Report of the Sixteenth WHOPES Working Group Meeting

WHO/HQ, GENEVA
22–30 JULY 2013

Review of:

PIRIMIPHOS-METHYL 300 CS
CHLORFENAPYR 240 SC
DELTAMETHRIN 62.5 SC-PE
DURANET LN
NETPROTECT LN
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SPINOSAD 83.3 MONOLAYER DT
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World Health Organization

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CONTROL OF NEGLECTED TROPICAL DISEASES
WHO PESTICIDE EVALUATION SCHEME

Report of the sixteenth WHOPES working group meeting: WHO/HQ, Geneva, 22-30 July 2013: review of Pirimiphos-methyl 300 CS, Chlorfenapyr 240 SC, Deltamethrin 62.5 SC-PE, Duranet LN, Netprotect LN, Yahe LN, Spinosad 83.3 Monolayer DT, Spinosad 25 Extended release GR.

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1. INTRODUCTION

The sixteenth meeting of the WHOPES Working Group, an advisory group to the WHO Pesticide Evaluation Scheme (WHOPES), was convened at the headquarters of the World Health Organization (WHO) in Geneva, Switzerland, from 22 to 30 July 2013. The objective of the meeting was to review pirimiphos-methyl 300 capsule suspension (CS) (Syngenta Crop Protection, Switzerland), chlorfenapyr 240 suspension concentrate (SC) (BASF, Germany) and deltamethrin 62.5 polymer-enhanced suspension concentrate (SC-PE) (Bayer CropScience, Germany) for indoor residual spraying against malaria vectors; Duranet long-lasting insecticidal mosquito net (LN) (Shobikaa Impex, India), Netprotect LN (Intelligent Insect Control, France) and Yahe LN (Fujian Yamei Industry, China) for malaria prevention and control; Spinosad 83.3 monolayer tablet for direct application (DT); and Spinosad 25 extended release granule (GR) (Clarke Mosquito Control Products, USA) for mosquito larviciding.

The meeting was attended by 13 scientists (see Annex 1: List of participants). Professor Dr Marc Coosemans was appointed as Chairman and Dr John Gimnig 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plenary and group sessions, in which the reports of the WHOPES supervised trials and relevant published literature and unpublished reports were reviewed and discussed (see Annex 2: References).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.

Declaration of interest

All invited experts completed a *Declaration of interests for WHO experts*, which was submitted and assessed by the WHO Secretariat prior to the meeting. The following interests were declared:

Dr Rajendra Bhatt and Dr Kamaraju Raghavendra's institute has received prescribed standard fees from eight manufacturers of pesticide products (Sumitomo Chemical India, BASF India, Syngenta Crop Protection India, Clarke Mosquito Control Products USA, Bayer CropScience India, Vestergaard Frandsen India, Chemtura India, BestNet Insect Controls India) in order to meet the costs of product evaluation.

Dr Fabrice Chandre's institute has received prescribed standard fees from Sumitomo Chemical Japan, Bayer CropScience Germany and SPCI France in order to meet the costs of evaluating their respective pesticide products. In addition, his travel to a malaria meeting in

Nairobi in 2009 was paid for by Bayer Environmental Sciences France.

Professor Dr Marc Coosemans' research unit has received grants from the European Union for mapping insecticide resistance in the Mekong Region, and from the Bill & Melinda Gates Foundation for studying the impact of repellents on malaria in Cambodia. The unit has also received repellents free of charge from SC Johnson & Son USA for use in the latter study.

Dr John Gimnig's research unit has received LNs from Clarke Mosquito Control Products USA, BASF Germany, Sumitomo Chemical Japan, Tana Netting Thailand and Vestergaard Frandsen Switzerland for use in field evaluations of such nets undertaken by its partner institutions in Kenya and Malawi.

Dr Stephen Magesa's former research institute received prescribed fees from BASF for testing a pesticide product manufactured by the company.

Dr Olivier Pigeon's research centre has received prescribed standard fees from BASF Germany, Bayer Germany, Syngenta Switzerland, Intelligent Insect Control France and Life Ideas Textiles Company China in order to meet the costs of physico-chemical studies of pesticide products manufactured by the respective companies.

Professor Dr Mark Rowland's unit has received grants from the Innovative Vector Control Consortium UK, Vestergaard Frandsen Switzerland and the President's Malaria Initiative USA for testing and evaluation of various pesticide products.

The interests declared by the experts were assessed by the WHO Secretariat. With the exception of Dr Chandre's declared personal interest, the declared interests were not found to be directly related to the topics under discussion at the meeting. It was therefore decided that all of the above-mentioned experts (with the exception of Dr Chandre) could participate in all evaluations, subject to the public disclosure of their interests.

In view of his declared personal interest, Dr Chandre did not participate in the evaluation of Bayer CropScience's deltamethrin polymer-enhanced suspension concentrate.

2. REVIEW OF PIRIMIPHOS-METHYL 300 CS

Pirimiphos-methyl 300 CS is a capsule suspension formulation containing 300 g of active ingredient per litre.

Pirimiphos-methyl is a broad-spectrum insecticide with contact and airborne (fumigant) actions. It is an acetyl-cholinesterase inhibitor. The Insecticide Resistance Action Committee (IRAC) of CropLife International has classified the compound in Group 1B[1].

Pirimiphos-methyl wettable powder (WP) and emulsifiable concentrate (EC) have previously been evaluated by WHO and are recommended for indoor residual spraying against malaria vectors at the dosage of 1–2 g AI/m^2, with 2–3 months of expected duration of effective action.[2] WHO specifications for pirimiphos-methyl technical material and EC formulation,[3] developed under the new procedure, are based on Syngenta data package and were published in April 2006. Currently, no WHO specification for pirimiphos-methyl WP is available.

The present review assesses the efficacy of pirimiphos-methyl CS (Actellic 300 CS, Syngenta Crop Protection, Switzerland) for indoor residual spraying against malaria vectors, comparing with previously published WHO recommendations for the EC formulation.

The following are extracts from the material safety data sheet of the manufacturer for Actellic 300 CS:

Acute oral LD_{50} (rat)	>5000 mg AI/kg
Acute dermal (rat)	>5000 mg AI/kg
Skin irritation (rabbit)	Non-irritating
Eye irritation (rabbit)	Minimally irritating
Skin sensitization (guinea-pig)	Not a skin sensitizer

[1] *Prevention and management of insecticide resistance in vectors of public health importance*, 2nd ed. CropLife International, Insecticide Resistance Action Committee, 2010 (also available at: http://www.afpmb.org/sites/default/files/whatsnew/2011/irac_manual.pdf).
[2] Available at http://www.who.int/entity/whopes/Insecticides_IRS_Malaria_09.pdf.
[3] Available at http://www.who.int/entity/whopes/quality/en/Pirimiphos_methyl_eval_may_06.pdf.

2.1 Safety assessment

The human risk assessment of pirimiphos-methyl 300 g AI/L CS for indoor residual spraying, provided by the manufacturer, was assessed by the Finnish Institute of Occupational Health (FIOH, 2011) on behalf of WHOPES. The WHO *Generic risk assessment model for indoor residual spraying of insecticides – first revision*[4] was used as a guiding document. The following assumptions were made in the assessment, that:

- technical material used in manufacture of pirimiphos-methyl CS complies with the WHO specification;
- the product is delivered in a 10L container, with a narrow opening (worst case);
- the dermal absorption of pirimiphos-methyl from the formulation is 0.56% and that of the diluted solution is 8.5%;
- breathing volume of the operator is 1.9 m^3/h;
- the average concentration of pirimiphos-methyl after spraying pirimiphos-methyl CS over the time between two sprayings (6 months) is 0.8 × target concentration;
- translodgeable part from the walls and floors onto the skin is 8.85% (maximum value from non-porous surfaces = worst case scenario);
- excretion in milk of pirimiphos-methyl is 1%, based on the observation of 0.4% in goats;
- of the 20% of product dissipated in 6 months, ½ is volatilized; the air exchange rate is 1/h, and residents stay indoors 12 hours/day. The ventilation rates for resident adults, children, toddlers and infants is 16.5, 12.4, 9.5 and 3.6 m^3/d, respectively. The temperature of the dwelling is 30 °C.

The hazard assessment conclusion based on the generic model is that when used for indoor residual spraying as instructed, pirimiphos-methyl 300 CS does not pose undue hazards to the spray operators or residents of the treated dwellings. Provided that operational guidelines are followed, routine cholinesterase monitoring of spraymen during indoor residual spraying programmes is not required. If WHO guidelines and label instructions on operator protection are not followed or inappropriate or malfunctioning equipment is used, exposure to pirimiphos-methyl is likely to exceed safe levels.

[4] Available at: http://whqlibdoc.who.int/publications/2011/9789241502177_eng.pdf.

2.2 Efficacy – background and supporting documents

2.2.1 Determination of the diagnostic dosage for pirimiphos methyl

Morgan and Hemingway (2012) calculated the discriminating dosage of pirimiphos-methyl against a range of susceptible mosquito strains. WHO insecticide test papers are normally prepared using non-volatile carrier oil. Pirimiphos-methyl is known to interact with some standard carrier oils such as olive oil used to make WHO impregnated papers. The pirimiphos-methyl papers were therefore prepared with acetone alone to facilitate even spreading of the liquid insecticide on the paper. A 1% w/v stock solution of pirimiphos-methyl was prepared in acetone and used to prepare a serial dilution of insecticide for paper impregnation ranging from 0.01% to 1%. Acetone was used as the diluent. Several strains of mosquito, characterized as susceptible to all insecticides, were tested in bioassays to establish the LC_{50} and LC_{99}. These strains included *Anopheles gambiae* (Kisumu), *An. arabiensis* (MOZ), *An. stephensi* (STS), *Culex quinquefasciatus* (Recife) and *Aedes aegypti* (Liverpool). Field-collected *An. funestus* from Malawi was also tested at the LC_{99} for *An. gambiae* to determine whether this diagnostic dosage was appropriate for this species.

Mortality values were consistent across all species tested. Data for each species were a good fit to a straight line in log-dosage probit mortality analysis. On the basis that the WHO-recommended discriminating dosage is set at double the LC_{99} of the least susceptible *Anopheles* species tested, then the discriminating dosage of pirimiphos-methyl was recommended as 0.25% papers made up as dry acetone papers without an oil carrier as described above.

Stability tests were undertaken on test papers at the LC_{50} (0.05%) and LC_{99} (0.08%) for *An. gambiae* (Kisumu) and with 0.1% and 0.2% for 2 months and were shown to be stable during this period. It is not clear whether the papers are stable for longer periods. Before test papers prepared without oil-based solvent are recognized by WHO, it is necessary to establish the absolute duration of stability. For consistency with other types of insecticide, it is preferable to identify alternative carrier oil that would not interact with the active ingredient.

2.2.2 Small-scale field trials

Akron, Benin

The efficacy of a capsule suspension formulation of pirimiphos-methyl (Actellic 300 CS) was compared with a standard emulsifiable concentrate formulation (Actellic 500 EC) and with the pyrethroid lambda-cyhalothrin (Icon 10 CS) in experimental huts in an area of southern Benin where *Anopheles gambiae* and *Culex quinquefasciatus* are highly resistant to pyrethroids and DDT (Rowland et al, 2013). Percentage mortality after 1 h exposure to 0.05% deltamethrin test papers and 24 h holding was 20% among *An. gambiae* and 17% among *Culex quinquefasciatus*. Using a locally derived diagnostic concentration of 0.5% pirimiphos-methyl in silicone oil, the wild *An. gambiae* was determined to be susceptible. The manufacturer confirmed to WHOPES that the product tested in Benin and the product submitted for WHOPES evaluation differed only in minor ways that related to production processing.

The walls of the experimental huts were lined with either cement or mud plaster and the ceilings with palm thatch, and sprayed with 0.5 or 1.0 g AI/m^2 pirimiphos-methyl CS or EC with compression sprayers. Cone bioassay tests using the susceptible *An. gambiae* Kisumu strain exposed for 30 min to the sprayed substrates induced >80% mortality for the following periods after spraying: on cement: CS 0.5 g AI/m^2: 9 months, CS 1.0: 9 months, EC 1.0: 3 months. On mud: CS 0.5 g AI/m^2: 3 months, CS 1.0: 6 months, EC 1.0: 3 months (Tables 2.1 and 2.2).

The mortality of wild free-flying *An. gambiae* in cement-lined huts sprayed with 0.5 and 1.0 g AI/m^2 pirimiphos-methyl CS exceeded 80% for a period of 8 and 9 months respectively, and that of *Culex* exceeded 80% for 1 and 3 months respectively. By contrast, in cement-lined huts treated with the EC formulation at 1 g AI/m2, the percentage mortality of *An. gambiae* and *Culex* did not reach 80% at any stage after spraying. In mud-walled huts sprayed with 1.0 g AI/m^2 pirimiphos-methyl CS, mortality exceeded 80% for 5 months for *An. gambiae* and for 1 month for *Culex*. With an application rate of 0.5 g AI/m^2 pirimiphos-methyl CS and 1.0 g AI/m^2 pirimiphos-methyl EC, mortality did not reach 80% at any stage after spraying.

It was concluded that pirimiphos-methyl CS applied at 1 g AI/m^2 is effective for 9 months in cement-walled huts and for over 3 months in mud walled huts using 80% mortality of free-flying *An. gambiae* as the criteria for the duration of residual effectiveness. Using this criterion

the EC 1.0 g AI/m^2 was ineffective from the outset. Using 50% mortality of free-flying mosquitoes as the criteria, the duration of residual effectiveness for the EC was 2 months for the cement, 1 month for the EC on mud, 10 months for the CS on cement and 7 months for the CS on mud.

Moshi, United Republic of Tanzania

The efficacy of pirimiphos-methyl 300 CS was compared with pirimiphos-methyl 500 EC in laboratory and in small-scale field trials in IRS sprayed huts at a target dose of 1 g AI/m^2 (Oxborough, 2013). Insecticides were applied using a calibrated controlled droplet applicator sprayer in the laboratory, and residual activity was examined using laboratory-susceptible *An. arabiensis* Dondotha strain in cone bioassay tests on sprayed cement, mud and plywood substrates. Mortality of *An. arabiensis* exceeded 80% for the following periods: plywood CS 12 months, EC 9 months; cement CS 7 months, EC 2 months; mud CS 4 months, EC <1 month (Tables 2.1 and 2.3) .

In the small-scale trial, the insecticides were applied using a Hudson sprayer to the inner surfaces of huts and residual activity examined using *An. arabiensis* Dondotha on mud plaster and thatch substrates. Mortality exceeded 80% for the following periods: palm thatch CS 7 months, EC <1 month; mud CS 2 months, EC <1 month.

Because an exposure period of 1 h was used rather than the WHO standard 30 min, the differences observed between CS and EC results are seen as relative but do not fulfill the requirements of the WHO standard for residual effectiveness.

Adama, Ethiopia

In a small-scale trial in Oromia region, the insecticides pirimiphos-methyl 300 CS, lambda-cyhalothrin (Icon 10CS) and bendiocarb 80% WP were applied using a Hudson sprayer to the inner walls of 4–5 houses per treatment and residual activity examined on a minimum of 2 houses per treatment as a trial to evaluate alternative insecticides for IRS (Balkew, 2010). Residual activity was examined using a laboratory colony of *An. gambiae* susceptible to all insecticides recommended for IRS in cone bioassay tests for 30 min on sprayed mud substrates. Mortality of pirimiphos-methyl CS 1 g AI/m^2 exceeded 80% for 6–7 months, mortality of lambda-cyhalothrin 20 mg AI/m^2 exceeded 80% at 5 months (when monitoring stopped) and that of bendiocarb 400 mg AI/m^2 exceeded 80% for 3 months (Table 2.2).

Chongwe and Kafue Districts, Zambia
In small-scale trials in two districts of Zambia, pirimiphos-methyl 300 CS was applied at 1.0 g AI/m^2 using a Hudson sprayer to mud and cement wall surfaces and deltamethrin 250 WG at 20 mg AI/m^2 to cement wall surfaces of houses (Chanda et al, 2013). Residual activity in cone bioassay tests using 2–3-day old *An. gambiae* Kisumu exceeded 80% mortality for 6–8 months on cement and for 6–7 months on mud surfaces. Residual activity on deltamethrin-sprayed cement surfaces lasted 6 months (Table 2.1).

Thiès, Senegal
In small-scale trials in two areas of Senegal, pirimiphos-methyl 300 CS and pirimiphos-methyl 500 EC were applied at dosages of 1 and 2 g AI/m^2 in 4 houses per treatment per area, and residual activity determined by cone bioassay using a susceptible strain of *An. gambiae* (Konate et al, 2013). Mortality on both dosages of CS exceeded 80% at 11 months when the trial was brought to an end. Mortality on both dosages of the EC exceeded 80% for 5 months (Tables 2.1 and 2.2).

2.3 Efficacy – WHOPES supervised trials

2.3.1 Small-scale field trials

Three small-scale field trials were implemented under WHOPES supervision: in South Africa against *An. arabiensis*, in India against *An. culicifacies* and in Viet Nam against *An. dirus*. The objective was to evaluate the residual activity of micro-encapsulated pirimiphos-methyl 300 CS on various local indoor surfaces against susceptible malaria vectors compared with pirimiphos-methyl 500 emulsifiable concentrate (EC) containing 500 g AI/L.

The specific objectives of the study were: (i) to evaluate efficacy of pirimiphos-methyl 300 CS at 0.5 and 1 g AI/m^2 doses and compare it with pirimiphos-methyl 500 EC at the dosages of 0.5 and 1 g AI/m^2 on the most common local indoor surfaces against mosquitoes; and (ii) to determine persistence over time of insecticidal action of pirimiphos-methyl 300 CS in comparison with pirimiphos-methyl 500 EC against mosquitoes.

In the WHOPES small-scale trials, a number of local houses are selected that are representative of local domestic structures and interior surfaces. Insecticide was applied by specially-trained spray operators. The residual activity of the treatment was determined using WHO cone bioassays carried out once per month until <80% mortality was reached for 2 consecutive months. Cones were placed on each wall at differing heights and unfed, female susceptible mosquitoes exposed for 30 min, after which they were held and provided with sugar solution for 24 h before mortality was recorded.

In the WHOPES supervised trials, after preparing the selected rooms for spraying, Whatman filter-papers of 10 cm diameter or 10 cm x 10 cm squares were attached at three different positions on the walls (top, middle and bottom) for quality assurance of spraying. The papers were removed after spraying, wrapped in aluminium foil and sent to the Walloon Agricultural Research Centre, WHO Collaborating Centre for Quality Control of Pesticides, Gembloux, Belgium (Annex 3). Upon receipt, they were stored into a deep freezer at –18 °C until the chemical analysis. Each filter-paper was weighed, the surface was accurately measured and the filter-paper was cut into small pieces before chemical analysis.

Mpumalanga, South Africa

Three structures per treatment were sprayed across 4 different villages as this area had few mud structures (Coetzee et al, 2012). The 4 spraymen were specially trained and provided with new spray pumps. Of the 45 structures sprayed with pirimiphos-methyl CS and EC, 96 filter-papers were submitted for chemical analysis. Susceptible female *An. arabiensis* (KGB strain) were used for the bioassays. Cone bioassays with *An. arabiensis* were carried out 7 days after spraying and once a month for 7 months until <80% mortality was reached for 2 consecutive months. Five cones were placed on each wall at differing heights and 10 unfed, female mosquitoes were exposed.

Spray operators reported on the strong smell of pirimiphos-methyl EC formulation, including headache, sneezing and excessive sweating. All participating households were satisfied with the treatments and no adverse events were reported.

Chemical analysis revealed that the ratio of actual to target dose of the two formulations and two application rates ranged from 0.88 to 2.28 on the two substrates. The ratio tended to be higher on the mud plaster than on cement substrates. The AI content variation between

filter-papers expressed as the relative standard deviation ranged from 23.3% to 58.7% (Table 2.4). The ratio was close to target for treatments with pirimiphos-methyl CS at 1 g AI/m², otherwise it tended to be higher than expected (Pigeon, 2012a).

Residual activity was longer on CS than on EC treatments and longer on mud plaster than on cement substrates (Tables 2.4 and 2.5). The CS at 1 g AI/m^2 achieved 5 months activity on mud plaster compared with 3 months on cement. CS at 0.5 g AI/m^2 was shorter lived, achieving 4 months activity on mud plaster and 0.25 months activity on cement. The EC treatments were effective for less than a month.

Gujarat, India

Pirimiphos-methyl CS and EC were sprayed at 0.5 and 1.0 g AI/m^2 in selected houses in Kheda district of Gujarat State using hand-operated Hudson compression sprayers (Srivastava et al, 2012). The most common interior surfaces in the study village were mud-plastered walls, lime-coated cemented walls and unpainted wood. A total of 36 houses, comprising three replicates of the afore-mentioned surfaces were sprayed with the 0.5 and 1.0 g AI/m^2 dosages of CS and EC formulations, plus 6 controls. Cone bioassays were conducted with laboratory-susceptible *An. culicifacies* females aged 2–5-days old.

Some householders reported sneezing and eye irritation after the CS but not the EC application.

Chemical analysis of filter-papers revealed that the ratio of actual to target dose ranged from 0.25 to 3.42 across the 12 treatments. The AI content variation between filter-papers expressed as the relative standard deviation ranged from 12.5% to 95.1% (Table 2.4). The ratio tended to be lower on the lime-coated cement than on mud plaster or wood substrates. The ratio was below 1 in 6 treatments and above 1 in the 6 others. The CS treatments tended to be overdosed and the EC treatments tended to be underdosed (Pigeon, 2012b).

Residual activity was consistently longer with pirimiphos-methyl CS than with EC treatments, and longer on wood and lime-coated cement than on mud plaster substrates (Tables 2.4 and 2.5). The CS at 1 g AI/m^2 achieved more than 8 months activity on all three substrates. Pirimiphos-methyl CS at 0.5 g AI/m^2 was shorter lived, achieving 4 months activity on lime-coated cement, 6 months on mud and more than 8 months on wood. The EC treatments were effective

for only 3 months (mud) to 5 months (lime-coated cement, wood) and raising the dosage to 1.0 g AI/m^2 failed to improve residual activity.

Hoa Binh, Viet Nam

The study was conducted in the village Suoi Bu, in Hoa Binh province, northern Viet Nam (Coosemans et al, 2012*)*. The most common house constructions were from brick or wood. A total of 24 representative houses were selected and randomly assigned to one of the 4 treatment arms (5 houses per arm: 3 concrete-brick houses, 2 wooden houses) and 2 control houses per surface. All brick walls were covered with a thin lime layer. Six experienced spraymen were given training on correct application procedures.

Indoor spraying was done using hand-operated compression sprayers (SEMCO sprayers – MR-8 bought in 2010 but with no Control Flow Valve, CFV) using new flat-fan nozzles (Type TEEJET N° 8002). The spraying was conducted in June 2011. To assess the accuracy of the spraying, 6 filter-papers per house at various wall heights were fixed to the selected surface before spraying. Prior to spraying, the perimeter of each filter-paper was traced on the wall, to enable the locating of cones bioassays around these spots. Filter-papers were stored at –18 °C before analysis.

All six spray operators were interviewed on the day of spraying and again the following morning and one week later. Perceived side-effects in inhabitants were recorded one week and one month after spraying. After spraying 2 or 3 houses per spray-round, several spraymen declared some adverse effects. All operators reported a bad odour and 3/6 reported sneezing and nausea. By the following morning and one week, later no further side-effects were reported.

Chemical analysis of filter-papers revealed that the ratio of actual to target dose ranged from 1.14 to 2.21 across the 8 treatments. The AI content variation between filter-papers expressed as the relative standard deviation (RSD) ranged from 17.6% to 49.5% (Table 2.4).

For the repeat trial conducted in 2012 (Van Roey et al, 2013a), the ratio of the actual to the target dose ranged from 0.70 to 0.78 with RSD ranging from 25.9% to 29.9% (Pigeon, 2012c,d). Bioassay tests conducted one week after IRS application on the brick or wood surfaces often gave 80–100% mortality. But within a month the activity of insecticide treatments on each of the sprayed surfaces had fallen far below the 80% threshold (Tables 2.4 and 2.5).

2.3.2 Large-scale field trials

The overall objective of the studies was to compare the persistence of residual action and impact on vectorial capacity of pirimiphos-methyl 300 CS (300 g AI/L) with pirimiphos-methyl 500 EC (500 g a.i./L) (as a positive control arm) applied by a single-round IRS at village-scale.

The specific objectives were: (i) to compare the persistence of insecticidal activity of pirimiphos-methyl CS at 1.0 g AI/m^2 dosage with the EC at 1.0 g AI/m^2 dosage applied on common surfaces in human dwellings against malaria vector species; (ii) to assess and compare the impact of the CS at 1.0 g AI/m^2 with the EC at 1.0 g AI/m^2 on the elements of vectorial capacity, viz. mortality, feeding success, survival rate, entry/exit rates and sporozoite rate/entomological inoculation rate of malaria vector; and to determine the acceptability and the perception of the effect of CS and EC treatments.

Gujarat, India

The study was carried out in 10 villages in Kheda, Vadodara and Panchmahals districts of Gujarat (Srivastava et al, 2013). Houses were of various types with either mud or brick walls and mud or cement-plastering, and with roofs made of either tiles or straw-thatching. Cattle-sheds were either in separate enclosures or shared a common roof with the human habitation (mixed dwellings). The preparatory phase of the trial was from October to December 2012, with post-intervention monitoring from January to August 2013. The meeting was presented with the post-intervention data for January to July 2013 and it was agreed that the data table be updated to include data up to August 2013 before publication of the report.

During the preparatory phase, the consent of the local authorities and communities was obtained, community meetings were held to explain the objectives and baseline susceptibility of *An. culicifacies* was determined. At the end of the baseline period, the sentinel villages were stratified according to ecology, entomology and malariogenic potential, and were randomly allocated to the two arms.

A team of scientists and technicians provided training to spray operators in proper spray techniques, equipment maintenance and daily calibration of pumps. Spray coverage was more than 96% of rooms in each arm and with no village achieving less than 92% coverage. To assess the accuracy of indoor spraying, 9 filter-papers

per surface type were attached at different heights before spraying. Once dry, they were removed, packed in aluminium foil and stored in refrigerator conditions before submission for chemical analysis. The residual activity of the treatment was determined using susceptible *An. culicifacies* in WHO cone bioassays carried out once per month until <80% mortality was reached for 2 consecutive months.

The entomological impact of the interventions was recorded each month on the following parameters: (i) vector density and behaviour, as determined by light-trap collections indoors, human landing catches indoors, exit trap collections and hand, floor sheet and pyrethrum space spray collections; and (ii) vectorial capacity, as determined by parous rates, sporozoite rates and entomological inoculation rate, and human blood index.

An. culicifacies comprised 7.3% of the baseline collection. *An. subpictus* was the most abundant species (86%), followed by *An. annularis* (1.7%), *An. fluviatilis* (1.3%) and *An. stephensi* (0.5%). *Culex* was 3.3%.

The perceptions of 20 households per village were recorded by questionnaire. A total of 526 and 570 people were interviewed from EC and CS arms respectively. None reported any adverse effect one week and one month after spraying.

Chemical analysis of filter-papers revealed that the average applied/target dose ratio ranged from 0.96 to 1.23 for pirimiphos-methyl 300 g AI/L CS at 1 g AI/m² and from 0.86 to 1.59 for pirimiphos-methyl 500 EC at 1 g AI/m². The ratio of actual to target dose on the three surfaces was close to one on the majority of surfaces for both treatment arms. For the CS, the ratio was 1.23 on wood, 0.99 on mud plaster and 0.96 on lime-coated cement. For the EC, the ratio was 0.86 on wood, 1.59 on mud plaster and 0.88 on lime-coated cement. The AI content variation between filter-papers was, however, high in both arms on each surface, with RSD ranging from 25.3% to 90.3% (Table 2.4) (Pigeon, 2013a).

Residual activity was consistently longer for CS than for EC for each type of substrate (Tables 2.4 and 2.6). Activity of CS lasted for 4 months on mud and cement plaster, while activity of EC lasted only for 2 months and 1 months on these substrates. Activity was longest on wood, with the CS lasting for 6 months and the EC for 2 months.

During the 2 months before spraying, the indoor density of *An. culicifacies* was similar between CS and EC arms. Post-spraying, the density diverged between the treatment arms. Initially density was lower in the EC arm but from 3 months to 6 months after spraying density was lower in the CS arm than in the EC arm, perhaps reflecting the longer residual activity of the CS.

The overall parous rate during the baseline period ranged from 23% to 37%. Post-intervention, the parous rates decreased. The hot, dry weather from April to June contributed to this. Parous rates and survival rates were reported as lower in the CS than in the EC arm during post-intervention months 4–6 when the activity of the two interventions might be expected to diverge, but no statistics were provided.

Walikunda, Gambia

This study was conducted in villages around the MRC entomological field site of Walikunda situated on the south bank of the River Gambia, about 270 km inland from the coast (D'Alessandro et al, 2013). It is an area of extensive rice cultivation throughout the year in irrigated and rain-fed fields in the rainy season, which occurs over a short period of 4 months of the year. The main malaria vector is *An. gambiae s.s.* Almost everybody sleeps under a bed net. A baseline collection of mosquitoes by pyrethrum spray collections (PSC) from 25 villages (spraying 4 rooms per village) established the variance in *Anopheles* density between villages, which was used to randomize the villages into 3 groups of 6 villages: pirimiphos-methyl 300 CS at 1g AI/m^2, pirimiphos-methyl 500 EC at 1 g AI/m^2 and DDT at 2 g AI/m^2.

The duration of the study was one year. The preparatory phase was from June to July 2012, with implementation of IRS done over 10 days in July 2011. Post-intervention monitoring and evaluation continued from mid-August until the end of February 2013 when field activities were completed.

In the study area, the majority of houses were constructed of mud bricks with tin or thatch roof. Some houses were made of cement bricks or cement plastered and with lime or paint coating. For monitoring, the selected houses had thatch roofs and open eaves for the monitoring of vector behaviour. A total of 5 mud-plastered and 5 cement-plastered houses were chosen per village. Cone bioassays confirmed no further residual activity from previous IRS applications.

Indoor spraying was done using hand-operated compression sprayers fitted with flat fan nozzles and a control flow valve according to WHO guidelines. Nozzles were calibrated daily before spraying each morning. To assess the accuracy of spraying, filter-papers were fixed at various heights and after spraying were stored in a refrigerator at +4 ^{o}C until sending for chemical analysis within 4 weeks of spraying. Residual activity of the treatments was determined using 2–5-day old susceptible *An. gambiae* in WHO cone bioassays on mud and cement plastered substrates. The entomological impact of the interventions was recorded each month in 4 villages per villages on: (i) vector density and behaviour as determined by light trap collections indoors, human landing catches indoors, exit trap collections and pyrethrum space spray collections; (ii) vectorial capacity as determined by parous rates, sporozoite rates, entomological inoculation rate and human blood index. Light-trap collections were done in different houses to the spray catch collections. An assessment of adverse events in spray operators was made by questionnaire on the day of spraying, the day after and one week later.

The *Anopheles* mosquito population was found to be fully susceptible to pirimiphos-methyl, deltamethrin and DDT. There was 9% survival to permethrin and 18% to bendiocarb test papers in WHO kits. Spray operators and inhabitants reported symptoms of headache, sneezing, irritation to eye and mucous membranes, and treatment odour.

Chemical analysis of filter-papers revealed that the average applied/target dose ratio ranged from 1.68 to 3.78 for pirimiphos-methyl 300 g AI/L CS at 1 g AI/m², from 2.07 to 2.98 for pirimiphos-methyl 500 EC at 1 g AI/m² and from 1.43 to 1.81 for DDT at 2 g AI/m². The AI content variation between filter-papers expressed as the relative standard deviation ranged from 19.8% to 77.2% (Table 2.4). The ratio of actual to target dose was therefore 2–4 times higher than one on the majority of surfaces. For the CS, the ratio was 1.68 on mud plaster and 3.78 on cement. For the EC, the ratio was 2.07 on mud plaster and 2.98 on cement. For DDT, the ratio was 1.30 on mud-plaster and 1.43 on cement (Pigeon, 2012e).

Residual activity was consistently longer for CS than for EC for both types of substrate. Activity of CS lasted for 3 months on mud and cement plaster but activity of EC lasted only for 1 month and 2 months on these substrates, respectively. The duration of residual activity on DDT-sprayed mud plaster and cement was similar to that of the CS formulation.

Species determination was carried out by PCR on all mosquitoes collected (1550). *An. arabiensis* formed 63% and *An. gambiae* ss formed 36%; 96% of *An. gambiae* ss was M form.

In the post-intervention period, 237 blood-fed mosquitoes (comprising 224 *An. gambiae* s.l. and 13 *An. funestus*) were collected and 110 (46%) had fed on humans. The mean proportion of human blood-fed females was higher in the pirimiphos-methyl EC (56%) arm than the CS (36%) arm. Of the 1455 *An. gambiae s.l.* and *An. funestus* tested for circumsporozoite protein (CSP), only 3 were positive (0.2%) and all 3 positives came from the pirimiphos-methyl CS arm, which gives a sporozoite rate of 0.4%.

The effort spent on human landing catches (2 villages once per month) and the number of mosquitoes collected for parous rate determination (149 in total) was insufficient to draw any conclusions.

Indoor density of *An. gambiae* s.l. was 1.6 times higher in sentinel houses of the CS than the EC arm, but without clearer evidence that density in the two arms was similar pre-intervention any difference post-intervention cannot be attributed to the treatments. The sentinel houses were treated with their respective CS and ES formulations. The mean number of anophelines caught in the exit traps per night was 3.9 in the CS arm and 2.4 in the EC arm. Of the mosquitoes collected in the exit traps, the proportion dying was 73% (117/205) in the CS arm and 45% (30/123) in the EC arm. This is an indication that the CS formulation induces mosquito mortality for a higher or longer duration than the EC and supports the inference that residual activity is greater in the CS than in the EC treatment arms.

2.4 Conclusions and recommendations

Pirimiphos-methyl 300 CS is a capsule suspension formulation containing 300 g of active ingredient per litre (Actellic 300 CS) produced by Syngenta Crop Protection, Switzerland. Pirimiphos-methyl is a broad-spectrum insecticide with a mode of action based on acetyl-cholinesterase inhibition. Pirimiphos-methyl 500 EC emulsifiable concentrate formulation has previously been evaluated by WHO and is recommended for indoor residual spraying against malaria vectors at the dosage of 1–2 g AI/m^2, with 2–3 months of

expected duration of effective action.[5] WHO specifications for the EC formulation were published in April 2006.[6]

The report reviews the efficacy and duration of activity of pirimiphos-methyl 300 CS in comparison with the pirimiphos-methyl 500 EC formulation for indoor residual spraying against malaria vectors in small-scale and large-scale field trials, and takes into consideration the previously published WHO recommendations for the EC formulation.

A series of background studies and WHOPES supervised trials were reviewed from Benin, Ethiopia, the Gambia, India, Senegal, South Africa, the United Republic of Tanzania, Viet Nam and Zambia. In every study except one (Viet Nam), the duration of residual activity of pirimiphos-methyl 300 CS, as measured by mortality $\geq$80% in cone bioassays, exceeded the duration of activity of the 500 EC formulation at dosages of 0.5 g AI/m^2 and 1 g AI/m^2. The duration of activity of the 500 EC ranged from 0–5 months depending on the study and the substrate, but typically lasted for 1–3 months. The duration of activity of pirimiphos-methyl 300 CS at 1 g AI/m^2 ranged from 3–9 months depending on the country of study and the substrate. In experimental hut trials in Benin, the duration of efficacy on free-flying mosquitoes (mortality >80%) was 9 months on cement-walled and 5 months on mud-walled huts. An application rate of 1 g AI/m^2 showed longer residual activity than 0.5 g AI/m^2 in the small-scale studies. Of the substrates tested, residual activity was longest on wood and thatch, longer on cement/s and plaster than on mud plaster, and shortest on lime-coated cement.

In the large-scale community randomized trials in India and the Gambia, the duration of residual activity of pirimiphos-methyl 300 CS was longer than that of pirimiphos-methyl 500 EC. In India, the post-intervention population density of *An. culicifacies* appeared to be lower in the CS than in the EC arm, but in the Gambia there was no difference in the population density of *An. gambiae* s.l. between CS and EC arms. In the Gambia, the mortality of *An. gambiae* s.l. in the exit trap collections was significantly greater in the CS than the EC arm.

[5] Available at http://www.who.int/entity/whopes/Insecticides_IRS_Malaria_09.pdf.
[6] Available at http://www.who.int/entity/whopes/quality/en/Pirimiphos_methyl_eval_may_06.pdf.

A tentative diagnostic dosage of 0.25% pirimiphos-methyl applied using acetone as the solvent carrier is adopted until further evidence is obtained.

Noting the above, the meeting recommended:

- that multi-centre studies on different well-characterized strains of different mosquito species, with priority on major malaria vectors, be carried out to establish the diagnostic concentration(s) for pirimiphos-methyl;

- that pirimiphos-methyl 300 CS be used for indoor residual spraying for malaria control at a recommended dose of 1.0 g AI/m^2 with an expected duration of residual activity of 4–6 months; and

- for quality assurance and determination of appropriate spray cycles under specific eco-epidemiologic settings, that national programmes be urged to monitor the residual activity of insecticides used for indoor residual spraying.

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

Table 2.1 Duration of residual activity in small-scale background IRS studies with pirimiphos-methyl 300 g AI/L CS. Cone bioassay tests in the United Republic of Tanzania and Zambia used 60-min exposure rather than the WHO-recommended 30-min exposure.

