% remained after 10 washes. By 30 washes, only 16% of the initial dose remained. For the PermaNet, 28% of the initial dose remained. However, the initial target dose for the PermaNet (55 mg/m^2 AI) is twice the initial target dose of the K-O TAB 1-2-3. The insecticide concentration of the K-O TAB 1-2-3 net that had double the loading dose retained insecticide better than nets with half the dose. However, the performance of this net in the WHO cylinder test and the median time-to-KD test were no

[12] The current WHO testing procedure recommends the use of cones for bioassays on mosquito nets.

different than that of K-O TAB 1-2-3 nets treated at 25 mg/m^2 AI.

The relationship between deltamethrin concentration and mosquito mortality in 3-minute exposure tests was non-linear, with very high mortality even at very low deltamethrin concentrations. However, median time to KD showed a strong linear relationship, with a longer time to KD at lower deltamethrin concentrations.

Malanville, Benin

Chandre et al. A (2005) conducted a small-scale field trial of the K-O TAB 1-2-3 in experimental huts in Malanville, Benin. A total of seven net types were tested: (i) untreated net; (ii) unwashed conventionally treated net (K-O TAB, 25 mg/m^2 AI); (iii) conventionally treated (K-O TAB, 25 mg/m^2 AI) net washed 10 times; (iv) unwashed K-O TAB 1-2-3 treated net; (v) K-O TAB 1-2-3 treated net washed 5 times; (vi) K-O TAB 1-2-3 treated net washed 10 times; and (vii) K-O TAB 1-2-3 treated net washed 20 times. The K-O TAB 1-2-3 treated nets were provided by the manufacturer.

Nets were washed in Savon de Marseille in 20 litres of soap solution (2 g/L) for 10 minutes and then rinsed twice to remove all the soap. WHO cone bioassays were conducted on each net before the start of the study. One net was sampled before the study for deltamethrin content, while all nets were tested at the end of the study.

The seven experimental huts in Malanville were 2.5 m long x 1.75 m wide x 2 m high. Mosquitoes could enter on three sides through window slits that were designed to allow for entry of mosquitoes but to limit their ability to exit. A veranda trap for capturing exiting mosquitoes was located on the fourth side. Volunteers slept in the huts from 20:00 to 06:00 hours five times per week over the month-long duration of the study. Volunteers were rotated among the huts each week. Nets were not rotated among huts, but preliminary tests indicated no significant variability in attractiveness of the huts. Outcomes measured included deterrency (number of mosquitoes entering hut relative to the hut with the untreated net), excito-repellency (proportion of mosquitoes in veranda trap), blood-feeding rate (proportion of mosquitoes in all compartments of hut that were blood-fed)

and overall mortality (proportion of mosquitoes that died within 24 hours).

Chemical analysis of the K-O TAB 1-2-3 treated nets indicated their deltamethrin concentrations were lower than the target dose (25 mg/m^2 AI). On five nets, the initial dose ranged from 14.7 to 21.6 mg/m^2 AI. After 10 washes, there was no detectable deltamethrin residues in four of the five samples tested. For the last sample, total deltamethrin concentration was measured at 2.7 mg/m^2 AI.

WHO cone bioassays indicated that the K-O TAB treated net caused high KD and mortality of susceptible *An. gambiae* before washing (KD = 98.5%; mortality = 100%). After 10 washes, there was no KD and little mortality on the K-O TAB treated net (10.3%). For the K-O TAB 1-2-3 treated net, KD was >90% through 10 washes, while mortality was >65%. By 20 washes, KD had fallen to 50.9% and mortality had declined to 7.5%. Reduction in efficacy may be explained by the lower deltamethrin target dose, as observed by chemical analysis.

For *An. gambiae*, 44 nights of collections were included for analysis. The average number of *An. gambiae* captured in each hut ranged from 5.0 to 6.1 females per night (total in control huts = 267). There was no significant difference in the entry rate among any of the treatments. However, the treated nets caused higher rates of exophily, lower rates of blood-feeding and higher rates of mortality compared with the untreated net. Exophily was 53.6% for the untreated net. For the treated nets, exophily was >60% for all treatment arms except the K-O TAB 1-2-3 treated net that had been washed 20 times. For this net, exophily was 45.8%. At 10 washes, the K-O TAB treated net cause 67.4% exophily, while the K-O TAB 1-2-3 net caused 64.7% exophily.

Blood-feeding was high for the untreated net (65.5%), particularly since the nets did not have holes. For the treated nets, blood-feeding was <40% for all treatments. Higher rates of blood-feeding were observed for the K-O TAB treated net washed 10 times (34.9%) and the K-O TAB 1-2-3 treated net washed 20 times (35.8%) compared with unwashed K-O TAB and K-O TAB 1-2-3 treated nets. Mortality was low for the untreated control net (2.2%) but was high for the unwashed K-O TAB treated net (89.4%) and the unwashed K-O TAB 1-2-3

treated net (92.3%). After 10 washes, mortality declined to 36.3% for the K-O TAB treated net and 38.5% for the K-O TAB 1-2-3 treated net. Mortality was 29.0% for the K-O TAB 1-2-3 treated net washed 20 times. When all mosquito species were included in the analysis, the results were similar. Blood-feeding increased and mortality decreased significantly on K-O TAB treated nets washed 10 times compared with an unwashed K-O TAB treated net. Similar observations were made on K-O TAB 1-2-3 treated nets that had been washed 20 times compared with an unwashed K-O TAB 1-2-3 treated net.

London, United Kingdom

N'Guessan et al. (2006) compared the efficacy of the K-O TAB and the K-O TAB 1-2-3 in WHO cone tests, cylinder tests and tunnel tests. A total of three different nets were tested: K-O TAB (treated by Bayer Environmental Science); K-O TAB 1-2-3 (treated by Bayer Environmental Science); and K-O TAB 1-2-3 (treated at LSHTM). Six samples were cut from each net (45 x 45 cm). One sample from each treatment was washed 0.5, 10, 15, 20 and 25 times. Samples were washed in 500 ml of soap solution (2 g/L, Le Chat, Savon de Marseille) for 10 minutes in a shaker bath at 30 °C and 155 movements per minute. For testing, the net was cut into several pieces. One piece was used for WHO cone and tunnel tests; three pieces were used for cylinder tests; the last piece was used for chemical analysis.

Chemical analysis of deltamethrin content on K-O TAB 1-2-3 treated nets indicated all test nets were close to the target dose (25 mg/m^2 AI). Washing reduced the amount of deltamethrin on the nets, but the decline was much greater on the K-O TAB treated net. On the K-O TAB treated net, there was <5mg deltamethrin per m^2 after just 5 washes and negligible amounts after 20 washes. For the K-O TAB 1-2-3 treated nets, the amount of deltamethrin remained above 5 mg/m^2 AI even after 25 washes.

Female *An. gambiae*, Kisumu strain, 2–5 days old, were used for all tests. Cone tests were conducted on netting that was 30 x 30 cm. At least 50 mosquitoes were exposed for 3 minutes. Cylinder tests were done by attaching 15 x 18 cm pieces of netting to 12 x 15 cm paper inserts and placing them into the exposure chamber of WHO cylinder tests. Batches of 10 mosquitoes were introduced into the holding chambers and then blown into the exposure chambers and held for 3 minutes.

At least nine replicates were performed for each treatment/wash interval. For both tests, KD was recorded 60 minutes after exposure and mortality was recorded 24 hours post-exposure.

The tunnel test was conducted by cutting nine holes of 1 cm in diameter in the test net and placing the netting 20 cm from one end of a tunnel (60 cm x 25 cm x 25 cm). A guinea-pig was placed in the short end of the tunnel and mosquitoes were introduced in the other end of the tunnel at 18:00. The test was stopped at 09:00 the next morning. Outcomes measured included the percentage of the total mosquitoes that passed through the netting, the percentage that failed to blood-feed and the percentage that died within 24 hours after exposure.

Initial comparisons aimed to determine if there were differences in the quality of the K-O TAB 1-2-3 treatment when done by Bayer Environmental Science or by the LSHTM. In the WHO cone test, mortality and KD were generally lower on the net treated by LSHTM, with the greatest difference observed at 25 washes. No differences were observed in these measures following a cylinder test. No differences were observed in the tunnel test in terms of percentage passing through the netting, percentage blood-feeding and percentage mortality.

In the cone test, mortality fell below 80% within 5 washes and was <20% after 15 washes for the K-O TAB. For the K-O TAB 1-2-3, mortality remained above 60% through 20 washes, declining to just below 50% after 25 washes. In the cylinder test, mortality of mosquitoes exposed to K-O TAB treated nets gradually declined to 56% after 15 washes before dropping below 10% at 20 and 25 washes. For the K-O TAB 1-2-3, mortality remained at or above 80% through 25 washes.

Mortality in the tunnel test was high for both the K-O TAB and the K-O TAB 1-2-3 after repeated washings. Mortality of mosquitoes exposed to the K-O TAB 1-2-3 in the tunnel test remained at 100% through 25 washes. For the K-O TAB, mortality was >95% through 15 washes before declining to 60% after 20 washes and 30% after 25 washes. The percentage of mosquitoes passing through the net and the percentage blood-feeding in the tunnel test were similar for nets treated with the K-O TAB or the K-O TAB 1-2-3 up to 15 washes. However, these indicators were lower for the net treated with the K-O TAB

1-2-3 at 20 and 25 washes compared with the K-O TAB treated net at 20 and 25 washes.

4.3 Efficacy – WHOPES supervised trials

Montpellier, France
Bonnet et al. (2005a) conducted laboratory tests to measure the optimum binder concentration for treatment of nets with K-O TAB 1-2-3, to measure the wash-resistance of the K-O TAB 1-2-3 and to compare the bioavailability of insecticide on coloured and white nets treated with the same insecticide and binder concentrations.

A total of 12 white, 2 blue and 2 green polyester nets were treated in the laboratory by manually dipping the nets in 500 ml of deionized solution containing one K-O TAB and the appropriate amount of binder. All samples were treated in Montpellier. Net samples (25 x 25 cm) were washed in 500 ml of soap solution (Savon de Marseille, 2 g/L) for 10 minutes at 155 movements per minute in a shaker bath followed by two rinses in 500 ml of deionized water.

Bioassays were conducted using the WHO cone test. A total of 50 female mosquitoes (*An. gambiae*, Kisumu strain, 2–5 days old) in five replicates of 10 mosquitoes were exposed for 3 minutes to each net sample. After exposure, mosquitoes were transferred to plastic cups and provided with sugar solution. KD was measured 60 minutes after exposure and mortality was measured 24 hours post-exposure.