Study site and species	Study arm	Exposure time (minutes)	Surface type	Residual activity (months)
United Republic of Tanzania *An. arabiensis* OP/pyr susceptible	Pirimiphos-methyl CS 1.0 g AI/m^2	60	Mud plaster	4
			Concrete cement	7
			Plywood	12
	Pirimiphos-methyl EC 1.0 g AI/m^2	60	Mud plaster	0.25
			Concrete cement	2
			Plywood	6
United Republic of Tanzania *Cx. quinquefasciatus* OP/pyr susceptible	Pirimiphos-methyl CS 1.0 g AI/m^2	60	Mud plaster	4
			Concrete cement	7
			Plywood	12
	Pirimiphos-methyl EC 1.0 g AI/m^2	60	Mud plaster	1
			Concrete cement	0.25
			Plywood	7

Table 2.1 contd Duration of residual activity in small-scale background IRS studies with pirimiphos-methyl 300 g AI/L CS. Cone bioassay tests in the United Republic of Tanzania and Zambia used 60-min exposure rather than the WHO-recommended 30-min exposure.

Study site and species	Study arm	Exposure time (minutes)	Surface type	Residual activity (months)
United Republic of Tanzania *Cx. quinquefasciatus* Pyr resistant OP susceptible	Pirimiphos-methyl CS 1.0 g AI/m^2	60	Mud plaster	3
			Concrete cement	6
			Plywood	12
	Pirimiphos-methyl EC 1.0 g AI/m^2	60	Mud plaster	0
			Concrete cement	0.25
			Plywood	4
Zambia *An. gambiae* susceptible	Pirimiphos-methyl CS 1.0 g AI/m^2	60	Mud plaster	6–7
			Concrete cement	6–8
Senegal *An. gambiae* susceptible (Kisumu)	Pirimiphos-methyl CS 1.0 g AI/m^2	30	Mud	>8
			Concrete	>8
	Pirimiphos-methyl CS 2.0 g AI/m^2	30	Mud	>8
			Concrete	>8
	Pirimiphos-methyl EC 1.0 g AI/m^2	30	Mud	2
			Concrete	1
	Pirimiphos-methyl EC 2.0 g AI/m^2	30	Mud	2
			Concrete	2

Table 2.1 contd Duration of residual activity in small-scale background IRS studies with pirimiphos-methyl 300 g AI/L CS. Cone bioassay tests in the United Republic of Tanzania and Zambia used 60-min exposure rather than the WHO-recommended 30-min exposure.

Study site and species	Study arm	Exposure time (minutes)	Surface type	Residual activity (months)
Benin *An. gambiae* susceptible (Kisumu)	Pirimiphos-methyl CS 0.5 g AI/m^2	30	Mud	0–3
			Concrete	6–9
	Pirimiphos-methyl CS 1 g AI/m^2	30	Mud	3–6
			Concrete	6–9
	Pirimiphos-methyl EC 1 g AI/m^2	30	Mud	0–3
			Concrete	0–3

Table 2.2 Cone bioassay results in small-scale background studies (% mortality after 24 hours' holding).

Study	Study arm	Type of surface	Mortality [%] post treatment for each month												Efficacy [months]
			1	2	3	4	5	6	7	8	9	10	11	12	
Rowland et al. (2013) Akron, Benin [1]	Lambda-cyhalothrin CS 500 mg AI/m^2	Cement	100	100	**100**	75	75	75	20	20	20	12	12	12	3
	Pirimiphos-methyl CS 500 mg AI/m^2	Cement	98	98	98	82	82	82	89	89	**89**	61	61	61	9
		Mud	100	100	**100**	57	57	57	40	40	40	31	31	31	3
	Pirimiphos-methyl CS 1 g AI/m^2	Cement	100	100	100	100	100	100	100	100	**100**	63	63	63	9
		Mud	100	100	100	100	100	**100**	77	77	77	67	67	67	6
	Pirimiphos-methyl EC 1 g AI/m^2	Cement	99	99	**99**	42	42	42	16	16	16	22	22	22	3
		Mud	100	100	**100**	30	30	30	5	5	5	9	9	9	3

The residual activity of the IRS applications was measured by standard WHO plastic cones placed on treated wall surfaces. Tests were performed monthly on susceptible laboratory colonies until the end of the trial or until no further treatment mortality was observed (i.e. mortality below 80%). Efficacy is given by the number of months in which mortality was ≥80.

[1] Laboratory-reared, insecticide susceptible *An. gambiae* s.s. Kisumu strain.

Table 2.2 contd Cone bioassay results in small-scale background studies (% mortality after 24 hours' holding).

Study	Study arm	Type of surface	Mortality [%] post treatment for each month												Efficacy [months]
			1	2	3	4	5	6	7	8	9	10	11	12	
Balkew (2010) Adama, Ethiopia [2]	Bendiocarb WP 400 mg AI/m^2	Mud	100	100	**100**	59	-	-	-	-	-	-	-	-	3
	Pirimiphos-methyl CS 1 g AI/m^2	Mud	98	81	75	96	100	84	**83**	-	-	-	-	-	≥7
	Lambda-cyhalothrin CS 20 mg AI/m^2	Mud	99	100	90	74	**90**	-	-	-	-	-	-	-	≥5

The residual activity of the IRS applications was measured by standard WHO plastic cones placed on treated wall surfaces. Tests were performed monthly on susceptible laboratory colonies until the end of the trial or until no further treatment mortality was observed (i.e. mortality below 80%). Efficacy is given by the number of months in which mortality was ≥80.

[2] Laboratory-reared, insecticide-susceptible *An. gambiae* s.l.

Table 2.2 contd Cone bioassay results in small-scale background studies (% mortality after 24 hours' holding).

Study	Study arm	Type of surface	Mortality [%] post treatment for each month												Efficacy [months]
			1	2	3	4	5	6	7	8	9	10	11	12	
Konaté et al. (2013) Thiès, Senegal [3]	Pirimiphos-methyl CS 1 g AI/m^2	Cement	100	98	90	80	97	**88**	68	-	-	-	-	-	6
		Mud	100	98	100	98	**95**	78	83	-	-	-	-	-	5
	Pirimiphos-methyl CS 2 g AI/m^2	Cement	98	92	100	116	100	68	**100**	-	-	-	-	-	≥7
		Mud	100	97	100	100	100	60	**93**	-	-	-	-	-	≥7
	Pirimiphos-methyl EC 1 g AI/m^2	Cement	100	68	22	30	**98**	23	53	-	-	-	-	-	1
		Mud	93	90	63	**80**	63	3	37	-	-	-	-	-	4
	Pirimiphos-methyl EC 2 g AI/m^2	Cement	100	100	73	53	**98**	47	42	-	-	-	-	-	2
		Mud	100	**98**	55	53	88	42	33	-	-	-	-	-	2

The residual activity of the IRS applications was measured by standard WHO plastic cones placed on treated wall surfaces. Tests were performed monthly on susceptible laboratory colonies until the end of the trial or until no further treatment mortality was observed (i.e. mortality below 80%). Efficacy is given by the number of months in which mortality was ≥80.

[3] Laboratory-reared, insecticide-susceptible *An. gambiae* s.s. molecular M form.

Table 2.3 Cone bioassay results in small-scale background studies (% mortality after 24 hours' holding) in which the exposure time was 1 h instead of 30 min.

Study	Study arm	Type of surface	Mortality [%] post treatment for each month											
			1	2	3	4	5	6	7	8	9	10	11	12
Oxborough et al. (2013) Moshi, United Republic of Tanzania, Laboratory study[1]	Pirimiphos-methyl CS 1 g AI/m^2	Cement	100	98	100	92	98	100	90	**85**	70	55	55	40
		Mud	100	97	100	**100**	65	48	58	45	62	33	20	8
		Plywood	100	100	100	100	100	100	100	100	100	100	100	**100**
	Pirimiphos-methyl EC 1 g AI/m^2	Cement	100	**88**	65	50	55	20	10	-	-	-	-	-
		Mud	38	33	53	38	44	5	1	-	-	-	-	-
		Plywood	100	100	100	100	100	100	90	95	**85**	70	60	33
Chanda et al. (2013) Chiawa, Zambia [2]	Deltamethrin WG 20 mg AI/m^2	Cement	100	100	100	100	100	**100**	79	71	56	-	-	-
	Pirimiphos-methyl CS 1 g AI/m^2	Cement	100	100	100	100	100	100	96	**91**	78	-	-	-
		Mud	100	100	100	100	100	**80**	78	65	40	-	-	-
Chanda et al. (2013) Shikabeta, Zambia [2]	Pirimiphos-methyl CS 1 g AI/m^2	Cement	100	100	100	100	100	**81**	71	66	59	-	-	-
		Mud	100	100	100	100	100	96	**80**	27	25	-	-	-

The residual activity of the IRS applications was measured by standard WHO plastic cones placed on treated wall surfaces. Tests were performed monthly on susceptible laboratory colonies until the end of the trial or until no further treatment mortality was observed (i.e. mortality below 80%). However, instead of the WHOPES-recommended exposure time of 30 minutes the mosquitoes were exposed for 60 min.

[1] Laboratory-reared, insecticide-susceptible *An. arabiensis* Dondotha strain; [2] Laboratory-reared, insecticide-susceptible *An. gambiae* s.l. strain.

Table 2.4 Chemical analysis of filter-papers collected from IRS studies with pirimiphos-methyl (PM) 300 g AI/L CS and duration of residual activity

Study site and species	Study arm	Surface type	No. papers analysed	Applied / target dose ratio	RSD[1]	Residual activity (months)
India *An. culicifacies* Susceptible to PM	Pirimiphos-methyl CS 0.5 g AI/m^2	Mud plaster	6	1.76	49.3%	6
		Lime-coated cement	6	0.62	55.1%	4
		Wood	6	2.03	59.3%	>8
	Pirimiphos-methyl CS 1.0 g AI/m^2	Mud plaster	6	2.01	39.0%	>8
		Lime coated cement	6	1.73	76.1%	>8
		Wood	6	3.42	27.1%	>8
	Pirimiphos-methyl EC 0.5 g AI/m^2	Mud plaster	6	0.63	12.5%	3
		Lime-coated cement	6	0.25	68.8%	4
		Wood	6	0.61	24.5%	5
	Pirimiphos-methyl EC1.0 g AI/m^2	Mud plaster	6	1.15	56.9%	3
		Lime-coated cement	6	0.31	45.5%	5
		Wood	6	0.91	95.1%	4

[1] RSD = relative standard deviation of the AI content between filter-papers.

Table 2.4 contd Chemical analysis of filter-papers collected from IRS studies with pirimiphos-methyl (PM) 300 g AI/L CS and duration of residual activity

Study site and species	Study arm	Surface type	No. papers analysed	Applied / target dose ratio	RSD[1]	Residual activity (months)
India *An. culicifacies* Susceptible to PM	Pirimiphos-methyl CS 1.0 g AI/m^2	Mud plaster	9	0.99	64.5%	4
		Lime-coated cement	9	0.96	25.3%	4
		Wood	9	1.23	90.3%	>6
	Pirimiphos-methyl EC 1.0 g AI/m^2	Mud plaster	9	1.59	71.5%	2
		Lime-coated cement	9	0.88	36.6%	1
		Wood	9	0.86	66.1%	2
South Africa *An. arabiensis* Susceptible to PM	Pirimiphos-methyl CS 0.5 g AI/m^2	Mud plaster	12	1.92	31.7%	4
		Cement	12	1.43	50.7%	0.25
	Pirimiphos-methyl CS 1.0 g AI/m^2	Mud plaster	12	0.98	56.3%	5
		Cement	12	0.88	61.9%	3
	Pirimiphos-methyl EC 0.5 g AI/m^2	Mud plaster	12	1.77	23.3%	0.25
		Cement	12	1.34	47.5%	0.25
	Pirimiphos-methyl EC 1.0 g AI/m^2	Mud plaster	12	2.28	31.8%	0
		Cement	12	1.56	58.7%	0

[1] RSD = relative standard deviation of the AI content between filter-papers.

Table 2.4 contd Chemical analysis of filter-papers collected from IRS studies with pirimiphos-methyl (PM) 300 g AI/L CS and duration of residual activity

Study site and species	Study arm	Surface type	No. papers analysed	Applied / target dose ratio	RSD[1]	Residual activity (months)
Gambia *An. gambiae* Susceptible to PM	Pirimiphos-methyl CS 1.0 g AI/m^2	Mud plaster	9	1.68	34.3%	3
		Cement	9	3.78	19.8%	3
	Pirimiphos-methyl EC 1.0 g AI/m^2	Mud plaster	9	2.07	26.1%	1
		Cement	9	2.98	55.0%	2
	DDT WP 2 g AI/m^2	Mud plaster	9	1.81	36.9%	3
		Cement	9	1.43	77.2%	3
Viet Nam 1 *Anopheles dirus s.s.* Susceptible to PM	Pirimiphos-methyl CS 0.5 g AI/m^2	Brick	18	1.61	43.9%	<1
		Wood	12	1.16	44.0%	<1
	Pirimiphos-methyl CS 1.0 g AI/m2	Brick	18	1.60	49.5%	<1
		Wood	12	1.54	27.5%	<1
	Pirimiphos-methyl EC 0.5 g AI/m^2	Brick	18	1.24	43.5%	<1
		Wood	12	1.74	45.2%	<1
	Pirimiphos-methyl EC 1.0 g AI/m^2	Brick	18	1.14	46.6%	<1
		Wood	12	2.21	17.6%	<1

[1] RSD = relative standard deviation of the AI content between filter-papers.

Table 2.4 contd Chemical analysis of filter-papers collected from IRS studies with pirimiphos-methyl (PM) 300 g AI/L CS and duration of residual activity

Study site and species	Study arm	Surface type	No. papers analysed	Applied / target dose ratio	RSD[1]	Residual activity (months)
Viet Nam 2 *Anopheles dirus s.s.* Susceptible to PM	Pirimiphos-methyl CS 1.0 g AI/m^2	Brick	8	0.70	25.9%	<1
		Wood	8	0.78	29.9%	<1

[1] RSD = relative standard deviation of the AI content between filter-papers.

Table 2.5 Residual activity against *Anopheles* spp. reported from WHOPES small-scale trials

Study	Study arm	Type of surface	Mortality [%] post treatment for each month								Efficacy [months]
			1	2	3	4	5	6	7	8	
Srivastava et al. (2012) Gujarat, India [1]	Pirimiphos-methyl CS 0.5 g AI/m^2	Lime-coated cement	100	100	96	**88**	58	77	-	-	4
		Mud plaster	100	98	99	85	85	**80**	41	60	6
		Wood	100	100	94	94	54	85	89	**88**	≥8
	Pirimiphos-methyl CS 1 g AI/m^2	Lime-coated cement	100	96	87	95	87	84	52	**81**	≥8
		Mud plaster	100	100	94	91	84	84	82	**88**	≥8
		Wood	100	98	95	95	88	82	74	**84**	≥8
	Pirimiphos-methyl EC 0.5 mg AI/m^2	Lime-coated cement	100	98	92	**90**	65	20	-	-	4
		Mud plaster	100	98	**98**	72	72	-	-	-	3
		Wood	100	100	88	87	**88**	28	26	-	5
	Pirimiphos-methyl EC 1 g AI/m^2	Lime-coated cement	100	98	84	91	**84**	42	36	-	5
		Mud plaster	100	96	**92**	72	69	-	-	-	3
		Wood	100	100	85	**91**	78	28	-	-	4

Table 2.5 contd Residual activity against *Anopheles* spp. reported from WHOPES small-scale trials

Study	Study arm	Type of surface	Mortality [%] post treatment for each month								Efficacy [months]
			1	2	3	4	5	6	7	8	
Van Roey et al. (2013a) Hoa Binh, Viet Nam [2]	Pirimiphos-methyl CS 1 g AI/m^2	Brick	14	-	-	-	-	-	-	-	<1
		Wood	28	-	-	-	-	-	-	-	<1
	Pirimiphos-methyl EC 1 g AI/m^2	Brick	17	-	-	-	-	-	-	-	<1
		Wood	**84**	-	-	-	-	-	-	-	1
Coetzee et al. (2012) Mpumalanga, South Africa [3]	Pirimiphos-methyl CS 0.5 g AI/m^2	Cement	76	76	36	-	-	-	-	-	<1
		Mud plaster	92	91	62	**80**	74	52	-	-	4
	Pirimiphos-methyl CS 1 g AI/m^2	Cement	99	90	**87**	52	60	59	-	-	3
		Mud plaster	100	100	100	99	**80**	76	63	-	5
	Pirimiphos-methyl EC 0.5 g AI/m^2	Cement	21	70	6	-	-	-	-	-	<1
		Mud plaster	25	58	3	-	-	-	-	-	<1
	Pirimiphos-methyl EC 1 g AI/m^2	Cement	22	15	10	-	-	-	-	-	<1
		Mud plaster	36	64	13	2	-	-	-	-	<1

The residual activity of the IRS applications was measured by standard WHO plastic cones placed on treated wall surfaces. Tests were performed monthly on susceptible laboratory colonies until the end of the trial or until no further treatment mortality was observed (i.e. mortality below 80%).

[1] Laboratory-reared, insecticide-susceptible *An. culicifacies* colony.

[2] Laboratory-reared, insecticide-susceptible *An dirus* s.s. colony.

[3] Laboratory-reared, insecticide susceptible *An. arabiensis* KGB strain.

Table 2.6 Residual activity against *Anopheles* spp. reported from WHOPES large-scale trials.

Study	Study arm	Type of surface	Mortality [%] post treatment for each month						Efficacy [months]
			1	2	3	4	5	6	
Srivastava et al. (2013) Gujarat, India [1]	Pirimiphos-methyl CS 1 g AI/m^2	Lime-coated cement	100	100	99	**83**	69	30	4
		Mud	100	93	98	**86**	68	33	4
		Wood	100	96	98	87	100	**87**	≥6
	Pirimiphos-methyl EC 1 g AI/m^2	Lime-coated cement	**97**	72	79	61	9	-	1
		Mud	97	**98**	77	51	13	-	2
		Wood	96	**87**	78	44	17	-	2
D'Alessandro et al. (2013) Walikunda, The Gambia [2]	DDT WP 2 g AI/m^2	Cement	93	88	**88**	44	59	49	3
		Mud	90	90	**94**	61	55	50	3
	Pirimiphos-methyl CS 1 g AI/m^2	Cement	99	89	**89**	41	32	46	3
		Mud	98	87	**91**	29	32	36	3
	Pirimiphos-methyl EC 1 g AI/m^2	Cement	94	**85**	77	24	31	18	2
		Mud	**89**	77	63	31	36	19	1

The residual activity of the IRS applications was measured by standard WHO plastic cones placed on treated wall surfaces. Tests were performed monthly on susceptible laboratory colonies until the end of the trial or until no further treatment mortality was observed (i.e. mortality below 80%).
[1] Laboratory-reared, insecticide susceptible *An. culicifacies* colony; [2] Laboratory-reared, insecticide susceptible *An. gambiae* s.s. colony.

3. REVIEW OF CHLORFENAPYR 240 SC

Chlorfenapyr 240 SC is a suspension concentrate formulation containing 240 g of active ingredient per litre.

Chlorfenapyr is an N-substituted halogenated pyrrole, a broad-spectrum insecticide with stomach and contact actions. The Insecticide Resistance Action Committee (IRAC) of CropLife International has classified the compound in Group 13, i.e. uncouplers of oxidative phosphorylation via disruption of proton gradient.[7]

The present review assesses the efficacy of chlorfenapyr 240 SC (Phantom/Mythic 240 SC, BASF, Germany) for indoor residual spraying against malaria vectors.

The following are extracts from the material safety data sheet of the manufacturer for Phantom/Mythic 240 SC:

Acute oral LD_{50} (rat)	560–567 mg AI/kg
Acute inhalation LC_{50} (rat)	0.571 mg AI/L (4 h)
Acute dermal (rabbit)	>2000 mg AI/kg
Skin irritation (rabbit)	Non-irritant
Eye irritation (rabbit)	Non-irritant
Skin sensitization (guinea-pig)	Not observed

3.1 Safety assessment

The human risk assessment of chlorfenapyr 240 g AI/L SC for indoor residual spraying, provided by the manufacturer, was assessed by the Finnish Institute of Occupational Health (FIOH, 2012) on behalf of WHOPES. The WHO *Generic risk assessment model for indoor residual spraying of insecticides – first revision*[8] was used as a guiding document. The following assumptions were made in the assessment, that:

[7] *Prevention and management of insecticide resistance in vectors of public health importance*, 2nd ed. CropLife International, Insecticide Resistance Action Committee, 2010 (also available at: http://www.afpmb.org/sites/default/files/whatsnew/2011/irac_manual.pdf).

[8] Available at http://whqlibdoc.who.int/publications/2011/9789241502177_eng.pdf.

- target dosage of chlorfenapyr is ≤250 mg AI/m^2;
- dermal absorption of chlorfenapyr from the formulation, from the diluted solution, and from the product dried on the surfaces, is as indicated by product-specific experimental study results, rather than the default 10%;
- inhalation exposure to gaseous chlorfenapyr of the operator and residents is negligible due the low vapour pressure (5.4×10^{-6} Pa at 25 C°);
- spraying represents moderate physical activity, and thus the breathing volume of an adult per hour is 1.9 m^3;
- translodgeable part from the walls and floors onto the skin is 11%;
- biological half-time of chlorfenapyr is 1 day;
- the half-time of chlorfenapyr on the wall does not exceed 6 months (worst-case scenario).

FIOH concluded that the characterization of the risks performed by the proposer closely follows the WHO generic risk assessment model and that where default assumptions are not accepted, justification is presented. The conclusion, in line with the generic model, is that when used for indoor residual spraying as instructed, chlorfenapyr 240 SC does not pose undue hazards to the spray operators or residents of the treated dwellings or to wildlife. If inappropriate or malfunctioning equipment is used, or the WHO guidelines and label instructions on operator protection are not followed, exposure may reach the upper range of safe levels.

3.2 Efficacy – background and supporting documents

3.2.1 Laboratory studies

3.2.1.1 Determination of the diagnostic concentration

Diagnostic concentration is defined by WHO as the concentration that kills all susceptible individuals in a population. Diagnostic concentration is twice the LC_{99} value obtained by exposing the fully-susceptible strain of a given species to an insecticide compound.

To determine the diagnostic dose and hence the susceptibility of malaria vector populations to chlorfenapyr, adult susceptibility tests were carried out in Benin, India and South Africa (Table 3.1). Laboratory-reared 3–5-day old sugar-fed mosquitoes were exposed to the range of 0.125–5.0% of chlorfenapyr-impregnated papers in

WHO tubes, following the standard WHO method.[9] LC_{50} and LC_{95} or LC_{99} were calculated using a log-dose probit regression analysis.[10]

Benin and United Kingdom

Exposure of *An. gambiae* Kisumu strain mosquitoes (a fully insecticide-susceptible strain) for 1 h to papers treated with a range of chlorfenapyr concentrations (0.125–4%) resulted in a LC_{95} of 4.6% after 24 h and of 1.0% after 72 h (N'Guessan et al, 2007). Due to the slow action of chlorfenapyr through tarsal contact, a 14-fold decrease of LC_{50} was found between 24 h and 72 h (0.6, 0.2 and 0.04% after 24, 48 and 72 h, respectively). Exposure to 4% chlorfenapyr induced 90–100% mortality after 24 and 48 h respectively in the Kisumu, Vkpr (pyrethroid-resistant, fixed for *kdr*) and Yao strains (multi-insecticide resistant, *Ace-1*^{R} allele 100%), indicating no cross-resistance between chlorfenapyr and *kdr* and *Ace-1*^{R} resistance mechanisms. The susceptibility to chlorfenapyr was the same between *An. stephensi* pyrethroid-susceptible and resistant strains, with LC_{95} slightly above 5% (only measured up to 24 h after exposure).

India

Four laboratory-reared mosquito strains – *An. culicifacies* species A (insecticide susceptible) and *An. culicifacies* species C (DDT resistant), *An. stephensi* Okhla strain (insecticide susceptible) and *Cx. quinquefasciatus* Mewat strain (resistant to DDT, propoxur and bendiocarb (0% mortality); malathion (8% mortality); lambda-cyhalothrin (33% mortality); deltamethrin (44% mortality); permethrin (62% mortality) and cyfluthrin (76% mortality) – were exposed to chlorfenapyr impregnated filter-papers (0.125–5%) for 60, 90, 120, 150 and 180 min and mortalities were recorded after 24, 48 and 72 h post-exposure holding periods (Raghavendra et al, 2011a,b).

Complete mortality in the susceptibility tests was found at 2.5% chlorfenapyr (exposure of 2 h and holding period of 48 h) and higher concentrations of chlorfenapyr, for both the *An. culicifacies* A and C strains and for *An. stephensi* Okhla strain. Their respective LC_{99} values of chlorfenapyr were 2.0, 2.4 and 2.1% (Table 3.1). Similarly, the LC_{99} value of the multi-resistant *Cx. quinquefasciatus* strain was 2.23%. Following the WHO guidelines, the authors considered 5%

[9] *Guidelines for testing mosquito adulticides for indoor residual sprahying and treatment of mosquito nets*. Geneva, World Health Organization, 2006 (available at: http://www.who.int/whopes/guidelines/en/).

[10] Finney DJ: Probit Analysis - A Statistical Treatment of the Sigmoid Response Curve. *Current Contents/Life Sciences* 1971, 18.

chlorfenapyr as the diagnostic dose for determining the susceptibility status of the above-mentioned species, using 2 h of exposure and 48 h of holding.

Under continued selection with pyrethroid insecticides, between 4% and 29% of a laboratory-reared strain of *Cx. quinquefasciatus* survived when exposed to 5% chlorfenapyr for 2 h. In contrast, field-collected *Cx. quinquefasciatus* at two different sites, which were reported resistant to DDT, malathion, bendiocarb and deltamethrin, showed 100% mortality, indicating absence of cross-resistance to chlorfenapyr.

South Africa
Two laboratory-reared *An. funestus* colonies – FANG (insecticide susceptible) and FUMOZ-R (colony selected for pyrethroid resistance, elevated P450 mono-oxygenases) – were exposed for 1 h to 0.0625–4% chlorfenapyr-impregnated filter-papers (Oliver et al, 2010). The dose mortality calculations from the susceptibility tests showed that the FUMOZ-R strain is less susceptible to chlorfenapyr than the FANG strain, a trend consistent at 24, 48 and 72 h post-exposure (Table 3.1). Despite LD ratios of 1.6 and 2.1 for the LC_{50} and LC_{95} values between the two strains, no significant difference was found for any of the mortality recording periods.

3.2.1.2 Residual activity on different substrates

Delhi, India
The residual activity of chlorfenapyr on different substrates was assessed during 6 months under laboratory conditions using cone bioassays (Raghavendra et al, 2011a,b). Bioassays were conducted at weekly intervals on three laboratory-reared species: *An. stephensi* Okhla strain and *An. culicifacies* A (Ghaziabad strain), both insecticide susceptible, and against the multi-insecticide resistant *Cx. quinquefasciatus* Mewat. Treatment of substrates, between the range 12.5–800 mg AI/m^2, was done with a compression spray pump. Five different substrates were used: mud, mud + lime, cement, cement + distemper paint and wood. Each substrate had 4 replicates per dose and controls. Four replicates of 10 female mosquitoes each were exposed in a cone for 30 min to each of the given substrates and doses. A different location on a substrate was used for exposure each time. After exposure, the mosquitoes were transferred into holding cups with sucrose solution to measure the mortality after 24, 48 and 72 h.

The efficacy of chlorfenapyr on different substrates treated at 12.5–200 mg AI/m^2 showed a drastic reduction in efficacy within 2 weeks after spraying for both insecticide-susceptible anopheline species and the multi-insecticide resistant *Cx. quinquefasciatus* (Table 3.2). Hence, bioassays were continued on substrates with dosages of 400, 600 and 800 mg/m^2.

The residual activity of chlorfenapyr on different substrates tested at target dosages of 400 and 800 mg AI/m2 was 24–34 weeks for anopheline species. Residual activity against Cx. *quinquefasciatus* was between 15 and 34 weeks at the same dosages.

3.2.2 Experimental hut studies

The experimental hut trials summarized below follow the design described by WHO.[11] Oxborough et al (2010) used experimental huts in Moshi, northern United Republic of Tanzania, typical of the East African region. The huts used by N'Guessan et al (2009) and Ngufor et al (2011) in Benin were typical of the West African region. In all the above-mentioned experimental hut trials, voluntary sleepers were rotated between the treatments and huts.

Moshi, United Republic of Tanzania
Oxborough et al (2010) conducted three experimental hut trials in Moshi, United Republic of Tanzania, to evaluate indoor residual spraying (IRS) of chlorfenapyr against wild, free-flying permethrin-resistant (80–90% mortality) *An. arabiensis* and pyrethroid-resistant *Cx. quinquefasciatus*. These were: (i) a 3-week study of chlorfenapyr efficacy at 500 mg AI/m^2 compared with an unsprayed hut; (ii) a 2-month study comparing the residual activity of chlorfenapyr 250 mg AI/m^2 with an alphacypermethrin-treated hut at 30 mg AI/m^2; and (iii) a 6-month study to evaluate the residual activity of chlorfenapyr IRS, 250 and 500 mg AI/m^2.

For both *An. arabiensis* and *Cx. quinquefasciatus*, the indoor residual spraying of chlorfenapyr at 500 mg AI/m^2 induced, respectively, 48% and 47% mortality, after 72 h holding (Table 3.3). In both cases, more than 80% of the total mortality occurred within 24 h of collection. There was a significant blood-feeding inhibition of *An. arabiensis* in

[11] *Guidelines for testing mosquito adulticides for indoor residual sprahying and treatment of mosquito nets*. Geneva, World Health Organization, 2006 (available at: http://www.who.int/whopes/guidelines/en/).

chlorfenapyr-treated huts compared with that of the untreated hut, while for *Cx. quinquefasciatus* there was none.

In the other trial, chlorfenapyr sprayed at 250 mg AI/m^2 killed a similar proportion of *An. arabiensis* as the pyrethroid alpha-cypermethrin sprayed at 30 mg AI/m^2 (51% and 47% respectively). However, for *Cx. quinquefasciatus*, chlorfenapyr induced a significantly higher mortality rate than with alpha-cypermethrin (mortality 56% and 17% respectively). Similar to the first trial, more than 80% of all dead mosquitoes, of both species, were killed within the first 24 h, while alpha-cypermethrin killed >90% in the same timespan. Both insecticides significantly reduced the blood-feeding in both species. The inhibition of *An. arabiensis* was greater for both insecticides than that of *Cx. quinquefasciatus*.

Chlorfenapyr at 250 and 500 mg AI/m^2 induced 41–48% mortality *in An. arabiensis* during the 6-month study of residual activity. For huts sprayed at 500 mg AI/m^2, mortality was consistent during the 6-month study. The mortality rates for *Cx. quinquefasciatus* were slightly higher (54%). More than 80% of mortality occurred within 24 h after collection.

Ladji, Benin

During an 8-week trial in Ladji, Benin, chlorfenapyr-impregnated net (100 mg AI/m^2) and chlorfenapyr IRS (1000 mg AI/m^2) were tested against the local population of *An. gambiae*, resistant to pyrethroids and DDT, and against *Cx. quinquefasciatus* resistant to pyrethroid, carbamate and organophosphate insecticides (N'Guessan et al, 2009). A total of 4 experimental huts were used: 2 to test a chlorfenapyr-treated net against an untreated net; and 2 to compare a hut sprayed with chlorfenapyr at 1000 mg AI/m^2 with an untreated hut. In order to mimic a torn net, test and control nets each had 80 holes of 4 cm^2.

Overall, the hut with the chlorfenapyr ITN induced, after 72 h, 54% mortality among *An. gambiae*, but a higher mortality (83%) was obtained in the chlorfenapyr IRS hut (Table 3.3). Mortality rates for C*x. quinquefasciatus* were lower than for *An. Gambiae*: after 72 h the ITN killed 34% and the IRS killed 46%. A trend of delayed mortality due to chlorfenapyr was found in the treated huts for both species and for both ITN and IRS. Secondly, both species showed a decline in mortality during the past 5 weeks. The chlorfenapyr ITN treatment induced a mortality of *An. gambiae* above 80% only during the first 2 weeks after treatment and up to 1 month for the IRS treatment.

Mortality declined progressively and after 2 months the mortality rate was down to 26% and 46% for respectively the ITN and the IRS treatments. *Cx. quinquefasciatus* mortality did not reach 80% during the first 2 weeks (69%) and it dropped to 18–20% for both ITN and IRS treatments after 8 weeks. The rate of decline in mortality was faster with the ITN than with the IRS treatments.

During the 8-week trial, blood-feeding inhibition of *An. gambiae* and *Cx. quinquesfasciatus* was very moderate in both IRS and ITN treatments, with the highly-holed nets presenting little or no barrier to host-seeking mosquitoes. Blood-feeding rates with the ITN during the initial 2 weeks were approximately 40% compared with approximately 80% in the control. For the IRS treatment, the difference in blood-feeding rates between treatment and control was initially less than with the ITN. The proportions of *An. gambiae* and *Cx. quinquefascitus* exiting into the verandahs of treated huts by dawn were similar to the proportions in the control verandahs.

Akron, Benin

In Akron, Benin, experimental huts studies were carried out to test: (i) chlorfenapyr IRS at 500 mg AI/m^2; (ii) deltamethrin-treated long-lasting insecticidal net (LN) at 55 mg AI/m^2; and (iii) combination of IRS chlorfenapyr 500 mg AI/m^2 with deltamethrin LN (55 mg AI/m^2) against pyrethroid and DDT resistant *An. gambiae* and against *Cx. quinquefasciatus* resistant to pyrethroid, carbamate and organophosphate insecticides (Ngufor et al, 2011). Both treated and untreated nets were holed with 80 holes of 2 cm^2 each or 6 holes of 4 cm^2 each to mimic badly or less torn nets (Table 3.3, nets with 80 holes not included).

The overall mortality of *An. gambiae* mosquitoes with chlorfenapyr IRS treatment was 57% and with the deltamethrin LN alone 50% (with 6 holes) and 37% (with 80 holes). The combination of IRS and LN induced a significantly greater mortality (83%) than the mortalities induced by LN or by IRS alone. The addition of IRS with chlorfenapyr to a hut with LN did not change blood-feeding rates. The majority of mosquitoes killed by the IRS, where no LN was present, had already blood-fed (87%), whereas only a minority of dead mosquitoes had managed to blood-feed when an LN was present in the IRS-treated hut (9.2% in ITN with 6 holes).

In comparison with *An. gambiae*, a smaller proportion of *Cx. quinquefasciatus* was killed (15%) by the deltamethrin LN. While chlorfenapyr IRS alone killed 32% of *Cx. quinquefasciatus*, combining

chlorfenapyr-IRS and deltamethrin LN induced a higher mortality (51%). Despite the low mortality rate, the blood-feeding inhibition induced by the deltamethrin LN was higher for *Cx. quinquefasciatus* than for *An. gambiae*.

3.3 Efficacy – WHOPES supervised trials

3.3.1 Laboratory study

3.3.1.1 Intrinsic insecticidal activity and irritant properties

Montpellier, France

The intrinsic insecticidal activity and irritant properties of chlorfenapyr were determined under standardized laboratory conditions[12] (Phase I) against three strains of *An. gambiae*: (i) Kisumu, insecticide-susceptible; (ii) VK-Per, pyrethroid resistant (homozygous for *kdr* 1014F mutation); and (iii) Acer-Kis, organophosphate/carbamate resistant (homozygous for Ace1R allele) (Rossignol et al, 2011).

The intrinsic toxicity of chlorfenapyr was tested by topical applications (on pronotum) on the above-mentioned strains. For each strain, a triplicate assay was done over 5–8 doses and each dose consisted of two samples of 25 females (n = 50). Mortality rates were recorded 24, 48 and 72 h following the tests. The study provides an estimation of the LD_{50} and LD_{95}. The LD_{50} and LD_{95} were determined in nanogram (ng) of chlorfenapyr/mg of female.

After 24 h, the LD_{50} of Kisumu (2.2 ng/fem) and VK-Per (2.3 ng/fem) was not significantly different. The LD_{50} from Acer-Kis (3.1 ng/fem) was significantly higher but the ratio with LD_{50} of the susceptible strain was only 1.5. No significant differences were observed between the LD_{95} of the three strains. After 72 h, the LD_{50} and LD_{95} between the three strains were not significantly different except for the LD_{95} of Kisumu and Acer-Kis (ratio=1.7).

No significant additional mortality occurred after 24 h on the susceptible *An. gambiae* Kisumu strain using topical applications. However, there was a significant increase in mortality – from 24 to 72

[12] WHO (2006). Guidelines for testing mosquito adulticides for indoor residual sprahying and treatment of mosquito nets. Geneva, World Health Organization (available at: http://www.who.int/whopes/guidelines/en/).

h – for the two resistant strains, indicating delayed mortality (ratio=2 maximum).

Noting the laboratory studies of Raghavendra et al. *(*2011a), the diagnostic concentration of 5% with 2 h exposure and 48 h holding was considered as a tentative value to study the irritant properties of chlorfenapyr.

Three replicates were done on the *An. gambiae* Kisumu-susceptible strain with papers impregnated at 2.5% and 5% of chlorfenapyr*.* Mosquitoes were exposed to impregnated papers for 2 h and mortality was recorded at 24 h and 48 h. At chlorfenapyr 5%, the mortality rates did not reach 100% after 24 h (63%) and 48 h (84%). Contrary to topical applications, tarsal contact induced a significant increase of mortality between 24 h and 48 h at both doses of 2.5% (Chi^2= 35.6, $P < 0.0001$) and 5% (Chi^2= 20.7, $P < 0.0001$). The delayed action of insecticide through tarsal contact may be due to the mode of action of chlorfenapyr and is in contrast to results obtained in wireball studies on chlorfenapyr-treated nets (N'Guessan et al, 2007) and the topical application study reported above. This study also indicates that the proposed diagnostic concentration (including exposure and holding times), as proposed by Raghavendra et al (2011a), may not be appropriate for *An. gambiae*.

The irritant effect of chlorfenapyr was tested using WHO cone bioassays. The irritant properties were determined using a technical grade of insecticide on filter-paper at the above-proposed diagnostic concentration (5%). After a brief observation period lasting for 60 s, the time elapsed between the first landing and the next take-off of the mosquito was recorded as the "time for first take-off". For each test, 50 mosquitoes were individually tested. Mosquitoes were grouped by classes of first take-off time, and cumulative frequencies were used to calculate the time for which 50% and 95% of the mosquitoes take off (FT_{50} and FT_{95}) using Probit analysis. Permethrin was used as a positive control.

On an untreated paper, the times for first take-off for Kisumu strain females were 15 s (FT_{50}) and 591 s (FT_{95}). Using permethrin 0.75%, the times for first take-off were significantly much shorter (FT50: 4 s; FT95: 18 s) confirming that this pyrethroid has a strong irritant effect on mosquitoes. While with chlorfenapyr 5%, the times for first take-off were not significantly different from those with untreated paper, i.e. 15 s (FT_{50}) and 309 s (FT_{95}), indicating that chlorfenapyr is not irritant for *An. gambiae*.

3.3.2 Small-scale field trials

Two small-scale chlorfenapyr field evaluations were carried out under WHOPES supervision: one in Malanville, Benin, on pyrethroid-resistant wild *An. gambiae* mosquitoes (Bouriama et al, 2012) and one in Hoa Binh, Viet Nam, on laboratory-reared pyrethroid-susceptible *An. dirus s. s.* (Van Roey et al, 2013b).

In Benin, the West African experimental huts were used to evaluate the efficacy of chlorfenapyr IRS in terms of mosquito mortality, blood-feeding inhibition, deterrence and induced exophily, according to WHO guidelines.[13] The huts (# 5) were made of concrete bricks, iron roof, a ceiling of polyethylene sheeting and a concrete base. Mosquito access was through four window slits, fixed at an angle to create a funnel with a 1 cm wide gap. A single verandah trap made of polyethylene sheeting and screening mesh projected from the back wall of each hut. Chlorfenapyr was sprayed using a hand-operated compression sprayer, equipped with a flat fan nozzle 80° swath and 0.76 l/min flow rate. The four walls, ceiling, the inside of the door and the roof were sprayed. Chlorfenapyr was evaluated in this experimental hut trial during 24 weeks. Every month, standard WHO cone bioassays were conducted, up to 6 months, to determine the residual efficacy of chlorfenapyr, using laboratory-reared, susceptible females of *An. gambiae* Kisumu strain.

In Viet Nam, a small-scale field trial was conducted to determine the residual activity of chlorfenapyr in 24 representative local houses made of different materials (12 wood and 12 brick). Wooden houses are built on stilts and the walls of all brick houses were covered with a thin lime layer. Spraying was done with a discharge of 550ml/min at 1.5 bar, using Semco© hand-operated compression sprayers equipped with a red constant flow valve and new flat-fan ceramic nozzles. Chlorfenapyr was evaluated in this household setting using WHO cone bioassays on laboratory-reared pyrethroid- and chlorfenapyr-susceptible *An. dirus s. s.* China Strain. Bioassays were conducted 1 week and 1 month after treatment, then every month thereafter until mosquito mortality dropped below 80%. When the mortality dropped below 80%, a confirmatory test was performed the week after.

[13] *Guidelines for testing mosquito adulticides for indoor residual sprahying and treatment of mosquito nets*. Geneva, World Health Organization, 2006 (available at: http://www.who.int/whopes/guidelines/en/).

In both trials, the following study arms were used: (i) negative control; (ii) chlorfenapyr at 150 mg AI/m²; (iii) chlorfenapyr at 250 mg AI/m²; and (iv) deltamethrin WG at 25 mg AI/m². Additionally in the Benin trial, bendiocarb WP was used at 400 mg AI/m² as another positive control.

Malanville, Benin

In this rice-growing area, *An*. *gambiae s.l*. is the main malaria vector, with 95% *An*. *gambiae s*.s, M form, and 5% *An*. *arabiensis*. Increased resistance of malaria vectors to pyrethroids (22% mortality to 0.75% permethrin in 2010) was already reported in Malanville,[14] showing enhanced oxidase activity and a prevalence of the 1014F *kdr* allele of 50%. These results were confirmed with a WHO tube test: *An. gambiae* population was resistant to 0.05% deltamethrin (15% mortality) and to 0.1% bendiocarb (95% mortality). Secondly, the molecular analyses among the live mosquitoes from the experimental hut trial proved that the *kdr* prevalence was 49% in the control hut and ranged from 47% to 65% in the treated huts.

The results of the chemical analysis of the filter-papers used to quality check spray operations are presented in Table 3.7 (see also Annex 3) (Pigeon, 2011a). The ratio of the average amount of insecticide applied, as determined by chemical assay, to that of the target dosage ranged between 0.89 and 1.27. The relative standard deviation of the content of insecticide among filter-papers was higher for chlorfenapyr (42–49%) compared with the two positive controls (25–29%).

To estimate the residual effect of the IRS treatment, cone bioassays were carried out every month using the laboratory-reared, susceptible *An. gambiae* Kisumu strain. Throughout the follow-up period after treatment, chlorfenapyr (150 and 250 mg AI/m²) did not induce any KD effect in bioassays, whereas deltamethrin and bendiocarb caused KD≥95% up to 2 months. Just after the treatment, the mortality ranged from 78% (chlorfenapyr 150 mg AI/m²) to 100% (bendiocarb) after 72 h (Table 3.4). Mortalities induced by chlorfenapyr (after 72 h holding) were always below the WHO cut-off point (80% mortality) except for chlorfenapyr 250 mg AI/m^2 just after treatment (84%). Bendiocarb 400 mg AI/m² managed to maintain mortality ≥80%.

[14] Djegbe I et al. Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S kdr mutation in *Anopheles gambiae* from West Africa. *Malaria Journal*, 2011,10:261.

Deltamethrin WG, throughout the 24-week study, outperformed chlorfenapyr 150 mg AI/m² and chlorfenapyr 250 mg AI/m², resulting in cone bioassay mortalities above 80%. Bendiocarb outperformed the two dosages of chlorfenapyr up to 12 weeks, but resulted in mortalities above 80%, only until 8 weeks. Only chlorfenapyr showed delayed mortality, with significant increase of mortality between 24 and 72 h post-exposures (Odds ratio 1.9 (95% CI 1.4–2.6 for 150 mg AI/m² and 1.5 (95% CI 1.1–2.1 for 250 mg AI/m²).

In the experimental hut trial, in an area of pyrethroid-resistant *An. gambiae* a negative binomial regression model showed that only deltamethrin induced a significant deterrent effect relative to the control. While in the huts treated with chlorfenapyr (150 and 250 mg AI/m²) and with bendiocarb significantly more *An. gambiae* females were collected compared with the untreated hut. Whereas deltamethrin was the only insecticide with a deterrent effect, logistic regressions showed that all treatments except chlorfenapyr 150 mg AI/m² induced higher exophilic behaviour than the control. All treatments induced a significant reduction of blood-feeding relative to the control.

Blood-feeding inhibition was similar and low for the two chlorfenapyr arms (10–14%; OR: 0.57–0.40) and for bendiocarb (16%; OR: 0.27) but was significantly higher in the deltamethrin arm (34%; OR 0.08)).

In contrast to the relatively high mortality rates of the laboratory-reared, susceptible *An. gambiae* mosquitoes, the mortality rates of the wild insecticide-resistant *An*. *gambiae s.l*. mosquitoes were overall lower. Deltamehtrin did not induce significantly higher mortality than the control. Moreover, only bendiocarb showed mortality rates above the WHO threshold (≥80% mortality up to 1 month). Overall mortalities after 72 h over the 6 months, corrected for the control, were 20, 32, 25 and 5% for respectively chlorfenapyr 150 mg AI/m², chlorfenapyr 250 mg AI/m², bendiocarb and deltamethrin (Fig 3.1). For the first 3 months post-treatment, overall mortalities were not much different (respectively 21, 33, 29 and 6%).

The regression model allowed to estimate and compare the mortality rates observed after 72 h induced by each treatment 3 months after indoor spraying and are respectively 0.2% [0.0–1.4%] for the control, 18% [15–21%] for bendiocarb 400 mg AI/m², 4% [2–7%] for deltamethrin 25 mg AI/m², 21% [18–25%] for chlorfenapyr 150 mg AI/m² and 32% [28–36%] for chlorfenapyr 250 mg AI/m².

Three months after spraying, chlorfenapyr 150 mg/m² induced significantly more mortality than deltamethrin 25 mg/m² (OR=0.144 [0.077–0.251]; $p<0.001$), whereas the difference was not significant with bendiocarb 400 mg/m² (OR=0.799 [0.620–1.025]; $p=0.078$). Three months after spraying, chlofenapyr 250 mg/m² induced significantly more mortality than both deltamethrin 25 mg/m² (OR=0.083 [0.044–0.145]; $p<0.001$) and bendiocarb 400 mg/m² (OR=0.461 [0.359–0.589]; $p<0.001$). Differences between the treatments with the mortalities observed at 24 h did not change when the mortalities were observed at 48 h and 72 h despite a general increase in mortality with the delay between exposure and observation periods.

None of the sleepers reported adverse side-effects with any of the insecticide treatments. However, three sleepers reported bad odours with chlorfenapyr 150 mg AI/m² (2/3) and chlorfenapyr 250 mg AI/m² (1/3). Most of the sleepers indicated that they spent a good night in the huts.

Hoa Binh, Viet Nam

The results of the chemical analysis of the filter-papers used to quality check spray operations are presented in Table 3.7 (see also Annex 3) (Pigeon, 2012f). The ratio of the average amount of insecticide applied, as determined by chemical assay, to that of the target dosage ranged between 0.96 and 1.32 with the exception of chlorfenapyr at 150 mg AI/m^2 on wood walls, which was 2.03. The relative standard deviation of the content of insecticide among filter-papers ranged from 24% to 51%.

One week after spraying, on both wood and brick surfaces, the two chlorfenapyr treatments, i.e. 150 and 250 mg AI/m^2, failed to comply with the WHOPES bioassay criteria of $\geq$80% mortality (Table 3.4) using the pyrethroid-susceptible *An. dirus* China strain. A week later, the low mortality rates (7.5–25%) were also observed in the confirmatory bioassay tests. Four and six weeks after spraying, no improvement was noted in the mosquito mortality for both dosages of chlorfenapyr. The reference insecticide, deltamethrin WG, performed only well on brick houses 1 week after treatment and not in wooden houses. Deltamethrin activity increased at 6 weeks after treatment on both wood and brick surfaces. No substantial increase of mortality was observed in cone bioassays of chlorfenapyr-treated houses between 48 and 72 h post-exposure.

After 1 week of spray operations and among the 20–25 inhabitants in each treatment arm, a few individuals reported some adverse effects. One individual in both the deltamethrin and chlorfenapyr 150 mg AI/m^2 study arm and two individuals from the chlorfenapyr 250 mg AI/m^2 study arm reported headache. Four individuals in the deltamethrin study arm reported experiencing a bad smell. In the chlorfenapyr 250 mg AI/m^2 study arm, one individual reported sneezing and another individual reported symptoms of nausea. One month after spraying, no more adverse events were recorded. No adverse effects were reported among the six spraymen.

3.3.3 Large-scale field trials

Two large-scale field trials were carried out under WHOPES supervision: one in the Gambia against *An. gambiae s.l*. population and one in India against *An. culicifacies* population. The overall objectives were to evaluate and compare the persistence and impact of a single round of indoor residual spraying of chlorfenapyr at village-scale. Two dosages of chlorfenapyr SC formulation, 150 mg AI/m^2 and 250 mg AI/m^2, were compared with a positive control respectively DDT WP 2 g AI/m^2 in the Gambia (D'Alessandro et al, 2012) and deltamethrin WG 25 mg AI/m^2 in India (Bhatt et al, 2013).

The indoor spraying was done in local households, described below, by using hand-operated compression sprayers fitted with 8002 E nozzles. In the study in India, a control flow valve (CFV) was fitted to control the discharge of the sprayers. The accuracy of spraying was assessed using Whatman No. 1 filter-papers, fixed with metal pins at three different wall heights before spraying and then removed once dry for chemical analysis.

In the Gambian study area, South Bank of the Central River Region, the majority of the houses are made out of mud bricks with tin or thatched roofs, and some houses are entirely made from cement bricks coated with lime. A total of 18 villages were selected and randomly allocated to the three study arms. For bioassays and stationing filter-papers for chemical assays, 6 houses per research arm were selected (3 mud-plastered and 3 lime-coated cement). Four randomly selected rooms in each selected village in each arm were chosen for entomological studies.

In the Indian study area in Kondagaon district, Chhattisgarh, the majority of the houses are brick-built, mud-plastered and with tiled-

roofs, and few houses have cement plastering and lime coating. Considering the suitability, houses were selected for monitoring of vector population behaviour studies. The 10 selected villages were randomized over the three study arms attributing 4 villages to each chlorfenapyr treatments and 2 villages to deltamethrin treatment. For bioassays and stationing filter-papers for chemical assay, 15 rooms were selected in each study arm (5 mud-plastered, 5 lime-coated cement and 5 with wood surfaces). Four houses were selected in each arm for entomological surveys.

Prior to both trials, the insecticide susceptibility of the local *An. gambiae s.l*. and local *An. culicifacies* mosquito populations were assessed using the WHO diagnostic test papers. The results showed that the *An. gambiae s.l*. mosquitoes in the Gambia were susceptible to chlorfenapyr (72-h mortality 96%, knowing that based on published studies diagnostic concentration of 5% is probably low for *An. gambiae*) and are resistant to DDT (24-h mortality 88%). The *Gambiae* complex is composed of 55% *An. gambiae s.s.* (70% the M Form and about 30% the S Form) and 44% *An. arabiensis* with no difference between the three study arms. In India, *An. culicifacies* (both wild-caught as well as F_1 progeny) were 100% susceptible to 5% chlorfenapyr and resistance to deltamethrin 0.05% (80% mortality).

At first, chlorfenapyr was evaluated in the household setting using WHO cone bioassays on laboratory-reared, insecticide-susceptible *An. gambiae s. s.* Yaoundé Strain in the Gambia, and in India with F_1 progeny of wild-caught, chlorfenapyr-susceptible, *An. culicifacies*. Bioassays were conducted on the most common wall structures: mud-wall, cement-plastered wall (lime-coated) and additionally in India on wooden surfaces. Five replicates of 10 mosquitoes were exposed to each dose and surface type at different positions for 30 min and mortality was recorded after holding them for 24, 48 and 72 h. The bioassays were performed 1 week and 1 month after treatment, then every month thereafter until mosquito mortality dropped below 80%. When the mortality dropped below 80%, a confirmatory test was performed the following week.

Secondly, within the household settings mosquitoes were sampled once a month from fixed sentinel rooms (treated) to measure the impact on mosquito density, parity and human blood-feeding.

Gambia

The chemical analysis of the filter-papers used to monitor the quality of spray operations indicated that the target doses were exceeded in

all three treatment arms (Table 3.7). The ratio of average applied dose to target dosage ranged from 2.61 to 4.39 with AI relative standard deviation among filter-papers ranging between 17% and 72% (Pigeon, 2012g).

The results of the cone bioassays on laboratory-reared, insecticide-susceptible *An. gambiae s. s.* Yaoundé Strain are summarized in Table 3.6. On mud walls, the 72 h mosquito mortality in both the chlorfenapyr arms was below 80% 1 week after spraying (75% and 76% for respectively 150 and 250 mg AI/m^2 treatments). The mortality remained below this threshold 2 and 3 months later (mortality 43–52%). In contrast, the DDT treatment mortality remained consistently above 80% up to 5 months after spraying. On cement walls, both chlorfenapyr arms provided >80% mortality 1 week post-spraying (85% and 83% respectively for 150 and 250 mg AI/m^2 treatments). After 1 month, however, mortality declined to 54% and 34% for respectively 150 and 250 mg AI/m^2 treatments. Mortality remained under 80% at 2 and 3 months post-spraying, although the chlorfenapyr 150 mg AI/m^2 still killed 54% of the mosquitoes after 3 months. For DDT, the mortality remained above the cut-off level for 4 months on cement, when comparative bioassays were stopped.

For each treatment arm, one village was randomly selected for human landing catches. In each of the selected villages, a single treated room was selected for indoor and outdoor collections, once a month, during 4 months. Two people were collecting indoors and two outdoors (sitting adjacent to each other) from 19:00 to 07:00 (or a total of 16 man/nights per arm). The mosquito densities were 15.7, 6.1 and 17.1 bites/man/night for chlorfenapyr 150 and 250 mg AI/m^2 and DDT respectively. Corresponding *An. gambiae s.l.* densities were 1.8, 0.1 and 1.6 bites/man/night.

In each study village, 4 rooms (2 treated and 2 untreated) were randomly chosen for light trap collections (LTC) performed once a month during 5 months or 120 LTC per arm. More mosquitoes were trapped in the chlorfenapyr arms as compared with the DDT arm. The mean number of mosquitoes per trap was 34.5, 26.1 and 4.4 for chlorfenapyr 150, and 250 mg AI/m^2 and DDT respectively. Corresponding *An. gambiae s.l.* densities were 1.0, 0.9 and 0.4 per trap. More mosquitoes, including *An. gambiae s.l.,* were collected in the treated rooms as compared with the untreated ones, as untreated rooms were unoccupied.

The pyrethrum spray collections (PSC) performed in 4 rooms per study village caught on average per catch and per room 1.7, 2.0 and 2.2 mosquitoes, and 0.6, 0.5 and 0.2 *An. gambiae s.l.* in respectively the chlorfenapyr 150 and 250 mg AI/m^2 and DDT treatment arms. Overall, there was no significant difference between PSC for the different treatment arms and for the different species collected. The exit trap collections were performed in the same rooms as for the PSC, allowing estimation of the total number entering into the room by adding PSC and ETC. The overall house entry rate per room and per collection was 1.5, 1.3 and 1.2 for *An. gambiae s.l.* for chlorfenapyr 150 and 250 mg AI/m^2 and DDT respectively, and 1.1 for *An. funestus* in each treatment arm. For *Culex* and *Mansonia*, entry rates varied between 1.5 and 2.0 per room.

Table 3.5 presents the immediate, 24, 48 and 72 h mortality rates of the mosquitoes caught in the exit trap collections. None of the treatments induced a mortality of 80%. Moreover, no substantial mortality increase was observed between 24 and 72 h.

Of the 705 *An. gambiae s.l.* tested for sporozoites, 6 (0.85%) were positives. The sporozoite rate was 0.9% (3/325), 0.5% (187) and 1.0% (2/193) for chlorfenapyr 150 and 250 mg AI/m^2 and DDT respectively. At the baseline survey, sporozoite rate was 1.1% (3/272).

Considering the adverse events for the spray operators immediately after spraying, there were few reports of headache, sneezing or bad smell; there was hardly any report the day after, except for one person who reported a bad smell. After one week, there was no complaint. For the inhabitants of the different treatment groups, several adverse events were recorded but no serious adverse event was reported and all treatment groups had similar answers to the questions asked.

Chattisgarh, India

The chemical analysis of the filter-papers used to monitor the quality of spray operations indicated under-dosing in all three treatment arms (Table 3.6). The ratio of average applied dose to target dosage ranged from 0.49 to 0.64 with the AI relative standard deviation among filter-papers ranging between 26% and 50% (Pigeon, 2013b).

The results of the cone bioassays on the chlorfenapyr-susceptible F1 progeny of *An. culicifacies* are summarized in Table 3.6. Chlorfenapyr 150 mg AI/m^2 provided residual activity up to 4 weeks on cement and wood. On mud, however, the residual activity was only 1 week. The

residual activity of chlorfenapyr 250 mg AI/m^2 on the three surfaces was 4 weeks. The positive control, deltamethrin WG sprayed at 25 mg AI/m^2, provided 8 weeks of residual activity on mud and cement and 4 weeks on wood.

In general, few mosquitoes and in particular malaria vectors were found resting inside houses or rooms in all the study villages, post-spray. A total of 1643 mosquitoes were collected, comprising 9 anopheline species and *Cx. quinquefasciatus* (17.3%). Among anophelines, *An. subpictus* was the predominant species (42.9%) followed by *An. culicifacies* (31.8%). Pre-intervention studies show the comparability of the three arms regarding the vector density.

During the 5 months post-intervention, the mean entry rate of mosquitoes per room was determined by floor sheet, hand catch, pyrethrum spray catch and exit traps in each room. The mean mosquito entry rate per room was 30.5, 24.5 and 13.5 (*An. culicifacies*: 6.1, 4.9 and 2.7) in chlorfenapyr 150 mg AI/m^2, chlorfenapyr 250 mg AI/m^2 and deltamethrin 25 mg AI/m^2 respectively. The mean entry rate per room in unsprayed houses was 58.5. The proportion of fed vector to total entry was calculated as 25.4% and 18.4% in chlorfenapyr 150 mg AI/m^2 and 250 mg AI/m^2 and 20.8% in deltamethrin 25 mg AI/m^2.

Immediate mortality in *An. culicifacies* was 1.6, 9.2 and 0% in chlorfenapyr 150 mg AI/m^2 and 250 mg AI/m^2 and deltamethrin 25 mg AI/m^2 respectively. Delayed mortality after 72 h holding was 30% in chlorfenapyr 150 mg AI/m^2, 45% in chlorfenapyr 250 mg AI/m^2 and 34% in deltamethrin 25 mg AI/m^2 arm.

The proportion of blood-fed to gravid females was recorded as 50.8% and 46.2% in chlorfenapyr 150 mg AI/m^2 and chlorfenapyr 250 mg AI/m^2, respectively, and 42.3% in deltamethrin 25 mg AI/m^2 sprayed villages. The feeding rates, i.e. total fed among captured mosquitoes in sprayed rooms, were 25.4, 16.3 and 20.8% in chlorfenapyr 150 mg AI/m^2 and 250 mg AI/m^2 and deltamethrin 25 mg AI/m^2 respectively.

The proportion of vector population found parous during the 5-month study period was 52.6, 33.3 and 12.5% in chlorfenapyr 150 mg AI/m^2 and 250 mg AI/m^2 and deltamethrin 25 mg AI/m^2 intervention arms respectively compared with 68.8% in unsprayed villages.

During the pre-intervention period, the human blood index and sporozoite rates were recorded as 0.026 (6/235) and 2.02% (7/346)

respectively. In the chlorfenapyr 250 mg AI/m^2 and deltamethrin 25 mg AI/m^2 arms of the study, no specimens were found positive for human blood as well as sporozoites during the 5-month study period.

3.4 Conclusions and recommendations

Chlorfenapyr 240 SC is a suspension concentrate formulation containing 240 g of active ingredient per litre.

Chlorfenapyr is an N-substituted halogenated pyrrole, a broad-spectrum insecticide with stomach and contact actions. It acts by targeting the oxidative pathway in insect mitochondria. The Insecticide Resistance Action Committee (IRAC) of CropLife International has classified the compound in Group 13, i.e. uncouplers of oxidative phosphorylation via disruption of proton gradient.[15]

The present review assesses the efficacy of chlorfenapyr 240 SC (Phantom/Mythic 240 SC, BASF, Germany) for indoor residual spraying against malaria vectors.

A provisional, tentative diagnostic concentration of 5% chlorfenapyr, with an exposure period of 2 h and a holding period of 48 h, has been established based on susceptibility studies on fully susceptible *An. culicifacies* A and *An. stephensi,* DDT-R *An. culicifacies* C and multi-resistant *Cx. quinquefasciatus* (Table 3.1). This concentration, however, did not provide 100% mortality in some other species tested, e.g. *An. gambiae* and *An. funestus,* which would require further investigations.

Based on several susceptibility studies, using different resistance strains, no cross-resistance has been observed in pyrethroid- and DDT-resistant mosquito populations.

Topical application of chlorfenapyr to the pronotum of *An. gambiae* has shown the relatively high insecticidal activity of this compound. However, exposure to chlorfenapyr through tarsal contact has revealed a slower action, which may be attributed to the mode of

[15] *Prevention and management of insecticide resistance in vectors of public health importance*, 2nd ed. CropLife International, Insecticide Resistance Action Committee, 2010 (also available at: http://www.afpmb.org/sites/default/files/whatsnew/2011/irac_manual.pdf).

action of this compound. Several studies have shown that mortality of mosquitoes exposed to chlorfenapyr through tarsal contact will reach its maximum 48–72 h post-exposure. This, however, requires further investigation, as some studies have reported no such delayed mortality. The compound has shown to have no significant irritant properties.

The study of residual activity of chlorfenapyr on different substrates in the laboratory suggested 400 mg AI/m^2 for field applications and that residual activity increased considerably between 200 and 400 mg AI/m^2 (factor of 10). Studies of the residual activity of chlorfenapyr SC on different common surfaces at 150 and 250 mg AI/m^2 in small- and large-scale studies have revealed residual activity of 0–4 and 0–9 weeks, respectively (Table 3.7).

Study of chlorfenapyr applications in experimental huts in Benin did not reveal any deterrent effect. Chlorfenapyr sprayed at 150 and 250 mg AI/m^2 resulted in low to moderate blood-feeding inhibition. Mortality of pyrethroid-resisant *An. gambiae* was higher in chlorfenapyr applications at 250 mg AI/m^2 than that of deltamethrin applied at 25 mg AI/m^2, but similar to that of bendiocarb at 400 mg AI/m^2.

Study of the impact of chlorfenapyr IRS applications on vector mortality in experimental huts at higher dosages of 500 and 1000 mg AI/m^2 provided mortalities of about 37–70% in susceptible *An. arabiensis* and DDT- and pyrethroid-resistant *An. gambiae*, compared with the control (Table 3.3).

In the large-scale trial in the Gambia, no important differences in *An. gambiae s.l.* densities, using different collection methods, were observed between the two chlorfenapyr arms (150 and 250 mg AI/m^2) and the DDT (2 g AI/m^2) arm, except for light-trap collections where fewer numbers of vectors were collected in DDT despite DDT resistance. The mortality of *An. gambiae s.l.* in exit traps was higher in the two chlorfenapyr arms (42 and 31%) compared with the DDT (25%) but still too low to have a significant impact on transmission as shown by the sporozoites rates. Interpretation of the results has to take into account that the applied doses were 2.6–4.4 higher than the target dosages.

In the large-scale trial in India, about half of the target doses were applied. The local malaria vector, *An. culicifacies*, is resistant to deltamethrin (80% mortality). No significant differences in indoor

vector densities, feeding rate, and mortality recorded after 72 hours were observed between the three study arms (chlorfenapyr 150 mg AI/m^2 and 250 mg AI/m^2 and deltamethrin 25 mg AI/m^2), but the number of vector mosquitoes collected was relatively low due to the relatively high zoophilic behaviour of *An. culicifacies* resting mainly in cattle sheds.

In neither of the WHOPES small- and large-scale field trials did spraymen or household inhabitants report any severe adverse effects from the chlorfenapyr.

Noting the above, the meeting recommended:

- that multi-centre studies on different well-characterized strains of different mosquito species, with priority on major malaria vectors, be carried out to establish the diagnostic concentration(s) for chlorfenapyr;

- that further evidence be gathered to assess the impact of indoor residual application of chlorfenapyr on malaria vector populations. The current estimate of the duration of effective action of chlorfenapyr on different surfaces at 250 mg AI/m^2 is 0–9 weeks; and

- that manufacturers develop novel formulations or methods of application of chlorfenapyr for vector control, noting the potential of the compound in addressing insecticide resistance in malaria vectors.

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

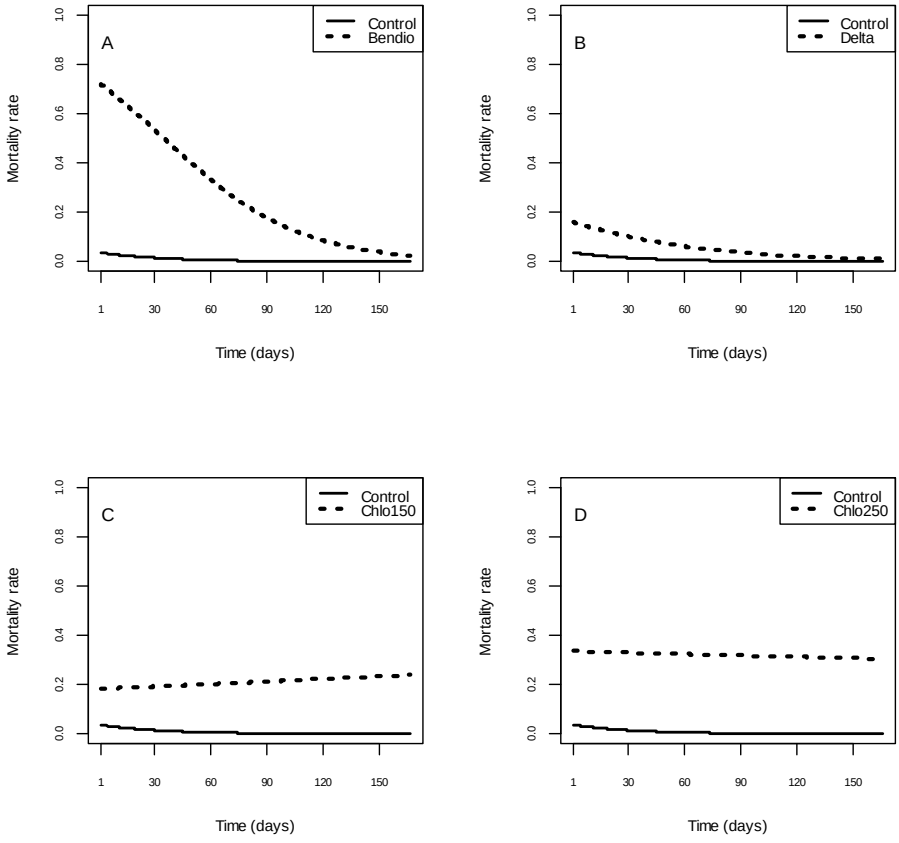

Figure 3.1 Residual activity of all insecticides compared to the control as measured in experimental huts; a) Bendiocarb WP 400 mg/m², b) deltamethrin WG 25 mg/m², c) Chlorfenapyr SC 150 mg/m²; d) Chlorfenapyr SC 250 mg/m². Curves were fitted according to equation parameters of the logistic regression illustrating the mortality after 72 h.

Table 3.1 Results of laboratory susceptibility studies using WHO test tubes

Reference	Species	Test paper	Exposure time (hours)	Mortality recording	LC_{50} (%) (95% CI)	LC_{99} (%) (95% CI)
N'Guessan et al. 2007	*An. gambiae* Kisumu (S)	0.125–4%	1	24 h	0.6 (0.5–0.7)	4.6* (3.5-6.7)
				72 h	0.04 (0.01–0.08)	1.0* (0.6–2.3)
	An. stephensi Beech (S)	0.125–4%	1	24 h	0.87 (0.7–1.08)	5.3* (3.5-10.5)
	An. stephensi Dub234 (pyrethroid R)	0.125–4%	1	24 h	0.99 (0.77–1.25)	5.1* (2.9–12.2)
Raghavendra et al. 2011a	*An. culicifacies* A Ghaziabad (S)	0.25–5%	2	48 h	0.41 (0.36–0.45)	2.0 (1.7–2.48)
	An. culicifacies C Jabalpur (DDT R)				0.67 (0.56–0.79)	2.39 (1.78–3.95)
	An. stephensi Okhla (S)				0.43 (0.31–0.54)	2.13 (1.57–3.55)
Raghavendra et al. 2011b	*Cx. quinquefasciatus* Mewat (S)	0.25–5%	2	48 h	0.37 (0.24–0.49)	2.23 (1.57–4.18)
Oliver et al. 2010	*An. funestus* FANG (S)	0.0625–4%	1	24 h	1.75	5.76*
				72 h	1.44	4.80*
	An. funestus FUMOZ-R (pyrethroid R-P450)			24 h	2.78	11.98*
				72 h	2.09	7.67*

S= insecticide susceptible, R= insecticide resistant; *= LC_{95}
Note: diagnostic concentration is double the LC_{99} value.

Table 3.2 Laboratory study of residual activity of chlorfenapyr on different surfaces (Raghavendra et al, 2011a,b). Figures represent the number of weeks with mortality above 80% on different substrates after a holding period of 72 h.

Species	Dosage mg AI/m^2	Type of surfaces				
		Mud	Mud + lime	Cement	Cement + distemper	Wood
An. culicifacies s.s. (S)	12.5–200	2	2	2	2	2
	400	24	24	24	24	24
	800	28	28	28	28	28
An. stephensi Okhla (S)	12.5–200	2	2	2	2	2
	400	34	34	34	34	34
	800	34	34	34	34	34
Cx. quinquefasciatus	12.5–200	2	2	2	2	2
Mewat (multi-insecticide	400	20	34	20	15	34
resistant)	800	20	34	30	25	34

Table 3.3 Summary of experimental hut trials of chlorfenapyr-treated nets (ITN) and indoor residual spraying of huts (IRS) with chlorfenapyr

Reference[1]	Mosquito species	Treatment arms	Total females caught	24-h mortality (%)	72-h mortality (%)	Blood-feeding (%)
Oxborough et al. 2010 United Republic of Tanzania	*An. arabiensis* (*S*)	Untreated hut	237	8.8[a]	10.9[a]	57.7[a]
		IRS Chlorfenapyr 500 mg AI/m^2	166	43.4[b]	47.6[b]	45.2[b]
	Cx. quinquefasciatus (partially pyrethroid R)	Untreated hut	279	6.1[a]	7.5[a]	36.6[a]
		IRS Chlorfenapyr 500 mg AI/m^2	284	44.4[b]	46.8[b]	37.7[a]
N'Guessan et al. 2009 Benin	*An. gambiae* pyrethroid & DDT R)	Untreated net	84	2.4[a]	9.5[a]	91.7[a]
		ITN Chlorfenapyr 100 mg AI/m^2	116	27.5[b]	53.5[b]	89.1[a]
		Unsprayed hut	199	1.0[a]	8.5[a]	98.0[a]
		IRS Chlorfenapyr 1000 mg AI/m^2	310	71.2[b]	82.9[b]	80.7[b]
	Cx. quinquefasciatus (pyrethroid, carbamate & organophosphate R)	Untreated net	317	1.3[a]	6.9[a]	80.4[a]
		ITN Chlorfenapyr 100 mg AI/m^2	355	21.1[b]	34.4[b]	73.2[a]
		Unsprayed hut	300	0.7[a]	4.7[a]	90.0[a]
		IRS Chlorfenapyr 1000 mg AI/m^2	439	38.9[b]	45.6[b]	82.7[a]

Table 3.3 contd Summary of experimental hut trials of chlorfenapyr-treated nets (ITN) and indoor residual spraying of huts (IRS) with chlorfenapyr

Reference[1]	*Mosquito species*	Treatment arms	Total females caught	24-h mortality (%)	72-h mortality (%)	Blood-feeding (%)
Ngufor et al. 2011 Benin	*An. gambiae* (pyrethroid & DDT R)	Untreated net	78	NA	5.1[a]	56.4[a]
		Deltamethrin LN (55 mg AI/m^2)	91		49.5[b]	12.1[b]
		IRS Chlorfenapyr 500 mg AI/m^2	263		56.7[b]	89.4[c]
		IRS Chlorfenapyr 500 mg AI/m^2 & Deltamethrin LN (55 mg AI/m^2)	105		83.0[c]	18.1[b]
	Cx. quinquefasciatus (pyrethroid, carbamate & organophosphate R)	Untreated net	533	NA	1.7[a]	33.8[a]
		Deltamethrin LN (55 mg AI/m^2)	1014		15.0[b]	5.4[b]
		IRS Chlorfenapyr 500 mg AI/m^2	1507		32.3[c]	76.8[c]
		IRS Chlorfenapyr 500 mg AI/m^2 & Deltamethrin LN (55 mg AI/m^2)	1260		51.0[d]	4.1[b]

[1] Duration of Oxborough et al. studies was over 3 weeks, that of N'Guessan et al. 8 weeks and Ngufor et al. 12 weeks.
NA = not available, LN = long-lasting insecticidal net, S= insecticide susceptible, R= insecticide resistant.
Percentages in the same column, per mosquito species, sharing a letter superscript do not differ significantly.

Table 3.4 Summary of cone bioassays to determine residual activity of insecticides in indoor residual spraying (% mortalities recorded 72 h post-exposure)

Mosquito species	Surface	Weeks	Control	Chlorfenapyr SC 150 mg AI/m²	Chlorfenapyr SC 250 mg AI/m²	Deltamethrin WG 25 mg AI/m²	Bendiocarb WP 400 mg AI/m²
An. gambiae **Kisumu (susceptible)**	Brick	0	0	78.1	84.4	97.8	100.0
		4	4	64.3	66.1	93.1	98.3
		8	2.0	50.9	59.3	92.6	92.6
Benin [1]		12	1.9	45.1	52.0	85.2	74.5
		16	0	34.6	42.3	85.7	48.9
		20	1.9	27.3	30.4	82.8	27.4
		24	2	23.5	28.8	81.4	25.4
An. dirus **China strain (susceptible)**	Brick	1	2.5	5.8	10.0	93.3	
		4	2.5	25	29.2	56.7	
		5	2.5	12.5	16.7	56.7	
Viet Nam [2]		6	3.3	18.3	14.2	96.7	
	Wood	1	6.7	19.2	14.2	54.2	
		4	2.5	9.2	5.8	29.2	
		5	2.5	8.3	5.0	24.2	
		6	5.8	24.2	22.5	81.7	

1: 4 bioassays/house, 1 house per treatment
2: 4 bioassays/house, 3 houses per treatment

Table 3.5 Mortality rates of exiting mosquitoes captured in exit traps in WHOPES large-scale (phase III) studies in the Gambia

	Treatment	Mosquito	N	Total mosquitoes in exit traps	Immediate mortality (%)	24-h mortality (%)	48-h mortality (%)	72-h mortality (%)
Gambia	Chlorfenapyr 150 mg AI/m^2	*An. gambiae s.l.*	120	52	32.7	42.3	42.3	42.3
		All mosquitoes	120	715	37.2	58.5	67,8	72.7
	Chlorfenapyr 250 mg AI/m^2	*An. gambiae s.l.*	120	13	23.1	30,8	30,8	30.8
		All mosquitoes	120	292	41.6	62.6	65.9	68.9
	DDT WP 2 g AI/m^2	*An. gambiae s.l.*	120	68	11.8	23.5	25.0	25.0
		All mosquitoes	120	166	22.3	39.8	46.4	46.4

Wild *An. gambiae s.l.* population is resistant to DDT (mortality 88%).
N = total number of exit trap collections.