In the first experiment, nets were treated with one K-O TAB plus binder concentrations ranging from 250 mg/m^2 to 1000 mg/m^2. Bioassays were conducted at 0, 10, 15, 20 and 25 washes, with one day between washes. Mortality on all formulations declined with increasing washes, from >93% at wash 0 to approximately 50% after 10 washes. By 20 and 25 washes, mortality was <10% on all formulations except for those treated with 750 and 1000 mg/m^2 of binder, where mortality was between 10 and 20%. KD was >80% for all formulations for up to 15 washes. After 15 washes, KD declined for all formulations, but the decline was highest for nets treated with 250, 450 and 666 mg/m^2 of binder. At the request of the manufacturer, the binder concentration was set at 666 mg/m^2.

Chemical analysis of deltamethrin content was done after 25 washes on nets treated with a K-O TAB and various amounts of binder. The highest concentrations of deltamethrin were on two nets treated with the K-O TAB plus 1000 mg/m^2 of binder (5.04 and 4.88 mg/m^2 AI). Of the remaining eight nets tested, only two other nets had deltamethrin concentrations higher than 3 mg/m^2.

In the second experiment, the regeneration time and wash resistance of the K-O TAB 1-2-3 with 666 mg/m^2 of binder were tested. Nets were washed up to three times, with bioassays conducted one day after each wash. Bioassays were also conducted three days and five days after the third wash. Mosquito mortality in the bioassays declined after each wash to 69%. The maximum bioavailability of insecticide on the net was reached in one day. Wash-resistance studies using a regeneration time of one day in which WHO cone bioassays were conducted after 1, 5, 10, 15 and 20 washes showed that mortality dropped below the WHOPES threshold of 80% after 15 washes. However, KD was >95% after 20 washes. Thus, the K-O TAB 1-2-3 met the criteria for WHOPES phase I studies.

In the second experiment using only the K-O TAB plus 666 mg/m^2 of binder, initial deltamethrin concentrations ranged from 21.2 to 30.6 mg/m^2. By 15 washes, the deltamethrin concentrations had fallen to an average of 11.3 mg/m^2 AI. By 20 washes, average deltamethrin concentrations had fallen to an average of 3.4 mg/m^2 AI.

Malanville, Benin

Hougard et al. (2006) conducted a small-scale field trial of the K-O TAB 1-2-3 in experimental huts in Malanville, northern Benin. A total of six net types were tested: (i) untreated net; (ii) unwashed conventionally treated net (K-O TAB, 25 mg/m^2 AI); (iii) conventionally treated net (K-O TAB, 25 mg/m^2 AI) washed until just before exhaustion (2 washes); (iv) unwashed K-O TAB 1-2-3 treated net; (v) K-O TAB 1-2-3 treated net washed 15 times; and (vi) K-O TAB 1-2-3 treated net washed 20 times.

All nets were treated in Benin. Nets to be treated with the K-O TAB 1-2-3 were washed before treatment, as recommended by the manufacturer.

Bioassays were conducted on the nets just before and just after being tested in the huts. The nets were tested in the experimental huts over 12 weeks (total of 60 nights), as described by Chandre et al. (2005). Six huts were employed, with nets and volunteers rotated independently among the huts in a Latin square design.

Chemical analysis of nets immediately after treatment and before washing indicated that the treatments were reasonably close to the target dose (25 mg/m^2 AI) for both the K-O TAB treated nets (20.5 to 26.7 mg/m^2 AI) and the K-O TAB 1-2-3 treated nets (21.2 to 25.1 mg/m^2 AI). The reported concentration of deltamethrin on the K-O TAB treated nets, washed two times, was unexpectedly low. After 15 or 20 washes, deltamethrin concentrations on the K-O TAB 1-2-3 treated nets were 0.8 and 1.5 mg/m^2 AI, respectively. After the trial, there was little change in deltamethrin concentrations for all nets except for the unwashed K-O TAB 1-2-3 treated net, which had an average of 12.8 mg/m^2 AI. However, of five values measured on the net, two were below the limit of quantification, while the other three were all above 16 mg/m^2 AI.

Initial WHO cone bioassays showed little KD or mortality on the K-O TAB 1-2-3 nets that had been washed 15 or 20 times. The K-O TAB treated net washed until just before exhaustion (two washes) caused 100% KD and 86.6% mortality. Similar results were obtained at the end of the 12-week evaluation except for the K-O TAB treated net washed two times, where KD and mortality declined significantly.

In the experimental hut study, 260 female *An. gambiae* were collected in the huts with the untreated control net. Blood feeding rate was 21%, exophily was 48% and mortality was low (2.3%). In the huts with the test nets, there was a significant reduction in the number of mosquitoes entering the huts for the unwashed K-O TAB and the unwashed K-O TAB 1-2-3 nets. However, there was no evidence for deterrency in the K-O TAB 1-2-3 nets that had been washed 15 or 20 times. Compared with the untreated net, there was a slight but significant increase in exophily from <50% to over 60% for the treated nets, regardless of washing. Mortality also significantly increased from <5% to over 40%. Mortality was 95.6% on the unwashed K-O TAB treated net and 72.0% on the K-O TAB

treated net washed two times. Mortality declined on the K-O TAB 1-2-3 treated nets from 84.2% on the unwashed net to 53.1% on the net washed 15 times and to 41.9% on the net washed 20 times. Similar trends were observed when the data were pooled across mosquito species.

The authors concluded that the K-O TAB 1-2-3 does not reduce hut entry, particularly after repeated washing. However, blood-feeding inhibition (BFI) and mortality still remained reasonably high after 15 (BFI = 79.5%; mortality = 58.5%) or 20 (BFI = 77.9%; mortality = 45.2%) washes.

Moshi and Muheza, United Republic of Tanzania

Malima et al. (2006) conducted an experimental hut trial at two sites in the United Republic of Tanzania, with six treatment arms. The treatment arms included: (i) untreated net; (ii) K-O TAB treated net (25 mg/m^2 AI) washed until exhaustion (6 washes); (iii) K-O TAB treated net (25 mg/m^2 AI) washed 20 times; (iv) unwashed K-O TAB 1-2-3 treated net; (v) K-O TAB 1-2-3 treated net washed 14 times; and (vi) K-O TAB 1-2-3 treated net washed 20 times.

All nets were treated in the United Republic of Tanzania. Nets to be treated with the K-O TAB 1-2-3 were washed before treatment, as recommended by the manufacturer.

Nets were treated individually, according to the manufacturer's instructions, in 0.5 L of water. Nets were thoroughly kneaded until saturated and then dried on plastic sheets, turning over every 15 minutes. Nets were washed in 10 litres of soap solution (2 g/L Savon de Marseille). The nets were soaked for 10 minutes, with manual rotation of the nets for 6 of the 10 minutes. The nets were rinsed twice in fresh water, dried in the shade and then stored at room temperature. The schedule for washing was designed to ensure all the washings were completed at the same time.

At both sites, six experimental huts were built with brick walls plastered with mud. The roof was made of iron sheets with a ceiling of hessian sackcloth. The eaves were left open. On two sides windows were fitted with window traps, but mosquitoes could enter or exit through the eaves. Veranda traps on the other two sides prevented mosquitoes from entering but captured mosquitoes that exited through the eaves. The

locations of the veranda traps and the window traps could be rotated so that for half the nights, the north and south verandas were left open, while the east and west verandas were left open the other nights.

The hut trial was run over 36 nights, with sleepers and treatment arms rotated through the huts. Each net was deliberately holed with six holes (4 cm x 4 cm). Six huts were employed with nets and volunteers rotated independently among the huts in a Latin square design. The primary outcomes were deterrency, induced exophily, BFI and mortality. Personal protection (PP) was estimated from deterrency and BFI by the formula $\%PP = 100(B_u–B_t)/B_u$, where B_u is the total number blood-fed in the huts with untreated nets and B_t is the total number blood-fed in the huts with the test nets. Insecticidal effect (IE) was estimated by the formula $\%IE = 100(K_t–K_u)/(T_u–K_u)$ where K_t is the number killed in huts with treated nets, K_u is the number killed in huts with untreated nets and T_u is the total collected from the huts with untreated nets.

Cone bioassays were conducted to determine the cut-off points for the K-O TAB treated net. A total of 10 WHO cone bioassays (50 mosquitoes per net) were conducted on nets before each washing and immediately before and after the experimental hut study. *An. gambiae* was the test mosquito at one site, while *An. arabiensis* was the test mosquito at the other site. For the K-O TAB treated nets, tests with *An. gambiae* indicated mortality fell below 80% after six washes, while KD fell below 95% after three washes. In tests with *An. arabiensis*, mortality fell below the cut-off after four washes and KD fell below the cut-off after two washes. The exhaustion point for the K-O TAB treated nets was set at six washes (28% KD and 60% mortality for *An. gambiae*).

The primary species at the experimental hut test site in Muheza included *An. funestus* and *An. gambiae*. All treated nets had a deterrent effect on *An. funestus* ranging from 19.4% to 46.6%, although there were no statistically significant differences among the deltamethrin treated nets (total number of *An. funestus* in control hut = 175). Exophily was very high in all treatments, including the huts with the untreated net where 86.9% of all mosquitoes were captured in the veranda traps. However, exophily was even higher for the treated nets and

only the K-O TAB 1-2-3 nets washed 20 times did not have a significantly higher rate of exophily than the untreated net. Blood-feeding was also reduced for *An. funestus* in the huts with treated nets. BFI was highest on the K-O TAB 1-2-3 treated nets and declined with increasing numbers of washes. Mortality in the huts with treated nets was also higher relative to that in huts with untreated nets. Furthermore, mortality was significantly higher in the K-O TAB 1-2-3 treated nets, regardless of washing, compared with the K-O TAB treated nets. Personal protection against *An. funestus* was around 70% for the K-O TAB 1-2-3 treated nets and just under 50% for the K-O TAB treated nets, regardless of the number of washes. The insecticide killing effect was also higher in the K-O TAB 1-2-3 treated nets compared with the K-O TAB treated nets.

Relatively few *An. gambiae* were collected at Muheza (total number of *An. gambiae* in control hut = 42), but the trends were similar. Deterrency ranged from 33.3% to 47.6% for the K-O TAB 1-2-3 treated nets. No deterrency was evident for the K-O TAB treated nets. Exophily was high for all treatment arms (>90%), but there was complete exophily for the K-O TAB 1-2-3 treated nets. BFI was greater than 60% for the K-O TAB 1-2-3 treated nets, regardless of washing. It was slightly less for the K-O TAB treated net washed 6 times (42.7%) and the K-O TAB treated net washed 20 times (59.8%), but the difference was not statistically significant. Mortality was highest in huts with the unwashed K-O TAB 1-2-3 treated net (88.0%). There was a significant decline in mortality, with increasing numbers of washes. All the K-O TAB 1-2-3 treated nets caused higher mortality than either of the K-O TAB treated nets, but the difference in mortality for the K-O TAB 1-2-3 treated nets at 20 washes (45.5%) was not significantly different from the K-O TAB treated net at either 6 (27.5%) or 20 washes (27.1%) owing to the low numbers collected.