Table 3.6 Summary of cone bioassay results in WHOPES supervised large-scale trials (% mortality after 72-h holding)

Study	Study Arm	Type of surface	% mortality after weeks post-spraying							
			1	4	5	8	9	12	13	20
D'Alessandro et al (2012) Gambia[1]	Chlorfenapyr SC 150 mg AI/m^2	Mud	74.8	54.4				52.1		
		Cement	84.5	54.2		43.6		54.2		
	Chlorfenapyr SC 250 mg AI/m^2	Mud	76.2	52.6		9.1		43.4		
		Cement	82.5	34.2		39.1				
	DDT WP 2 g AI/m^2	Mud	100	91.1				87.5		100
		Cement	100	97.3		83.3		100		
Bhatt et al (2013) India[2]	Chlorfenapyr SC 150 mg AI/m^2	Mud	85.1	77.1	48.9					
		Cement	89.2	91.6		31.6	9.2*			
		Wood	86.9	81.7		48.1	41*			
	Chlorfenapyr SC 250 mg AI/m^2	Mud	80.2	96.7		47.0*	38.3*			
		Cement	88.9	100		66.1*	17.9*			
		Wood	88.7	100		76.6*	83.1*	44.1	75.9	
	Deltamethrin WG 25 mg AI/m^2	Mud	100	85.1		81.7*		68.8	55.9	
		Cement	100	97.7		79.3*	73.7*			
		Wood	94	82.7		74.0*	46.8*			

1: laboratory-reared, insecticide-susceptible *An. gambiae s. s.* Yaoundé Strain.
2: F_1 progeny of wild-caught, chlorfenapyr-susceptible *An. culicifacies*.
* Mortality at 48 h.

Table 3.7 Summary of results of residual activity of insecticides in small- and large-scale WHOPES supervised trials as well as results of chemical assay of filter-papers used for monitoring quality of spray operations. Residual activity is reported as number of weeks with mortality ≥ 80% in cone bioassays.

Study site and species	Study arm	Surface type	No. papers analysed[1]	Applied/ target dose ratio	RSD[2]	Residual activity (weeks)
Benin *An. gambiae*, Kisumu Small-scale studies	Chlorfenapyr SC 150 mg AI/ m^2	Brick	5	0.95	49.1%	0
	Chlorfenapyr SC 250 mg AI/ m^2	Brick	5	1.27	41.7%	0
	Deltamethrin WG 25 mg AI/m2	Brick	5	0.89	25.3%	24
	Bendiocarb WP 400 mg AI/ m^2	Brick	5	0.84	28.8%	8
Viet Nam *An. dirus*, China Small-scale studies	Chlorfenapyr SC 150 mg AI/m^2	Wood	12	2.03	24.1%	0
		Brick	12	1.24	51.0%	0
	Chlorfenapyr SC 250 mg AI/m^2	Wood	12	1.04	31.4%	0
		Brick	12	0.96	30.9%	0
	Deltamethrin WG 25 mg AI /m^2	Wood	12	1.32	35.3%	0
		Brick	12	1.10	51.2%	1

[1] Number of papers analysed in Benin, 1 house per arm and 5 papers per room; Viet Nam, 3 rooms per arm and 4 papers per room; India, 5 houses per arm and 3 papers per room; the Gambia, 3 rooms per arm and 3 papers per room.
[2] RSD = relative standard deviation of the AI content between filter-papers.

Table 3.7 contd Summary of results of residual activity of insecticides in small- and large-scale WHOPES supervised trials as well as results of chemical assay of filter-papers used for monitoring quality of spray operations. Residual activity is reported as number of weeks with mortality ≥ 80% in cone bioassays.

Study site and species	Study arm	Surface type	No. papers analysed[1]	Applied/ target dose ratio	RSD[2]	Residual activity (weeks)
India *An. culicifacies*, s.l. Large-scale studies	Chlorfenapyr SC 150 mg AI/m^2	Mud plaster	15	0.58	28.4%	1
		Lime-coated cement	15	0.61	46.7%	4
		Wood	15	0.61	37.5%	4
	Chlorfenapyr SC 250 mg AI/m^2	Mud plaster	15	0.52	28.5%	4
		Lime-coated cement	15	0.49	25.9%	4
		Wood	15	0.63	40.2%	9
	Deltamethrin WG 25 mg AI/m^2	Mud plaster	15	0.54	49.8%	8
		Lime-coated cement	15	0.50	31.7%	8
		Wood	15	0.64	42.9%	4
Gambia *An. gambiae* Large-scale studies	Chlorfenapyr SC 150 mg AI/m^2	Mud plaster	9	2.82	36.7%	0
		Lime-coated cement	9	2.61	52.7%	1
	Chlorfenapyr SC 250 mg AI/m^2	Mud plaster	9	3.98	19.1%	0
		Lime-coated cement	9	3.87	17.2%	1
	DDT WP 2 g AI/m^2	Mud plaster	9	2.70	33.4%	≥20
		Lime-coated cement	9	4.39	72.4%	≥12

[1] Number of papers analysed in Benin, 1 house per arm and 5 papers per room; Viet Nam, 3 rooms per arm and 4 papers per room; India, 5 houses per arm and 3 papers per room; the Gambia, 3 rooms per arm and 3 papers per room.
[2] RSD = relative standard deviation of the AI content between filter-papers.

4. REVIEW OF DELTAMETHRIN 62.5 SC-PE

Deltamethrin 62.5 polymer-enhanced suspension concentrate (SC-PE) is an adjuvanted aqueous suspension concentrate formulation containing 62.5 g of active ingredient per litre intended for extended residual activity on treated surfaces due to the addition of a specific polymer.

Deltamethrin is a broad-spectrum insecticide with contact and stomach actions. The Insecticide Resistance Action Committee (IRAC) of CropLife International has classified the compound in Group 3A[16] – sodium channel modulators.

Deltamethrin wettable powder (WP) and water dispersible granules (WG) have previously been evaluated by WHO and are recommended for indoor residual spraying against malaria vectors at the dosage of 0.02–0.025 g AI/M^2, with 3–6 months of expected duration of effective action.[17] WHO specifications for deltamethrin technical material and WP and WG formulations,[18] developed under the new procedure, are based on data packages of several manufacturers, including that of Bayer Crop Sciences, which has served as reference profile.[19]

The present review assesses the efficacy of deltamethrin polymer-enhanced 62.5 suspension concentrate (SC-PE) (K-Othrine Polyzone, Bayer Crop Sciences, Germany) for indoor residual spraying against

[16] *Prevention and management of insecticide resistance in vectors of public health importance*, 2nd ed. CropLife International, Insecticide Resistance Action Committee, 2010 (also available at: http://www.afpmb.org/sites/default/files/whatsnew/2011/irac_manual.pdf).

[17] Available at http://www.who.int/entity/whopes/Insecticides_IRS_Malaria_09.pdf.

[18] Available at http://www.who.int/entity/whopes/quality/Deltamethrin_eval_specs_WHO_November_2012.pdf.

[19] The impurity, toxicological and ecotoxicological profiles upon which the original specification for a technical grade active ingredient is based. The reference profiles are used for the determination of equivalence. A reference profile is not amended by the data supporting additional technical grade active ingredients that are subsequently judged to be equivalent but, following a review of specifications by the JMPS, a new reference profile may supersede an earlier one. Generally, the reference profile of impurities relates to the technical grade active ingredient supported by the most complete toxicological and ecotoxicological profiles.

malaria vectors, comparing with previously published WHO recommendations for the WG formulation.

4.1 Safety assessment

The human risk assessment of deltamethrin 62.5 g AI/L SC-PE for indoor residual spraying, provided by the manufacturer, was assessed by the Finnish Institute of Occupational Health (FIOH, 2010) on behalf of WHOPES. The WHO *Generic risk assessment model for indoor residual spraying of insecticides*[20] was used as a guiding document.

Assuming, that:

- the dermal absorption of deltamethrin, as reported by the manufacturer, is <0.2%, rather than the risk assessment model default value of 10%;

- in line with the generic risk assessment model, due to the low vapour pressure the inhalation exposure of the operator to gaseous deltamethrin, using appropriate personal protection, is negligible;

- breathing volume is in line with the risk assessment model 4 m3/h and the daily exposure time is 6 h;

- translodgeable part from the walls and floors onto the skin is in line with the risk assessment model, 50%; and

- the disappearance half-time of deltamethrin is 0.23 d.

The WHO risk assessment model indicates that if the WHO guidelines vis-à-vis equipment and operator protection are followed, the exposures are low and there is no undue hazard to either the operator or residents. Even in the case of incomplete compliance with the package instructions and WHO guidelines, the risks are minimal (model-predicted exposures are clearly less than the acceptable

[20] *A generic risk assessment model for indoor residual spraying of insecticides.* Geneva, World Health Organization, 2010 (WHO/HTM/NTD/WHOPES/2010.5, available at http://whqlibdoc.who.int/publications/2010/9789241599559_eng.pdf).

exposure level (AEL)). Only in case of gross negligence may the AELs be exceeded.

FIOH concluded that when deltamethrin 62.5 SC-PE is used for indoor residual spraying as instructed, it does not pose undue hazards to the spray operators or residents of the treated dwellings. Only if grossly inappropriate and malfunctioning equipment is used, and the WHO guidelines for spraying and label instructions on operator protection are ignored, exposure may reach and even slightly exceed AEL levels.[21]

4.2 Efficacy – background and supporting documents

4.2.1 Small-scale trials

Moshi, United Republic of Tanzania

A small-scale study was done in the United Republic of Tanzania to compare the initial efficacy and residual activity of deltamethrin 62.5 SC-PE at 25 mg AI/m² and 50 mg AI/m² and DDT WP 75% at 2 g AI/m² on different wall surfaces (Rowland, 2010a). Treatments were sprayed in experimental huts, on walls surfaced with mud, cement/sand concrete, palm thatch or plywood. WHO cone tests (30 min exposure) were done using a laboratory strain of *An. arabiensis* (Doldotha strain) 1 day after spraying and every 4 weeks for 52 weeks (Table 4.1).

On palm thatch, deltamethrin SC-PE at 25 mg AI/m² and 50 mg AI/m² gave high mortality (>80%) throughout the study, with respectively 100% and 96% after 12 months. The mortality with DDT was 89% on day 1 after treatment but decreased below 80% at 1 month and throughout the remaining 12 months of the study. On plywood, mortality of *An. arabiensis* was >80% after 12 months for deltamethrin SC-PE at 25 mg AI/m² and 50 mg AI/m². DDT showed a gradual decline in activity with <80% mortality after 7 months. On concrete, deltamethrin SC-PE applied at 50 mg AI/m² showed the highest and most prolonged mortality with >80% *An. arabiensis* killed after 10 months in two replicate studies. Mortality with deltamethrin SC-PE

[21] The revised WHO risk assessment model (2011) has revised the defaults for the respiratory volume and exposure time of the operator, as well as that for the translodgeability of the product from the walls and floors. If the new defaults are applied in this case, the exposure even in the realistic worst case scenario remains below the AEL.

applied at 25 mg AI/m² was less consistent, decreasing below 80% within 2 months in one study and after 11 months in a second. Mortality with DDT was below 80% throughout both studies. On mud, deltamethrin SC-PE at 50 mg AI/m² killed <80% after 16 weeks. Deltamethrin SC-PE at 25 mg AI/m² killed <80% after 8 weeks. DDT fared worse, inducing <40% mortality after 4 weeks.

In conclusion, on plywood and palm thatch that are non-porous, organic materials deltamethrin SC-PE at 50 and 25 mg AI/m² killed >80% of *An. arabiensis* for more than 1 year. On concrete, deltamethrin SC-PE killed >80% of *An. arabiensis* after 11 months at 50 mg AI/m² but was less consistent at 25 mg AI/m^2. On mud, deltamethrin SC-PE killed >80% of *An. arabiensis* for 1 month at 25 mg AI/m² and 2 months at 50 mg AI/m² (Table 4.1).

Moshi, United Republic of Tanzania

A Phase II study was conducted in the United Republic of Tanzania to compare the efficacy and residual activity of deltamethrin SC-PE at 25 mg AI/m² and DDT WP 75% at 2 g AI/m² against free-flying wild *An. arabiensis* mosquitoes in experimental huts with interior wall surfaces of mud or cement/sand plaster and ceilings of palm thatch (Rowland, 2010b). Low-level permethrin resistance has been detected in the local population of *An. arabiensis*. Treatments were sprayed onto walls and ceilings using a Hudson sprayer at an application rate of 40ml/m². Fixing a guidance pole to the spray lance ensured a consistent distance from the wall, a regular vertical swath width and a 5 cm overlap between swaths. Swaths were marked out with chalk to aid timing and accuracy. No filter-papers were fixed to the walls during spraying to enable confirmation of dosage by chemical analysis.

Using a laboratory-susceptible *An. arabiensis* strain (Doldotha strain), a decrease in residual activity with time was observed by cone bioassay on the sprayed surfaces. Deltamethrin SC-PE showed >80% mortality up to 7 months on cement, 2 months on mud and over 7 months on thatch. Thereafter, cone mortality varied between 50–70% on cement and 25–60% on mud until the end of the 12-month trial. DDT WP showed a similar trend to deltamethrin except that mortality was rather lower at each interval, with DDT mortality >80% after 4 months on cement, 1 month on mud and 6 months on thatch (Table 4.1).

Against the free-flying, wild vector population, similar levels of performance were initially observed for both deltamethrin SC-PE and

DDT with mortality 40–55% for the first 2 months after spraying. Between months 4 and 9, mortality was higher in all treatment arms with >60% mortality across all formulations. During months 10–12, mortality decreased to 30–50% at which point the study was ended. There was no difference between deltamethrin SC-PE and deltamethrin WP in the trend or level of mosquito mortality on either mud or cement walls. The proportion of mosquitoes exiting was least and blood-feeding was greater during the months of highest mortality.

Because residual mortality was higher on thatch than on mud or cement, a rotation of covering and uncovering the ceiling with plastic sheet was undertaken in all huts to separate the contribution of ceiling and wall treatments to mortality of free-flying mosquitoes in the hut trial. The ceiling treatment made only a 25% difference to DDT WP mortality and a <15% difference to deltamethrin SC-PE mortality.

There was a paradox between the decline in residual activity as measured in cone bioassays and mortality in free-flying mosquitoes peaking midway through the trial. The toxicity of DDT and pyrethroids is known to have a negative temperature coefficient. The site of the hut trial in the Kilimanjaro region experiences seasonal weather with hot and cool dry periods, long rains and short rains. Climatic data recorded at the hut site showed a negative correlation between percentage mosquito mortality per month and mean ambient temperature inside the huts. Temperature-dependent mortality may be due to the negative temperature coefficient of DDT and pyrethroids together with seasonal changes in the behaviour of *An. arabiensis* inside huts, which affects resting duration and insecticide uptake from pyrethroid and DDT sprayed surfaces during the cooler months.

Mpumalanga, South Africa

A phase I study was done in the Mpumalanga Province of South Africa to compare the efficacy and residual activity of deltamethrin SC-PE at 25 mg AI/m², deltamethrin WG at 20 mg AI/m², and lambda-cyhalothrin at 25 mg AI/m² (Icon CS) on mud and cement interior surfaces against a colony of *An. arabiensis* susceptible to insecticides recommended for IRS (Brooke et al, 2013). A total of 24 houses were sprayed, 4 per insecticide formulation and substrate; 2 unsprayed houses per substrate type were used as controls.

Spraying was done using calibrated Hudson Xpert pumps and 8002 nozzles for porous surfaces, by spray operators who routinely conduct spraying for the Mpumalanga Malaria Control Programme.

Cone bioassays were performed using a susceptible strain of *An. arabiensis* 1 week post-spraying and once a month thereafter until <80% mortality was recorded for 2 consecutive months. Five cones were placed on each wall at differing heights, and 10 unfed, 2–4-day old female mosquitoes were exposed for 30 min in each cone. Mortality was recorded 24 h post-exposure.

When mortality declined below 80% for 2 consecutive months, bioassays were stopped. Bioassays on deltamethrin WG cement surfaces were discontinued 5 months post-spraying, and bioassays on deltamethrin WG mud surfaces were discontinued 8 months post-spraying. Bioassays data on deltamethrin SC-PE surfaces were available up to 8 months at the time of the review meeting. The meeting agreed to update Table 4.1 to include bioassay data up to 12 months before publication deadline of the report. Lambda-cyhalothrin performed below expectations: bioassays were discontinued after 2 months post-spraying for cement and mud surfaces treated with lambda-cyhalothrin (Table 4.1).

Based on linear regression, there was a small but significant decrease in mortality with time for the deltamethrin SC-PE cement and mud surfaces by 8 months post-spraying (P<0.05). The deltamethrin SC-PE continued to perform equally well on mud and cement surfaces up to and including 12 months post-spraying and there was no significant difference in overall induced mortality between the two substrates. Deltamethrin SC-PE significantly outperformed deltamethrin WG and lambda-cyhalothrin.

4.3 Efficacy – WHOPES supervised trials

4.3.1 Small-scale trial

Hoa Binh, Viet Nam

A phase I study was done in Viet Nam to compare the initial efficacy and residual activity of deltamethrin SC-PE at 15, 20 and 25 mg AI/m², and deltamethrin WG at 25 mg AI/m² within huts constructed of brick or wood (Van Roey et al, 2013c). The study was conducted in the Mo A village (Hoa Binh province) where 30 houses (15 concrete brick houses and 15 wooden houses) were randomly assigned to the 4 treatment arms or the control arm. The insecticides were applied using Semco hand-operated compression sprayers equipped with a red control flow valve and new flat-fan nozzles with a ceramic tip (Type TEEJET N°8002VR).

Cone bioassays were performed using a colony of *Anopheles dirus s.s.* (China strain) from NIMPE (Hanoi, Viet Nam), which is fully susceptible to deltamethrin. In each house, 4 replicates were performed, one around each surface delineated by the position of the filter-paper used to assess the quality of treatment. Three-day old non-blood-fed females were exposed in the cones (10/cone) for 30 min. Bioassays were done 1 week and 1 month after treatment, then every month thereafter until mortality dropped below 80%, at which point a confirmatory test was performed the week after to confirm the low performance.

On the wooden surface 1 week after spraying, all 3 SC-PE treatments and the WG gave mortality below the WHOPES efficacy criteria. A week later, the low mortality rates were confirmed. At 1 and 2 months post-spraying, none of the treatments gave ≥80% mosquito mortality on the wooden surface. As a result, none of the deltamethrin-treated wooden houses induced a mortality complying with the WHOPES efficacy criteria (Tables 4.2 and 4.3).

On brick walls, the 4 deltamethrin treatments complied with the efficacy criteria during the first 2 months. After 3 months, the deltamethrin SC-PE 15 mg AI/m^2 induced mortality above 80%, but not the other treatments. At month 4, the deltamethrin SC-PE 15 mg AI/m^2 treatment dropped below the 80% mortality threshold, which was confirmed 1 week later.

To assess the accuracy of indoor spraying, 4 filter-papers (10 cm x 10 cm) were attached in each house at three different wall heights (respectively 1 paper at 0.5 m, 2 at 1 m and 1 at 1.5 m from the floor). Once the papers had dried post-spraying, each sample was placed individually in labelled aluminium foil and sent to the WHO Collaborating Centre in Gembloux, Belgium for chemical analysis (Annex 3). The average of the applied to target dose ratio was 0.73–0.88 for deltamethrin 62.5 g AI/L SC at 15 mg AI/m², 0.83–0.95 for deltamethrin 62.5 g AI/L SC at 20 mg AI/m², 0.62–0.92 for deltamethrin 62.5 g AI/L SC at 25 mg AI / m² and 1.34–1.63 for deltamethrin 25% WG at 25 mg AI/m². The AI content variation between filter-papers expressed as the relative standard deviation ranged from 29.7% to 42.7% (Table 4.2) (Pigeon, 2012h).

No correlation was found between the applied dosage of deltamethrin as checked by the chemical analysis and the mortality in the bioassays. No improvement in residual activity was observed by

increasing the dosage from 15 mg AI/m^2 to 20 mg AI/m^2 to 25 mg AI/m^2 (Tables 4.2 and 4.3).

After spraying, no adverse effects were reported by the 6 spraymen. None of the 218 inhabitants sleeping in control or treated houses reported side-effects 1 week or 1 month after spraying.

4.3.2 Large-scale trials

Assam, India

A Phase III study was done in India to compare the residual activity of deltamethrin SC-PE at 20 mg AI/m² and 25 mg AI/m², with deltamethrin WG at 25 mg AI/m² on the commonest types of indoor surfaces against malaria vectors (Prakash et al, 2012). The impact of these treatments on the entomological parameters influencing the vectorial capacity of wild populations was also investigated.

The study was carried out in Assam in 9 villages of which 7 belonged to Dolamara Mini Primary Health Centre (MPHC) (Karbi Anglong district), and 2 belonged to Bokakhat Community Health Centre (Golaghat district). The villages were selected based on their similarity for eco-epidemiological factors, with a distance at least of 1.5–2.0 km apart from each other and the predominance of *An. minimus* as the malaria vector.

Baseline mosquito densities were evaluated from May to July 2011 using CDC light traps in 3–4 sentinel houses per village. The densities of *An. minimus* (including 10% of *An. aconitus* + *An. varuna*) per trap night in the 3 treatment arms (3 villages/arm) were 5.2 ± 3.5 (Deltamethrin SC-PE 20 mg AI/m^2), 7.2 ± 5.2 (Deltamethrin SC-PE 25 mg ai/m^2) and 5.6 ± 3.8 (Deltamethrin WG 25 mg AI/m^2), and did not show statistically significant differences.

Indoor spraying of the households with deltamethrin SC-PE or WG in the study villages was carried out in the second fortnight of July 2011 using Hudson hand-held compression sprayers with red control flow valves. Coverage of 98–99% of targeted houses (94 to 117 houses/arm) and 94–98% of the targeted dwelling rooms (251 to 309 rooms/arm) was achieved during the spray.

After spraying, mosquito collections were done fortnightly in 4 fixed rooms in each village. The collection methods were floor sheet collection, indoor resting collection, exit trap collection and pyrethrum

spray catches. Light trap collections were done in 2 other rooms/village once a month. Another house was selected for human landing catches, outdoors and indoors.

Before spraying, the susceptibility to deltamethrin 0.05% of wild *An. minimus* was estimated, using WHO test tubes with 2 replicates of 10 wild-caught females. Results were 95% knockdown after 1 h exposure and 100% mortality after 24 h holding period, indicating susceptible status of this sample of *An. minimus* s.l.

A total of 84 filter-papers used to monitor the quality of spraying (75 treated, 9 controls) were sent to the WHO Collaborating Centre in Gembloux, Belgium for chemical analysis (Annex 3). The average of the applied to target dose ratio was 0.80–1.39 for deltamethrin SC-PE at 20 mg AI / m², 0.70–1.07 for deltamethrin SC-PE at 25 mg AI/m² and 0.65–1.31 for deltamethrin WG at 25 mg AI/m². The AI content variation between filter-papers expressed as the relative standard deviation ranged from 25.6% to 86.7% (Table 4.2) (Pigeon, 2011b).

The residual insecticidal activity of deltamethrin treatments was determined using laboratory-reared 2–5-day old females of an *An. stephensi* strain susceptible to deltamethrin on the three locally sprayed surfaces: mud-plastered walls, cement walls and lime-coated cement walls, using WHO cone bioassays (30 min exposure). Bioassays were done after 1 week and 1 month of spraying, and thereafter every month until mosquito mortality dropped below 80%. Whenever the mortality dropped below 80% in a village or on a substrate, bioassays were performed a week later to confirm the low performance. When the mortality in a village or a specific substrate was lower than 80% on two consecutive tests, further testing was stopped.

Deltamethrin SC-PE applied at 20 mg AI/m^2 remained effective (mortality >80%) for 6 months on all the different sprayed surfaces. Deltamethrin SC-PE applied at 25 mg AI/m^2 gave 5 months on cement and lime-coated cement walls and 6 months on mud plastered walls, and therefore did not result in a longer or higher efficacy than 20 mg AI/m^2. Deltamethrin WG applied at 25 mg AI/m^2 remained effective (mortality >80%) for 6 months on cement and lime-coated cement walls and for 4–5 months on mud-plastered walls (Tables 4.2 and 4.3).

During the post-spraying period (August 2011 – April 2012), overall mean mosquito densities per trap night declined compared with the

baseline period in the three arms SC-PE 20 mg AI/m^2, SC-PE 25 mg AI/m^2 and WG 25 mg AI/m^2 respectively to 1.7, 1.9 and 1.2 for *An. minimus*; 4.2, 3.4, 5.8 for other anophelines; 16.8, 19.7, 31.0 for culicines, and 24.9, 30.4 and 40.1 for total mosquitoes. None of these differences were significant.

Before spraying, the parous rate in *An. minimus* was not significantly different between villages. A difference was only observed during the first quarter of the post-spray period, since the parous rates in the SC-PE 20 mg AI/m^2 arm (29.6%) were significantly lower than the WG 25 mg dose (58.3%) as well as the deltamethrin SC-PE 25 mg arm (53.8%). In the remaining two quarters of the post-spray period, the parous rates were not statistically different.

The exit rate (% of mosquitoes collected in exit traps relative to total entry) of total mosquitoes was significantly lower, i.e. 19% in houses sprayed with deltamethrin SC-PE 20 mg compared with 37.7% in deltamethrin SC 25 mg and 34.7% in deltamethrin WG 25 mg sprayed houses, but this difference was not significant when considering only anopheline mosquitoes (*An. minimus* included). Overall immediate mortality of mosquitoes (no. of mosquitoes collected dead on the floor sheets and exit traps) in the sprayed rooms was similar during the post-spray period in the three study arms but was significantly different in the case of *An. minimus*. A significantly higher immediate mortality of *An. minimus* was found in the deltamethrin SC-PE 25 mg arm (0.58/room/night) compared with the deltamethrin WG 25 mg arm (0.51/room/night).

The blood-feeding preferences, indoor densities and human landing catches did not differ between treatments arms.

During the night after treatment, 9 out of 444 inhabitants reported an adverse effect for which no medical attention was sought as it vanished spontaneously after a few hours. No other adverse event was reported during the study.

Chiapas, Mexico

A phase III study was done in Mexico to compare the residual activity of deltamethrin SC-PE at 20 mg AI/m^2 and 25 mg AI/m^2 with deltamethrin WG at 25 mg AI/m^2 on the commonest types of indoor surfaces against malaria vectors (Rodriguez et al, 2013). The impact of these treatments on the entomological parameters influencing the vectorial capacity of wild populations was also investigated.

The study was carried out in 20 villages of the coastal plain of Chiapas, Mexico. The physical structure of the houses consisted of walls predominantly made of block, cement or brick (72.3%, n=392), palm or bamboo (15.8%, n=86) and wood (10.7%, n=58); the floor in most households was made of cement (90%) while the roof was made from zinc sheet (44%) and straw or palm (42%). Villages were randomly allocated to 3 treatment arms (SC-PE 20 mg AI/m², SC-PE 25 mg AI/m², WG 25 mg AI/m²) and to the control arm.

To assess the quality of spray application, a total of 135 filter-papers were sent to the WHO Collaborating Centre in Gembloux, Belgium for chemical analysis (Annex 3). The average of the applied to target dose ratio was 0.97 for deltamethrin SC-PE at 20 mg AI/m², 1.09 for deltamethrin SC-PE at 25 mg AI/m² and 0.54 for deltamethrin WG at 25 mg AI/m². The AI content variation between filter-papers expressed as the relative standard deviation ranged from 26.1% to 35.6% (Table 4.2) (Pigeon, 2013c).

Adult anophelines were monitored in every village using light traps and window traps (in 4 houses/village), and indoor resting collections and human landing catches during the pre- and post-spray stages. There were two collection periods during both pre- and post-spray periods. Larval surveys were done in all water bodies near to villages.

Baseline mosquito densities were evaluated in all villages in February–March 2012 using human landing catches outdoors and indoors during 3 half-nights (18:00–24:00) and 1 full night (18:00–06:00). Densities were very low, with a total of 381 anopheline females collected from 20 villages; these comprised 93% *An. albimanus* and 7% *An. vestitipennis*.

A batch of *An. albimanus* females from each locality was allowed to lay eggs and the F_1 generation was used to determine the resistance status of the populations before spraying. Susceptibility tests were done using WHO cylinders with deltamethrin 0.05% papers on about 300 F_1 mosquitoes per village. All populations showed a mortality rate <97%, 24 h after exposure and 3/20 villages showed mortality <90%.

The residual insecticidal activity of deltamethrin treatments was determined on wood walls, cement-plastered walls and cement brick walls using WHO cone bioassays (30 min exposure). Tests were done fortnightly using 1-day old *An. albimanus* mosquitoes (laboratory-susceptible strain) carried out in all selected villages.

Deltamethrin SC-PE applied at 20 mg AI/m^2 remained effective (mortality >80%) for at least 7 months on all the different sprayed surfaces. Deltamethrin SC-PE applied at 25 mg AI/m^2 was effective for 6 months on wood walls and cement-plastered walls and at least 7.5 months on cement brick. Deltamethrin WG applied at 25 mg AI/m^2 remained effective (mortality >80%) for at least 7.5 months on all the different sprayed surfaces. No significant differences in residual effect were observed between arms when compared up to 6 months. Increased mortalities were observed during the final series of bioassays in all arms, probably because houses or cone positions were changed when some villagers became reluctant to participate and when houses used for testing were changed. No significant differences were detected between the types of treated wall surface (Tables 4.2 and 4.3).

During the entire study period, low mosquito densities were recorded during human landing catches outdoors and indoors. The effect of deltamethrin treatment was observed after the intervention in the villages where mosquito abundance was previously the highest. A significant decrease in mosquito density was observed in 5 villages (1 with SC-PE 20 mg AI/m^2, 3 with SC-PE 25 mg AI/m^2 and 1 with WG 25 mg AI/m^2) when collected by human landing catches indoors and outdoors. Before spraying, the overall parity rate was 83%. After spraying, it decreased significantly in all treated villages to 44% in WG 25 AI/m^2 and SC-PE 20 AI/m^2 arms and to 39% in SC-PE 25 AI/m^2 arms.

The data obtained using light traps, window traps, indoor resting collections and larval surveys were too low or too limited to draw any conclusions.

About 30% of the 415 people interviewed in the treated villages mentioned headache and 24% had cough after spraying; overall, only 2% of those interviewed suggested that the insecticide was the main cause of the symptoms, while 98% considered that they were due to other etiologies. There was no difference in rates of reported adverse events among the different study arms.

4.4 Conclusions and recommendations

Deltamethrin 62.5 polymer-enhanced suspension concentrate (SC-PE) is an adjuvanted aqueous suspension concentrate formulation containing 62.5 g of active ingredient per litre intended for extended

residual activity on treated surfaces due to the addition of a specific polymer.

The present review assesses the efficacy of deltamethrin polymer-enhanced 62.5 suspension concentrate (SC-PE) (K-Othrine Polyzone, Bayer CropSciences, Germany) for indoor residual spraying against malaria vectors and reviews the data from the background small-scale field studies in the United Republic of Tanzania and South Africa, and WHOPES supervised small-scale studies (Viet Nam) and large-scale trials (India and Mexico).

In background small-scale studies in the United Republic of Tanzania, the efficacy and duration of residual activity were greater with deltamethrin SC-PE than with DDT 75% WP. The efficacy and residual activity of deltamethrin SC-PE against free-flying mosquitoes in experimental huts were similar to those of DDT WP, and the percentage mortality per month of both insecticides appeared correlated with mean ambient temperature. In the small-scale study in South Africa, the residual activity of deltamethrin WG was effective for less than 6 months whereas SC-PE remained effective for 12 months on sprayed surfaces in this particular trial.

In the trial in Viet Nam, the residual efficacy of the SC-PE formulation (15 mg AI/m², 20 mg AI/m², 25 mg AI/m²) was 2–3 months on brick and was similar to the WG formulation at 25 mg AI/m².

The Indian trial (9 villages) demonstrated that deltamethrin SC_PE applied at 20 mg AI/m^2 was effective for 6 months on cement walls, lime-coated cement walls and mud-plastered walls. Deltamethrin SC-PE applied at 25 mg AI/m^2 did not result in a longer or higher efficacy than 20 mg AI/m^2. Deltamethrin WG applied at 25 mg AI/m^2 was also effective for 6 months, and hence the performances of the two formulations were similar in this trial. Although significantly higher immediate mortality of *An. minimus* was found in exit traps with deltamethrin SC-PE 25 mg AI/m² compared with deltamethrin WG 25 mg AI/m², the blood-feeding preferences, indoor densities and human landing catches did not differ between all treatments arms.

The Mexico trial gave similar results: deltamethrin SC-PE applied at 20 mg AI/m^2 remained effective for at least 7 months on wood, cement brick and cement-plastered walls, and deltamethrin SC-PE applied at 25 mg AI/m^2 did not increase the residual efficacy. The treatments with deltamethrin WG were equal in efficacy to the SC-PE formulation. Parity rates decreased significantly after spraying and

mosquito density was reduced in villages where mosquito abundance was previously the highest. However, the other entomological parameters were too limited to draw any conclusion.

No significant adverse effect was reported by spraymen or inhabitants in any of the sprayed villages.

Noting the above, the meeting concluded:

- that the residual efficacy of deltamethrin WG and SC-PE applied at 25 mg AI/m^2 was similar and recommended the use of deltamethrin SC-PE for indoor residual spraying against malaria vectors at a target dose of 20–25 mg AI/m^2 with an expected residual efficacy of 6 months; and

- for quality assurance and determination of appropriate spray cycles under specific eco-epidemiologic settings, that national programmes be urged to monitor the residual activity of insecticides used for indoor residual spraying.

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

Table 4.1 Cone bioassay results in small-scale background studies (% mortality after 24 h holding).

Study	Study arm	Type of surface	Mortality [%] after months post-spraying												Efficacy [months]
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
Rowland (2010a)	DDT	Cement	**85**	45	47	58	35	18	26	16	48	25	44	-	<1
	WP 75	Mud	38	28	27	35	29	13	23	32	8	20	19	-	<1
	2 g AI/m^2	Palm thatch	44	76	52	40	39	61	17	23	26	33	40	40	<1
Moshi, United Republic of Tanzania[1]		Plywood	100	100	89	100	87	92	**80**	77	49	79	79	70	7
	Deltamethrin	Cement	100	100	97	95	94	76	90	96	94	95	**86**	-	≥11
	SC-PE	Mud	79	69	57	47	64	55	19	31	40	53	34	-	<1
	25 mg AI/m^2	Palm thatch	95	100	100	94	98	98	98	97	100	100	98	**100**	≥12
		Plywood	77	100	86	87	78	88	83	93	78	94	98	**85**	≥12
	Deltamethrin	Cement	99	100	100	100	94	80	96	90	94	**90**	78	-	10
	SC-PE	Mud	90	97	76	**85**	53	50	45	48	72	40	30	-	4
	50 mg AI/m^2	Palm thatch	96	100	98	100	98	92	96	98	100	100	100	**96**	≥12
		Plywood	91	100	91	94	91	98	81	67	100	100	98	**100**	≥12
Rowland (2010b)	DDT	Cement	98	100	78	**85**	42	49	99	42	67	34	57	-	4
	WP 75	Mud	**99**	66	22	34	11	12	35	22	38	20	38	-	1
	2 g AI/m^2	Palm thatch	100	-	-	100	100	**100**	76	-	-	-	-	45	6
Moshi, United Republic of Tanzania[1]	Deltamethrin	Cement	99	98	91	90	82	67	**100**	71	83	54	58	-	7
	SC-PE	Mud	100	**90**	49	39	62	29	22	39	41	73	28	-	2
	25 mg AI/m^2	Palm thatch	100	-	-	96	94	94	**90**	-	-	-	-	72	7

Table 4.1 contd Cone bioassay results in small-scale background studies (% mortality after 24 h holding).

Study	Study arm	Type of surface	Mortality [%] after months post-spraying												Efficacy [months]
			1	2	3	4	5	6	7	8	9	10	11	12	
Brooke et al (2013)	Deltamethrin SC-PE 25 mg AI/m^2	Cement	95	96	92	96	86	96	91	85	78	97	**97**	-	≥11
		Mud	98	97	95	96	98	98	71	85	95	94	**95**	-	≥11
Mpumalanga, South Africa [2]	Deltamethrin WG 20 mg AI/m^2	Cement	98	95	**89**	59	61	-	-	-	-		-	-	3
		Mud	99	**84**	76	76	73	80	64	31	-		-	-	2
	Lambda-cyhalothrin CS 25 mg AI/m^2	Cement	78	50	-	-	-	-	-	-	-		-	-	<1
		Mud	78	58	-	-	-	-	-	-	-		-	-	<1

[1] Laboratory-reared pyrethroid susceptible *An. arabiensis* Dondotha strain.
[2] Laboratory-reared pyrethroid susceptible *An. arabiensis* KGB strain.