For *An. arabiensis* at Moshi, there was low deterrence in all the treatment arms (total number of *An. arabiensis* in control hut = 392). Exophily was highest in the K-O TAB 1-2-3 washed to exhaustion and the K-O TAB washed to exhaustion. However, all the treated nets induced significantly higher exophily compared with the untreated control net. Compared with the untreated control net, blood-feeding was reduced by >70% in the K-O TAB 1-2-3 treated nets, regardless of washing, while blood-feeding was reduced by 61.8% in huts with the K-O

TAB net washed 6 times and by 65.7% in the huts with the K-O TAB net washed 20 times. Mortality was similar on the K-O TAB 1-2-3 treated nets compared with the K-O TAB treated nets. However, all treated nets induced significantly higher mortality than the untreated control net.

BFI and mortality of *An. funestus* were significantly higher for the K-O TAB 1-2-3 treated nets washed 20 times compared with a K-O TAB treated net washed to exhaustion (six times). The trend was similar in *An. Gambiae*, but there were no significant differences in BFI or mortality owing to the low numbers collected. For *An. arabiensis*, there were no significant differences in BFI or mortality.

In WHO cone bioassays conducted on mosquito nets before the hut trial (but after the washing of the nets) and after the hut trial was completed, there was a significant reduction in mortality and KD of *An. gambiae* after completion of the hut trial, suggesting some loss of efficacy due to normal handling of the nets.

4.4 Conclusions and recommendations

The K-O TAB 1-2-3 is a “dip-it-yourself” kit for treatment of washed polyester mosquito nets. The target dose (25 mg/m^2 AI) is similar to a K-O TAB but includes a binder that resists repeated washing. K-O TAB, a water dispersible tablet (WT) formulation of deltamethrin, has previously been evaluated by WHOPES and has been recommended for treatment of mosquito nets. The company has disclosed the nature of the binder to WHOPES, and this information will be treated as confidential. The company has certified that it will inform WHO of any change to the binder, as this may require the re-assessment of the safety and efficacy of K-O TAB 1-2-3 and therefore WHO's recommendation on the product.

The WHO assessment of the compliance of the manufacturer's assessment of exposure to and risks of treatment, washing and sleeping under K-O TAB 1-2-3 treated nets, is in line with the WHO generic risk assessment model.

Several laboratory studies have demonstrated high efficacy after up to 20 washes, particularly for KD of *An. gambiae*.

There was some variability in the performance of nets in different laboratories, with mortality dropping significantly below the cut-off value of 80% in at least two of the laboratory studies. However, in all three laboratories studies, K-O TAB 1-2-3 met the WHOPES requirement for KD ($\geq$95% after 20 washes). After washing, maximum bioavailability of K-O TAB 1-2-3 treated nets was reached in one day at room temperature.

In the small-scale field study (experimental huts) in the United Republic of Tanzania, mortality and BFI of *An. funestus* were higher with K-O TAB 1-2-3 washed 14 and 20 times compared with the exhausted conventional treated net with KO TAB and were similar for *An. arabiensis*. However, overestimation of the efficacy of K-O TAB 1-2-3 cannot be excluded as the exhausted conventional treated net used as a reference was washed beyond the cut-off value rather than before.

In the WHOPES supervised study in Benin, the performance of K-O TAB 1-2-3 treated nets, washed 20 times, was not equal to or better than those conventionally treated with K-O TAB and washed just before exhaustion (two times). The mortality and BFI of *An. gambiae* in huts with K-O TAB 1-2-3 treated nets, washed 20 times, were 41.9% and 74.8%, compared with 72.0% and 59.9% in huts with nets conventionally treated with K-O TAB and washed two times.

Considering safety, efficacy and wash-resistance of K-O TAB 1-2-3 in laboratory and small-scale field studies, the meeting concluded that:

- K-O TAB 1-2-3 is an innovative technology for treatment of *washed, polyester* mosquito nets in the field. Polyester mosquito nets treated with K-O TAB 1-2-3 can be washed normally up to 15 washes, after which they should be re-treated;

- treatment of mosquito nets with K-O TAB 1-2-3 does not convert mosquito nets to “long-lasting insecticidal mosquito nets", as defined by WHOPES, which requires that a long-lasting insecticidal mosquito net washed 20 times or more should perform equally to or better than a conventionally treated net washed until just before exhaustion.

The meeting recommended:

- that time-limited *interim recommendation* is given to K-O TAB 1-2-3 for treatment of washed white and coloured polyester mosquito nets and requested WHOPES to coordinate large-scale field studies (WHOPES Phase III) to confirm its efficacy and wash resistance, as well as operational acceptability, as a requirement for development of *full recommendations* on its use;

- that efficacy and wash-resistance of K-O TAB 1-2-3 on different types of polyester and on other type of fabrics should be determined.

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.[13]

[13] WHO specifications for public health pesticides are available on the WHO homepage on the Internet at http://www.who.int/whopes/quality/en/.

5. REVIEW OF INTERCEPTOR®

Interceptor® net is an alpha-cypermethrin long-lasting insecticidal (coated) mosquito net (LN) manufactured by BASF (Germany), with the target dose of 200 mg of alpha-cypermethrin per square metre of the polyester fabric.

5.1 Safety assessment

The human risk assessment for washing and sleeping under the treated nets, provided by the manufacturer, was assessed by the FIOH on behalf of the WHO Programme on Chemical Safety. The WHO *Generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*[14] was used as a guiding document.

Using the manufacturer's experimental data, and applying the Generic Model, washing of a bednet and sleeping under the net were estimated to result in exposures which are below the appropriate guidance values (acute reference dose for the washing of the net and long-term AEL for sleeping under the net) for an adult, child, and newborn. The major contributor to the exposure of the newborn sleeping under the net is the chewing of the net.

FIOH concluded that, while the manufacturer's assessment and the FIOH assessment are procedurally somewhat different, their bottom-line conclusions are in agreement in estimating that the washing of the net or sleeping under the net do not pose undue hazards to the exposed adults, children or newborns.

[14] *A generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*. Geneva, World Health Organization, 2004 (WHO/CDS/WHOPES/GCDPP/2004.6 and WHO/PCS/04.1; available at http://whqlibdoc.who.int/hq/2004/WHO_PCS_04.1.pdf).

5.2 Efficacy – Background and supporting document

Duchon et al. (2005) determined the wash resistance and efficacy of the Interceptor nets in the laboratory for two series (S-60 and S-61) of net samples provided by the manufacturer. After 20, 25, and 30 standard washes (WHO washing procedure), samples were tested in bioassays, and in experimental tunnels using a fully susceptible *An. gambiae s.s*. strain (Kisumu). For one sample of each series, KD effect after 20 washes was 100% but decreased below 95% after 25 washes. Mortality was below 80% for all assays. Both series met the WHO criteria at 20 washes (≥80% mortality and/or ≥95% KD). Tunnel tests were used to measure the efficacy in terms of BFI and mortality. One sample of each series was tested and mortality was above 80% after 20 and 25 washes; only the sample of series S-60 provided satisfactory results after 30 washes (82%). Reduction of blood-feeding was 100% after 20 washes and still above 90% after 30 washes for series S60. Despite relatively low mortality observed with the cone tests, these nets showed high efficacy in terms of mortality rates and BFI in tunnel tests.

5.3 Efficacy – WHOPES supervised trials

5.3.1 Laboratory studies

Bonnet et al. (2005b) determined the efficacy and wash resistance of Interceptor® according to the WHO guidelines for laboratory testing of LNs.[15] Two series (S-60 and S-61), each of eight samples (25 x 25 cm) were used for that purpose. For each series, two samples were tested without any wash, others were standard washed 5, 10 or 20 times by replicates of two. Bioassays were carried out 1, 2, 4 and 8 days after the end of the washing cycle. KD was observed after 3 minutes of exposure and 60 minutes of holding, and mortality after 24 hours. Both series of nets showed good performances against susceptible *An. gambiae s.s.* (Kisumu strain) in terms of efficacy and wash resistance. The KD was above the critical

[15] *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets*. Geneva, World Health Organization, 2005 (document WHO/CDS/WHOPES/GCDPP/2005.11; available at http://www.who.int/whopes/guidelines/en/).

value of 95%, in one hour post-exposure, for all tests even after 20 washes. Mortality dropped down below the cut-off value of 80% at 20 washes. Samples of the Interceptor showing an efficacy below the cut-off point were heated at 60 °C for 1 hour. Maximum bioavailability was reached after 1 day at 25 °C. Heating for 1 hour at 60 °C did not increase insecticide bioavailability.

5.3.2 Experimental hut studies

Interceptor® nets were evaluated in experimental huts in two locations, Benin and the United Republic of Tanzania, using wild, free-flying, pyrethroid-susceptible malaria vectors. Efficacy was evaluated in terms of BFI, deterrence, induced exophily and mortality. The design of the experimental huts was slightly different between the two localities: in Benin, the mosquitoes could only escape outside to a single veranda trap, while in the United Republic of Tanzania they could escape via the eaves to screened or open verandas. To adjust unrecorded escapes in the latter case, numbers of mosquitoes collected in screened verandas were multiplied by two.

In each locality, six trap huts were used and six treatment arms were tested. Each week, treatment arms were rotated through the huts according to a Latin square scheme. Five nets were used per treatment arm and each net was tested one night a week. Each net was deliberately holed with six holes (4 cm x 4 cm) to simulate a torn net. One additional net per treatment arm was used for chemical analyses and bioassays.

Malanville, Benin

Chandre et al. (2006) tested the following six treatment arms: (i) unwashed Interceptor® net; (ii) Interceptor® net washed 20 times (WHO criteria for a long-lasting insecticidal net); (iii) polyester net conventionally treated with alpha-cypermethrin SC10% at 40 mg/m² AI (WHO recommended concentration); (iv) polyester net conventionally treated with alpha-cypermethrin SC10% at 200 mg/m² AI (same concentration as Interceptor®)[16] and washed until just before exhaustion; (v) polyester net conventionally treated with alpha-cypermethrin SC10% at 200

[16] The high dose of alpha-cypermethrin on conventionally treated nets was a modification of the WHO guidelines for net treatment and was used to provide an equivalent comparison to the Interceptor net.

mg/m² AI (same come concentration as Interceptor®) and washed 20 times; and (vi) untreated net (same fabric and mesh size as Interceptor®).

Before any washing, all nets were fully effective (one hour post-exposure KD and 24-hour post-exposure mortality of 100%) in bioassays. The positive controls (treatment arm number 4) were nets conventionally treated and washed to just before exhaustion. The cut-off point of a net conventionally treated with alpha-cypermethrin at 200 mg/m² was here considered to be six washes (KD 1 hour: 92%, mortality 24 hours: 79%).

The initial bio-efficacy of the nets to be used in each arm was studied before field evaluation. The unwashed LN and unwashed conventionally treated net (alpha-cypermethrin at 40 mg/m²) were fully effective with 100% KD after 60 minutes post-exposure and 100% mortality in 24 hours post-exposure. With Interceptor® LN, a significant impact of washing (20 times) was observed on mortality (56%) but not on KD (97%). Nets conventionally treated with the same concentration as in the LN (200 mg/m²) lost completely their efficacy (KD: 2% and mortality 17%) after 20 washes.

Chemical residue analysis showed that the initial dosages were below the expected dosages, not only for conventionally treated nets (155 mg/m² instead of 200 mg/m² and 24.2 mg/m² instead of 40 mg/m²) but also for Interceptor nets (125 and 168 mg/m² instead of 200 mg/m² AI). After 14 weeks of use in experimental huts, no significant decrease of alpha-cypermethrin content on unwashed nets due to normal handling and use was observed. For Interceptor®, after 20 washes and field utilization, 40.8 mg/m² of alpha-cypermethrin remained on the net. For used nets, conventionally treated with 200 mg alpha-cypermethrin/m², only 2.5 mg/m² AI remained after six washes and no insecticide was detected in conventionally treated nets (200 mg alpha-cypermethrin/m²) washed 20 times.
During a 14-week collecting period, 89 *An. gambiae* and 789 other mosquito species, mainly *Mansonia africana* and *M. uniformis*, were collected in the control huts.

All treated nets significantly reduced the entry rates of female mosquitoes (15–30%). However, this was usually not significant for *An. gambiae* s.s. because of the low number of females

collected in the control arm. A slight increase of exophily was also noticed ranging from 10% to 35%.

High mortality (above 95%) and high BFI (above 90%) were observed for *An. gambiae s.s.* as well as for the other mosquito species. Personal protection of LN washed 20 times was almost 100%. Moreover, the fact that nets did not strongly reduce the entry rates of female mosquitoes, but kill almost all of them, suggested that these nets could have a strong mass effect of mosquito populations if used at large scale. The overall insecticidal effect of Interceptor® nets on *An. gambiae* after 20 washes, taking into account the number of mosquitoes that were not killed by the deterrency of the treatment, was 63%.

The bioassay of nets after field evaluation indicated no significant reduction in efficacy of the nets after 67 nights of use compared with the observations made before the start of the study. Interceptor® and conventionally treated nets at the target dose (40 mg/m²) remained fully effective, with 100% KD and 99-100% mortality. No significant reduction in KD and mortality was noted with the Interceptor® washed 20 times, the nets conventionally treated at 200 mg/m² and washed 20 times, and those washed until cut-off point, compared with bioefficacy of the nets observed before the start of the experimental hut studies.

Although a very low amount of insecticide was reported in the chemical analysis of conventionally treated nets washed 20 times, significant mortality was still noted.

Muheza, United Republic of Tanzania

Rowland et al. (2006) tested the following six treatment arms: (i) unwashed Interceptor®; (ii) Interceptor® washed 20 times (criteria for a long-lasting insecticidal net); (iii) Interceptor® washed 30 times; (iv) polyester net conventionally treated with alpha-cypermethrin SC10% at 200 mg/m² AI (same concentration as Interceptor®)[17] and washed until just before exhaustion; (v) polyester net conventionally treated with alpha-

[17] The high dose of alpha-cypermethrin on conventionally treated nets was a modification of the WHO guidelines for net treatment and was used to provide an equivalent comparison to the Interceptor® net.

cypermethrin SC10% at 200 mg/m² AI (same concentration as Interceptor®) and washed 30 times; and (vi) untreated net (same fabric and mesh size as Interceptor®).

Before washing, the one-hour KD observations and the 24-hour post-treatment mortality readings from the bioassays were 100% on all treated nets. After washing the conventionally treated net 20 times, the one-hour KD fell to 33% and mortality to 66%, while one-hour KD and 24-hour mortality stayed close to 100% with the Interceptor® washed 20 times.

Chemical analysis showed that the initial dosages were below the expected dosages, and this was true for conventionally treated nets (around 150 mg/m² instead of 200 mg/m² AI) and for LN manufactured nets (140 mg/m² instead of 200 mg/m² AI). After 66 days of use in experimental huts, no decrease of alpha-cypermethrin on unwashed nets was observed. For post - trial Interceptor®, the concentration had fallen to about 40 mg/m² AI after 20 washes and to 21 mg/m² AI after 30 washes. For used nets conventionally treated with 200 mg/m² AI, only 1–3 mg/m² AI remains after 20 washes and <1 mg/m² AI after 30 washes.

During the 66 night collections, 147 *An. gambiae*, 70 *An. funestus* and 86 *Culex* spp. were collected in control huts. There was no evidence of an important deterrence effect with *An. gambiae*, *An funestus* or *Culex* mosquitoes by any of the treated nets. In the control arm, and in contrast to *Culex* (50%), the majority (more than 80%) of the *Anopheles* escaped to the veranda traps. Exophily rates of the two *Anopheles* species were slightly higher (up to 95%) with treated nets, especially when washed. Insecticide-induced exophily was more evident with *Culex* and reached as high as 95% with unwashed Interceptor® nets.

Interceptor® induced high mortality of *An. gambiae* that entered the hut. This fell significantly with the number of washes (0 washes: 93%, 20 washes: 79%, 30 washes: 66%). Conventionally treated nets washed 20 and 30 times scored significantly lower for mortality (20 washes: 46.1%, 30 washes 46.9) than the washed Interceptor®. Mortality rates for *An. funestus* were similar to those of *An. gambiae* with respect to traditional treated nets (20 washes: 55%; 30 washes 48%) but

rather lower than that of *An. gambiae* with respect to Interceptor® (0 washes: 76%, 20 washes: 61%, 30 washes: 59%). Mortality was much lower with *Culex* (around 15%) than with the two *Anopheles* species, and no significant difference was observed between the different treatment arms. This is presumably due to pyrethroid resistance commonly observed in *Culex* species.

The Interceptor® outperformed the conventional treated nets in terms of BFI especially for *An. gambiae* and *Culex*. For *An. gambiae*, 47% were blood-fed with the untreated nets for only 12% with Interceptor® at 0 washes. Blood-feeding increased in *An. gambiae* with the number of washes and was significantly lower for Interceptor® than for conventionally treated nets at 20 washes (respectively 16 and 32%) but was similar at 30 washes (21% and 29% respectively).

An. funestus showed significantly lower blood-feeding rates with the Interceptor® than with the conventionally treated net at the 20 wash cut-off point (14% and 27% respectively) but not at 30 washes (21% and 25% respectively).

The overall personal protection was high with unwashed Interceptor® (*An. gambiae*: 80%, *An. funestus* 61%, *Culex*: 89%). At 20 washes, Interceptor® provided a much higher protection than the conventionally treated nets (*An. gambiae*: 72 vs 12%, *An. funestus* 57 vs 0%, *Culex*: 78 vs 37%).

Although a very low amount of insecticide was reported in the chemical analysis of conventionally treated nets washed 20 times, significant mortality was still noted.

5.4 Conclusions and recommendations

Interceptor® net is an alpha-cypermethrin long-lasting insecticidal (coated) mosquito net manufactured by BASF (Germany), with the target dose of 200 mg of alpha-cypermethrin per square metre of the polyester fabric.

The WHO assessment of the compliance of the manufacturer's assessment of exposure to and risks of washing and sleeping under Interceptor® nets was in line with the WHO generic risk

assessment model and, although procedurally somewhat different, their conclusions were in agreement.

Laboratory studies demonstrated for two net series of the manufacturer good and similar performances against *An. gambiae* in terms of efficacy and wash resistance. The Interceptor® nets met the WHOPES Phase I criteria of a KD effect above the 95% after 20 washes. Mortality dropped below the cut-off value of 80% after 20 washes. Maximum bioavailability was achieved within one day at 25 °C and heating did not increase bioavailability.

Field studies demonstrated a better or equal impact of Interceptor® washed 20 times on mortality and BFI of prominent malaria vectors compared with that of the conventionally treated nets (200 mg/m^2 AI) washed until just before exhaustion. This confirms that the Interceptor® fulfils the WHOPES main efficacy criteria of Phase II studies. Even after 30 washes, the overall killing effect of Interceptor® was still higher than that of conventionally treated nets washed 20 and 30 times. It should be noted that the high dose of alpha-cypermethrin on conventionally treated nets was a modification of the WHO guidelines for net treatment (recommended dose of 20–40 mg alpha-cypermethrin/m^2) and was used to provide an equivalent comparison to the Interceptor net.

Interceptor® nets provided good personal protection against malaria vectors, and also against *Culex* and *Mansonia* mosquitoes, in experimental hut studies. Interceptor® did not substantially reduce the entry rates of female mosquitoes but induced relatively high mortality in malaria vectors (60–95%).

Considering safety, efficacy and wash-resistance of Interceptor® nets in laboratory and small-scale field studies, it is recommended:

- that a time-limited *interim recommendation* is given for the use of Interceptor® as a long-lasting insecticidal mosquito net for the prevention and control of malaria;

- that WHOPES should coordinate large-scale field studies (WHOPES Phase III) of Interceptor® to confirm its long-lasting efficacy, longevity and fabric integrity, as a

requirement for development of *full recommendations* on the use of the product;

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.[18]

[18] WHO specifications for public health pesticides are available on the WHO homepage on the Internet at http://www.who.int/whopes/quality/en/.

6. EXTENSION OF WHO SPECIFICATIONS FOR LONG-LASTING INSECTICIDAL NETS

Unlike most other insecticide formulation types, apparently similar LN products may be based on different technologies, with the result that a specification developed for one manufacturer's product may not provide a reliable means for testing the acceptability of another manufacturer's product. For this reason, additional information is required to extend existing WHO specifications for LNs to additional products (i.e. to determine their equivalence) or, where appropriate, to develop separate specifications. The minimum requirements for assessing the equivalence of LNs are currently as follows:

1. The manufacturer must certify to WHO that the active ingredient incorporated into the LN complies with the existing WHO specification for technical material (TC). Where the existing specification has been developed under the new procedure, this means that the active ingredient must be manufactured by a company whose technical material has been evaluated by the FAO/WHO Joint Meetings on Pesticide Specifications (JMPS) and has consequently been recommended for inclusion in the WHO specification for the TC.