Table 4.2 Chemical analysis of filter-papers collected from IRS studies with deltamethrin 62.5 g AI/L SC in WHOPES supervised studies and duration of residual activity

Study site and species	Study arm	Surface type	No. papers analysed	Applied/target dose ratio	RSD[1]	Duration of bioefficacy (months)
India *An. stephensi* susceptible to deltamethrin	Deltamethrin SC-PE 20 mg AI/m^2	Mud plaster	9	0.80	25.6%	6
		Cement	9	1.39	41.4%	6
		Lime-coated cement	6	0.88	49.1%	6
	Deltamethrin SC-PE 25 mg AI/m^2	Mud plaster	9	0.70	40.7%	6
		Cement	9	1.07	65.1%	5
		Lime-coated cement	9	0.98	43.2%	5
	Deltamethrin WG 25 mg AI/m^2	Mud plaster	9	0.65	82.6%	4
		Cement	9	1.01	46.8%	6
		Lime-coated cement	6	1.31	86.7%	6
Mexico *An. albimanus* susceptible to deltamethrin	Deltamethrin SC-PE 20 mg AI/m^2	Block/cement/brick/ palm/ bamboo/wood	45	0.97	35.6%	>7
	Deltamethrin SC-PE 25 mg AI/m^2	Block/cement/brick/ palm/bamboo/wood	45	1.09	26.1%	6
	Deltamethrin WG 25 mg AI/m^2	Block/cement/brick/ palm/bamboo/wood	45	0.54	32.4%	6

Table 4.2 contd Chemical analysis of filter-papers collected from IRS studies with deltamethrin 62.5 g AI/L SC in WHOPES supervised studies and duration of residual activity

Study site and species	Study arm	Surface type	No. papers analysed	Applied/target dose ratio	RSD[1]	Duration of bioefficacy (months)
Viet Nam *An. dirus* susceptible to deltamethrin	Deltamethrin SC-PE 15 mg AI/m^2	Wood	12	0.88	36.9%	0
		Brick	12	0.73	33.6%	3
	Deltamethrin SC-PE 20 mg AI/m^2	Wood	12	0.95	40.1%	0
		Brick	12	0.83	29.7%	2
	Deltamethrin SC-PE 25 mg AI/m^2	Wood	12	0.62	42.7%	0
		Brick	12	0.92	41.9%	2
	Deltamethrin WG 25 mg AI/m^2	Wood	12	1.63	33.1%	0
		Brick	12	1.34	37.0%	2

[1] RSD = relative standard deviation of the AI content between filter-papers.

Table 4.3 Cone bioassay results in WHOPES supervised small-scale and large-scale studies (% mortality after 24 h holding)

Study	Study arm	Type of surface	Mortality [%] after months post-spraying							Efficacy [months]
			1	2	3	4	5	6	7	
Van Roey et al (2013c) Hoa Binh, Viet Nam [1]	Deltamethrin SC-PE 15 mg AI/m^2	Brick	89	93	**92**	58	-	-	-	3
		Wood	13	15	-	-	-	-	-	<1
	Deltamethrin SC-PE 20 mg AI/m^2	Brick	75	**95**	72,5	-	-	-	-	2
		Wood	3	26	-	-	-	-	-	<1
	Deltamethrin SC-PE 25 mg AI/m^2	Brick	96	**97**	71	-	-	-	-	2
		Wood	18	55	-	-	-	-	-	<1
	Deltamethrin WG 25 mg AI/m^2	Brick	96	**94**	62	-	-	-	-	2
		Wood	18	55	-	-	-	-	-	<1
Prakash et al (2012) Asam, India [2]	Deltamethrin SC-PE 20 mg AI/m^2	Cement	100	94	94	96	85	**85**	53	6
		Lime-coated cement	100	93	85	88	73	**84**	48	6
		Mud plaster	100	92	96	91	64	**85**	57	6
	Deltamethrin SC-PE 25 mg AI/m^2	Cement	100	86	87	95	**89**	72	-	5
		Lime-coated cement	100	93	89	87	**86**	70	-	5
		Mud plaster	100	86	88	88	78	**83**	76	6
	Deltamethrin WG 25 mg AI/m^2	Cement	100	94	93	94	93	**89**	57	6
		Lime-coated cement	100	90	85	87	88	**80**	63	6
		Mud plaster	100	90	81	**82**	53	77	53	4

Table 4.3 contd Cone bioassay results in WHOPES supervised small-scale and large-scale studies (% mortality after 24 h holding)

Study	Study arm	Type of surface	Mortality [%] after months post-spraying							Efficacy [months]
			1	2	3	4	5	6	7	
Rodríguez et al (2013) Chiapas, Mexico [3]	Deltamethrin SC-PE 20 mg AI/m^2	Brick	99	100	100	99	83	85	**84**	≥7
		Cement	100	99	99	99	90	89	**90**	≥7
		Wood	98	99	99	97	87	88	**90**	≥7
	Deltamethrin SC-PE 25 mg AI/m^2	Brick	99	99	99	97	91	**86**	68	6
		Cement	100	99	98	96	92	**86**	74	6
		Wood	99	99	99	98	92	**88**	67	6
	Deltamethrin WG 25 mg AI/m^2	Brick	98	99	99	94	93	**86**	64	6
		Cement	99	99	98	94	93	**87**	59	6
		Wood	99	99	99	98	94	**87**	65	6

The residual activity of the IRS applications was measured by standard WHO plastic cones placed on treated wall surfaces. Tests were performed monthly on susceptible laboratory colonies until the end of the trial or until no further treatment mortality was observed (i.e. mortality below 80%).

[1] Laboratory-reared, insecticide-susceptible *An. dirus* s.s. NIMPE strain.

[2] Laboratory-reared, insecticide-susceptible *An. stephensi* strain.

[3] Laboratory-reared, insecticide-susceptible *An. albimanus* strain

5. REVIEW OF DURANET LN

Duranet is manufactured by Shobikaa Impex, India,[22] as an alpha-cypermethrin long-lasting (incorporated into filaments) insecticidal net (LN). Alpha-cypermethrin is incorporated into 150-denier, monofilament, high-density polyethylene fibres, with the target dose of 5.8 g AI/kg, corresponding to 261 mg of alpha-cypermethrin per square metre of the fabric (weight 45 g/m^2).

Safety assessment and WHO interim recommendations for the use of Duranet LN in malaria prevention and control were published in 2008.[23] The updated version of WHO interim specifications for alpha-cypermethrin long-lasting (incorporated into filaments) insecticidal net was published in 2011.[24] The manufacturer has confirmed to WHO that the alpha-cypermethrin technical material used in making Duranet LN complies with WHO specifications and is solely from the source supported by the WHO specifications.[25]

The present assessment includes a review of relevant background information as well as the results of WHOPES supervised large-scale studies as requirements for development of full recommendations.

5.1 Efficacy – background and supporting documents

Kisumu, Kenya

A trial of the Duranet LN comparing the PermaNet 2.0 LN (Vestergaard-Frandsen, polyester net with deltamethrin coated on the fibres at a target dose of 55 mg AI/m^2) with nets that were conventionally treated with deltamethrin at a target dose of 25 mg AI/m^2 was conducted in Kisian village just outside Kisumu town in western Kenya (Gimnig et al, 2013a). The residents of the area were primarily of the Luo ethnic group. Most residents engaged in

[22] In a letter dated 30 April 2012, Clarke Mosquito Control Products, Inc. (USA) informed WHOPES that it has sold Duranet product to Shobikaa Impex Pvt Ltd, India, effective 26 March 2012.

[23] *Report of the eleventh WHOPES Working Group Meeting, WHO/HQ, Geneva,10–13 December 2007*. Geneva, World Health Organization, 2008; available at http://www.who.int/whopes/recommendations/wgm/en/.

[24] Available at: http://www.who.int/whopes/quality/newspecif/en/.

[25] Available at: http://www.who.int/entity/whopes/quality/en/Alphacypermethrin_WHO_specs_eval_Jan_2013.pdf.

subsistence farming although some were employed in Kisumu town. Most houses were constructed of sticks and mud with a corrugated metal roof. However, some houses had grass thatch roofs or cement walls. The village had participated in a similar study previously.

Nets were distributed in July 2007. A total of 60 nets of each type were distributed. The nets were randomly assigned to sleeping spaces so that if more than one sleeping space was in a single house, it was possible that the household received two different types of nets. After distribution, study participants were visited monthly and asked questions about net use and washing practices. The nets were followed at 4–5-month intervals, at which time bioassays were conducted on the nets in the field. Bioassays were conducted by fixing three plastic WHO cones to the top and two sides of the net. A total of 10 mosquitoes were then introduced into each cone and exposed for 3 min. The mosquito strain used was *An. gambiae*, pink-eye. The strain was susceptible to deltamethrin in WHO resistance assays (98% mortality in WHO cone assays), but molecular tests conducted after the start of the study showed the 1014S *kdr* allele to be nearly fixed in the colony.

Nets that had bioassay mortality of <50% on two consecutive bioassays were considered to have failed. These nets were removed from the study and the study participants were provided with new nets. Differences in the duration of effectiveness were estimated in a proportional hazards survival model. Nets were followed until they were considered to have failed or until the end of the study in January 2012.

During 4.5 years of follow-up, 22 Duranet LNs, 52 PermaNet 2.0 LNs and 58 conventionally treated nets had failed. The median time to failure was 562 days (1.5 years) for a conventionally-treated net, 919 days (2.5 years) for a PermaNet 2.0 LN and 1519 days (4.2 years) for a Duranet LN. In a survival analysis, the risk of failure was significantly lower for a PermaNet 2.0 LN relative to the conventionally-treated net (HR=0.132, $P<0.001$). The risk of failure of a Duranet LN was also significantly lower than that of a conventionally-treated net (HR=0.040, $P<0.001$) and it was significantly lower than that of a PermaNet 2.0 LN (HR=0.302, $P<0.001$). However, the results should be interpreted with caution as the Duranet LN was used less frequently than a conventionally-treated net or a PermaNet 2.0 LN as reported in monthly follow-up visits. Net use the previous night as reported during the monthly visits was significantly lower for the Duranet LN (60.4%) compared

with a conventionally-treated net (78.3%). However, the reported usage rates of the Duranet LN were not significantly different from those of the PermaNet 2.0 LN (64.9%). The Duranet LNs were also washed less frequently than either the PermaNet 2.0 or the conventionally-treated net, although these differences were not statistically significant.

Report rates of side effects during the first 6 months of the study were generally low. Among adults interviewed, 5.2% of Duranet LN users reported symptoms that might be associated with net use compared with 5.0% of PermaNet 2.0 LN users and 1.5% of users of conventionally-treated nets. The symptoms reported included rashes, burning/itching skin, runny nose, sneezing, coughing and headaches.

Although the study did not follow WHOPES-recommended guidelines,[26] the authors concluded that the Duranet LN meets or exceeds the phase III requirements for an LN. However, they also noted that baseline chemical analyses were not available to confirm that the nets were within specifications, and recommended that the results be interpreted with this caveat.

5.2 Efficacy – WHOPES supervised trials

Rourkela, India

A study to compare Duranet LN with a conventionally-treated net was conducted in the Sundargarh District of Orissa State in India (Sharma et al, 2013). The study area was characterized by undulating forested hills bisected by rocky streams. Residents of the area subsist on forest produce and farming, including rice farming. The climate of the area is tropical, with mean annual temperatures ranging from 22 °C to 27 °C and annual rainfall averaging 160–200 cm per year. Most rainfall occurs during the two monsoon seasons from June to September and from December to January. Malaria transmission in this area is perennial with the main vectors *An. culicifacies* and *An. fluviatilis*.

The study was conducted in 5 hamlets in the Bisra block of Sundargarh District. Each village was enumerated with a list of all

[26] *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets.* Geneva, World Health Organization, 2005 (available at: http://whqlibdoc.who.int/hq/2005/WHO_CDS_WHOPES_GCDPP_2005.11.pdf).

households, including their exact geo-location and the name, age, sex and education of all residents. A baseline survey was carried out in the selected villages to determine the socioeconomic status of the study participants, current use and perceptions of nets, and the number of sleeping spaces.

A total of 440 households were enrolled in the study. Before distribution, the households were randomly assigned to receive either Duranet LNs or polyester nets (75 denier) that were conventionally-treated with alphacypermethrin at target dose of 40 mg AI/m^2. Some 300 households were allocated to receive Duranet LNs and 140 were allocated to receive conventionally-treated nets. A total of 874 nets (734 Duranet LNs and 140 ITNs) were distributed among the 440 households. However, only 300 Duranet LNs and 140 ITNs were marked and eligible for follow-up.

At 6-month intervals, 30 nets were removed from each arm of the study and replaced with new nets. After 1 year, the ITN arm was dropped from the study. At the final follow-up (36 months post-distribution), 50 nets were sampled. Annual surveys of all households were conducted at 12, 24 and 36 months to assess physical presence/absence of the study nets as well as their fabric integrity. At 1, 6, 18 and 30 months after distribution, houses selected for net sampling were interviewed to determine the net utilization patterns (use and washing frequency), user perceptions of adverse or beneficial effects and the physical integrity of the nets.

Holes were measured and categorized as size 1 (smaller than a thumb), size 2 (larger than a thumb but smaller than a fist) and size 3 (larger than a fist). The proportionate hole index (pHI) was estimated with weights of 1, 23 and 196 for holes of size 1, 2 and 3 as recommended by WHOPES. [27]

Bioassays were done at baseline and every 6 months for up to 3 years for the Duranet LN. For the ITN arm, bioassays were done at baseline, 6 months and 12 months. For bioassays, 10 pieces of netting (30 cm x 30 cm) were cut from each sampled net at five different positions (Annex 4) as recommended by WHOPES . Five pieces were used for bioassays and five were retained for chemical

[27] *Guidelines for laboratory and field-testing of long-lasting insecticidal nets.* Geneva, World Health Organization, 2013 (available at http://www.who.int/iris/bitstream/10665/80270/1/9789241505277_eng.pdf).

assays. Standard cone bioassays were conducted on each sample of netting. The netting material was fixed between two pieces of acrylic sheets. One sheet had 4 holes cut in it to hold plastic WHO cones in place on the netting. Five female *An. culicifacies* mosquitoes (2–5-days old) from a laboratory-reared strain of known susceptibility to pyrethroids were introduced into each cone and exposed for 3 min. After exposure, mosquitoes were placed in plastic cups and provided with cotton-wool moistened with 10% glucose solution. Knockdown was recorded at 60 min and mortality at 24 h after exposure. Control bioassays were conducted with mosquitoes exposed to untreated netting. If control mortality was 5–20% on a given day, the results for that day were adjusted using Abbott's formula. If control mortality was >20%, bioassays for that day were repeated. Bioassays were conducted at 25 $\pm$ 2 °C and 75 $\pm$ 10% RH.

Nets with a knockdown rate of <95% and a bioassay mortality of <80% were subjected to tunnel tests as described by WHOPES guidelines using a rabbit as the bait. A total of 100 mosquitoes were introduced into the longer section of the tunnel at 18:00 and mortality and blood-feeding were measured at 09:00 hours the following morning. Mortality was measured by pooling the mortality rates of mosquitoes from all sections of the tunnel. Blood-feeding inhibition was measured by comparing the proportion of blood-fed mosquitoes (alive or dead) in treated and control tunnels.

At baseline, 30 Duranet LNs and 30 conventionally-treated nets were randomly selected for chemical analysis. At 12 months, 30 conventionally-treated nets and at 36 months, 50 Duranet LNs were randomly selected for chemical analysis. For chemical analysis, four 30 cm x 30 cm pieces of netting (Annex 4, excluding position 1) were wrapped in aluminium foil and sent to the Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium (WHO Collaborating Centre for Quality Control of Pesticides). Each combined net sample was cut into 5–10 mm squares, homogenized and an analytical portion was weighed for determination of alpha-cypermethrin using the CIPAC method 454/LN/M/3.2. Alpha-cypermethrin was extracted by heating under reflux for 30 min in xylene with dioctyl-phthalate as an internal standard. The alpha-cypermethrin content was measured by gas chromatography with a flame ionization detector (GC-FID) (Pigeon, 2009; 2011c; 2013d) using all samples collected. The average target dose and tolerance limit for the baseline and average content for the subsequent samplings were determined.

After 36 months, 4 nets were reported to have been lost to the study and attrition was estimated at 0.46% for the entire 3 years. Net use was highest 6 months after distribution, with 93.4% of respondents reporting using nets year-round and every night. Thereafter, the percentage of respondents reporting using nets year round and every night ranged between 53.3% and 78.0%. During the follow-up surveys, 17% of households reported washing nets once per year and 60.0% reported washing them twice per year. The remaining households (23.4%) reported washing their nets 3 or more times per year.

After 6 months of use, 40% of the conventionally-treated nets had at least 1 hole, while 20% of the Duranet LNs had at least 1 hole. At 1 year, 56.7% of the conventionally-treated nets and 26.7% of the Duranet LNs had at least 1 hole. The mean pHI for the Duranet LN was 2.4 at 6 months and rose to a maximum of 138.0 at 30 months. At 36 months, the mean pHI was 92.9. The median pHI was 0 up to 24 months. At 36 months, the median pHI was 212 (Table 5.1).

The baseline average alpha-cypermethrin content was 1.23 g AI/kg (34.6 mg AI/m^2) for the conventionally-treated nets and 5.12 g AI/kg (249.3 mg AI/m^2) for the Duranet LN. All the Duranet LNs were within the specification of 5.8 g AI/kg $\pm$ 25% (range: 4.35–7.25 g AI/kg) (Figure 5.1). After 1 year, the average alpha-cypermethrin content of the conventionally-treated nets had dropped to 0.32 g AI/kg (8.6 mg AI/m^2), a loss of 74% of the initial chemical content. After 3 years, the average alpha-cypermethrin content of the Duranet LN was 2.69 g AI/kg (158.7 mg AI/m^2), a loss of 47% of the initial chemical content (Table 5.3) (Pigeon, 2009; 2011c; 2013d).

Cone bioassays with *An. culicifacies* against the Duranet LN and the conventionally-treated nets indicated that both met the WHO criteria at baseline. By 6 months, mean knockdown was 66.2% and mean mortality was 61.6% for the conventionally-treated nets. For the Duranet LNs, mean knockdown remained above 90% for all rounds except the 24-month follow-up when knockdown was 85.1% and the 36-month follow-up when mean knockdown was 73.0%. Mortality was above 85% for all rounds. Over the course of the 3-year study, 18 Duranet LNs did not meet the WHO criteria based on the cone test and were further subjected to the tunnel test. There were 4 nets that did not meet the WHO criteria at 18 months, 1 at 24 months and 13 at 36 months. However, all 18 nets met the WHO criteria based upon the tunnel test (Table 5.2).

During the 1-month follow-up survey, 64.2% of Duranet LN users and 49.1% of the conventionally-treated net users reported transient side-effects. The main complaints included skin irritation, itching, suffocation and rashes on the body. However, the effects were transitory and only noted on the first usage.

The authors concluded that the Duranet LN was well accepted and, after regular use by the community, maintained its biological efficacy for 3 years based on the WHO cone test and the tunnel test.

Ada-Foah, Ghana

A study to compare the Duranet LN with conventionally-treated nets was carried out in five communities in the Ada-Foah area of eastern Ghana (Boakye et al, 2013a). The villages were mapped and out of 1071 households enumerated, 440 were selected for the study. Of these, 300 households were randomly allocated to receive Duranet LNs while 140 were randomly allocated to receive conventionally-treated nets. The conventional nets were 75-denier polyester nets treated with alphacypermethrin by study staff at a target dose of 40 mg AI/m^2.

At 1 week after distribution, 30 Duranet LNs and 30 conventionally-treated nets were randomly selected for chemical analysis. At 36 months, 50 Duranet LNs were randomly selected for chemical analysis. Since the alpha-cypermethrin content of the conventionally-treated nets was very low at baseline, further chemical analyses were not done. Four pieces of netting (30 cm x 30 cm) were cut from each net at positions 2–5 according to WHOPES guidelines (Annex 4). The four subsamples were wrapped together in aluminum foil and submitted to the Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium for chemical analysis as described above. The average target dose and tolerance limit for the baseline and average content for the subsequent samplings were determined.

At 1 month after distribution and every 6 months thereafter, 30 households were randomly selected for interview. An adult participant in the selected households was interviewed on net utilization practices, and washing frequency and methods. The physical presence of coded nets was recorded and, where nets were no longer present, the reason for their loss was noted. The frequency of adverse effects from using the nets was assessed at 1 week, 1 month, 6 months and 12 months after distribution.

Fabric integrity was examined in 30 nets per arm at 6-month intervals up until 24 months after distribution. At 30 and 36 months after distribution, 100 Duranet LNs were sampled. Sampled nets were draped over a frame and holes counted and categorized according to size (size 1 = smaller than a thumb, size 2 = larger than a thumb but smaller than a fist, size 3 = larger than a fist but smaller than a head, size 4 = larger than a head) and location (top, upper sides, lower sides). The proportionate hole index (pHI) was calculated according to WHOPES guidelines by applying weights of 1, 23, 196 and 576 to size 1, size 2, size 3 and size 4 holes, respectively.

Cone bioassays were conducted on net samples (25 cm x 25 cm) that were cut from sampled nets at 6-month intervals after distribution. Some 30 nets were tested at each time-point except for the 30- and 36-month follow-ups when 50 nets were tested. Four samples were used for each net from positions 2–5 according to WHOPES recommended guidelines. Five unfed female mosquitoes, 2–5-days old from a susceptible colony (*An. gambiae s.s*., Kisumu strain) were introduced into the cones and exposed for 3 min, after which mosquitoes were removed and placed in paper cups with access to sugar solution. The test was replicated so that 10 mosquitoes were used for each net sample. Knockdown was measured at 60 min after exposure and mortality was measured at 24 h after exposure.

For nets that failed to meet the WHOPES criteria for the cone test (mortality >80% or knockdown >95%), the tunnel test was performed. The test was done in a 60 cm tunnel (25 cm wide by 25 cm high) made of clear plastic and divided into thirds and covered on each end with netting. A 20 cm x 20 cm piece of netting was placed between two pieces of cardboard and placed at 20 cm from one end of the tunnel. Nine holes 1 cm in diameter were cut in the netting material. A guinea-pig was restrained in the short end of the tunnel and 100 mosquitoes were introduced at the opposite end of the tunnel. The experiment was done in the evening and at the end of the test, the mosquitoes were scored according to whether they passed through the netting, whether they successfully blood-fed and whether they survived the exposure period.

After 12 months, the overall attrition rates for coded nets were 40% for the conventionally-treated nets and 20% for the Duranet LNs. Attrition of the non-coded nets was higher, suggesting study participants were more likely to retain nets that they knew were being followed. The overall survivorship rate was 70% at 18 months and

73.3% at 24 months. A total of 83% of nets were recoded as present at 36 months.

The percentage of nets with at least one hole was 13.3% at 6 months after distribution. This figure increased to 33.3% at 24 months after distribution. At 30 months after distribution, 67.0% of nets had at least one hole. During the final survey at 36 months after distribution, 86.3% of nets had at least one hole. The average number of holes on the Duranet LN was 1.6 during the 6-month follow-up. The average number of holes per net rose to 6.2 by the final survey, at 36 months after distribution. The mean pHI for the Duranet LN was 27.0 after 6 months of use. By 24 months of use, the mean pHI had risen to 175.8 but fell to 83.2 and 87.1 at 30 and 36 months, respectively. The median pHI was 0 up to 30 months. At 36 months, the median pHI was 9 (Table 5.1).

In cone bioassays, the conventionally-treated nets performed poorly at baseline when only half of the nets met the WHOPES criteria. The combined pass rate with both the cone test and the tunnel test was 53.3%. At 6 and 12 months post-distribution, only 36.7% and 27.7% of conventionally-treated nets met the criteria of either the cone test or the tunnel test. For the Duranet LN, all nets met the WHOPES criteria for the cone test until 1 year of follow-up. However, at 18 and 24 months, the pass rate fell to 73.3% and 53.3% respectively. With the tunnel test, the combined pass rate was 76.7% for both the 18 and 24 month follow-ups. As the study was conducted in a coastal community, it was hypothesized that many people used their nets outside on the beach where they became very dirty and were washed more frequently. It was also hypothesized that the nets may have been washed with sea water. The community was educated on the need to avoid using the nets at the beach and the combined pass rate at the 30 and 36 month follow-ups was 84.0% and 82.0%, respectively (Table 5.2).

The average alpha-cypermethrin content of the conventionally-treated nets was 0.31 g AI/kg (9.4 mg AI/m^2) at 1 week after distribution, which was significantly below the target dose of 40 mg AI/m^2. Further chemical analysis of the conventionally treated nets at year 1 was not done. The average alpha-cypermethrin content of the Duranet LN was 4.71 g AI/kg (247.8 mg AI/m^2) at 1 week after distribution and was within the specifications tolerance limits (4.35–7.25 g AI/kg). Of the 32 samples tested, 27 were within the specification of 5.8 g AI/kg $\pm$ 25%. Five Duranet samples were below the lower specification limit (Figure 5.1) After 3 years, the average alpha-cypermethrin

content of the Duranet LN was 1.52 g AI/kg (97.9 mg AI/m^2), a loss of 68% of the initial chemical content (Table 5.3) (Pigeon, 2010a; 2011d; 2013e).

In surveys conducted 1, 6 and 12 months after distribution, 83.0% to 88.9% of respondents reported using the Duranet LN every night. During the same surveys, 65.3% to 86.3% of respondents reported using the conventionally treated nets every night. From 18 to 36 months after distribution, reported net use ranged from 81.3% to 90.3%. After 1 month of net use, 26.3% of respondents reported washing their nets at least once. After the 24-month survey, at least half of respondents reported washing their nets at least once per month. Most respondents reported using local or commercial bar soaps to wash their nets.

The authors concluded that the physical integrity and community acceptance of the Duranet LN was good. The Duranet LN fell below WHOPES criteria for the bio-efficacy at 18 and 24 months, but this was considered to be an effect of excessive washing as the nets were used outdoors where they became very dirty. After a community education campaign, the bioefficacy rose to above WHOPES thresholds for LNs at 30 and 36 months. The Duranet LN was therefore considered to have met the criteria for an LN after 36 months of use.

5.3 Conclusions and recommendations

Duranet LN is a long-lasting insecticidal mosquito net manufactured by Shobikaa Impex Pvt., Ltd. The net is treated with alpha-cypermethrin incorporated into monofilament polyethylene fibers (150 denier) at a target dose of 5.8 g AI/kg corresponding to 261 mg AI/m^2 of netting.

WHOPES published interim recommendations for the Duranet LN in 2008 based on phase I laboratory testing and phase II experimental hut studies. Full WHO recommendations require further evidence of the efficacy, durability and acceptability of the Duranet LN under routine household use over a period of 3 years. Longitudinal, randomized household trials to evaluate its efficacy, longevity and fabric integrity over a period of 3 years are required as part of phase III testing. WHOPES guidelines recommend that after 3 years of routine household use, at least 80% of nets tested meet the cut-off

criteria for either the WHO cone bioassay test or the tunnel test. However, criteria for fabric integrity have yet to be established.

This report reviews the data from phase III testing of the Duranet LN according to WHOPES guidelines in two countries.

In two WHOPES supervised trials, the loss of physical integrity was measured by the percentage of nets with holes and the proportionate hole index (pHI). Among the two sites, the percentage of Duranet LNs with at least one hole after 6 months of use ranged from 13.3% to 20%. After 36 months of use, that percentage ranged between 74.0% and 86.3%. The mean pHI ranged between 2.3 and 27.0 after 6 months of use, and between 87.1 and 92.9 after 36 months of use. The median pHI was 0 up to 24 months in Ghana and up to 30 months in India. At 36 months, the median pHI was 212 in India and 9 in Ghana.

The alpha-cypermethrin content declined over the 3 years of use in each of the two WHOPES supervised trials. The average loss of insecticide ranged from 47% to 68% over the course of 36 months. After 18 and 24 months of use in Ghana, the percentage of nets that met WHOPES criteria by either the cone test or the tunnel test was 76.7%. However, this was thought to be due to outdoor use and excessive washing. At 30 and 36 months, 84.0% and 82.0% of nets met the WHOPES criteria. In India, 100% of nets met the WHOPES criteria for either the cone test or the tunnel test through 36 months of use.

Noting the above, the meeting recommended:

- that based on WHOPES LN guidelines, which are largely based on efficacy criteria, and noting the overall bio-efficacy of the Duranet LN, full recommendation be granted;

The meeting also recommended:

- that national programmes monitor and evaluate the performance of LNs, including the Duranet LN, under local conditions following procedures recommended in WHO guidelines to select the most suitable LN for their local setting.

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

Table 5.1 Physical integrity of Duranet LNs over time in two study sites. For each site, the number of nets examined (N), the percentage with any holes, the mean proportionate hole index (pHI) (standard deviation in bracket) and the median pHI (interquartile range in bracket) are presented.

		Months after distribution					
		6	**12**	**18**	**24**	**30**	**36**
India	N	30	30	30	30	30	50
	% with any holes	20.0	26.7	36.7	43.3	46.7	74.0
	Mean pHI	2.4 (7.1)	28.9 (67.8)	81.6 (160.6)	29.6 (71.7)	138.0 (259.2)	92.9 (148.4)
	Median pHI	0 (0–0)	0 (0–1)	0 (0–0)	0 (0–2)	0 (0–249)	212 (0–196)
Ghana	N	30	30	30	30	100	100
	% with any holes	13.3	23.0	20.0	33.3	67.0	86.3
	Mean pHI	27.0 (108.0)	36.1 (93.3)	102.0 (227.9)	175.8 (329.6)	83.2 (157.9)	87.1 (167.4)
	Median pHI	0 (0–5)	0 (0–0)	0 (0–0)	0 (0–143)	4 (0–75)	9 (0–75)

Table 5.2 Number and percentage of Duranet LNs meeting WHOPES criteria according to the cone test or the tunnel test in two study sites (for each site, the total number (N), the number passing the cone test (Cone) and the number passing the tunnel test (Tunnel) are presented; the percentage passing according to either the cone test or the tunnel test are presented in the bottom row for each site (%)).

Site		**Months after distribution**					
		6	**12**	**18**	**24**	**30**	**36**
India	N	30	30	30	30	50	50
	Cone	30	30	26	29	30	37
	Tunnel	–	–	4	1	–	13
	%	**100**	**100**	**100**	**100**	**100**	**100**
Ghana	N	30	30	30	30	50	50
	Cone	30	30	22	16	38	35
	Tunnel	–	–	1	7	4	6
	%	**100**	**100**	**76.7**	**76.7**	**84.0**	**82.0**

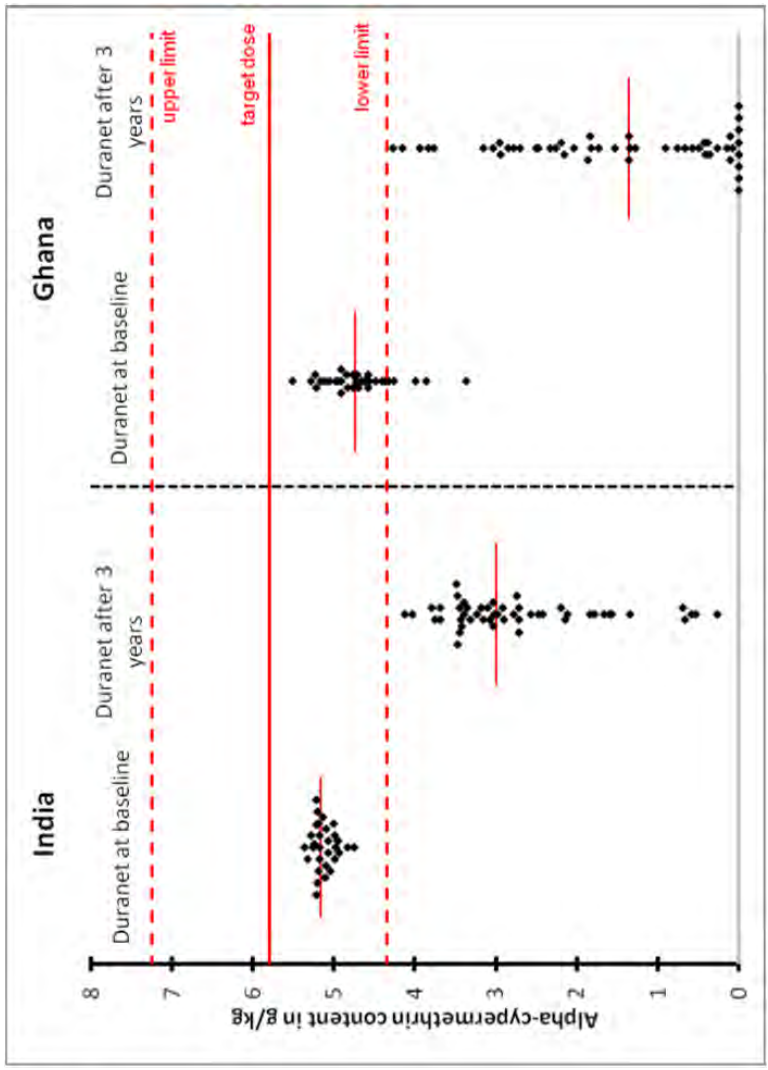

Figure 5.1 Alpha-cypermethrin content (in g AI/kg) on individual Duranet samples at baseline and after 3 years of household use in India and Ghana. The target dose (5.8 g AI/kg) and the upper (7.25 g AI/kg) and lower (4.35 g AI/kg) limits are for alpha-cypermethrin content at baseline, indicated as solid and dashed lines respectively.

Table 5.3 Mean (95% confidence limits) alpha-cypermethrin content in g AI/kg in Duranet and percentage AI lost over time in the two study sites.

Site	Test	Months after distribution	
		0	**36**
India	N	30	50
	Mean	5.12 (5.07–5.17)	2.69 (2.41–2.97)
	% lost	-	47%
Ghana	N	32	50
	Mean	4.71 (4.55–4.87)	1.52 (1.11–1.93)
	% lost	–	68%

6. REVIEW OF NETPROTECT LN

Netprotect is manufactured by Bestnet A/S, Denmark, as a deltamethrin long-lasting (incorporated into polyethylene) insecticidal net (LN). Deltamethrin is incorporated into 118-denier, monofilament polyethylene fibres of different densities, with the target dose of 1.8 g AI/kg, corresponding to 68.4 mg of deltamethrin per square metre of the fabric (weight 38 g/m2).

WHO has been advised that as from 21 October 2005, the owner of Netprotect, Intelligent Insect Control, has licensed the manufacture and commercialization of Netprotect to Bestnet Europe Ltd and later Bestnet A/S.

Safety assessment and WHO interim recommendations for the use of Netprotect LN in malaria prevention and control were published in 2008. [28] WHO interim specifications for deltamethrin long-lasting (incorporated into polyethylene) insecticidal net were published in 2010. [29] The manufacturer has confirmed to WHO that the deltamethrin technical material used in making Netprotect LN complies with WHO specifications and is solely from the source supported by the WHO specifications.[30]

The present assessment includes a review of relevant background information as well as the results of WHOPES supervised large-scale studies as requirements for development of full recommendations.

6.1 Efficacy – background and supporting documents

Kanyaboli, Kenya

A field study of the Netprotect LN was conducted in western Kenya in a rice-growing irrigation area near Yala swamp (Odhiambo et al, 2013). Annual rainfall in the area averages 1400 mm, with peaks of rainfall occurring in March to April and November to December. Most residents live in family compounds consisting of one or more house

[28] *Report of the eleventh WHOPES Working Group Meeting, WHO/HQ, Geneva,10–13 December 2007*. Geneva, World Health Organization, 2008; available at: http://www.who.int/whopes/recommendations/wgm/en/.
[29] Available at: http://www.who.int/whopes/quality/newspecif/en/.
[30] Available at:http://www.who.int/entity/whopes/quality/Deltamethrin_eval_specs_WHO_November_2012.pdf.

structures separated by farmland. Most houses have mud walls and thatched roofs with open eaves that allow the unimpeded entry and exit of mosquitoes. Other than rice farming, most people practice subsistence agriculture with maize, millet and cassava as the staple crops.

Two villages that were 2 km apart were selected for the study. A random sample of 150 houses in 150 compounds was selected in each of two villages; and in December 2007, Netprotect LNs were distributed to cover all sleeping spaces in the selected households in one village while untreated nets were distributed to the other village. After 6 months, Netprotect LNs were distributed to the control village.

Each month, 10 houses were randomly selected in each village for pyrethrum spray catches. Mosquitoes were retained for species identification by polymerase chain reaction (PCR) and for blood-meal analysis by enzyme-linked immunosorbent assay (ELISA). Malaria cases were detected passively at the village clinic that had been established by a commercial rice farm. Study participants seeking treatment were identified and reported by the clinic personnel. Malaria was identified by a rapid diagnostic test (RDT, ParaCheck PF test kits). Participants who were positive by the RDT were treated according to Kenyan national guidelines. In accordance with Kenya national guidelines, however, all children aged under 5 years who were clinically diagnosed with malaria were treated regardless of the RDT result.

The residual activity of Netprotect LNs was determined using cone bioassays on 10 randomly selected nets hanging in the field at 1-month intervals beginning 3 months after distribution. The bioassays continued for 6 months. A bioassay cone was attached to the one side of the net and 10 mosquitoes from a susceptible strain (*An. gambiae* Kisumu strain) were exposed for 3 min. This was repeated 10 times so that a total of 100 mosquitoes were exposed to each net. After exposure, the mosquitoes were held in paper cups with access to 10% sucrose solution. Knockdown was recorded at 60 min and mortality at 24 h.

After 18, 28 and 38 months of use, 34–38 nets were collected from the field and returned to the laboratory for further examination. Nets were draped over a frame and holes counted and categorized as <1 cm, 1–2 cm, 3–5 cm, 5–10 cm or >10 cm. These were subsequently recategorized according to WHOPES guidelines and the proportionate hole index (pHI) estimated. Five samples were taken

from each net and cone bioassays were conducted on two randomly selected samples. For the 18-month follow-up, the samples were tested against *An. cracens* (formerly *An. dirus* B). A total of 50 mosquitoes were tested against each sample. For samples collected at 38 months, 10 nets were randomly selected and 30 cm x 30 cm pieces were cut from the nets. Cone bioassays with 10–14 female *An. gambiae* Kisumu strain were conducted as described above.

Chemical content analysis was done on nets collected at baseline and after 9, 24 and 36 months. Five pieces were taken from each net, combined, cut into small pieces and a subsample was drawn for extraction in xylene under reflux. For nets collected at 9 and 24 months, deltamethrin was quantified by high performance liquid chromatography with a UV detector (HPLC-UV) after exchange of xylene to the mobile phase. This analytical method is the method recommended by CIPAC. For nets collected at 36 months, deltamethrin was quantified by gas chromatography with a flame ionization detector (CG-FID). The analytical method used for baseline samples was not reported.