2. Laboratory testing to determine regeneration and wash resistance of the LN, as well as its efficacy, according to the WHO *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets* (document WHO/CDS/WHOPES/GCDPP/2005.11).[19]

3. The manufacturer must state whether the active ingredient is incorporated within the filament polymer in the spinning process, or is incorporated into a polymer applied to the outside of filaments; or is applied/incorporated in some other way. If, exceptionally, any detailed information on manufacture of the treated netting is required, it will be treated as confidential by WHO.

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[19] *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets*. Geneva, World Health Organization, 2005 (document WHO/CDS/WHOPES/GCDPP/2005.11; available at http://www.who.int/whopes/guidelines/en/).

4. The manufacturer must provide data to show the applicability of the existing clauses and tests for active ingredient retention/release index in washing and storage stability.

6.1 Objectives

The objectives of this study were to determine the regeneration, wash resistance and efficacy of three LNs of Netto Group (Thailand), Hiking Group Shandongtex Genfont (HGSG, China) and Tianjin Yorkool (China), as part of requirements for extension of WHO specifications for deltamethrin long-lasting (coated) insecticidal mosquito net.[20] Performances of the new deltamethrin-LN products were compared with those of the reference LN (i.e. *PermaNet*®) under the same specification. The evaluation included the following two steps: (i) to determine whether a period of time is required for regeneration of activity after washing, in comparison with the regeneration curves of the reference LN; and (ii) to determine wash resistance and efficacy of the candidate LNs after 0, 1, 5, 10 and 20 washes, in comparison with the reference LN.

6.2 Materials and methods

The procedures to evaluate the efficacy of the LN products are described in the following sections.

Net equipment
The WHO Collaborating Centre in Montpellier, France (LIN/IRD laboratory) received from each of the manufacturers four LNs. Five pieces of netting (25 x 25 cm) from each net were cut and used for the laboratory study (5 x 4 = 20 nettings). Four net samples were used for the regeneration study and 12 other samples for the wash resistance evaluation. The last 4 samples were stored at 4 °C and served as reference samples. The corresponding reference LN (*PermaNet*®) was provided by Vestergaard Frandsen Company.

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http://www.who.int/whopes/quality/deltamethrin_eval_july_2006.pdf).

Chemical analysis for deltamethrin content
The chemical residue analysis of unwashed and washed netting samples were carried out by the WHO Collaborating Centre, in Gembloux, Belgium (Pesticides Research Department of the Walloon Agricultural Research Centre). One piece of 10 cm x 10 cm (100 cm^2) was cut from every net sample, weighed and then analysed, using the analytical method MEREPERMA. Deltamethrin was extracted from the sample by heating under reflux for 60 minutes with 40 mL xylene. The extract was let to reach ambient temperature and quantitatively transferred into a 50 mL volumetric flask. The flask was filled up to volume with xylene. A 10-times dilution was achieved in xylene. The final extract was finally analysed for determination of deltamethrin by Capillary Gas Chromatography.

Biological material
Non-blood fed females of *Anopheles gambiae s.s* (kisumu strain) were used during the evaluation. Kisumu strain is a standard susceptible strain originated from Kenya colonized for many years in the laboratory and free of any detectable insecticide resistance mechanisms.

Regeneration time
The time required for full regeneration of biological efficacy was measured using WHO cone tests following 2-day intervals (1, 3, 5, and 7 days) on netting samples washed three times consecutively and stopped once mortality and KD effect reached the initial level (0 wash).

Wash resistance and efficacy
Wash resistance of the candidate and reference LNs was studied by carrying out bioassays on samples washed 0, 1, 5, 10, 15 and 20 times, using WHO standard washing procedure (see footnote 19). Mosquito KD after 60 minutes and mortality at 24 hours were used for comparison (requirement: $\geq$80% mortality and/or $\geq$95% KD).

The efficacy of the 20-times washed candidate nets that fell below the cut-off point were subjected to tunnel test, using susceptible *An. gambiae* mosquitoes and following the WHOPES guidelines (see footnote 19) (requirement: ≥80% mortality and/or ≥90% BFI).

Mortality of treated samples was corrected using Abbott's formula[21] when mortality rates in control batches exceeded 5%.

6.3 Results and discussion

6.3.1 Deltamethrin long-lasting (coated) insecticidal mosquito net of Netto Group

Chemical residue analysis
The average and the standard deviation of the total content of deltamethrin in chemical residue analysis of Netto LNs were 2.10 (±0.14), 1.75 (±0.06), 1.49 (±0.09), 1.48 (±0.05), 1.28 (±0.05) and 1.11 (±0.04) g/kg for unwashed and 1-, 3-, 5-, 10- and 15-times washed nets, i.e. overall retention of 83.3, 71, 70.5, 61, and 52.9% of loading dose, respectively, and an average retention per wash of 91.4%, using WHO washing procedure.

Regeneration time
Regeneration time of the deltamethrin LN of Netto was studied (Bonnet et al., 2006a) and was compared with that of *PermaNet*® (Table 3). Mortality of *An. gambiae* in bioassays carried out on unwashed nets was 100%, but decreased to 78% after three consecutive washes ($P < 0.05$). A plateau of mortality (above WHO threshold) was reached after one day of storage (regeneration time). No statistical difference was noted in the mortality of mosquitoes in bioassays of three-times washed nets stored at 30 °C for 1 and 7 days ($P > 0.05$). The KD effect was above the WHO threshold of ≥95% after 0 and 3 washes Considering the overall data, the time period required to reach a plateau was 1 day, i.e. regeneration time of 1 day.

Wash resistance and efficacy
The result of bioassays carried out on unwashed and washed LNs, in comparison with *PermaNet*®, is presented in Table 4 (Bonnet et al., 2006a). The interval between washes was according to the regeneration time determined in the previous study and was one day. Each result represented a mean of four replicates.

[21] Corrected mortality (%) = ((% survival control – % survival treated)/(% survival control)) * 100.

The mortality of mosquitoes significantly decreased under the WHO threshold after 5 washes (71%, P<0.05). The mortality rates were then 28%, 5% and 3% after 10, 15 and 20 washes, respectively. KD effect remained maximal until 5 washes (100%) but significantly decreased under the WHO cut-off point of ≥95% (21%) after 15 washes.

The efficacy as determined by mortality, BFI and reduction of entry rates in tunnel tests was recorded for samples washed 20 times (Table 5). Each result represented a mean of two replicates. These were 61% ±17, 94% ±2 after 12-hour exposure, and 61%, respectively.

Overall assessment of deltamethrin LN of Netto Group
This study showed that the total deltamethrin content of unwashed long-lasting (coated) insecticidal mosquito net of Netto Group was within the WHO specifications (1.8 g/kg ±25%). Using WHO standard washing procedure, the average deltamethrin retention index per wash was 91.4%.

The bio-efficacy of the LN was different from the reference product under the same specification (*PermaNet®*) with mortality and KD significantly below WHO thresholds after 15 washes. Consequently, this LN does not meet the WHO requirements for extension of LN specifications through laboratory studies. Noting the performance of the product, it is unlikely that it would meet the minimum WHO requirements, should it be tested in experimental hut studies.

6.3.2 Deltamethrin long-lasting (coated) insecticidal mosquito net of Hiking Group

Chemical residue analysis
The average and the standard deviation of the total content of deltamethrin in chemical residue analysis of Hiking Group LNs were 1.75 (±0.03), 1.67 (±0.07), 1.56 (±0.05), 1.41 (±0.09), 1.24 (±0.03), 1.14 (±0.05) and 1.09 (±0.06) g/kg for unwashed and 1, 3-, 5-, 10-, 15- and 20-times washed nets, i.e. overall retention of 95.5, 89.1, 80.6, 70.9, 65.1, 62.3% of loading dose, respectively, and an average retention per wash of 96.5%, using WHO washing procedure.

Regeneration time
Regeneration time of the deltamethrin LN of Hiking Group was studied (Duchon et al., 2006) and compared with that of *PermaNet*® (Table 3). Initial mortality as determined by bioassay of unwashed nets was high (95%) but declined significantly (8%) after only three washes. A plateau of mortality was noted after 3 days of storage (no significant difference in mortality was noted between 3 and 7 days, P >0.05). Initial KD was 100% but fell to 54% after three washes. Three days storage was needed to reach the WHO KD threshold (87% ±11). Considering the overall data, the time period required to reach a plateau, i.e. regeneration time, was 3 days.

Wash resistance and efficacy
The result of bioassays carried out on unwashed and washed LNs, in comparison with *PermaNet*®, is presented in Table 4 (Duchon et al., 2006). The interval between washes was according to the regeneration time determined in the previous study and was three days. Each result represented a mean of four replicates.

The initial mortality was good against *An. gambiae* (95% mortality and 100% KD effect). After only five washes, both mortality and knock-down effect decreased below the WHO threshold (2% mortality and 35% KD effect). After 20 washes, the LNs induced only 1% for both mortality and KD effect against susceptible mosquitoes.

The efficacy as determined by mortality, BFI and reduction of entry rates in tunnel tests was recorded for samples washed 20 times (Table 5). Each result represented a mean of two replicates. These were 79% ±4%, 94% reduction ±2%, and 30%, respectively.

Overall assessment of deltamethrin LN of Hiking Group
This study showed that the total deltamethrin content of unwashed long-lasting (coated) insecticidal mosquito net of Hiking Group was within the WHO specifications (1.8 g/kg ±25%). Using WHO standard washing procedure, the average deltamethrin retention index per wash was 96.5%.

The bio-efficacy of the LN was different from the reference product under the same specification (*PermaNet*®), with regeneration time of 3 days, and with mortality and KD that

were significantly below WHO thresholds in 5-, 10-, 15- and 20-washes. Consequently, this LN does not meet the WHO requirements for extension of LN specifications through laboratory studies. Noting the performance of the product, it is unlikely that it would meet the minimum WHO requirements, should it be tested in experimental hut studies.

6.3.3 Deltamethrin long-lasting (coated) insecticidal mosquito net of Yorkool

Chemical residue analysis
The average and the standard deviation of the total content of deltamethrin in chemical residue analysis of Yorkool LNs were 1.82 (±0.04), 1.9 (±0.06),1.77 (±0.06), 1.76 (±0.05), 1.67 (±0.04), 1.53 (±0.04) and 1.43 (±0.03) g/kg for unwashed and 1, 3-, 5-, 10-, 15- and 20-times for washed nets, i.e. overall retention of 104.4, 97.3, 96.7, 91.8, 84.1, 78.6% of loading dose, respectively, and an average retention per wash of 99.9%, using the WHO washing procedure.

Regeneration time
Regeneration time of the deltamethrin LN of Yorkool was studied (Bonnet et al., 2006b) and compared with that of *PermaNet*® (Table 3). The initial mortality was low (16%) but did not significantly differ after three washes (11%, P>0.05). A plateau of mortality was reached after 1 day's storage, indicating that no reactivation occurred with this LN. The KD effect was high and did not vary between 0 and 3 washes (83% vs 87% respectively, P >0.05). Considering the overall data, the time required to reach a plateau, i.e. regeneration time was 1 day.

Wash resistance and efficacy
The result of bioassays carried out on unwashed and washed LNs, in comparison with *PermaNet*®, is presented in Table 4 (Bonnet et al., 2006b). The initial mortality was very low (16% at both 0 and 1 wash), demonstrating a low bioavailability of deltamethrin on the net surface. After 5 and 10 washes, mortality increased to 49% and 65% respectively but still remained under the WHO threshold (P <0.05). Finally, mortality fell to 34% and 6% after 15 and 20 washes respectively. The KD effect remained high until 15 washes but significantly decreased under the cut off point (62%) after 20.

An adequate amount of active ingredient must be present on the surface of the LN, for efficacy, but the majority must reside within the coating of the LN, to avoid excessive losses during washing and to provide a reservoir from which the surface is replenished with active ingredient. The lower efficacy of unwashed nets compared with nets washed 5 and 10 times suggests loss of the coating polymer and increased availability of the insecticide on the surface.

The efficacy as determined by mortality, BFI and reduction of entry rates in tunnel tests was recorded for samples washed 20 times (Table 5). Each result represented a mean of two replicates. These were 74% ±8%, 94% reduction ±4%, and 48%, respectively.

Overall assessment of deltamethrin LN of Yorkool
This study showed that the total deltamethrin content of unwashed long-lasting (coated) insecticidal mosquito net of Yorkool Tianjin was within WHO specifications (1.8 g/kg ± 25%). Using the WHO standard washing procedure, the average deltamethrin retention index per wash was 99.9%.

This study showed that the bio-efficacy and wash-resistance of deltamethrin long-lasting (coated) insecticidal mosquito net of Yorkool are different from the reference product (*PermaNet*®) in terms of regeneration and wash resistance and do not meet the bio-efficacy requirements for the extension of WHO specifications.

WHO recommendations on the use of the product for malaria prevention and control can only be granted subject to satisfactory performance of the nets in experimental hut studies.

Table 3. Regeneration time as determined by mortality and knock-down, KD (average and confidence interval) of *Anopheles gambiae* females in bioassays of unwashed and 3-times washed nets stored at 30 °C for 1 to 7 days, for the three candidate long-lasting (coated) insecticidal mosquito nets (LNs) in comparison with the reference LN (*PermaNet*®)

LN	*unwashed*		*3 washes + 1 day*		*3 washes + 3 days*		*3 washes + 5 days*		*3 washes + 7 days*	
	% mort	*% KD*	*% mort*	*% KD*	*% mort*	*% KD*	*% mort*	*% KD*	*% mort*	*% KD*
Hiking	95 *(93–97)*	100 –	8 *(3–13)*	54 *(43–65)*	27 *(15–39)*	87 *(76–98)*	48 *(35–61)*	97 *(94–100)*	35 *(26–41)*	94 *(91–97)*
Netto	100 –	100 –	78 *(63–93)*	98 *(96–100)*	85 *(73–97)*	100 –	91 *(85–97)*	100 –	85 *(75–95)*	99 *(98–100)*
Yorkool	16 *(9–23)*	83 *(79–87)*	11 *(4–18)*	87 *(79–95)*	23 *(17–29)*	91 *(90–92)*	16 *(11–21)*	98 *(96–100)*	14 *(7–21)*	91 *(87–95)*
Perma-net	100 –	99 *(97–100)*	100 –	100 –	98 *(95–100)*	100 –	99 *(97–100)*	100 –	91 *(89–93)*	99 *(97–100)*

Table 4. Wash resistance as determined by mortality and knock-down, KD (average and confidence interval) of *Anopheles gambiae* females in bioassays of unwashed and 1- to 20-times washed nets, for the three candidate long-lasting (coated) insecticidal mosquito nets (LNs) in comparison with the reference LN (*PermaNet*®)

LN	*unwashed*		*1 wash*		*5 washes*		*10 washes*		*15 washes*		*20 washes*	
	% Mort	*% KD*	*% Mort*	*% KD*	*% Mort*	*% KD*	*% Mort*	*% KD*	*% Mort*	*% KD*	*% Mort*	*% KD*
Hiking	**95** ***(93-97)***	**100** ***100***	57 *(30-76)*	**98** ***(97-99)***	2 *(0-4)*	35 *(31-39)*	6 *(2-10)*	51 *(36-66)*	5 *(0-10)*	12 *(1-23)*	1 *(0-3)*	1 *(0-3)*
Netto	**100** **-**	**100** **-**	**100** **-**	**100** **-**	71 *(63-79)*	**100** **-**	28 *(13-43)*	**86** ***(73-99)***	5 *(2-8)*	21 *(9-33)*	3 *(0-6)*	18 *(1-35)*
Yorkool	16 (9-23)	83 *(79-87)*	16 (11-21)	**100** **-**	49 (42-56)	**100** **-**	65 (52-78)	**100** **-**	34 (26-42)	**100** **-**	6 (3-9)	62 (49-75)
Perma-net	**100** **-**	**100** **-**	**100** **(99-100)**	**100** **-**	**100** **-**	**100** **-**	**97** **(95-99)**	**100** **-**	**78** **(69-87)**	**100** **-**	23 (9-37)	**87** **(73-100)**

In bold, are indicated the values which fulfil WHO requirements (≥80% mortality and ≥95% KD)

Table 5. Efficacy as determined by mortality, blood feeding inhibition (BFI) and reduction of entry rates (average and range), in tunnel apparatus, of three candidates deltamethrin long-lasting (coated) insecticidal mosquito nets (LNs) washed 20-times, in comparison with the reference LN (*PermaNet®*)

LN	*Control*			*Deltamethrin LNs*		
	% mortality	*% entry*	*% blood feeding*	*% corrected Mortality*	*% entry reduction*	*% BFI*
Hiking	19 *(11–27)*	71 *(62–80)*	54 *(44–64)*	**79 (75–83)**	30 (0–61)	**94 *(92–96)***
Netto	12 *(6–18)*	83 *(76–90)*	67 *(58–76)*	61 (44–78)	61 (41–81)	**94 *(92–96)***
Yorkool	20 *(17–23)*	80 *(60–100)*	56 *(52–60)*	**74 *(66–82)***	48 (37–59)	**96 (92–100)**
Perma-net	22 *(14–30)*	84 *(77–91)*	57 *(47–67)*	**77 (72–82)**	69 (60–78)	**97 *(93–100)***

In bold, are indicated the values which fulfil WHO requirements (≥80% mortality and ≥90% BFI)

7. MULTICENTRE STUDY TO IDENTIFY SIMPLE AND RELIABLE METHODS TO DETERMINE THE BIOEFFICACY OF PYRETHROID-TREATED NETTING

7.1 Background information

The WHOPES Meeting on the Development of Guidelines for Testing and Evaluation of Long-lasting Insecticidal Mosquito Nets held at WHO/HQ in Geneva on 4–7 April 2005[22] noted some drawbacks with the current methods to measure the efficacy of insecticide-treated nets and suggested that an alternative testing method be developed. The main concern with existing methods was that insecticides with strong excito-repellent properties, such as permethrin, would be at a disadvantage in cone tests, as exposed mosquitoes might spend more time resting on the cone (and therefore less time on the treated net) than they would if exposed to a less repellent insecticide. Another concern is that not every research laboratory is able to run tunnel tests, as these involve use of animals. Increasingly, there are restrictions on the use of animals in laboratory testing, quite apart from the expense of keeping mammals. If a test can be devised that circumvents the need for animals and gives a fair indication of LN potential, this will be both an improvement on the present system and would enable more centres to become involved in LN testing.

For these reasons, use of the WHO cylinders (test tubes) used in testing the susceptibility of mosquitoes to insecticides, was suggested as a potential alternative method.

In view of the above, WHOPES organized a multi-centre study to compare three test methods to identify simple, reliable methods for determining the bioefficacy of insecticide-treated nets. Five laboratories agreed to participate in the study: Institut de Recherche pour le Développement (LIN/IRD), Montpellier, France; LSHTM, London, UK; Centers for Disease Control and Prevention (CDC), Atlanta, USA; School of Public Health, Teheran University of Medical Science (SPH), Teheran,

[22] *Report of the meeting on the development of guidelines for testing and evaluation of long-lasting insecticidal mosquito nets. WHO/HQ, Geneva, 4–7 April 2005*. Geneva, World Health Organization, 2005 (document WHO/CDS/WHOPES/GCDPP/2005.14; available at http://www.who.int/whopes/gcdpp/publications/en/).

Islamic Republic of Iran; and Vector Control Research Unit, University of Sains Malaysia (VCRU), Penang, Malaysia.[23]

7.2 Materials and methods

Two polyester (75 denier) mosquito nets (15 m²) were conventionally treated with either permethrin EC (500 mg/m^2 AI) or deltamethrin SC (25 mg/m^2 AI) by the WHO Collaborating Centre in Montpellier, France.[24] A third net was left as untreated (negative control). Eight pieces from the same net (40 x 40 cm) were sent to each participating laboratory for cone and cylinder bioassays and tunnel testing before and after every wash made by the participating laboratory (e.g. 0x, 1x, 2x, 3x, 4x), following WHO standard washing procedures as outlined below.

The nets were washed and dried once a week, with cone and tube bioassays and tunnel tests performed on one randomly selected net for each insecticide and before the next washing. Each selected 40 x 40 cm net was cut into the following pieces (see Figure 1) and used as specified below: (i) a 25 x 25 cm net to be used in cone bioassay, followed by tunnel test; (ii) a 25 x 15 cm net to be used for cylinder bioassay; and (iii) a 15 x 15 cm for chemical analysis.

WHO washing procedure: Net samples (25 cm x 25 cm) were individually introduced into 1-l beakers containing 0.5 l deionized water, with 2 g/l soap Savon de Marseille added just before and fully dissolved. Beakers were immediately introduced into a water bath at 30 °C and shaken for 10 minutes at 155 movements per minute. The samples were then removed and rinsed twice for 10 minutes in clean, deionized water in the same shaking conditions as stated above. Nets were dried at room temperature and stored at 30 °C in the dark between washes.

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[23] WHOPES wishes to thank Dr Vincent Corbel (LIN/IRD); Dr Mark Rowland (LSHTM); Dr John Gimnig (CDC); Dr Hassan Vatandoost (SPH); and Dr Zairi Jaal (VCRU) for their participation in the study.

[24] Ten pieces (25 x 25 cm) of the net were cut (two on each side by side samples collected for bioassays) and subjected to chemical assay by the WHO Collaborating Centre to confirm the target dose of the insecticide and the homogeneity of treatments.