A total of 807 indoor resting *Anopheles* mosquitoes were collected during the first 6 months of the study. Of these, 82.5% were collected from the control houses. *An. funestus* was the most common mosquito accounting for 69.9% of the anophelines collected. The remaining anopheline mosquitoes were all identified as *An. arabiensis*. Malaria incidence was also lower in the intervention households. A total of 1350 people lived in intervention compounds. Between January and July 2007, 220 were clinically diagnosed with malaria and 67 were confirmed by RDT. A total of 1234 people lived in the control compounds and between January and July 2007, 670 were clinically diagnosed with malaria while 277 were confirmed by RDT. The incidence of clinically-diagnosed malaria in the control areas was estimated at 41.3% while that in the intervention area was estimated at 16.3%. Malaria incidence peaked in April with 88 cases in the control areas. However, the peak was not detectable in the intervention area. After Netprotect LNs were distributed to the control village, the peak was not detectable in the following years in either area.

Net damage was observed in 34–38 nets collected at 18, 28 and 38 months after distribution. The percentage of nets with any holes at each time point ranged between 82.3% and 85.3%. The total number of holes observed was 115 (3.0 per net) at 18 months, 99 (2.9 per net) at 28 months and 409 (12.0 per net) at 38 months. The pHI was 333

at 18 months. This figure fell to 114 at 28 months and rose to 381 at 38 months.

In cone bioassays conducted in the field, knockdown ranged between 80% and 100% from 2 to 8 months after distribution. Mortality was 100% until 6 months after distribution when it fell to 80%. Average knockdown on two subsamples of nets collected from the field after 18 months and tested against *An. cracens* was 85.7%, while average mortality was 69.5%. Only 28 of 42 nets (66.7%) passed based on the WHOPES criteria. In analysis of variance, it was found that the position of the net sample was not statistically significant, but nets that were classified as "very dirty" had significantly lower mortality. When three nets classified as very dirty were washed and tested again, mortality declined to near zero for two of the nets but rose from 10% to 96% on the third. Furthermore, the authors noted that mortality of *An. cracens* in cone bioassays is often lower than that of *An. gambiae* Kisumu strain. In cone bioassays against 10 nets with *An. gambiae* (Kisumu strain) after 38 months of use, the mortality was >80% on all but one net. However, knockdown was 100% on that net, indicating that it also met the WHOPES criteria for the cone bioassay.

Baseline chemical analysis indicated that the nets (n = 4) had an average deltamethrin content of 1.9 g AI/kg, which was within the specifications for the Netprotect LN. Nevertheless, the *R*-alpha isomer was not reported and it is therefore possible that this value represents the sum of deltamethrin plus the *R*-alpha isomer. For nets sampled at 9 months (n = 5), the average deltamethrin content was 1.17 g AI/kg plus 0.21 g AI/kg of *R*-alpha isomer. By 22 months (n = 22), the average deltamethrin content was 0.73 g AI/kg plus 0.30 g AI/kg of *R*-alpha isomer. By 36 months (n = 31), the average deltamethrin content was 0.49 g AI/kg plus 0.48 g AI/kg of *R*-alpha isomer.

The authors concluded that the Netprotect LN reduced mosquitoes in houses and malaria incidence. Cone bioassays with *An. cracens* indicated a large number of net failures after 18 months of use, although the authors reported that nets underperform when tested against this species compared with *An. gambiae* s.s. Cone bioassays with a susceptible strain of *An. gambiae* showed all nets (n=10) to meet WHOPES efficacy criteria after 38 months of use in the field.

Kisumu, Kenya

A trial to compare the Netprotect LN with the PermaNet 2.0 LN (Vestergaard-Frandsen, polyester net with deltamethrin coated on the fibres at a target dose of 55 mg AI/m^2) and nets that were conventionally treated with deltamethrin at a target dose of 25 mg AI/m^2 was conducted in Kisian village just outside Kisumu town in western Kenya (Gimnig et al, 2013b). The residents of the area were primarily of the Luo ethnic group. Most residents engaged in subsistence farming, although some were employed in Kisumu town. Most houses were constructed of sticks and mud with a corrugated metal roof. However, some houses had grass thatch roofs or cement walls. The village had participated in a similar study previously.

Nets were distributed in July 2007. A total of 60 nets of each type were distributed. The nets were randomly assigned to sleeping spaces so that if more than one sleeping space was in a single house, it was possible that the household received two different types of nets. After distribution, study participants were visited monthly and asked questions about net use and washing practices. The nets were followed at 4–5 month intervals, at which time bioassays were conducted on the nets in the field. Bioassays were conducted by fixing three plastic WHO cones to the top and two sides of the net. A total of 10 mosquitoes were then introduced into each cone and exposed for 3 min. The mosquito strain used was *An. gambiae*, pink-eye. The strain was fully susceptible to deltamethrin in WHO resistance assays, but molecular tests conducted after the start of the study showed the 1014S *kdr* allele to be nearly fixed in the colony.

Nets that had bioassay mortality of <50% on two consecutive bioassays were considered to have failed. These nets were removed from the study and the study participants were provided with new nets. Differences in the duration of effectiveness were estimated in a proportional hazards survival model. Nets were followed until they were considered to have failed or until the end of the study in January 2012.

During 4.5 years of follow-up, 42 Netprotect LNs, 52 PermaNet 2.0 LNs and 58 conventionally treated nets had failed. The median time to failure was 562 days (1.5 years) for the conventionally-treated net, 919 days (2.5 years) for the PermaNet 2.0 LN and 923 days (2.5 years) for the Netprotect LN. In a survival analysis, the risk of failure was significantly lower for a PermaNet 2.0 LN relative to the conventionally-treated net (HR=0.144, P<0.001). The risk of failure of a Netprotect LN was also significantly lower than that of a

conventionally treated net (HR=0.125, *P*<0.001). Net use the previous night as reported during the monthly visits was lower for the Netprotect LN (71.1%) compared with a conventionally-treated net (78.3%) but higher than a PermaNet 2.0 LN (64.9%). However, the reported usage rates of the Netprotect LN were not significantly different from those of either the PermaNet 2.0 LN (*P*=0.302) or the conventionally treated net (*P*=0.068). The Netprotect LNs were also washed less frequently than either the PermaNet 2.0 or the conventionally-treated net, although these differences were not statistically significant.

Report rates of adverse effects during the first 6 months of the study were generally low. Among adults interviewed, 2.3% of Netprotect LN users reported any symptoms that might be associated with net use compared with 5.0% of PermaNet 2.0 LN users and 1.5% of users of conventionally-treated nets. The symptoms reported included rashes, burning/itching skin, runny nose, sneezing, coughing and headaches.

Although the study did not follow WHOPES-recommended guidelines and the median time to failure for the Netprotect was <3 years according to criteria used in the study, the Netprotect LN performed as well as or better than the PermaNet 2.0 LN, which has a full WHOPES recommendation. The authors therefore concluded that the Netprotect LN should also be considered to meet the phase III requirements for an LN. However, they also noted that baseline chemical analyses were not available to confirm that the nets were within specifications and recommended that the results be interpreted with this caveat.

6.2 Efficacy – WHOPES supervised trials

Veal Veng District, Cambodia

A trial to compare the Netprotect LN with a conventionally-treated net (75 denier polyester nets treated with deltamethrin by study staff at a target dose of 25 mg AI/m^2) and the PermaNet 2.0 LN (100 denier polyester LN with deltamethrin coated on the fibers at a target dose of

55 mg AI/m^2)[31] was conducted in 7 villages in Veal Veng District, Cambodia (Van Roey et al, 2013d). The villages were enumerated and all households were randomly selected to receive one of the three net types. A total of 762 households were enrolled in the study. Of these, 148 received conventionally-treated nets, 305 received Netprotect LNs and 309 received PermaNet 2.0 LNs.

Eight surveys were conducted after the distribution of nets. At 1 week and 1 month after distribution, 30 households were visited per arm to assess net perceptions and adverse events. At week 1 and every 6 months thereafter, 30 nets were sampled per study arm to assess physical integrity and bioefficacy of the nets. At 1 week and 6, 24 and 36 months after distribution, chemical analysis was performed to assess deltamethrin content on the nets.

Net survivorship/attrition was determined during each survey. In addition, at 12, 24 and 36 months, all remaining households were surveyed to assess net attrition rates, net perceptions and use. In the calculation of survivorship and attrition, households that had moved from the study area were removed from the analysis.

Holes were counted on the sampled nets by draping them over a frame and recording the number, size and location (top, upper side, lower side) of the holes. Holes were categorized as small (smaller than a thumb; <2 cm in diameter), medium (larger than a thumb but smaller than a fist; 2–10 cm in diameter) or larger (larger than a fist; >10 cm in diameter). The proportionate hole index (pHI) was calculated by applying weights of 1, 23 and 196 to the small, medium and large holes respectively and summing across each net.

Cone bioassays were conducted on four pieces of each sampled net at positions 2–5 according to WHOPES guidelines. A total of 5 unfed female mosquitoes, 2–5 days old from a colony of pyrethroid susceptible *An. dirus* s.s. were exposed in the cones for 3 min and then held for 24 h. Knockdown was recorded at 60 min and mortality at 24 h. Two replicates were done on each sample for a total of 10

[31] The inclusion of the PermaNet 2.0 was not part of the WHOPES study. The investigators evaluated PermaNet 2.0 in parallel as part of their own study and at their own cost. As the revised guidelines for testing of LNs (WHOPES 2013) recommend the inclusion of a WHOPES-recommended LN as a positive control, data from the PermaNet 2.0 study are also presented in this report for scientific interest and have no bearing on the WHOPES recommendation of Netprotect LN.

mosquitoes exposed per sample. An untreated negative control net was run each day. If control mortality was 5–20%, the results were adjusted using Abbott's formula. If control mortality was >20%, the results from that day were discarded.

At the 36 month follow-up, if mortality in the cone bioassays was <80% and knockdown was <95%, the net was subjected to a tunnel test according to WHOPES guidelines using a mouse as bait.

Insecticide content was measured on nets sampled at 1 week, 6 months, 12 months and 36 months after distribution (Pigeon, 2010b,c; 2011e,f,g; 2013f,g). Chemical analysis was done on 4 pieces of netting from positions 2–5, excluding position 1 as it is exposed to excessive abrasion, according to WHOPES recommendations (Annex 4). Each sample was individually packed in aluminium foil, labelled according to net ID and position, and sent to the Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium (WHO Collaborating Centre for Quality Control of Pesticides) for analysis. Extraction was carried out by heating the samples under reflux for 60 min in xylene in presence of dipropyl phthalate as an internal standard, and insecticide content was measured by gas chromatography using flame ionization detection (GC-FID). The content of both the biological active *S*-isomer (deltamethrin) and the inactive *R*-alpha isomer was measured on all samples. The average target dose and tolerance limit for the baseline and average content for the subsequent samplings were determined.

The GC-FID method used in this study was not the same as the CIPAC HPLC-DAD method recommended in the WHO interim specification 333/LN/3 (August 2010) for Netprotect, but at the time of analysis of baseline Netprotect samples (July 2010), the WHO specification was not yet published and the CIPAC HPLC-DAD method was not fully approved and published by CIPAC (provisional method). The CIPAC HPLC-DAD method was published later in 2012 in the CIPAC Handbook N. The HPLC-DAD method (CIPAC 333/LN/(M2)/3) involves extraction by refluxing with xylene in presence of dibutyl phthalate as internal standard, solvent exchange to the mobile phase and determination by high performance liquid chromatography with UV diode array detection (HPLC-DAD).

The same GC-FID method was used for Neprotect samples at baseline and after 6 months, 1 year and 3 years of use, as well as for the conventionally-treated nets and the PermaNet 2.0 samples.

The GC-FID method used in this study was fully validated on its specificity, linearity of chromatographic response, repeatability, reproducibility, accuracy and limit of quantification, and the CRA-W in Gembloux received the ISO 17025 accreditation for this method. The epimerization of deltamethrin to the *R*-alpha isomer during the extraction with hot xylene and in the GC injection inlet was checked during the method validation and concurrently with the analysis of samples by performing recoveries and analysis of reference quality control samples. Results showed that the epimerization of deltamethrin does not occur during the analytical procedure.

The GC-FID method was compared with the HPLC-DAD method (CIPAC 333/LN/(M2)/3) on 23 samples of Netprotect used as a reference quality control sample concurrently with the analysis of Netprotect samples. Results obtained for deltamethrin content and the *R*-alpha isomer content showed that the two methods provide very similar results.

During net distribution, 148 conventionally-treated nets, 866 PermaNet 2.0 LNs and 1086 Netprotect LNs were distributed and exchanged for existing nets. Over the entire study period, 1435 households were visited in 8 surveys. During the first 2 years of the study, more than 80% of net owners reported using their nets year-round and every night. There were no differences in the rates of net use among the different net types. By the 36 month follow-up, net use dropped to about 60% for both the PermaNet 2.0 LN and the Netprotect LN. Over the course of the study, the PermaNet 2.0 LN was reported to have been washed more frequently than the Netprotect LN. The average wash frequency per year reported in surveys at 1, 2 and 3 years respectively was 3.4, 1.7 and 1.3. There were no differences in the washing frequencies of the Netprotect LN and the conventionally-treated net. Nearly all nets (98.8%) were washed with cold water and a local detergent or soap (96%).

After 2 years of use, 62% of Netprotect LNs and 77% of PermaNet 2.0 LNs had survived. Most nets that were lost were due to reasons other than poor fabric integrity. By 36 months, survivorship dropped to 31% for the Netprotect LN and 36% for the PermaNet 2.0 LN. The attrition rate for nets lost to damage was 24% for the Netprotect LN and 20% for the PermaNet 2.0 LN. Functional survivorship was >90% during the first 2 years of use and declined to 60% for both the Netprotect LN and the PermaNet LN after 3 years. Throughout the 3 years, there were no differences in survivorship or in the different causes of attrition between the study arms.

During the 1 week, 6 month and 12 month surveys combined, 52% of conventionally-treated nets had holes. This was significantly higher than the PermaNet 2.0 LN (28%) or the Netprotect LN (26%). During the entire 3 years, there was no difference in the proportion of holes on the Netprotect LN compared with the PermaNet 2.0 LN. At 36 months, 84.6% of the Netprotect LNs and 92.9% of the PermaNet 2.0 LNs had at least one hole. The mean pHI on the conventionally-treated nets rose to 209 at 12 months. However, this was largely determined by a few nets with many holes. The median pHI was 6.5. For the PermaNet 2.0 LN, the mean (median) pHI rose from 137.4 (0.5) at 12 months to 169.4 (35.5) at 24 months and 442.5 (303.5) at 36 months. For the Netprotect LN, the mean (median) pHI was 22.3 (0) at 12 months, 107.8 (43) at 24 months and 573.9 (154) at 36 months (Table 6.1).

Knockdown and mortality were generally very low for the conventionally-treated nets. During the 1 week follow-up, only 66.7% of nets met the WHOPES criteria for the cone test. By the 12-month follow-up, only 41.4% met the criteria. For the Netprotect, the proportion of nets that met the WHOPES efficacy criteria for the cone test varied throughout the study. More than 95% of nets met the WHOPES criteria at baseline and 6 months after distribution. The percentage meeting the WHOPES criteria for the cone test fell to 63.3% at 12 months, 72.4% at 24 months and 69.2% at 36 months. However, at 18 and 24 months, 93.1% and 85.7% met the WHOPES criteria based on the cone test alone. For the PermaNet 2.0 LN, more than 85% of the nets met the WHOPES criteria based on the cone test alone at each follow-up (Table 6.2).

At the 36-month follow-up, all nets that failed by the cone test were subjected to a tunnel test. The tunnel test was conducted on 4 PermaNet LNs and 8 Netprotect LNs. None of them passed based on the tunnel test.

Chemical analysis of samples collected at 1 week after distribution showed high variability in the conventionally-treated nets and much lower variability in the PermaNet 2.0 LNs and the Netprotect LNs (Pigeon, 2010b,c). The average deltamethrin content of the conventionally-treated nets was 0.67 g AI/kg (20.9 mg AI/m^2) at baseline, which was lower than the target dose of 25 mg AI/m^2 but within the tolerance limits of $\pm$25%. Baseline average deltamethrin content for the Netprotect LNs was 1.39 g AI/kg (58.7 mg AI/m^2), which is at the lower tolerance limit of the specifications (range: 1.35–

2.25 g AI/kg) (Figure 6.1). The mean dose at 1 week after distribution was 1.60 g AI/kg (71.1 mg AI/m^2) for the PermaNet 2.0 LNs. Of 30 nets sampled, 1 PermaNet 2.0 LN was below specifications while 8 were above specifications (Figure 6.2). By 6 and 12 months, the conventionally-treated net had lost 39% and 63% of its original dose, respectively (Table 6.3). By 6, 12 and 36 months, the Netprotect LN had lost 20%, 39% and 65% respectively of its original dose, while the PermaNet 2.0 LN had lost 12%, 33% and 51% respectively of its original dose. Furthermore, the Netprotect had a high amount of the inactive *R*-alpha isomer. The average *R*-alpha isomer content in Netprotect samples was 0.46 g AI/kg, 0.57 g AI/kg, 0.45 g AI/kg and 0.34 g AI/kg respectively at baseline, and after 6, 12 and 36 months . The ratio of R-alpha isomer to deltamethrin was 0.33:1 at baseline, 0.51:1 at 6 months, 0.53:1 at 12 months and 0.69:1 at 36 months. In contrast, the ratio of *R*-alpha isomer to deltamethrin of the PermaNet 2.0 LN was 0.07:1 at baseline, 0.03:1 at 6 months, 0.06:1 at 12 months and 0.03:1 at 36 months (Table 6.3) (Pigeon, 2010b,c; 2011e,f,g; 2013f,g).

After 1 week, the frequency of any adverse event reported by study participants was 48% overall. At 3 months, 40% of all surveyed participants reported an adverse event. Surprisingly, 76% of surveyed participants reported an adverse event at 6 months after net distribution. However, there were no differences in the rates of reported adverse events among the different treatment arms. At the 1 week follow-up, itching was the most common complaint, while bad smell was the most common complaint at the 3 and 6 month follow-ups. Only one person reported any adverse event after 12 months.

The authors concluded that the Netprotect LN has similar rates of survivorship and physical integrity to the PermaNet 2.0 LN, however its bioefficacy was generally lower. It did not meet WHOPES efficacy criteria and this was likely due to the isomerization of deltamethrin to the inactive R-alpha isomer during manufacturing as well as over time during routine use.

Ada-Foah, Ghana

A study to compare the Netprotect LN with conventionally-treated nets was carried out in 5 communities in the Ada-Foah area of eastern Ghana (Boakye et al, 2013b). The villages were mapped and of the 870 households mapped, 440 were selected for the study. Of these, 300 households were randomly allocated to receive Netprotect LNs while 140 were randomly allocated to receive conventionally-treated nets. The conventional nets were 100-denier polyester nets

treated with deltamethrin SC (K-Othrine) by study staff at a target dose of 25 mg AI/m^2.

A total of 30 Netprotect LNs and 30 conventionally-treated nets were randomly selected for chemical analysis at 1 week after distribution. In addition, 27, 29 and 50 Netprotect LNs were randomly selected for chemical analysis at 12, 24 and 36 months respectively after distribution. Since the deltamethrin content of the conventionally-treated nets was very low at baseline, further chemical analysis at year 1 was not done. Four pieces of netting (30 cm x 30 cm) were cut from each net at positions 2–5 according to WHOPES guidelines (Annex 4). Position 1 was not included as it is subjected to excessive abrasion. The four subsamples were wrapped together in aluminium foil and submitted to the Walloon Agricultural Research Centre, Gembloux, Belgium for chemical analysis as described above (Pigeon, 2010d; 2013h,i). The average target dose and tolerance limit for the baseline and average content for the subsequent samplings were determined.

At 1 month after distribution and every 6 months thereafter, 30 households were randomly selected during each follow-up survey. To assess net survivorship and attrition, the physical presence of coded nets was recorded and, where nets were no longer present, the reason for their loss was noted. An adult participant in the selected households was interviewed on net utilization practices, and washing frequency and methods. The frequency of adverse effects from using the nets was assessed at 1 week, 1 month, 6 months and 12 months after distribution.

Fabric integrity was examined in 30 nets per arm at 6-month intervals up until 24 months after distribution. At 30 and 36 months after distribution, 100 Netprotect LNs were sampled. Sampled nets were draped over a frame and holes counted and categorized according to size (size 1 = smaller than a finger/thumb, size 2 = larger than a finger/thumb but smaller than a fist, size 3 = larger than a fist) and location (top, upper sides, lower sides). A proportionate hole index (pHI) was calculated according to WHOPES guidelines by applying weights of 1, 23, and 196 to size 1, size 2 and size 3 holes, respectively.

Cone bioassays were conducted on nets sampled at 6-month intervals after distribution. Four samples (25 cm x 25 cm) were cut from each sampled net from positions 2–5 according to WHOPES-recommended guidelines for use in cone bioassays (Annex 4). Five

unfed female mosquitoes, 2–5-days old from a susceptible colony (*An. gambiae* s.s., Kisumu strain) were introduced into the cones and exposed for 3 min. After exposure, the mosquitoes were removed and placed in paper cups with access to sugar solution. The test was replicated so that 10 mosquitoes were used for each net sample. Knockdown was measured at 60 min after exposure and mortality was measured at 24 h after exposure.

For nets that failed to meet the WHOPES criteria for the cone test (mortality $\geq$80% or knockdown $\geq$95%), the tunnel test was performed. The test was done in a 60 cm tunnel (25 cm wide by 25 cm high) made of clear plastic. The tunnel was divided into thirds and covered on each end with netting. A 20 cm x 20 cm piece of netting was placed between two pieces of cardboard and placed at 20 cm from one end of the tunnel. Nine holes 1 cm in diameter were cut in the netting material. A guinea-pig was restrained in the short end of the tunnel and 100 mosquitoes were introduced at the opposite end of the tunnel. The experiment was done in the evening, and at the end of the test the mosquitoes were scored according to whether they passed through the netting, whether they successfully blood-fed and whether they survived the exposure period.

After 12 months, the annual attrition rates for coded nets were 2.9% for the conventionally-treated nets. None of the coded Netprotect LNs were lost in the 12 months after distribution. Attrition of the non-coded LNs was 1.7%. The overall survivorship rate was 83.3% at 18 months and 87.8% at 24 months. Overall, 68.4% of nets survived for the full 3 years of the study.

The percentage of Netprotect LNs with at least one hole was 26.7% at 6 months after distribution. This figure increased to 40.0% at 24 months after distribution. At 30 months after distribution, 72.0% of Netprotect LNs had at least one hole while 78.0% of Netprotect LNs had at least one hole at 36 months. The average number of holes on the Netprotect LN was 0.7 during the 6 month follow-up. The average number of holes per net rose to 2.8 by the final survey at 36 months after distribution. The mean pHI for the Netprotect LN was 10.9 after 6 months of use. By 12 months of use, the mean pHI had risen to 16.1. However, the mean pHI declined to 11.8 at 18 months, 5.0 at 24 months and 6.5 at 30 months. The median pHI was 0 until 24 months. By 36 months, the median pHI was 4 (Table 6.1).

In cone bioassays, the conventionally-treated nets performed poorly at baseline when only 10% of the nets met the WHOPES criteria

based on a combination of the cone test and the tunnel test. At 6 months post-distribution, only 3.3% of conventionally-treated nets met the criteria of either the cone test or the tunnel test and by 12 months, none of the conventionally treated nets passed by either criterion. For the Netprotect LN, all nets met the WHOPES criteria for the cone test until 1 year of follow-up. However, during the remaining follow-ups, the pass rate based on the cone test alone was <60%. With the tunnel test, the combined pass rate was <65% for all follow-ups conducted after 18 months (Table 6.2).

The average deltamethrin content of the conventionally-treated nets was only 0.05 g AI/kg (2.3 mg AI/m^2) at 1 week after distribution, which was significantly below the target dose of 25 mg AI/m^2. Further chemical analysis of the conventionally-treated nets was therefore not done. The average deltamethrin content of the Netprotect LN was 1.40 g AI/kg (58.1 mg AI/m^2) at 1 week after distribution, which is at the lower tolerance limit of the specifications (range: 1.35–2.25 g AI/kg) (Figure 6.3). At 12, 24 and 36 months, the average deltamethrin content was 0.63 g AI/kg (28.4 mg AI/m^2), 0.44 g AI/kg (21.1 mg AI/m^2) and 0.36 g AI/kg (17.6 mg AI/m^2), showing a loss of 55%, 69% and 74% respectively. However, the Netprotect LN had a high amount of the inactive *R*-alpha isomer of deltamethrin. The average *R*-alpha isomer content in Netprotect LN samples was 0.42 g AI/kg, 0.48 g AI/kg, 0.40 g AI/kg and 0.33 g AI/kg and the ratio of R-alpha isomer to deltamethrin was 0.30:1, 0.76:1, 0.91:1 and 0.92:1 at baseline and after 12, 24 and 36 months respectively (Table 6.3) (Pigeon, 2010d; 2013h,i).

In surveys conducted 6 and 12 months after distribution, 86.0–88.6% of respondents reported using the Netprotect LN every night. During the same surveys, 86.6–90.0% of respondents reported using the conventionally-treated nets every night. For the remaining surveys, more than 85% of respondents reported using the Netprotect LNs every night. The average frequency of washing Netprotect reported by households in surveys at months 1, 6 and 12 was 1, and at months 18, 24, 30 and 36 respectively was 3, 2, 2 and 4. Most respondents reported using local or commercial bar soaps to wash their nets.

The authors concluded that the physical integrity and community acceptance of the Netprotect LN was good. However, the Netprotect LN fell below WHOPES criteria for bio-efficacy at 18 months and remained below these criteria for the remainder of the study.

6.3 Conclusions and recommendations

Netprotect LN is manufactured by Intelligent Insect Control. The net is treated with deltamethrin incorporated into monofilament polyethylene fibres (118 denier) at a target dose of 1.8 g AI/kg corresponding to 68.4 mg AI/m^2 of netting.

WHOPES published interim recommendations for the Netprotect LN in 2008 based on phase I laboratory testing and phase II experimental hut studies. Full WHO recommendations require further evidence of the efficacy, durability and acceptability of the Netprotect LN under routine household use over a period of 3 years. Longitudinal, randomized household trials to evaluate its efficacy, longevity and fabric integrity over a period of 3 years are required as part of phase III testing. WHOPES guidelines recommend that after 3 years of routine household use, at least 80% of nets tested meet the cut-off criteria for either the WHO cone bioassay test or the tunnel test. However, criteria for fabric integrity have yet to be established.

This report reviews available background information for 2 studies that were not supervised by WHOPES, as well as data from phase III testing of the Netprotect LN in two WHOPES supervised trials in Cambodia and Ghana.

In one study, the nets were distributed in one village and entomological and epidemiological impact observed over 6 months. Of the 807 mosquitoes captured, 82.5% were from the control village. Furthermore, malaria incidence in the control village was estimated at 41.3% compared with 16.3% in the village that received Netprotect nets. The pHI was 333 at 18 months, 114 at 28 months and 381 at 38 months. In cone bioassays conducted in the field up to 8 months after net distribution, mortality in mosquitoes was $\geq$80% throughout but was 80% after 6 months of use. Nets that were sampled at 18 months and tested against *An. cracens* had 69.5% mortality. Of these, only 66.7% passed efficacy criteria according to the cone test. At 38 months, 10 nets were tested against *An. gambiae* and all passed according to the cone test. Although the nets passed at 38 months, the study did not follow WHOPES guidelines, small number of nets were sampled for cone bioassays at 36 months (n=10) as well for chemical analysis at the baseline (n=4), the deltamethrin *R*-alpha isomer was not reported at the baseline and the results at 18 months raised concerns about the efficacy of the Netprotect.

In a second study, 60 Netprotect were distributed and followed at 4–5-month intervals over 4.5 years and compared with a PermaNet 2.0 and a conventionally-treated net. Nets were removed from the field if bioassay mortality fell below 50% in two consecutive bioassays. In a survival analysis, the Netprotect had a median survival time of approximately 2.5 years. This was significantly greater than that of a conventionally-treated net and similar to that of the PermaNet 2.0. However, the study did not follow WHOPES guidelines and chemical analysis of nets distributed at baseline was not done; the results should therefore be interpreted with caution.

The loss of physical integrity in the Netprotect LNs was measured by the percentage of nets with holes and the proportionate hole index (pHI). In two WHOPES supervised trials, the percentage of Netprotect LNs with at least one hole after 6 months of use ranged from 25.8% to 26.7%. After 36 months of use, that percentage ranged between 78.0% and 96.6%. The mean pHI ranged between 6.7 and 10.9 after 6 months of use and between 16.0 and 573.9 after 36 months of use. The median pHI was 0 up to 12 months in Cambodia and 24 months in Ghana. After 36 months, the median pHI was 154 in Cambodia and 16.0 in Ghana. In the trial in Cambodia, there were no differences in the loss of physical integrity over time between the Netprotect LN and a WHOPES-recommended LN used as a positive control.

The deltamethrin content in Netprotect declined over the 3 years of use in the two WHOPES supervised trials. The average loss of deltamethrin ranged from 65% to 74% over the course of 36 months. The R-alpha isomer content was high at baseline in both studies and the proportion of the R-alpha isomer relative to deltamethrin continued to increase throughout the study, suggesting isomerization of the biologically active deltamethrin to the biologically inactive R-alpha isomer occurred during manufacture, and continued to occur after distribution to study participants.

After 18 and 30 months of use in Cambodia, the percentage of nets that met WHOPES criteria by either the cone test or the tunnel test was 93.1% and 85.7%, respectively. However, less than 80% of the Netprotect LN met the WHOPES criteria for the cone test at the 12, 24 and 36 month follow-ups. At the 36 month follow-up, the nets that did not meet the criteria by the cone test were subjected to the tunnel test. However, none of these nets met the WHOPES criteria for the tunnel test. In Ghana, the percentage of Netprotect LNs meeting WHOPES criteria for either the cone test or the tunnel test was 100% at 6 and 12 months after distribution. However, less than 70% of

Netprotect LNs met the WHOPES criteria for either the cone test or the tunnel test during the remaining follow-ups.

Taking into account all the available information, the meeting concluded:

- that sufficient evidence is not available to grant full recommendation to Netprotect. The meeting recommended that until more evidence on performance of the product is available from large-scale studies, the WHO interim recommendation on the use of the product be withdrawn; and

- that national programmes currently using Netprotect for malaria prevention and control be urged to monitor efficacy and performance of Netprotect under local conditions, using WHO guidelines [32] and provide any feedback to the WHOPES secretariat.

[32] Available at: http://whqlibdoc.who.int/publications/2011/9789241501705_eng.pdf.

Table 6.1 Physical integrity of Netprotect LNs over time in two study sites. Data on the PermaNet 2.0 from Cambodia are also presented for comparison. For each site, the number of nets examined (N), the percentage with any holes, the mean proportionate hole index (pHI) (standard deviation in bracket) and the median pHI (interquartile range in bracket) are presented.

Site and LN		Months after distribution					
		6	**12**	**18**	**24**	**30**	**36**
Cambodia Netprotect	N	31	30	30	30	27	26
	% with any holes	25.8	50	63.3	83.3	70.4	96.6
	Mean pHI	6.7 (16.5)	22.3 (64.7)	109.6 (170.9)	107.8 (183.9)	229.8 (351.6)	573.9 (965.6)
	Median pHI	0 (0–0.5)	0 (0–2.75)	15 (0–141.5)	43 (6–81.5)	49 (0–341.8)	154 (13.8–484)
Cambodia PermaNet	N	31	30	30	30	29	28
	% with any holes	22.6	56.7	63.3	86.7	96.6	92.9
	Mean pHI	59.0 (265.8)	137.4 (526.8)	77.0 (155.7)	169.4 (235.2)	367.6 (603.5)	442.5 (713.3)
	Median pHI	0 (0–0)	0.5 (0–23.3)	2 (0–52.5)	35 (7–246.5)	80 (11–330)	303 (66–520.5)
Ghana Netprotect	N	30	30	30	30	100	100
	% with any holes	30.0	25.9	23.3	40.0	71.0	78.0
	Mean pHI	10.9 (40.8)	16.1 (42.5)	11.8 (40.0)	5.0 (13.9)	6.5 (22.1)	16.0 (23.8)
	Median pHI	0 (0–2)	0 (0–10)	0 (0–0)	0 (0–1)	1 (0–2)	4 (2–27)

Table 6.2 Number and percentage of Netprotect LNs meeting WHOPES criteria according to the cone test or the tunnel test in two study sites. Data on the PermaNet 2.0 from Cambodia are also presented for comparison. For each site, the total number (N), the number passing the cone test (Cone) and the number passing the tunnel test (Tunnel) are presented; the percentage passing according to either the cone test or the tunnel test are presented in the bottom row for each site (%).

Site and LN	Test	Months after distribution					
		6	**12**	**18**	**24**	**30**	**36**
Cambodia*	N	27	30	29	29	28	26
Netprotect	Cone	27	19	27	21	24	18
	Tunnel	–	–	–	–	–	0
	%	**100**	**63.3**	**93.1**	**72.4**	**85.7**	**69.2**
PermaNet 2.0	N	28	27	28	30	29	30
	Cone	28	23	26	28	25	26
	Tunnel	–	–	–	–	–	0
	%	**100**	**85.2**	**92.9**	**93.3**	**86.2**	**86.7**
Ghana	N	30	30	30	30	50	50
Netprotect	Cone	30	30	18	12	30	28
	Tunnel	–	–	1	1	2	3
	%	**100**	**100**	**63.3**	**43.3**	**64.0**	**62.0**

*In the Cambodia study, the tunnel test only performed on nets that did not meet the criteria for the cone test at 36 months.

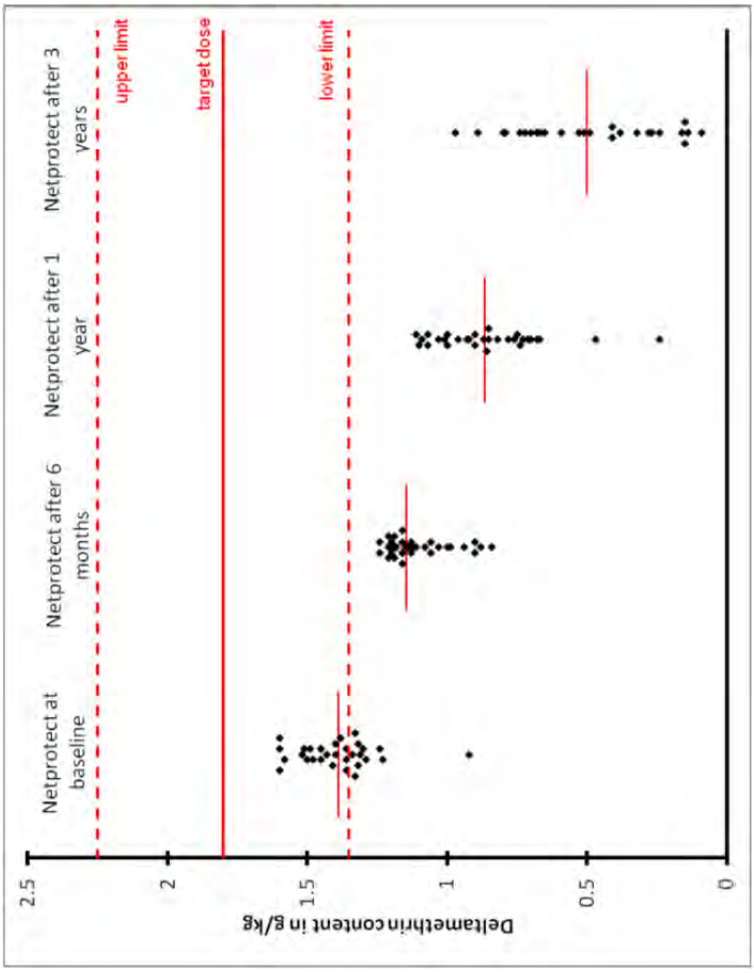

Figure 6.1 Deltamethrin content (in g AI/kg) on individual Netprotect samples at baseline and after 6 months, 1 year and 3 years of household use in Cambodia. The target dose (1.8 g AI/kg) and the upper (2.25 g AI/kg) and lower (1.35 g AI/kg) acceptable limits are for deltamethrin content at baseline, indicated as solid and dashed lines respectively.

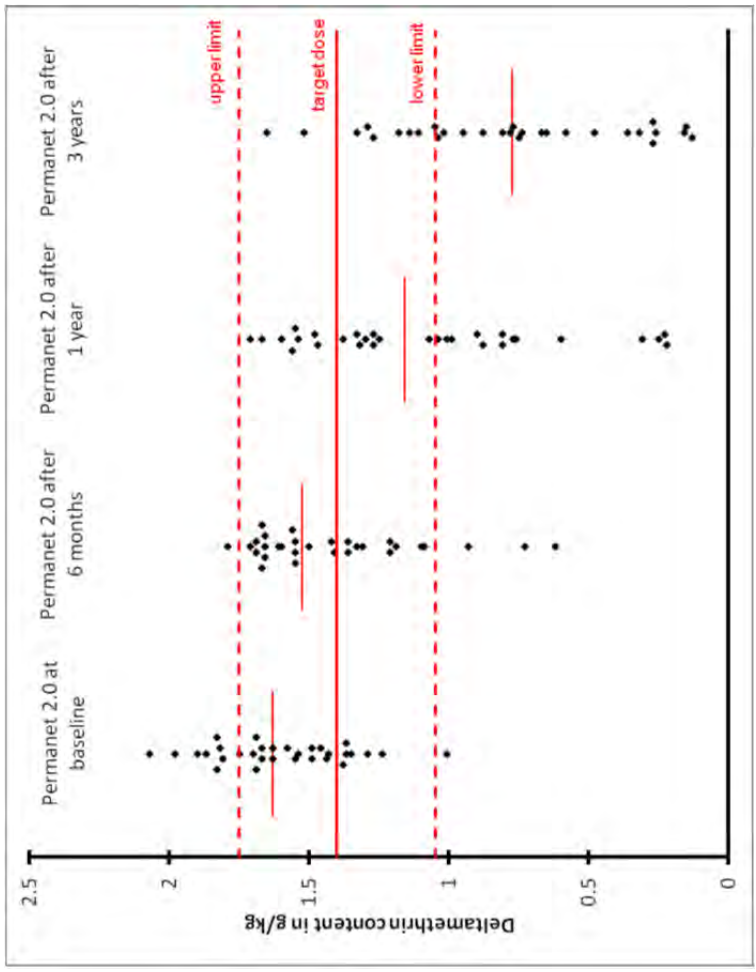

Figure 6.2 Deltamethrin content (in g AI/kg) on individual PermaNet 2.0 samples at baseline and after 6 months, 1 year and 3 years of household use in Cambodia. The target dose (1.4 g AI/kg) and the upper (1.75 g AI/kg) and lower (1.05 g AI/kg) acceptable limits are for deltamethrin content at baseline, indicated as solid and dashed lines respectively.