Bioassay: Non-blood fed, 2–5-day-old susceptible female *Anopheles* (species to be stated in the test report) mosquitoes were exposed to netting samples for 3 minutes, after which they were held for 24 hours with access to sugar solution. KD was measured after 60 minutes post-exposure and mortality after 24 hours.

In cone bioassays, 5 mosquitoes were introduced into a cone at a time. At least 50 mosquitoes were tested on a netting sample (25 x 25 cm).

In cylinder bioassays, 20 female mosquitoes were introduced into a cylinder at a time and at least 60 mosquitoes were tested (3 x 20) on a netting sample. The 25 x 15 cm net was folded to 12.5 x 15 cm and introduced in the cylinders, lining the inside surface of the cylinder. Three metallic clips were used instead of two to maintain properly the netting within cylinders. The top screen of the cylinder (see A in Figure 1) was made of the same fabric (same treatment, same number of washes). The cylinder was kept vertical during the 3-minute exposure.

The average mortality and KD for the 5 x 10 mosquitoes in cone bioassay and 3 x 20 mosquitoes in cylinder bioassays were reported, for each insecticide separately (Tables 1 and 2). Mosquitoes exposed to untreated nets were used as controls. Bioassays were carried out at 25 $\pm$ 2 °C and 75 $\pm$ 10% RH.

Tunnel test: Efficacy (mortality and BFI) of netting samples were studied in the laboratory, by releasing non-blood fed female anopheline mosquitoes, aged 5–8 days, in a tunnel (square section 25 x 25 cm) made of glass, 60 cm length (Figure 2). At each end of the tunnel, a 25-cm square cage was fitted (extension) and covered with polyester netting. At one third of the length (20 cm within the tunnel), a disposable cardboard frame was placed with the treated netting sample. The surface of netting "available" to mosquitoes is 400 cm^2 (20 x 20 cm) with nine holes, each 1 cm in diameter: one hole was located at the centre of the square and the eight others were equidistant and located at 5 cm from the border.

In the shorter section of the tunnel, a bait (e.g. guinea-pig for *An. gambiae*) was placed, unable to move. In the cage at the end of the longer section of the tunnel, 100 females were introduced at 18:00. Females were free to fly in the tunnel but

had to make contact with the piece of netting and locate the holes in it before passing through to reach the bait.

The following morning, at 09:00, the mosquitoes were removed by hand using a suction glass tube and counted separately from the two sections of the tunnel and the immediate mortality was recorded. Live females were placed in plastic cups with honey solution; delayed mortality was recorded after 24 hours. During tests, cages were maintained at 27 ± 2 °C and 80 ± 10% relative humidity under subdued light.

One tunnel with untreated netting was always used as a negative control. BFI is assessed by comparing the proportion of blood-fed females (alive or dead) in treated and control tunnels. Overall mortality was measured by pooling the immediate and delayed (24-hour) mortalities of mosquitoes from the two sections of the tunnel. The proportion of mosquitoes able to pass through the netting was recorded. By comparison with control, an eventual reduction in penetration was calculated. This reduction provides an indication of the repellent effect of the insecticide and extent to which this effect correlates with mortality.

Chemical assay: Samples (15 x 15 cm) were collected for each insecticide (see Figure 1) unwashed and after each washing on each of the sample as well as at the end of the last testing and sent to WHOPES for chemical assays, wrapped separately in aluminium foils, and labelled (type of insecticide, name of the laboratory, date of sampling, number of washes).

7.3 Results

7.3.1 Chemical analysis

The results of chemical residue analysis of nettings samples (Agriculture Research Centre, Gembloux, Belgium, 2005), treated with permethrin or deltamethrin, confirmed the accuracy of the manual treatments, with an average of 25.6 mg/² AI (24.6–26.8) for deltamethrin and 501.7 mg/m² AI (407.8–555.4) for permethrin.

7.3.2 Bioassay results

The results of the efficacy testing of the nets by different participating institutions are summarized below. Mortality of treated samples was corrected by using the Abbott formula, when mortality rates in control batches ranged from 5% to 20% (tests showing mortality >20% were rejected). The following cut-off points were used in assessment of the data: cone and cylinder test: 80% mortality or 95% KD; tunnel test: 80% mortality or 90% BFI.

LIN/IRD, Montpellier, France

Susceptible females of *An. gambiae* (Kisumu strain) were tested. The results are summarized in Table 6.

Permethrin

Low mortalities were recorded with unwashed treated nets under cones (27%) and, to a lesser extent, under cylinders (60%). When considering both KD and mortality (Table 11), samples tested under cones went below the WHO threshold after four washes, while the same sample tested under cylinders or tunnels were still fulfilling the WHO requirement at seven washes: additional washes would have been necessary to determine the cut-off point.

Deltamethrin

Mortality and KD still fulfil the WHO requirements practically at the same number of washes (six washes for cones and seven washes for cylinders and tunnels). As for permethrin, additional washes would have been necessary to show a significant decrease of efficacy.

LSHTM, London, United Kingdom

Susceptible females of *An. gambiae* (Kisumu strain) were tested. The results are summarized in Table 7.

Permethrin

A low percentage mortality is recorded with the unwashed treated net under cones (27%). With both KD and mortality (Table 11), samples tested under cones fall below the WHO threshold after four washes, while the same sample tested under cylinders falls below the WHO threshold after 10 washes.

Under tunnels, both mortality and BFI still meet the WHO requirements at 12 washes.

Deltamethrin
Mortality and KD fall below the WHO threshold after four washes for both cones and cylinders. In tunnels, mortality and BFI decrease under the cut-off point after six washes.

VCRU, Penang, Malaysia
Susceptible females of *An. sinensis* were tested. The results are summarized in Table 8.

Permethrin
A low mortality is observed with unwashed nets under cones (18%). However, the level of KD and mortality was irregular and always under the WHO threshold. Efficacy of treatments tested under cylinders and tunnels falls below the WHO threshold after two and three washes respectively (Table 11).

Deltamethrin
Mortality and KD still meet the WHO requirement at practically the same number of washes (three washes for cones and four washes for cylinders and tunnels).

SPH, Teheran, Islamic Republic of Iran
Susceptible females of *An. stephensi* (IND-strain) were tested. The results are summarized in Table 9.

Permethrin
Mortality and KD (Table 11) are still within the WHO requirement at four washes for cones and five washes for cylinders. As for cones, results in tunnels still undergo the WHO threshold at four washes.

Deltamethrin
When considering both KD and mortality, samples tested under cones fall below the WHO threshold after five washes, while the same sample tested under cylinders still fulfils the WHO requirement at six washes. Additional washes would have been necessary to show a significant decrease of efficacy. Mortality under tunnels decreases under the WHO threshold after only one wash.

CDC, Atlanta, USA
Susceptible females of *An. gambiae* (Kisumu strain) were tested. The results are summarized in Table 10 (no data in tunnel were provided).

Permethrin
Samples tested under cones fall below the WHO threshold after only one wash, while the same sample tested under cylinders are below the WHO threshold after five washes (Table 11).

Deltamethrin
Samples tested under cones fall below the WHO threshold after three washes, while the same sample tested under cylinders still fulfils the WHO requirement at seven washes. Additional washes would have been necessary to show a significant decrease of efficacy in cylinders.

7.4 Conclusions and recommendations

The cylinder provides only insecticide-treated surfaces on which the mosquito may rest, whereas the cone and tunnel are a composite of insecticidal and non-insecticidal surfaces. The cylinder is a forced contact test, whereas cones and tunnels provide opportunities to avoid contact. For less irritant insecticides (such as deltamethrin), the efficacy in cones and cylinders would be expected to be similar. For more irritant insecticides (such as permethrin), the efficacy in cones would be expected to be lower. Although tunnels comprise a mix of insecticidal and non-insecticidal surfaces, the exposure time is overnight and mortality might therefore be expected to be higher than in cones.

These predictions were confirmed by the results of the multi-centre study.

For deltamethrin, cones and cylinders were similar in indicating the maximum number of washes before the cut-off point based on composite thresholds of mortality and KD. Mortality (Figure 1) and KD (Figure 2) in cones and cylinders were similar up to 3–4 washes and were higher in cylinders thereafter.

For permethrin, cones and cylinders consistently showed differences in the maximum number of washes before reaching

the cut-off point, based on composite thresholds of mortality and KD. For cones, the threshold was reached after an average 3.3 washes; for cylinders it was reached after ≥6 washes (Table 11). There was a declining trend in mortality in cones after 1–2 washes, but this trend was not evident in cylinders until 10 washes (Figure 1). Differences in KD between cones and cylinders become apparent at eight washes (Figure 2). With permethrin treated nets, there is low mortality in the unwashed nets but this increases after one wash, presumably due to a reduction in irritability.

For the tunnel test, there was considerable variability between centres ($P<0.001$, multivariate analysis). For deltamethrin, the mortality trend was similar to that of cylinders and showed a decline in mortality only after seven washes (Figure 1). For permethrin, mortality in tunnel remained high until 12+ washes, but in cylinders mortality started to decline after 8 washes (Figure 1). For highly irritant insecticides, the tunnel test may overestimate the true efficacy of washed ITN and hence may not discriminate between ITN and LN at <20 washes (but this requires confirmation in experimental huts).

Because of this limitation with the tunnel test (together with the restrictions on animal use in laboratories), the use of cylinders could also be considered for determination of the efficacy of pyrethroid-treated nets. However, this requires further calibration and development of cut-off values.

In addition, more studies are needed to better understand the relationship between the different bioassay techniques and experimental huts.

Until more information is available, cone bioassays remain the standard for measuring the efficacy of pyrethroid-treated nets.

Table 6. Results of multi-centre study, LIN/IRD, Montpellier, France – mortality (24 hours post-exposure), knock-down (KD, 60 minutes post-exposure) and blood feeding inhibition (BFI) of *Anopheles gambiae* in cone, cylinder and tunnel bioassays of permethrin (perm) and deltamethrin (delta)-treated nets

	0 Wash		1 Wash		2 Washes		3 Washes		4 Washes		5 Washes		6 Washes		7 Washes	
	KD or BFI[a] (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)
Perm																
Cone	98	27	100	82	100	86	100	45	**100**	**47**	88	22	98	35	73	20
Cylinder	100	60	100	100	100	94	100	94	100	94	100	88	100	78	**100**	**93**
Tunnel	100	94	100	88	97	92	100	90	-	-	-	-	-	-	**96**	**90**
Delta																
Cone	100	81	100	100	100	78	100	92	98	75	96	34	**95**	**33**	88	35
Cylinder	100	100	100	100	100	100	100	100	100	94	94	96	100	100	**100**	**96**
Tunnel	94	89	97	95	97	98	100	95	-	-	-	-	-	-	**100**	**100**

[a] KD for cone and cylinder bioassays and BFI for tunnel tests (in comparison with a control).
Note: Figures in bold indicate the last wash meeting WHO threshold for mortality, KD or BFI.

Table 7. Results of multi-centre study, LSHTM, London, UK – mortality (24 hours post-exposure), knock-down (KD, 60 minutes post-exposure) and blood feeding inhibition (BFI) of *Anopheles gambiae* in cone, cylinder and tunnel bioassays of permethrin (perm) and deltamethrin (delta)-treated nets

	0 Wash		1 Wash		2 Washes		3 Washes		4 Washes		6 Washes		8 Washes		10 Washes		12 Washes	
	KD or BFI[a] (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)
Perm																		
Cone	100	26.8	100	80	100	72	100	66.7	**98.1**	**49.1**	83.3	18	59.4	26.6	15.8	15.9	38.6	12.3
Cylinder	100	100	100	100	98.2	98.2	98.1	98.1	100	98.1	100	84.8	100	93.6	**98.6**	**76.8**	50.7	26.1
Tunnel	100	100	100	99	100	100	100	100	100	100	100	100	100	100	98.4	100	**100**	**100**
Delta																		
Cone	100	100	100	100	100	97.9	100	81	**100**	**52.1**	41.7	26.7	3.7	9.3	0	0	0	3.8
Cylinder	100	100	100	100	100	100	100	100	**100**	**100**	83.6	68.8	71.7	55	48.3	19	7.3	7.3
Tunnel	99	98	100	100	100	100	100	98.8	100	93.3	**90.4**	**96.9**	71.1	77	56.1	58.7	30.6	8.7

[a] KD for cone and cylinder bioassays and BFI for tunnel tests (in comparison with a control).
Note: Figures in bold indicate the last wash meeting WHO threshold for mortality, KD or BFI.

Table 8. Results of multi-centre study, VCRU, Penang, Malaysia – mortality (24 hours post-exposure), knock-down (KD, 60 minutes post-exposure) and blood feeding inhibition (BFI) of *Anopheles sinensis* in cone, cylinder and tunnel bioassays of permethrin (perm) and deltamethrin (delta)-treated nets

	0 Wash		1 Wash		2 Washes		3 Washes		4 Washes		5 Washes	
	KD or BFI[a] (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)
Perm												
Cone	52	18	72	52	92	46	60	68	24	18	22	18
Cylinder	100	78	100	100	**100**	**73.3**	26.7	60	36.7	30	28.3	31.7
Tunnel	-	63	-	94	-	84	**-**	**96**	-	46	-	18
Delta												
Cone	90	74	80	94	88	92	**84**	**80**	58	42	40	28
Cylinder	93.3	100	100	100	100	100	93.3	95	**98.3**	**98.3**	65	43.3
Tunnel	-	69	-	92	-	68	-	92	**-**	**90**	-	20

[a] KD for cone and cylinder bioassays and BFI for tunnel tests (in comparison with a control).
Note: Figures in bold indicate the last wash meeting WHO threshold for mortality, KD or BFI.

Table 9. Results of multi-centre study, SPH, Teheran, Islamic Republic of Iran – mortality (24 hours post-exposure), knock-down (KD, 60 minutes post-exposure) and blood feeding inhibition (BFI) of *Anopheles stephensi* in cone, cylinder and tunnel bioassays of permethrin (perm) and deltamethrin (delta)-treated nets

	0 Wash		1 Wash		2 Washes		3 Washes		4 Washes		5 Washes		6 Washes	
	KD or BFI[a] (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)
Perm														
Cone	-	90	-	87	-	92.2	-	85.06	**-**	**81.6**	-	75	-	78
Cylinder	-	100	-	83.4	-	90	-	100	-	100	**-**	**80**	-	73.3
Tunnel	-	81	-	77	-	96	-	86	**-**	**100**	-	16	-	3
Delta														
Cone	-	99.23	-	93.3	-	86.3	-	86.06	-	88.9	**-**	**86.6**	-	70
Cylinder	-	100	-	98.2	-	95.9	-	93.1	-	100	-	96.2	**-**	**94.1**
Tunnel	**-**	**93**	-	48	-	41	-	58	-	-	-	62	-	66

[a] KD for cone and cylinder bioassays and BFI for tunnel tests (in comparison with a control).
Note: Figures in bold indicate the last wash meeting WHO threshold for mortality, KD or BFI.

Table 10. Results of multi-centre study, CDC, Atlanta, USA – mortality (24 hours post-exposure), knock-down (KD, 60 minutes post-exposure) and blood feeding inhibition (BFI) of *Anopheles gambiae* in cone, cylinder and tunnel bioassays of permethrin (perm) and deltamethrin (delta)-treated nets

	0 Wash		1 Wash		2 Washes		3 Washes		4 Washes		5 Washes		6 Washes		7 Washes	
	KD or BFI[a] (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)	KD or BFI (%)	Mort-ality (%)
Perm																
Cone	86.9	30	**98**	**24**	88	30	90.7	27.8	26.2	4.6	3.6	3.6	5.4	9.1	3.9	0
Cylinder	100	100	100	95	100	67.3	-	-	-	-	-	-	-	-	25.5	16.4
Tunnel	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Delta																
Cone	100	100	100	100	100	100	**100**	**100**	62.1	51.7	48.8	46.3	36.7	46.9	51.8	33.9
Cylinder	95.6	100	100	100	96.3	100	-	-	-	-	-	-	-	-	98	80.4
Tunnel	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] KD for cone and cylinder bioassays and BFI for tunnel tests (in comparison with a control).

Note: Figures in bold indicate the last wash meeting WHO threshold for mortality, KD or BFI.

Table 11. Summary of the results of multi-centre study – maximum number of washes of permethrin- or deltamethrin-treated nets that fulfill the WHO requirement for mortality, knock-down or blood feeding inhibition

Participating laboratory	Permethrin		Deltamethrin	
	Cone bioassay	Cylinder bioassay	Cone bioassay	Cylinder bioassay
LIN	4	≥7	6	≥7
LSHTM	4	10	4	4
VCRU	–	2	3	4
SPH	4	5	5	6
CDC	1	5	3	≥7
Average	3.3	≥6	4.2	≥5.6

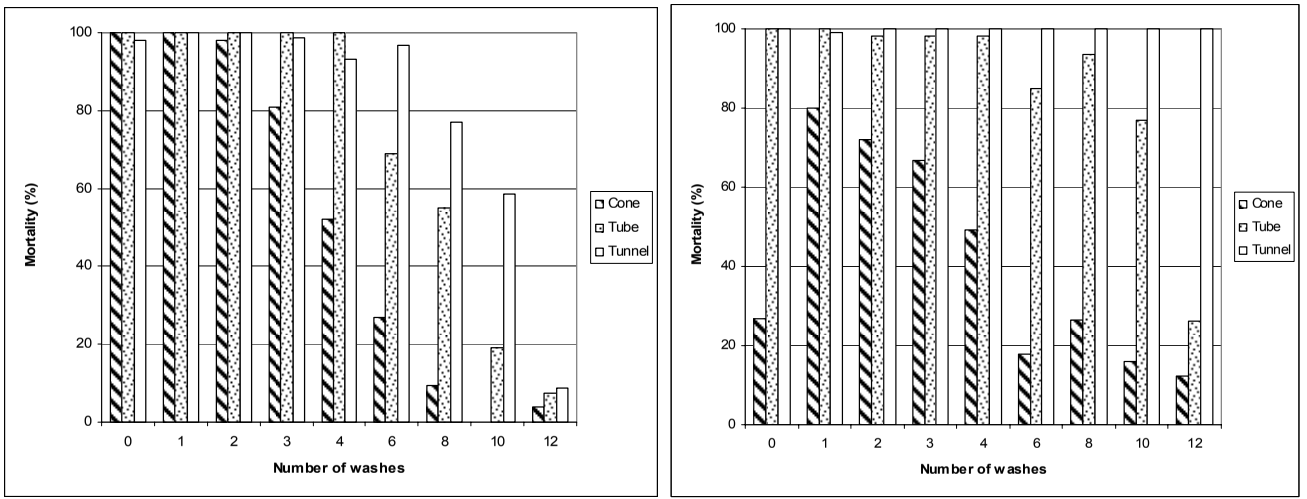

Figure 1. Summary of the results of a multi-centre study – average percentage mortality (24 hours post-exposure) of *Anopheles* species in cone, cylinder (tube) and tunnel bioassays of deltamethrin- (left) and permethrin (right)-treated nets

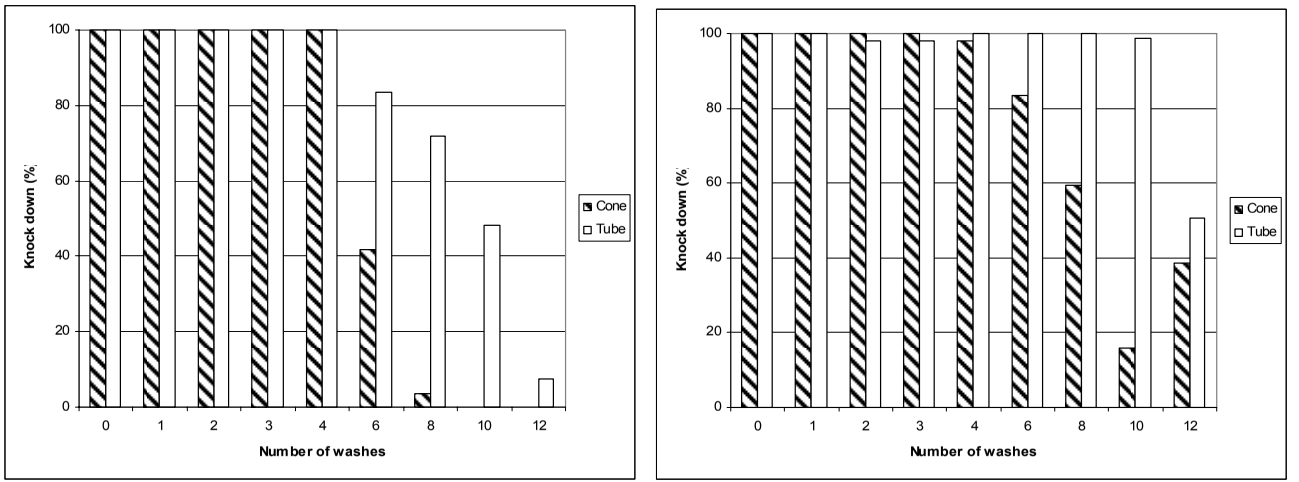

Figure 1. Summary of the results of a multi-centre study – average percentage knock-down (60 minutes post-exposure) of *Anopheles* species in cone and cylinder (tube) bioassays of deltamethrin- (left) and permethrin (right)-treated nets

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