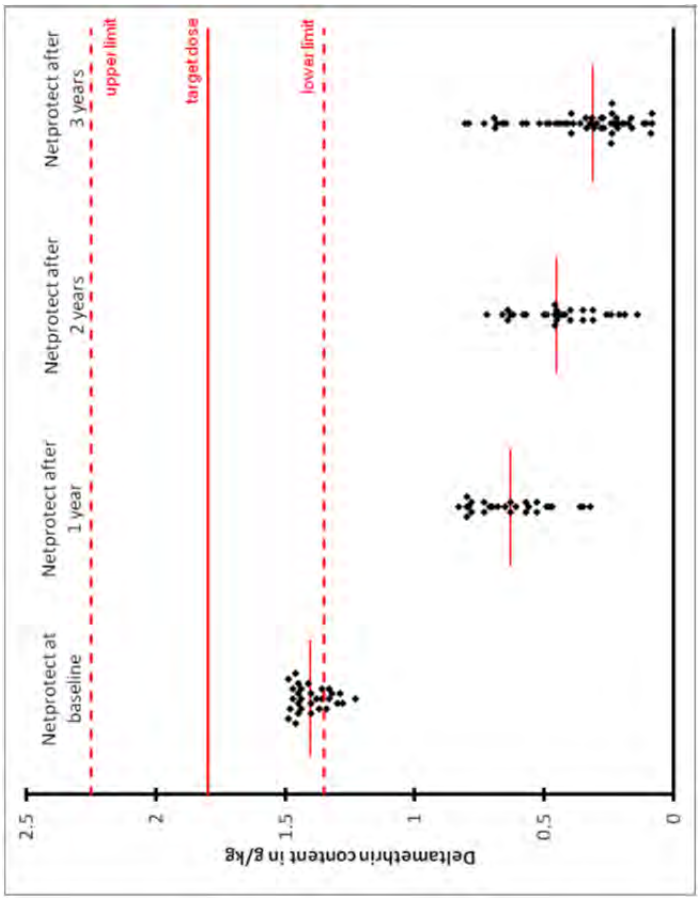

Figure 6.3 Deltamethrin content (in g AI/kg) on individual Netprotect samples at baseline and after 1, 2 and 3 years of household use in Ghana. The target dose (1.8 g AI/kg) and the upper (2.25 g AI/kg) and lower (1.35 g AI/kg) acceptable limits are for deltamethrin content at baseline, indicated as solid and dashed lines respectively.

Table 6.3 Mean (95% confidence limits) deltamethrin content (in g AI/kg) in Netprotect and PermaNet 2.0 and percentage AI lost over time in the two study sites. The R-alpha isomer content (g AI/kg) and the ratio of the R-alpha isomer to deltamethrin are also presented.

Site and LN	Test	Months after distribution				
		0	6	12	24	36
Cambodia	N	30	32	30	-	26
Netprotect	Mean	1.39 (1.34–1.44)	1.11 (1.07–1.15)	0.85 (0.78–0.92)	-	0.49 (0.39–0.59)
	% lost	-	20%	39%	-	65%
	R-alpha	0.46	0.57	0.45	-	0.34
	Ratio	0.33:1	0.51:1	0.53:1	-	0.69:1
Cambodia	N	30	30	30	-	30
PermaNet 2.0	Mean	1.60 (1.51–1.69)	1.41 (1.30–1.52)	1.08 (0.91–1.25)	-	0.79 (0.63–0.95)
	% lost	-	12%	33%	-	51%
	R-alpha	0.11	0.04	0.06	-	0.02
	Ratio	0.07:1	0.03:1	0.06:1	-	0.03:1
Ghana	N	30	-	27	29	50
Netprotect	Mean	1.40 (1.37–1.43)	-	0.63 (0.57–0.69)	0.44 (0.38–0.50)	0.36 (0.30–0.42)
	% lost	-	-	55%	69%	74%
	R-alpha	0.42	-	0.48	0.40	0.33
	Ratio	0.30:1	-	0.76:1	0.91:1	0.92:1

7. REVIEW OF YAHE LN

Yahe LN is a deltamethrin, long-lasting (coated onto filaments) insecticidal net manufactured by Fujian Yamei Industry, China. Deltamethrin is coated on 75-denier, knitted, multi-filament polyester fibres at the target dose of 1.85 g AI/kg netting material, corresponding to 55.5 mg of deltamethrin per square metre of the fabric (weight 30 g/m^2).

Yahe LN has been subject to WHOPES review for extension of WHO interim specification 333/LN/1 (netting and net) (September 2010). The outcome of the regeneration, wash resistance and efficacy studies of Yahe LN in laboratory, as part of the requirements for extension of WHO specifications, were published in the *Report of the fourteenth WHOPES Working Group Meeting, WHO/HQ, Geneva, 10-14 April 2011*.[33] The meeting noted that Yahe LN complied with the WHO interim specifications with reference to total content of deltamethrin and retention index. However, there was high variability in the deltamethrin content in the netting samples tested. In addition, in contrast to the reference LN, the biological efficacy of the Yahe LN before the first wash did not meet WHO criteria. After the first wash, the Yahe LN net showed KD consistently above the 95% threshold. However, the mortality rates of the Yahe LN never exceeded 86% at any wash point and were always lower than mortality rates of the reference LN.

The fourteenth WHOPES Working Group Meeting concluded that Yahe LN does not meet WHO requirements for extension of specifications and shall be treated as an independent product, i.e. to be subjected to standard WHOPES phase II studies as a requirement for obtaining WHOPES interim recommendations on its use in malaria prevention and control.

The Meeting also advised WHOPES to invite the manufacturer to provide supporting data on homogeneity of deltamethrin content.

The present assessment includes a review of the results of WHOPES supervised phase II (experimental hut) studies as a requirement for development of interim recommendations.

[33] Available at: http://www.who.int/whopes/recommendations/wgm/en/

7.1 Efficacy – WHOPES supervised trials

Mae Sot District, Thailand

Washed and unwashed Yahe LNs were evaluated in experimental huts in Tum Sua village in Mae Sot District in western Thailand to determine their effects on free-flying, wild *An. minimus* mosquitoes for their ability to deter entry, repel or drive mosquitoes out of houses, induce mortality and inhibit blood-feeding (Duchon et al, 2013). The PermaNet 2.0 LN was used as a comparison product. The study site was bordered by fruit orchards and agricultural fields on the east and by intact forest on the west. In mosquito collections conducted between 2008 and 2010, *An. minimus* was the most commonly collected mosquito followed by *An. dirus* (28%) and *An. maculatus* (1%). WHO susceptibility tests conducted using *An. minimus* indicated full susceptibility to deltamethrin.

Six experimental huts constructed in the west African design [34] were used in the study. Mosquitoes entered the huts through 1 cm wide entry slits constructed from pieces of wood fixed at an angle to prevent mosquitoes from exiting. The back side of the house had a screened veranda that was 2 m long x 1.5 m wide x 1.5 m high.

Six treatment arms were tested: (i) an untreated polyester net; (ii) unwashed Yahe LN; (iii) Yahe LN washed 20 times; (iv) unwashed PermaNet 2.0 LN; (v) PermaNet 2.0 LN washed 20 times; and (vi) polyester conventional net treated with deltamethrin at a target dose of 25 mg AI/m^2.

Six nets were used per treatment arm and, to simulate worn nets, all nets had 6 holes (4 cm x 4 cm) cut in them with 2 holes on each of the long sides and 1 on each of the short sides.

Before the start of the study, baseline bioassays were done on all 6 nets in each treatment arm. The nets in the two treatment arms that were to be washed were then washed 20 times according to the standard WHOPES guidelines. [34] After washing, bioassays were again done on all 6 nets in each treatment arm. The nets were then used in the hut study, and at the end of the hut study bioassays were done on all 6 nets in each treatment arm. Bioassays were done using a susceptible strain of *An. minimus*. Bioassay cones were attached

[34] *Guidelines for laboratory and field-testing of long-lasting insecticidal nets*. Geneva, World Health Organization , 2013 (available at: http://www.who.int/whopes/guidelines/en/).

to the net at 5 positions (Annex 4) and 5 mosquitoes were exposed in the cones for 3 min. After exposure, the mosquitoes were transferred to holding cages and knockdown recorded at 60 min and mortality at 24 h post-exposure. The bioassays were repeated twice at each position so that 10 mosquitoes were exposed at each position and 50 mosquitoes were exposed on each net.

The nets were washed according to a WHOPES recommended procedure. Washing was done in bowls containing 10 litres of water and 2 g/litre of soap. Each net was manually agitated for 3 min, allowed to soak for 4 min and agitated again for 3 min. The nets were then rinsed twice in clean water using the same procedures and dried horizontally in the shade.

Before testing, preliminary catches were made for 1 week using untreated nets to ensure that there were no differences in the huts or the sleepers in their attractiveness to mosquitoes.

Each week, one net type was assigned to a specific hut. Each of the 6 nets in the different treatment arms was then tested in that hut on one night for 6 consecutive nights. Sleepers rotated through the huts each night. At the end of the 6 nights, the huts were cleaned and ventilated and the nets were then rotated to different huts. This was repeated until all nets were tested once in each hut. Two complete rotations were made so that the trial lasted for a total of 12 weeks.

Each night, volunteers would enter the huts at 18:00 and remain inside under the test nets until 06:00. In the morning, the dead mosquitoes were collected from inside the nets and from the floor of the hut and the veranda trap. Resting mosquitoes were then collected using aspirators from inside the nets and from the walls and roof of the hut and veranda trap. Mosquitoes were recorded by location, as dead or alive and as fed or unfed. Live mosquitoes were placed in small cups and provided with sugar solution to assess delayed mortality.

The primary outcomes of the trial were deterrence (reduction in hut entry relative to the control huts), induced exophily (the proportion of mosquitoes captured in the exit traps), blood-feeding inhibition (the reduction in blood-feeding of mosquitoes compared with the control huts) and immediate mortality (the proportion of mosquitoes found dead in the morning).

A seventh replicate net was used in each arm for the chemical assays. Five pieces (30 cm x 30 cm) were removed from these nets at baseline from the positions as described in Annex 4. After washing was completed, 5 additional pieces were removed from each net. After the trial was completed, 5 pieces were taken from each of the nets used in the hut trial. All pieces were labelled, wrapped in aluminium foil and stored in sealed bags at 4 °C. The samples were then sent to the WHO Collaborating Centre at Gembloux, Belgium for chemical analysis using the CIPAC method 333/LN/(M)/3.

At baseline before washing, the PermaNet 2.0 LN and the conventionally-treated net met the WHOPES thresholds for the cone bioassay for both mortality (≥80) and knockdown (≥95). Average knockdown on the Yahe LN ranged from 60% (washed 20 times) to 73% (unwashed), while average mortality ranged from 58% (unwashed) to 69% (washed 20 times). After the nets were washed but before the hut trial, knockdown was 100% on all treated nets except for the unwashed Yahe LN where knockdown was 88%. Mortality was ≥98% for all treated nets except the unwashed Yahe LN where mortality was 86%. After the hut trial, knockdown was >96% on all treated nets except for the unwashed Yahe LN where knockdown was 88%. Mortality was 100% for both washed and unwashed PermaNet 2.0 LN. Mortality was 89% for the conventionally-treated net, 56% for the unwashed Yahe LN and 82% for the Yahe LN washed 20 times (see Table 7.1).

The average number of *Anopheles* mosquitoes entering the huts ranged from 0.43 per night for the unwashed PermaNet 2.0 LNs to 0.65 per night for the conventionally-treated net. There were no statistically significant differences between any of the study arms. Exophily ranged from 24% in the huts with the untreated net to 48% in the huts with the PermaNet 2.0 LN washed 20 times. Exophily was significantly higher in huts with the PermaNet 2.0 LN washed 20 times compared with all treatment arms except for the unwashed Yahe LN and the unwashed PermaNet 2.0 LN. No other statistically significant differences in exophily were observed. Blood-feeding was consistently low in all treatment arms, presumably due to the low anthropophily of *An. minimus*. Blood-feeding was low across all treatments, ranging from 6% to 13%, and there were no statistically significant differences among the treatment arms. Mortality was 6% in the huts with the untreated nets and was significantly lower than mortality in all other treatment arms, where mortality ranged from 32% huts with the unwashed PermaNet 2.0 LN to 57% in the huts with the conventionally-treated net. Mortality was significantly higher

in the huts with the conventionally-treated net compared with the unwashed PermaNet 2.0 LN, but no other statistically significant differences were detected (Table 7.3). In terms of mortality, the Yahe LN washed 20 times performed as well as either a PermaNet 2.0 LN washed 20 times or an unwashed conventionally-treated net.

Results were similar for free-flying, wild culicine mosquitoes. The number of females caught per night ranged from 0.68 to 0.88 per hut and did not differ by treatment arm. Exophily ranged from 27% to 57% among the different treatment arms. Exophily was highest in huts with unwashed PermaNet 2.0 LNs and was significantly higher than huts with conventionally-treated nets or unwashed nets. Blood-feeding rates ranged from 2% to 7% and were not different among any treatment arms. Mortality was 6% in the huts with untreated nets and was significantly lower than mortality in all other treatment arms, where mortality ranged from 41% to 54%. There were no statistically significant differences among the arms with treated nets. In terms of mortality, the Yahe LN washed 20 times performed as well as a PermaNet 2.0 LN washed 20 times or an unwashed conventionally-treated net.

The average deltamethrin content in three unwashed Yahe LN was 2.21, 2.33 and 2.46 g AI/kg. After washing 20 times, the deltamethrin content in the Yahe LN fell to 1.88 g AI/kg for an overall retention of 77% of the initial deltamethrin content. After the trial, the average deltamethrin content of the Yahe LN was 1.27 g AI/kg (Table 7.4). Of three unwashed Yahe LNs, (the unwashed Yahe LN, the unwashed Yahe LN after other nets had been washed and the Yahe LN to be washed 20 times), two were above the maximum tolerance limit of 2.31 g AI/kg (Tables 7.4 and 7.5) (Pigeon, 2013j).

The authors concluded that the Yahe LN performed less well than the PermaNet 2.0 LN in terms of mortality in WHO cone assays. However, the unwashed Yahe LN and the Yahe LN washed 20 times performed as well as the PermaNet 2.0 LN and as well as the unwashed conventionally-treated net in the experimental hut study in terms of mortality. However, the authors noted that the experimental hut studies should be interpreted with caution due to the low numbers of mosquitoes captured during the course of the study. The results also may have been affected by the higher than acceptable levels of deltamethrin on some of the unwashed Yahe LNs.

Muheza, United Republic of Tanzania

The efficacy of the Yahe long-lasting net was evaluated in veranda trap experimental huts in Muheza (United Republic of Tanzania) against wild, free-flying, host-seeking *An. gambiae* and *Cx. quinquefasciatus* (Tungu et al, 2013). Characterization of the *An. gambiae* population identity by PCR showed that 93% of the 425 samples assayed were *An. gambiae* s.s. and 7% were *An. arabiensis*. Furthermore, a *kdr* assay on 180 *An. gambiae* s.s. samples indicated that 35% (64) were homozygous wild type; 24% (43) were heterozygous and 41% (73) homozygous for the *kdr* 1014S allele.

Six treatment arms were included in the study as follows: (i) untreated polyester net; (ii) unwashed Yahe LN; (iii) Yahe LN washed 20 times; (iv) unwashed PermaNet 2.0 LN; (v) PermaNet 2.0 LN washed 20 times; and (vi) polyester net, conventionally treated with deltamethrin at 25 mg AI/m², washed until just before exhaustion.

Three nets were used for each treatment arm. Nets were washed according to standard WHOPES recommendations. Nets were placed in 10 L of water with 2 g/L of soap (Savon de Marseille) and washed using manual agitation for 6 min within a total 10 min washing/soaking period. Agitation was done by stirring the net with a wooden pole at 20 rotations per min. Nets were rinsed twice (10 litres per rinse) and dried between consecutive washes.

The point of exhaustion was determined by washing three conventionally-treated nets (treated at 25 mg AI/m^2) according to the above protocol and conducting bioassays after each wash cycle. The point of exhaustion was defined as the maximum number of washes a net could withstand before mortality of mosquitoes exposed in standard WHO cone assays fell below 80% and knockdown fell below 95%. Cone bioassays using *An. gambiae* (Kisumu strain) showed that knockdown on the conventionally-treated net fell below 95% after the second wash while mortality fell below 80% after four washes. The point of exhaustion for the conventionally-treated net was therefore set at three washes.

Bioassays were conducted on netting material taken from each of the treatment arms before washing, after washing and after completion of the trial. In bioassays conducted before washing, knockdown and mortality scored were 100% for all treatment arms. After washing, mortality was 90% on the Yahe LN, 96% on the PermaNet 2.0 and 91% on the conventionally-treated net. By the end of the trial, mortality was 98% for the Yahe LN, 96% for the PermaNet 2.0 and

88% for the conventionally-treated net. After washing and after the trial, knockdown fell below 95% on all the treated nets. However, mortality remained above the WHOPES threshold of 80% for all treated nets at all stages of the trial (see Table 7.2).

An additional net in each treatment arm was not tested in the huts but used for chemical analysis by HPLC using the CIPAC method 333/LN/(M)/3 as described above (Pigeon, 2013k).

Six experimental huts were used in the study and were made to the traditional East African veranda trap design. The huts were made of concrete walls smeared with mud and an iron roof with a wooden ceiling lined with hessian cloth. The eaves were open on all sides to allow passage of mosquitoes. Two verandas placed on opposite sides were screened to capture mosquitoes that exited through the eaves or windows, while the verandas on the remaining two sides were left open to allow for mosquito entry. The screens on the verandas were rotated periodically to reduce any biases introduced by the position of the veranda screens. The huts were built on concrete plinths and surrounded by a water-filled moat to prevent the entry of ants or other scavengers.

Before the start of the trial, the nets were deliberately holed to simulate a torn net. Six holes, 4 cm x 4 cm, were cut in each net with two holes on each long side of the net and one hole at each end. The three nets were used for each treatment arm and the sleepers were rotated through the huts in a Latin square design. Each net was tested in each hut on at least 2 nights. Mosquitoes were collected each morning from the floors, walls, exit traps and inside the nets and were scored as dead or alive, blood-fed or unfed. Live mosquitoes were held for 24 h to assess delayed mortality. For data analysis, the number of mosquitoes captured in the veranda traps was doubled to account for mosquitoes escaping through the open verandas. The trial was run for 36 nights.

The average number of *An. gambiae* collected per night ranged from 8.0 to 15.2. Significantly fewer *An. gambiae* were caught from huts with unwashed PermaNet 2.0 and unwashed Yahe LN than from huts with Yahe LN washed 20 times and PermaNet 2.0 washed 20 times. The number of *An. gambiae* caught in huts with LNs washed 20 times or conventionally-treated nets washed to exhaustion did not differ from those caught in the control hut with an untreated net. The exit rate of *An. gambiae* from huts with untreated nets was 73.9% and was significantly lower than the exit rate in all other treatment arms.

Exit rates for *An. gambiae* from the treated huts ranged from 87.5% to 94.1%. The rate of exit from huts with the Yahe LN washed 20 times was significantly lower than that from huts with the unwashed PermaNet 2.0 LN. No other statistically significant differences in exit rates were observed (see Table 7.3).

In the untreated control huts, 25.8% of *An. gambiae* were blood-fed. Significantly more mosquitoes had fed in the untreated control huts compared with all other treatment arms. Among the treated nets, the proportion of *An. gambiae* that had fed was significantly lower in the huts with unwashed PermaNet 2.0 LNs compared with huts with Yahe LNs washed 20 times. No other differences were detected among the treated nets. Mortality was lowest in the untreated control huts and was significantly lower compared with all other treatment arms. Mortality was highest in the huts with unwashed Yahe LNs and was significantly higher compared with all other treatment arms. No other differences in mortality among the different treatment arms were detected. The Yahe LN washed 20 times performed as well as either a PermaNet 2.0 LN washed 20 times or conventionally-treated net washed to exhaustion as measured by blood-feeding inhibition and mortality (see Table 7.3).

For *Cx. quinquefaciatus*, the entry rates ranged from 3.3 to 5.6 per hut per night. The entry rate was significantly higher in the huts with untreated nets and the huts with the Yahe LN washed 20 times compared with huts with the unwashed Yahe LN. No other differences in hut entry were observed. Exit rates of *Cx. quinquefaciatus* ranged from 51.1% to 88.8%. Exit rates in the control hut were significantly lower than all other treatment arms. The exit rate from huts with the conventionally-treated nets washed to exhaustion was significantly lower than the exit rate from huts with unwashed PermaNet 2.0 LNs. No other differences in exit rates were detected.

In control huts, the rate of blood-feeding was 42.9%, which was significantly higher than that observed in all other treatment arms where rates of blood-feeding ranged from 4.5% to 25.2%. The rate of blood-feeding in huts with unwashed PermaNet 2.0 LN was significantly lower compared with all other treatment arms. Blood-feeding rates in the huts with Yahe LNs washed 20 times and PermaNet 2.0 LNs washed 20 times were 12.4% and 12.8% respectively. These were significantly lower compared with the huts with unwashed Yahe LNs, where the blood-feeding rate was 25.2%. Mortality rates of *Cx. quinquefaciatus* ranged from 2.2% in the control

hut to 8.4% in the huts with the unwashed Yahe LNs. Mortality was significantly higher in the huts with unwashed Yahe LNs compared with the control huts. No other statistically significant differences were observed.

The average deltamethrin content in three unwashed Yahe LNs was 2.28, 2.42 and 2.70 g AI/kg. After washing, the deltamethrin content in the Yahe LN fell to 1.47 g AI/kg. This corresponds to an overall retention of 61% of the initial deltamethrin content. After the trial, the average deltamethrin content of the Yahe LNs was 1.66 g AI/kg (Table 7.4). Of three unwashed Yahe LNs, (the unwashed Yahe LN, the unwashed Yahe LN after other nets had been washed and the Yahe LN to be washed 20 times), two were above the maximum tolerance limit of 2.31 g AI/kg. Furthermore, the within-net variability of one of these nets exceeded the maximum recommended limit of 20% (Tables 7.4 and 7.5) (Pigeon, 2013k).

The Yahe LN washed 20 times showed similar efficacy in terms of blood-feeding inhibition and mortality of *An. gambiae* compared with a conventionally-treated net washed to exhaustion. However, these results must be interpreted with care as several Yahe LNs were above specifications and one Yahe LN exhibited unacceptably high within-net variation (Table 7.5).

7.2 Conclusions and recommendations

Yahe LN is a long-lasting insecticidal net manufactured by Fujian Yamei Industry, China. Deltamethrin is coated on 75-denier, knitted multi-filament polyester fibers at the target dose of 1.85 g AI/kg netting material, corresponding to 55.5 mg of deltamethrin per square metre of the fabric (weight 30 g/m^2).

Yahe LN was previously reviewed by WHOPES for extension of WHO interim specification 333/LN/1 (netting and net) (September 2010). The outcome of the regeneration, wash resistance and efficacy studies of Yahe LN in laboratory, as part of the requirements for extension of WHO specifications, were published in the *Report of the fourteenth WHOPES Working Group Meeting, WHO/HQ, Geneva, 10–14 April 2011*.[35] The meeting noted that the Yahe LN met WHOPES criteria for knockdown in the cone test. However, mortality rates in cone bioassays were always lower than those of the

[35] Available at: http://www.who.int/whopes/recommendations/wgm/en/

reference LN and within-net deltamethrin content exhibited high heterogeneity. Therefore, the fourteenth WHOPES Working Group Meeting concluded that Yahe LN does not meet WHO requirements for extension of specifications and should be considered an independent product requiring evidence of efficacy from phase II experimental hut studies. The Meeting also advised WHOPES to invite the manufacturer to provide supporting data on homogeneity of deltamethrin content.

In Phase II trials, the Yahe LN washed 20 times should have similar or better efficacy than a net conventionally treated with the WHO-recommended dose of the corresponding insecticide and washed until just before exhaustion. More recent WHOPES guidelines recommend that new LNs be compared with a WHOPES-recommended LN. Candidate LNs that perform as well as or better than a conventionally-treated net washed to exhaustion or a reference LN washed 20 times in terms of blood-feeding inhibition and mortality may be given an interim recommendation. As part of both phase I and phase II studies, LNs should be within specifications for insecticide content and the heterogeneity of insecticide content should not exceed recommended limits (i.e. relative standard deviation for AI within-net variability should not be more than 20%).

Two WHOPES supervised experimental hut studies of the Yahe LN comparing the PermaNet 2.0 LN with a conventionally-treated net were conducted in Thailand and the United Republic of Tanzania. It was not possible to conclude on blood-feeding inhibition in the Thailand study due to low mosquito densities and low rates of blood-feeding. The mortality of *An. minimus* in the huts with a Yahe LN washed 20 times (53%) was not significantly different from the mortality in huts with a PermaNet 2.0 LN washed 20 times (48%) or an unwashed conventionally-treated net (57%).

In the United Republic of Tanzania, the rate of blood-feeding by *An. gambiae* s.l. in huts with the Yahe LN washed 20 times (17.3%) was not significantly different compared with 12.4% for the PermaNet 2.0 LN washed 20 times and 12.8% for the conventionally-treated net washed to just before exhaustion; these differences being not statistically significant. Mortality of *An. gambiae* s.l. in huts with the Yahe LN washed 20 times was 30.3% compared with 29.7% for the PermaNet 2.0 LN washed 20 times and 26.8% for the conventionally-treated net washed to just before exhaustion.

In chemical analysis of the Yahe LN, overall retention of deltamethrin after 20 washes was 61% in the United Republic of Tanzania and 77% in Thailand. However, of 6 unwashed nets, 4 were above specifications. One net had high AI within-net variability (RSD = 30.6%) that was above the maximum recommended threshold.

Noting the above, the meeting recommended:

- that the manufacturer provide the evidence and supporting information on acceptable within-net variation in deltamethrin content and on quality assurance of Yahe LN; and

- that WHOPES conduct a minimum of one additional phase II study with the Yahe LN within the product specifications, as a requirement for an interim recommendation.

Table 7.1 Knockdown (% KD) and mortality (% Mort.) of *An. minimus* in WHO cone bioassays in Thailand. N is the sample size for each set of bioassays.

Treatment	Before washing			After washing, before field testing			After field testing		
	% KD	% Mort.	N	% KD	% Mort.	N	% KD	% Mort.	N
Untreated polyester net	0	2	50	0	0	53	0	0	51
Yahe LN, unwashed	73	58	48	88	86	49	88	56	50
Yahe LN, washed 20 times	60	69	52	100	98	54	100	82	50
PermaNet 2.0 LN, unwashed	100	100	51	100	100	52	100	100	51
PermaNet 2.0 LN, washed 20 times	98	100	51	100	100	55	100	100	50
Conventionally-treated net*	100	100	54	100	100	52	96	89	45

*The conventionally-treated net was unwashed.

Table 7.2 Knockdown (% KD) and mortality (% Mort.) of *An. gambiae* in WHO cone bioassays in the United Republic of Tanzania. N is the sample size for each set of bioassays.

Treatment	Before washing			After washing, before field testing			After field testing		
	% KD	% Mort.	N	% KD	% Mort.	N	% KD	% Mort.	N
Untreated polyester net	0	0	50	0	0	50	0	0	50
Yahe LN, unwashed	100	100	50	98	100	50	84	98	50
Yahe LN, washed 20 times	100	100	50	90	90	50	74	98	50
PermaNet 2.0 LN, unwashed	100	100	50	88	100	50	92	84	50
PermaNet 2.0 LN, washed 20 times	100	100	50	90	96	50	90	96	50
Conventionally-treated net*	100	100	51	84	91	51	84	88	51

*The conventionally-treated net was washed until just before exhaustion (3 times).

Table 7.3 Summary results obtained for free-flying, wild *Anopheles* in experimental huts in Thailand and the United Republic of Tanzania. Values in the same row sharing the same letter superscript do not differ significantly (*P*>0.05)

Treatment	Site	Un-treated net	Yahe LN, unwashed	Yahe LN, washed 20 times	PermaNet 2.0 LN, unwashed	PermaNet 2.0 LN, washed 20 times	CTN un-washed*
Females/night	Thailand	0.47[a]	0.51[a]	0.47[a]	0.43[a]	0.51[a]	0.65[a]
	Tanzania	12.4[a]	11.2[b,c]	15.1[a]	8.0[c]	15.2[a]	12.6[a,b]
Exophily (%)	Thailand	24[a]	32[a,b]	24[a]	35[a,b]	48[b]	26[a]
	Tanzania	73.9[a]	90.6[b,c]	87.5[c]	94.1[b]	90.9[b,c]	89.2[b,c]
Blood fed (%)	Thailand	6[a]	3[a]	6[a]	13[a]	11[a]	6[a]
	Tanzania	25.8[a]	13.4[b]	17.3[b]	5.9[c]	12.4[b]	12.8[b]
Mortality (%)	Thailand	6[a]	46[b,c]	53[b,c]	32[b]	48[b,c]	57[c]
	Tanzania	10.3[a]	40.2[b]	30.3[c]	31.5[c]	29.7[c]	26.8[c]

*For the Thailand trial, the CTN was unwashed while for the United Republic of Tanzania trial, the conventionally-treated net was washed to exhaustion (3 washes).

Table 7.4 Deltamethrin content and retention in Yahe LN and PermaNet 2.0 tested in WHOPES phase II trials. Target dose and tolerance limit for deltamethrin in baseline Yahe LN of 75 denier = 1.85 g AI/kg ± 25% for 75 denier yarn [1.39–2.31 g AI/kg]; target dose and tolerance limit for deltamethrin in baseline PermaNet 2.0 of 100 denier = 1.4 g AI/kg ± 25% for 100 denier yarn [1.05–1.75 g AI/kg].

Treatment	United Republic of Tanzania				Thailand			
	DM content (g AI/kg) before washing	DM content (g AI/kg) after washing	DM retention (% of wash 0)	DM content (g AI/kg) after testing	DM content (g AI/kg) before washing	DM content (g AI/kg) after washing	DM retention (% of wash 0)	DM content (g AI/kg) after testing
Yahe LN 0 wash	2.28	2.70	–	2.35	2.21	2.33	–	1.91
Yahe LN 20 washes	2.42	1.47	61	1.66	2.46	1.88	77	1.27
PermaNet 2.0 0 wash	1.52	1.56	–	1.08	1.31	1.34	–	1.19
PermaNet 2.0 20 washes	1.47	0.56	38	0.36	1.29	0.82	64	0.58
CTN, exhausted	0.42	0.02	–	0.03	0.45	0.51	–	0.74
Untreated net	-	-	-	-	< 0.01	< 0.01	-	< 0.01

CTN = conventionally-treated polyester net; DM = deltamethrin

Table 7.5 Deltamethrin (DM) average content, within-net and between-net variations, expressed as the relative standard deviation (RSD), in unwashed Yahe LN and PermaNet 2.0 tested in WHOPES phase II trials. The compliance of the mean deltamethrin content with the specification is also presented for each net. Target dose and tolerance limit for deltamethrin in baseline Yahe LN of 75 denier = 1.85 g AI/kg ± 25% for 75 denier yarn [1.39–2.31 g AI/kg]; target dose and tolerance limit for deltamethrin in baseline PermaNet 2.0 of 100 denier = 1.4 g AI/kg ± 25% for 100 denier yarn [1.05–1.75 g AI/kg].

LN		United Republic of Tanzania			Thailand			DM between-net variation (RSD)
		DM content (g AI/kg)	Compliance with specification	DM within-net variation (RSD)	DM content (g AI/kg)	Compliance with specification	DM within-net variation (RSD)	
Yahe LN	1	2.28	Yes	16.3%	2.21	Yes	10.4%	
Yahe LN	2	2.42	No	13.1%	2.46	No	9.2%	6.57%
Yahe LN	3	2.70	No	30.6%	2.33	No	9.4%	
PermaNet 2.0	1	1.52	Yes	11.5%	1.31	Yes	8.4%	
PermaNet 2.0	2	1.47	Yes	11.5%	1.29	Yes	9.0%	7.49%
PermaNet 2.0	3	1.56	Yes	12.7%	1.34	Yes	10.4%	

8. REVIEW OF SPINOSAD 83.3 MONOLAYER DT

Spinosad 83.3 monolayer DT is a single-layer tablet for direct application (25 mm in diameter and weight approximately 6 g) containing 83.3 g/kg spinosad (approximately 500 mg of spinosad per tablet). Spinosad is biologically derived from the fermentation of the bacterium *Saccharopolyspora spinosa* (Actinomycetales), a naturally occurring soil organism. Spinosad contains a complex of the active ingredients spinosyn A and D. The Insecticide Resistance Action Committee of CropLife International has classified spinosad under Group 5, describing its mode of action as nicotinic acetylcholine receptor (nAchR) allosteric activators.[36]

Spinosad monolayer DT is manufactured by Clarke Mosquito Control Products, USA, and uses spinosad technical material (TC) of Dow AgroSciences, which has served as a reference profile for developing WHO specification 636/TC February 2007.[37] Spinosad monolayer DT is designed for easy and direct application (i.e. not intended for dispersion in water prior to application) and is intended for use as a multiple-brood larvicide in small bodies of water as well as natural and artificial depressions (e.g. ornamental ponds, sewers, catch basins, storm drains), with expected residual activity of 30 days.

Spinosad 0.5% GR (granule), 12% SC (suspension concentrate), 7.48% DT(tablet for direct application) and 20.6% EC (emulsifiable concentrate) have previously been evaluated by WHO for mosquito larviciding.[38, 39, 40] A WHO safety assessment of spinosad[41] and recommendations for its use, as well as WHO specifications for

[36] *Prevention and management of insecticide resistance in vectors of public health importance*, 2nd ed. CropLife International, Insecticide Resistance Action Committee, 2010 (also available at: http://www.afpmb.org/sites/default/files/whatsnew/2011/irac_manual.pdf).
[37] Available at: http://www.who.int/whopes/quality/newspecif/en/.
[38] Available at: http://whqlibdoc.who.int/hq/2007/WHO_CDS_NTD_WHOPES_2007_1_eng.pdf.
[39] Available at: http://whqlibdoc.who.int/hq/2008/WHO_HTM_NTD_WHOPES_2008.1_eng.pdf.
[40] Available at: http://whqlibdoc.who.int/publications/2011/9789241502160_eng.pdf.
[41] Available at: http://whqlibdoc.who.int/hq/2007/WHO_CDS_NTD_WHOPES_2007_1_eng.pdf.

quality control of the named formulations, have previously been published.[42]

The 7.48% DT (Natular DT 60) formulation, previously evaluated by WHOPES, is a two-layer small round tablet (12 mm in diameter and weight approximately 1.35 g) containing nominal 74.8 g/kg spinosad (approximately 101 mg of spinosad per tablet), designed for control of container-breeding mosquitoes.

The present review assesses the efficacy of spinosad 83.3 monolayer DT (Natular T30, Clarke Mosquito Control Products, USA) for mosquito larviciding in small natural or artificial bodies of water.

8.1 Efficacy – background and supporting documents

Kisumu, Kenya

Nabie Bayoh et al (2009) evaluated spinosyn-based Natular formulations against *Anopheles gambiae* larvae in naturalistic habitats in western Kenya. These formulations were Natular T30 (Spinosad 83.3 monolayer DT); Natular G30 (Spinosad 25 extended release GR); and Natular EC an emulsifiable concentrate formulation and Vectobac WG granules. The Natular formulations were manufactured by Clarke Mosquito Control Products, USA, and Vectobac WG was provided by Valent Biosciences Corporation, USA. The trial was carried out in 2009 in Kisumu, Kenya. Naturalistic habitats were created by digging pits of 1.52 m diameter and 0.76 m depth. All pits were lined with polyethylene sheet to prevent loss of water through percolation into the ground. A layer of 2 cm of soil was added on top of the sheets and water was poured into the pits and level maintained at a constant depth of 35 cm (1.8 m^2 area of water surface). The following treatments were applied in a randomized design: (i) Natular T30 8.33% w/w formulation at the rate of 1 tablet per habitat, (ii) Natular G30 2.5% w/w formulation at the rate of 1.12 g product/m^2, (iii) Natular EC 120 g AI/L at the rate of 0.021 ml/m^2, (iv) VectoBac WG (374 g /kg; 3000 ITU) at 0.3 g product//m^2 and (v) untreated control. For each of the treatment and control arms, five replicates were tested. Screened bioassay cages (small circular cups with netting at the bottom) containing 30 third-instar larvae of *An. gambiae* s.s. Kisumu strain were floated in water in each pit. Five separate cohorts of larvae were introduced, i.e. on day 0, 8, 15, 22 and 29 post-treatment. Each cohort was held in water for 7 days.

[42] Available at: http://www.who.int/whopes/quality/newspecif/en/.

Larval mortality was recorded at 1, 2 and 3 cohort-days after introducing the larvae into floating cages.

Larval mortality in control was acceptably low for all cohorts. For the first cohort corresponding to 1–3 days post-treatment, larval mortality in pits treated with Natular T30 was 5% on day 1 and increased to 75% on day 3. Mortality in the second cohort of larvae was about 50% on day 10 post-treatment, which rose to nearly 100% on day 12. For the succeeding time intervals corresponding to 17–19, 24–26 and 32–34 days post-treatment, mortality was >80% after 72 h of exposure in the treated habitats. This showed that the active ingredient of the Natular T30 tablets released slowly during the initial few days, explaining the lower larval mortality in the first cohort of larvae and at the 24-h exposure point. Larval mortality in pits treated with spinosad GR for the first time interval (days 1–3 post-treatment) was highest across the 24 and 48 h and reached 100% at 72 h. In the later time intervals (subsequent cohorts, 2–5), mortality gradually declined and reached 60% after 72 h of exposure at 32–34 day interval after treatment. Treatment with Natular EC formulation resulted in >80% mortality in the cohort exposed from days 1 to 3 post-treatment. However, mortality in the second cohort from days 10 to 12 was <80% and further tests were terminated. Similarly, treatment of habitats with Vectobac WG formulation showed only short-term mortality during the first few days after treatment, which declined to <80% cut-off efficacy level in the second time interval. Thus, for both Natular EC and Vectobac WG formulations, active ingredient appeared to be short-lived in the habitats, compared with the Natular T30 formulation.

8.2 Efficacy – WHOPES supervised trials

Haikou, China

Liu et al (2013) undertook small-scale field trials to determine the optimum application dose of Spinosad monolayer DT formulation for evaluation in the Phase III trials. The trials were carried out in natural open bodies of clean water (2–3 m^2 plots in paddy fields) with breeding of *An. sinensis* and *Culex tritaeniorhynchus* and against *Cx. quinquefasciatus* breeding in small water bodies with aquatic plants (2–4 m^2), sewage water habitats (2–5 m^2) and wastewater pools (2–5 m^2). Spinosad monolayer DT was applied at 25, 50, 75 and 100 mg AI/m^2 in these habitats of various sizes, i.e. 20 m^2, 10 m^2, 6.7 m^2 and 5 m^2, respectively so that intact tablet could be applied directly (note: the tablet should not be broken into pieces in order to retain its slow release properties). The application of spinosad monolayer DT was

done manually over the water surface and the tablet(s) was released in the centre of the habitats. For each dosage tested and habitat, 3–5 replicates were taken and a similar number of untreated control habitats were concurrently monitored for comparison.

Larval samples were taken using a dipper (500 ml capacity) twice within one week before the treatment and again on days 1, 2, 3 and 7 and then twice every week post-treatment, and continued until one month or until the density of late instars in treated habitats levelled to that of the control. Three to five dips were taken for each replicate, and the number of larvae (first + second instars; third + fourth instars) and pupae were counted separately. Reduction in larval and pupal densities in treated habitats for each sampling point on post-treatment days was determined based on the population of these stages relative to their pre-treatment densities and taking into account the densities in the untreated habitats (Mulla et al, 1971). The dose of spinosad monolayer DT at which >80% larval and pupal mortality were observed for a longer duration was selected as the dose for evaluation in the Phase III trial for each habitat.

Application of spinosad monolayer DT in paddy fields at 25, 50, 75 and 100 mg AI/m^2 doses reduced the larval density by 82–100% (Table 8.1). Effective control of *An. sinensis* was immediately achieved within a day post-treatment and lasted up to 3 days in many replicates. The reduction in the larval density of *Cx. tritaeniorhynchus* was 84–100% in most treatment replicates, starting on day 2 until day 7 post-treatment at various doses. In small pools with aquatic plants, treatment at the above-mentioned dosage provided 81–100% reduction of *Cx. quinquefasciatus* larvae starting on day 2 until 31 days post-treatment. In open sewage water pools, spinosad monolayer DT applied at 25, 50 and 75 mg AI/m^2 dosage reduced the larval density by 85–100% with an effective duration of control starting on day 1 or 2 until day 14 after treatment. Application at 100 mg AI/m^2 reduced the larval density by 94–100% with an effective duration of control starting on day 1 and lasting until day 31. In wastewater pools, 84–100% larval control was observed with an effective duration of control starting on day 2 until day 14 after treatment at the four dosages tested.

Based on the outcome of the Phase II trials, the 50 mg AI/m^2 dose of spinosad monolayer DT was found optimum for further testing in Phase III trials in various habitats, such as paddy fields with clean water with breeding of *An. arabiensis* and *Cx. tritaeniorhynchus,* as well as open polluted water bodies such as small pools with aquatic

plants, and sewage pools and wastewater pools with breeding of *Cx. quinquefasciatus*. Effect on non-target organisms such as fish and tadpoles was observed and recorded.

In the Phase III trials, 25 replicates of each selected habitat were treated with the DT formulation and 12 replicates were taken as control. Application of spinosad monolayer DT at 50 mg AI/m^2 dose in paddy plots provided 34–71% and 4–73% control of the late-instar larvae of *An. sinensis* and *Cx. tritaeniorhynchus,* respectively during the 13-day follow-up post-treatment. In polluted water habitats (small pools with aquatic plants, sewage pools and wastewater pools) with breeding of *Cx. quinquefasciatus*, spinosad application at 50 mg AI/m^2 dose resulted in effective control (81–100%) for up to one week (i.e. from day 3 until day 7 post-treatment in small pools with aquatic plant; after 1 day in sewage water pools and from day 11 until day 15 in wastewater pools).

Pondicherry, India

Sadanandane et al (2013) carried out small- and large-scale field trials of spinosad monolayer DT in an urban area against *Culex* species breeding profusely in open street drains and disused wells containing debris with high organic matter.

The U-shaped open street drains were made of brick either cement-lined or unlined and carried domestic wastewater round the year. At their terminal end they form small pools of wastewater. Due to dumping of garbage and lack of proper gradient, they are often found choked with debris leading to slowing of water flow or stagnation of water that supported profuse breeding of *Cx. quinquefasciatus.* Disused wells polluted with floating debris and organic matter were the source of breeding of *Cx. tritaeniorhynchus*, the vector of Japanese encephalitis.

In the small-scale trials for dose determination, larval and pupal samples were collected from different types of habitats for adult mosquito emergence in the laboratory to verify prevalence of mosquito species. Mosquito immatures were sampled from drains using enamel dippers (350 ml) and from disused wells using a galvanized iron bucket (2 L) tied with a rope. Three dips were taken from each habitat replicate and samples of immatures were counted by stages and later returned to the same habitats.

Spinosad monolayer DT was applied manually over the water surface of drains at four dosages of 25, 50, 100 and 150 mg AI/m^2. Five

replicates of drains were selected for each dose. An equal number of replicates were run in parallel as untreated control for comparison. Every segment of 10 m length of drain was considered a replicate. However, while applying the tablets, the drain at its entire length was treated with one dose. Separate drains were selected for each dosage as well as for control. Spinosad was also tested at three dosages of 50, 100 and 150 mg AI/m^2 in disused wells. Four replicates of disused wells each were included for each dose and control.

Larval (instar-wise) and pupal counts were made twice during the week preceding the application of spinosad DT, again on days 1, 2, 3 and 7 post-treatment and thereafter every week until the pupal density in the treated habitats equalled to that in the untreated control. The reduction of larval and pupal densities during post-treatment period was estimated by comparing the pre- and post-treatment densities in the treated habitats with the corresponding densities in the untreated habitats using Mulla's formula.

Application of spinosad monolayer DT at 25, 50, 100 and 150 mg AI/m^2 dosages gave 0 to 15% control of *Cx. quinquefasciatus* late instars in drains. Its application in disused wells at 50, 100 and 150 mg AI/m^2 caused 1–60% control of *Cx. tritaeniorhynchus* larvae. Application of spinosad monlyaer DT therefore did not yield the desired level (≥80%) of control of immatures of *Culex* species in these particular habitats due to the product's inability to release spinosad active ingredient into the water column due to heavy organic layer covering.

Mwea, Kenya

Mathenge (2013) carried out field trials of spinosad monolayer DT in Mwea division of Kirinyaga County in Central Kenya, which is a rice irrigation scheme 100 km north-east of Nairobi. Small-scale trials for dose determination were carried out in rice paddies with *An. arabiensis* and in septic pits with *Cx. quinquefasciatus*. Several plots measuring 5 m x 5 m (25 m^2) were made using mud moulds in a large rice farm. The plots were left for a few days to allow mosquito breeding and build up of larval densities. Septic pits selected for the trial were open and unlined, measuring around 0.28 m^3, and received domestic wastewater.

The immatures of mosquito species were sampled using enamel dippers, counted by stage and returned to the habitats. Temperature and pH of water in each habitat was recorded. Pre-treatment larval and pupal densities were monitored by taking 3–5 samples per replicate twice in the week preceding the treatment.

Spinosad monolayer DT was applied manually in rice paddies at 25, 50, 75 and 100 mg AI/m^2, while in septic tanks it was applied at 25, 50 and 100 mg AI/m^2. Three replicates each of the treatment and control habitats were taken. As the tablets were to be applied intact, most septic pits did not have an area meeting the dosage requirement according to the manufacturer's label recommendation, therefore septic pits in closest range of the surface area required that various dosage were chosen. Post-treatment larval monitoring was done on day 2 and 7 and thereafter once every week until densities of late instars and pupae in the treated habitats levelled control. Reduction in larval counts was calculated using Mulla's formula described earlier.

Application of spinosad DT in rice paddies at 25 and 50 mg AI/m^2 dosage caused about 80% reduction of late-instar *An. arabiensis* larvae on day 35 post-application. Reduction in the larval density in rice paddies treated at 75 and 100 mg AI/m^2 ranged from 21% to 65% until day 35 post-treatment. The 25 mg AI/m^2 dose of spinosad monolayer DT applied in septic pits was found to give effective control of late-instar larvae of *Cx. quinquefasciatus* from 85% to 92% starting on day 7 until day 21 days post-treatment (Table 8.1). However, in septic pits treated at the dosage of 50 and 100 mg AI/m^2, the duration of effective control (81–85%) was observed on day 21 post-treatment for one day only.

Based on these observations, 25 mg AI/m^2 dose was further tested in a Phase III trial in plots of rice paddies. *An. arabiensis* was the main species found breeding in paddy fields. Pre-treatment larval counts were made twice a week. Spinosad monolayer DT was applied manually in 25 replicates of rice plots of 5m x 5 m size, while 12 replicates of rice plots were taken as control. The density of mosquito immatures was monitored from the treated as well as control habitats on day 2 and 7, and thereafter once every week post-treatment. During the first 14 days, 0–61% control of late instar larvae was observed. Thereafter from day 21 until day 31 post-treatment, 90–100% reduction of late-instar larvae was observed.

8.3 Conclusions and recommendations

Spinosad 83.3 monolayer DT (Clarke Mosquito Control Products, USA) is a single-layer tablet formulation, designed for easy and direct application for the control of mosquito breeding. Each tablet is of 25 mm diameter, weighs approximately 6 g and contains about 500 mg of spinosad (8.33%). Spinosad contains a complex of the active ingredients spinosyn A and D, and is biologically derived from the fermentation of the naturally occurring soil bacterium *Saccharopolyspora spinosa*. The tablet is to be applied intact (unbroken) so as to maintain the integrity of the chemical content and its slow-release property. The manufacturer's label recommendation is for application of spinosad DT at the rate of one tablet per 100 ft^2 or 9.3 m^2 area (i.e. 53.8 mg AI/m^2 or 538 g AI/ha) in various habitats.

The release of spinosad active ingredients depends upon the rate of dissolution of the tablet in water. If the tablet is covered by obstructions such as debris, organic matter, vegetation or loose sediment, normal release and dispersion of the active ingredients may be inhibited, resulting in reduced efficacy against mosquito larvae.

Spinosad monolayer DT was tested in simulated conditions as well as in small- and large-scale field trials in various habitats with varying levels of organic matter.

In a simulated field trial in artificial pits filled with clean water (1.52 m diameter; 0.35 m water depth; 1.8 m^2 surface area) in Kenya, spinosad monolayer DT applied at the rate of 1 tablet per pit (approximately 4 times the label recommended dose) caused 100% mortality of cohorts of third-instar larvae of *An. gambiae* s.s. for up to 30 days. As the tablets are to be applied intact, it is noted that in situations where water bodies are smaller than 9.3 m^2 area, 1 tablet per habitat may result in higher doses but give quick and longer efficacy.

Small-scale field trials of spinosad monolayer DT at four dosages of 25, 50, 75 and 100 mg AI/m^2 in paddy fields in China provided 82–100% control of *An. sinensis* larvae for 3 days and 84–100% control of *Cx. tritaeniorhynchus* larvae for 7 days post-application. Similarly, in small-scale field trials in Kenya, out of four dosages evaluated in rice paddies against *An. arabiensis*, only 25 and 50 mg AI/m^2 were effective on day 35 post-application, providing 80–81% control.

In large-scale trials in paddy fields in Kenya and China, dosages of 25 and 50 mg AI/m^2 respectively were tested vis-à-vis the manufacturer's label-recommended dose of 53.8 mg AI/m^2. In Kenya, 25 mg dose provided effective control of *An. arabiensis* larvae (>90% reduction in late larval instars) starting from only day 21 until day 31 post-treatment. The initial time taken to manifest full efficacy seems possibly due to time taken in sufficient release of the active ingredients in paddy plots having organic matter and muddy water. In China, unlike the efficacy seen in small-scale trials in rice paddies, spinosad DT at 50 mg dose produced 34–71% and 4–73% control of the breeding of *An. sinensis* and *Cx. tritaeniorhynchus* in paddy fields. Investigators attributed it largely to the tablets being embedded in mud during the test procedures.

In polluted water bodies such as pools with aquatic plants and wastewater pools having breeding of *Cx. quinquefasciatus,* spinosad monolayer DT tested at 25, 50, 75 and 100 mg AI/m^2 doses in small-scale trials in China produced 81–100% reduction in larval density starting on day 2 until day 31 post-treatment. In sewage pools, effective control of *Cx. quinquefasciatus* larvae (>80%) started after 1 or 2 days and lasted until day 14 at 25, 50 and 75 mg AI or up to day 31 at 100 mg AI/m^2 dose. In a large-scale trial in small pools of water with aquatic plants and wastewater pools with breeding of *Cx. quinquefasciatus*, application of spinosad monolayer DT at 50 mg AI/m^2 dose provided effective control (84–100% reduction) of larval density for 4 days starting on day 3 until day 7 (small pools) and starting on day 11 until day 15 (wastewater pools).

In small- and large-scale trials of spinosad monolayer DT in India, applied at 25, 50, 100 and 150 mg AI/m^2 in polluted drains with breeding of *Cx. quinquefasciatus* and at 50, 100 and 150 mg AI/m^2 in disused wells, containing high organic matter with breeding of *Cx. tritaeniorhynchus,* provided only modest control of larvae with 0 to 60% reduction in late-instar larvae. The efficacy might have been affected by flowing water in drains and deep-water column in wells apart from high organic matter layering in these habitats. In Kenya, spinosad monolayer DT applied at 25, 50 and 100 mg AI/m^2 in small-scale field trials in septic pits with breeding of *Cx. quinquefasciatus* provided 7–21 days of effective control (>80% reduction) of late-instar larvae. The lowest tested dose of 25 mg AI/m^2 was found effective up to 21 days in controlling the mosquito breeding.

The meeting noted:

- that spinosad 83.3 monolayer DT caused very low control of *Cx. quinquefasciatus* breeding in open water bodies, such as slow flowing drains, and disused wells containing high levels of organic matter.

- that the efficacy of spinosad 83.3 monolayer DT may be drastically reduced in certain situations where the tablet is covered with silt or mud.

Considering the above, and noting the safety and efficacy of spinosad 83.3 monolayer DT, the meeting:

- recommended the use of spinosad 83.3 monolayer DT in paddy fields for the control of anopheline larvae at 25–50 mg AI/m^2 dosage (1 tablet per 10–20 m^2 area; 250–500 g AI/ha) with an expected duration of efficacy of up to about 21–31 days; and

- recommended the use of spinosad 83.3 monolayer DT in open bodies of water such as wastewater pools, small pools with aquatic plants, septic pits, sewage pools at 25–50 mg AI/m^2 dosage (1 tablet per 10–20 m^2 area; 250–500 g AI/ha) for the control of culicine mosquitoes with an expected duration of efficacy of up to about 2 to 31 days depending upon the level of pollution.

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

Table 8.1 Efficacy of spinosad 83.3 monolayer DT formulation as tested against mosquito larvae of different species in various habitats. The duration of efficacy is from the day when effective control of late instars (>80% reduction in density) was first observed post-treatment until the day it lasted.

Habitat	Dose (active ingredient mg AI/m^2)	Species	Post-treatment day		Percent reduction in late-instar larvae	Country and location
			Effective control first observed	Effective Control lasted		
Paddy fields	25[a]	*An. sinensis*	1	1	90	China, Haikou
	50[a]		2	3	100	
	50[b]		0	–	34–71	
	75[a]		2	3	82–98	
	100[a]		3	3	100	
Paddy fields	25[a]	*An. arabiensis*	35	35	81	Kenya, Mwea
	50[a]		35	35	80	
	75[a]		0	–	21–65	
	100[a]		0	–	51–64	
	25[b]		21	31	90–100	

Table 8.1 contd Efficacy of spinosad 83.3 monolayer DT formulation as tested against mosquito larvae of different species in various habitats. The duration of efficacy is from the day when effective control of late instars (>80% reduction in density) was first observed post-treatment until the day it lasted.

Habitat	Dose (active ingredient mg AI/m^2)	Species	Post-treatment day		Percent reduction in late-instar larvae	Country and location
			Effective control first observed	Effective Control lasted		
Paddy fields	25[a]	*Cx. tritaeniorhynchus*	3	3	95	China, Haikou
	50[a]		3	3	94	
	50[b]		0	–	4–73	
	75[a]		2	7	84–100	
	100[a]		2	2	100	
Small pools with aquatic plants	25[a]	*Cx. quinquefasciatus*	3	31	89–100	China, Haikou
	50[a]		7	31	81–100	
	50[b]		3	7	90–100	
	75[a]		2	31	98–100	
	100[a]		7	31	100	

Table 8.1 contd Efficacy of spinosad 83.3 monolayer DT formulation as tested against mosquito larvae of different species in various habitats. The duration of efficacy is from the day when effective control of late instars (>80% reduction in density) was first observed post-treatment until the day it lasted.

Habitat	Dose (active ingredient mg AI/m^2)	Species	Post-treatment day		Percent reduction in late-instar larvae	Country and location
			Effective control first observed	Effective Control lasted		
Sewage pools	25[a]	*Cx. quinquefasciatus*	2	14	94–100	Cnina, Haikou
	50[a]		1	14	85–100	
	50[b]		1	1	81	
	75[a]		3	14	95–100	
	100[a]		1	31	94–100	
Wastewater pools	25[a]	*Cx. quinquefasciatus*	2	3	100	China, Haikou
	50[a]		2	7	100	
	50[b]		11	15	84–88	
	75[a]		2	7	90–100	
	100[a]		2	14	87–100	

Table 8.1 contd Efficacy of spinosad 83.3 monolayer DT formulation as tested against mosquito larvae of different species in various habitats. The duration of efficacy is from the day when effective control of late instars (>80% reduction in density) was first observed post-treatment until the day it lasted.

Habitat	Dose (active ingredient mg AI/m^2)	Species	Post-treatment day		Percent reduction in late-instar larvae	Country and location
			Effective control first observed	Effective Control lasted		
Septic pits	25[a]	*Cx. quinquefasciatus*	7	21	85–92	Kenya, Mwea
	50[a]		1	1	81	
	100[a]		1	1	85	
Open drains with polluted water	25[a]	*Cx. quinquefasciatus*	0	–	0–4	India, Pondicherry
	50[a]		0	–	0–0.3	
	100[a]		0	–	0–11	
	150[a]		0	–	0–15	
Disused wells	50[a]	*Cx. tritaeniorhynchus*	0	–	1–14	India, Pondicherry
	100[a]		0	–	11–60	
	150[a]		0	–	16–55	

[a]Small-scale (Phase II) trial

[b]Large-scale (Phase III) trial

9. REVIEW OF SPINOSAD 25 EXTENDED RELEASE GR

Spinosad 25 extended release GR is a granule formulation containing 25 g AI/kg spinosad. Spinosad is biologically derived from the fermentation of the bacterium *Saccharopolyspora spinosa* (Actinomycetales), a naturally occurring soil organism. Spinosad contains a complex of the active ingredients spinosyn A and D. Spinosad extended release GR is intended for direct application to open bodies of water (fresh- or wastewater) for control of mosquito larvae. The Insecticide Resistance Action Committee of CropLife International has classified spinosad under Group 5, describing its mode of action as nicotinic acetylcholine receptor (nAchR) allosteric activators.[43]

Spinosad extended release GR is manufactured by Clarke Mosquito Control Products, USA, and uses spinosad technical material (TC) of Dow AgroSciences, which has served as a reference profile for developing WHO specification 636/TC February 2007.[44]

Spinosad 0.5% GR (granule), 12% SC (suspension concentrate), 7.48% DT (tablet for direct application) and 20.6% EC (emulsifiable concentrate) have previously been evaluated by WHO for mosquito larviciding[45,46,47] at the dosage of 20–500 g AI/ha.[48] A WHO safety assessment of spinosad[49] as well as WHO specifications for quality

[43] *Prevention and management of insecticide resistance in vectors of public health importance*, 2nd ed. CropLife International, Insecticide Resistance Action Committee, 2010 (also available at: http://www.afpmb.org/sites/default/files/whatsnew/2011/irac_manual.pdf).
[44] Available at: http://www.who.int/whopes/quality/newspecif/en/.
[45] Available at: http://whqlibdoc.who.int/hq/2007/WHO_CDS_NTD_WHOPES_2007_1_eng.pdf.
[46] Available at: http://whqlibdoc.who.int/hq/2008/WHO_HTM_NTD_WHOPES_2008.1_eng.pdf.
[47] Available at: http://whqlibdoc.who.int/publications/2011/9789241502160_eng.pdf.
[48] Available at: http://www.who.int/whopes/Mosquito_Larvicides_Sept_2012.pdf.
[49] Available at: http://whqlibdoc.who.int/hq/2007/WHO_CDS_NTD_WHOPES_2007_1_eng.pdf.

control of the above said formulations, have previously been published. [50]

The present review assesses the efficacy of spinosad 25 extended release GR (Natular G30, previously known as Natular XRG, Clarke Mosquito Control Products, USA) for mosquito larviciding in open bodies of water (fresh- or wastewater) for control of mosquito larvae.

9.1 Efficacy – background and supporting documents

Kisumu, Kenya

Nabie Bayoh et al (2009) evaluated spinosyn-based Natular formulations against *Anopheles gambiae* larvae in naturalistic habitats in western Kenya. These formulations were Natular T30 (Spinosad 83.3 monolayer DT), Natular G30 (Spinosad 25 extended release GR) and Natular EC, an emulsifiable concentrate formulation, and Vectobac WG granules. The spinosad formulations have been developed by Clarke Mosquito Control Products.

The trial was carried out in 2009 in Kisumu, Kenya. Naturalistic habitats were created by digging pits of 1.52 m diameter and 0.76 m depth. All pits were lined with polyethylene sheet to prevent loss of water through percolation into the ground. A layer of 2 cm of soil was added on top of the sheets and water was poured into the pits and level maintained at a constant depth of 35 cm (1.8 m^2 area of water surface). The following treatments were applied in a randomized design: (i) Natular T30 8.33% w/w formulation at the rate of 1 tablet per habitat, (ii) Natular G30 2.5% w/w formulation at the rate of 1.12 g product/m^2, (iii) Natular EC 120 g AI/L at the rate of 0.021 ml/m^2, (iv) VectoBac WG (374 g/kg; 3000 ITU) at 0.3 g product/m^2 and (v) untreated control.

For each of the treatment and control arms, five replicates were tested. Screened bioassay cages (small circular cups with netting at the bottom) containing 30 third-instar larvae of *An. gambiae* s.s. Kisumu strain were floated in water in each pit. Five separate cohorts of larvae were introduced, i.e. on day 0, 8, 15, 22 and 29 post-treatment. Each cohort was held in water for 7 days. Larval mortality was recorded at 1, 2 and 3 cohort-days after introducing the larvae into floating cages.

[50] Available at: http://www.who.int/whopes/quality/newspecif/en/.

Control mortality was acceptably low for all cohorts. Mortality in first cohort of 30 larvae in pits treated with spinosad extended release GR was highest at day 1 and 2 post-release and reached 100% at day 3. By day 26 when four cohorts had been successively released, the larval mortality was still 90%. In the last cohort released on day 29 post-treatment, larval mortality declined and reached to 60% after 3 days of sampling post-release of larvae.

In this simulated trial, compared with the efficacy of Natular G30 formulation, larval mortality in the Natular T30 formulation in the first cohort was only 5% at day 1 post-treatment and increased to 75% at day 3. Mortality in the second cohort of third-instar larvae, released on day 8 post-treatment, was about 50% after 2 days post-treatment and rose to nearly 100% by day 4 post-releases (i.e. corresponding to day 12 post-treatment). For each succeeding cohort of larvae, corresponding to 17–19, 24–26 and 32–34 days post-treatment, larval mortality at 3 days of exposure was more than 80%. This indicated that the active ingredients of Natular T30 tablets were slowly released during the initial few days of treatments, explaining why there was lower larval mortality in the first cohort/time interval and at the 24 h exposure point.

These results indicate that, in contrast to the tablet formulation, active ingredients from the granule formulation did not sustain as long as the tablet formulation, although it provided some control of larvae up to day 34. Treatment with Natular EC formulation resulted in >80% mortality in the first cohort of larvae during 1 to 3 days post-treatment. However, mortality in the second cohort released on day 8 up to the next 4 days, corresponding to days 10 to 12 post-treatment, was <80%, so further tests were terminated. Similarly, treatment of habitats with Vectobac WG formulation showed only short-term efficacy during the first few days after treatment and mortality in the second larval cohort declined to <80%. Natular EC and Vectobac WG produced short efficacy compared with the Natular GR and Natular DT formulations.

9.2 Efficacy – WHOPES supervised trials

Haikou, China

Liu et al (2013) undertook small- and large-scale field trials to determine the optimum application dose of Spinosad extended release GR. Trials were carried out against *An. sinensis* and *Cx. tritaeniorhynchus* breeding in paddy fields (2–3 m^2 trial plots) and

against *Cx. quinquefasciatus* breeding in small pools (2–4 m^2 area) infested with water hyancinths, in sewage pools (2–5 m^2) and in wastewater pools (2–5 m^2). Spinosad extended release GR was applied manually on the water surface at 10, 20, 40 and 80 mg AI/m^2.

Sampling of larvae and pupae was done using a dipper (500 ml capacity) twice in the week preceding the treatment and again on days 1, 2, 3 and 7 and then twice every week post-treatment. The sampling continued for one month or until the density in treated sites reached that of control level or until on two consecutive sampling days the reduction in late larval instars was <80%. Three to five dips were taken in each replicate and larvae (first and second instars; third and fourth instars) and pupae were counted separately and returned to the same habitats. The reduction of larval and pupal densities on post-treatment days was estimated by comparing the pre- and post-treatment densities in the treated habitats with the corresponding densities in the untreated habitats using Mulla's formula.[51] The dosage at which >80% larval and pupal mortality was observed for a longer duration in clean and polluted water habitats was selected as the optimum field dosage for the large-scale field trial.

For each habitat, 3 replicates were treated with spinosad extended release GR and 3–9 replicates were taken as control. In the small-scale trials, application of spinosad extended release GR at four dosages (10, 20, 40 and 80 mg AI/m^2) in paddy fields reduced the larval density of *An. sinensis* by 82–100%. The treatment provided effective control of larvae ranging from 1–3 days post-treatment at 10 and 20 mg AI/m^2 doses, 1–17 days at 40 mg AI/m^2 and 1–28 days at 80 mg AI/m^2, thus providing longest duration of effective control up to 28 days (Table 9.1). The reduction in the larval density of *Cx. tritaeniorhynchus* in paddy fields was 90–100%, with duration of effective control ranging from 1–7 days at 10 and 20 mg AI/m^2, 1–21 days at 40 mg AI/m^2 and 1-31 days at 80 mg AI/m^2. Overall, the dosage of 40 and 80 mg AI/m^2 provided longest effective control of up to 21 and 31 days, respectively.

In small pools infested with aquatic plants (water hyacinths), the application of spinosad extended release GR at the four above-said dosages provided 81–100% control of *Cx. quinquefasciatus* larvae starting on day 1 or 2 post-treatment until day 17–31 (Table 9.1). Longer duration of residual efficacy of 31 days was found at 20, 40

[51] Mulla MS et al. Control of chironomid midges in recreational lakes. *Journal of Economic Entomology,* 1971, 62:300–307.

and 80 mg AI/m^2 dosages. In sewage pools with organic matter, 10 and 20 mg AI/m^2 dosages provided 2–14 days of effective control with 86–100% larval mortality. At 40 and 80 mg AI/m^2, the effective control of late instars (82–100%) was achieved after 1 or 2 days post-treatment lasting until day 28 or 31. In wastewater pools, 85–100% larval control was also observed after 1 or 2 days post-treatment, which lasted until day 10–28. Here again, longer residual efficacy until 17 and 28 days was seen at 40 mg AI/m^2 and 80 mg AI/m^2 dosages compared with 10 mg AI/m^2 or 20 mg AI/m^2.

Based on the outcome of the small-scale trials, 40 mg AI/m^2 dose of spinosad extended release GR was selected for the large-scale trials in various habitats.

The habitats selected for large-scale trials were paddy fields with clean water and with breeding of *An. sinensis* and *Cx. tritaeniorhynchus,* and polluted water bodies such as small pools infested with aquatic weed (water hyacinth), sewage pools and wastewater pools with breeding of *Cx. quinquefasciatus*. Larval and pupal samples were collected from various habitats for adult mosquito emergence in the laboratory to re-confirm the breeding of target mosquito species. Mosquito larvae and pupae were sampled using a dipper (500 ml), counted by stage and returned to the same habitats. Observation on the prevalence of non-target organisms such as fish and tadpoles was made. Spinosad extended release GR was applied manually over the water surface.

For each habitat, 25 replicates were treated with spinosad extended release GR and 12 replicates were taken as control. Application of spinosad extended release GR at 40 mg AI/m^2 dose in paddy fields provided 82–100% control of *An. sinensis* and *Cx. tritaeniorhynchus* larvae, starting on 1 day after treatment and lasting up to 7–8 days. Its application in small pools with weeds produced 99–100% control of *Cx. quinquefasciatus* just 1 day after treatment until 21 days. In sewage pools and wastewater pools with organic matter, spinosad extended release GR caused 84–90% control of *Cx. quinquefasciatus* for 1–2 days.

Pondicherry, India

Sadanandane et al (2013) carried out small- and large-scale field trials of spinosad extended release GR formulation in an urban area against *Culex* species breeding profusely in open street drains and disused wells containing high organic matter.

Open street drains (cement lined, U-shaped or unlined) carry wastewater from houses throughout the year and end up in small pools of wastewater. Due to dumping of garbage and lack of proper gradient, they are often found choked leading to slow flow or stagnation of water. Drains had profuse breeding of *Cx. quinquefasciatus,* while disused wells, polluted with floating debris and garbage, were found with breeding of *Cx. tritaeniorhynchus*, the vector of Japanese encephalitis.

In the small-scale trials for dose determination, larval and pupal samples were collected from different types of habitats for adult mosquito emergence in the laboratory to verify mosquito species breeding in them. Mosquito immatures were sampled from drains by using enamel dippers (350 ml) and from disused wells by using a galvanized iron bucket (2 L) tied to a rope. Three dips were taken from each habitat replicate and samples of immatures were counted by stage and later returned to the same habitats.

Spinosad extended release GR was applied manually over the water surface at four dosages of 25, 50, 100 and 150 mg AI/m^2. Five replicates of drains were selected for each dosage and an equal number of replicates were run in parallel as untreated control for comparison. Every segment of 10 m length of the drains was considered a replicate. However, while applying the formulation, the drain at its entire length was treated with one dose. Separate drains were selected for each dosage as well as for control. Four replicates of disused wells were selected for each treatment dosage and for control.

Larval (instar-wise) and pupal counts were made twice a week for 1 week prior to application of spinosad extended release GR formulation and on days 1, 2, 3 and 7 post-treatment and thereafter every week until the pupal density in the treated habitats reached the level equal to that in the untreated habitats.

The reduction of larval and pupal densities during post-treatment period was estimated by comparing the pre- and post-treatment densities in the treated habitats with the corresponding densities in the untreated habitats using Mulla's formula. The dosage at which >80% reduction of larval and pupal density was observed for a longer duration was selected for large-scale field trial.

Application of spinosad extended release GR formulation at 25, 50 and 100 mg AI/m^2 dosages in drains gave effective control of *Cx.*

quinquefasciatus from day 1 to 7, when the reduction in late-instar larval density ranged from 80% to 99% (Table 9.1). At the dose of 150 mg AI/m^2, the duration of effective control observed was from day 1 to day 21 and there was 100% reduction in the density of late larval instars. Application of spinosad extended release GR in disused wells at 25 and 50 mg AI/m^2 produced only 5–57% control of *Cx. tritaeniorhynchus* larvae. At 100 and 150 mg AI/m^2 dosages, the duration of effective control was for 1–7 days with 85–100% reduction in larval density.

Based on the outcome of the small-scale field trials of spinosad extended release GR, the dose of 150 mg AI/m^2 was further tested in a large-scale trial in drains and disused wells. In all, 24 disused wells and 11 drains (55 segments) were selected for the trial. Larval and pupal densities in the selected habitats were monitored twice in the week preceding the day of treatment. Thereafter, 18 wells and 8 drains with a minimum of 40 segments (replicates) were treated with spinosad extended release GR. Fifteen segments of 3 drains were kept as controls.

The impact on larval pupal densities was monitored on days 1, 2, 3 and 7 post-treatment and thereafter at weekly intervals in treated and control habitats until the mean pupal density in the treated habitats equalled that in the untreated controls.

Application of spinosad extended release GR in wastewater drains provided effective control of *Cx. quinquefasciatus* larvae for 1–14 days with 88–100% reduction in the density of late larval instars. In wells with high organic matter and more depth of water, spinosad GR gave effective control of *Cx. tritaeniorhynchus* for 2–28 days with 90–99% reduction in larval density.

Mwea, Kenya

Mathenge (2013) carried out field trials in Mwea division of Kirinyaga County in Central Kenya, which is a rice irrigation scheme 100 km north-east of Nairobi. A small-scale trial of spinosad extended release GR was carried out in paddy fields and septic pits with breeding of *An. arabiensis* and *Cx. quinquefasciatus,* respectively. Immatures of the two mosquito species were sampled using enamel dippers, counted by stage and returned to same habitats. The temperature and pH of water in each type of habitat were recorded. Pre-treatment larval and pupal densities were monitored by taking 3–5 samples per replicate twice in the week preceding application of spinosad extended release GR.

Spinosad extended release GR was applied manually by hand-casting over water surfaces at 25, 50 and 100 mg AI/m^2. A minimum of three replicates of each habitat were treated at each dosage or kept as control. Post-treatment larval monitoring was done on second and seventh day and thereafter once every week until late instars and pupal densities in the treated habitats reached the level of untreated ones. Reduction in larval counts was analysed using Mulla's formula.

Application of spinosad extended release GR in paddy fields (rice paddies) at 25 mg AI/m^2 dosage provided effective control of late-instar larvae of *An. arabiensis* (87–100% reduction) after day 14 post-treatment that lasted up to day 35 (Table 9.1). At 50 and 100 mg AI/m^2 dosages, 89–100% control of late instar larvae was observed on day 7 and lasted until day 28 post-treatment.

In septic pits with breeding of *Cx. quinquefasciatus*, 25 mg AI/m^2 dose was found to produce effective larval control (86–88%) after day 14 and lasting until day 28 (Table 9.1). At the dosage of 50 and 100 mg AI/m^2, effective control of 81–97% was observed after day 7 or 21 post-treatment and lasting until day 28 or 35, respectively.

Thus, treatment of rice paddies and septic pits with spinosad extended release GR at 25 mg AI/m^2 dose gave effective control after 14 days until 35 days post-treatment and after 14 days until 28 days, respectively. This dose was therefore selected for further testing in large-scale trials.

In the large-scale trials, treatment was applied at the dose of 25 mg AI/m^2 in 25 plots each of paddy fields (rice paddies) in the farming area that had breeding of mainly An*. arabiensis,* and in septic pits in institutional areas with breeding of *Cx. quinquefasciatus.* Twelve plots of paddy fields and 12 speptic pits were kept as control. The density of immature stages was monitored from the treated as well as control paddies.

Application of spinosad extended release GR formulation in paddy fields provided 88–100% reduction of late-instar larvae of *An. arabiensis* and effective control was observed after day 7 until day 31 post-treatment (Table 9.2). In septic pits, effective control of Cx. *quinquefascuiatus* (87–100% reduction in late-instar larval density) was observed after day 14 until day 31 post-treatment.

9.3 Conclusions and recommendations

Spinosad 25 extended release GR (Clarke Mosquito Control Products, USA) is a granule formulation for the control of mosquito larvae. The formulation contains 25 g/kg spinosad (spinosyn A and spinosyn D) and is intended for direct application to open bodies of water (fresh or wastewater). The label-recommended dose of spinosad 25 extended release GR is 140–560 g AI/h (14–56 mg AI/m^2).

In a simulated field trial carried out in artificial pits filled with clean water, spinosad extended release GR at 28 mg AI/m^2 (1.12 g product/m^2) caused 100% mortality after 72 h of application in the first cohort of third instar *An. gambiae* larvae released soon after treatment. In the subsequent cohorts of larvae, 90% mortality was observed until 26 days post-treatment and the mortality later declined to 60% at day 34 post-treatment.

In China, small-scale field trials of spinosad extended release GR carried out at 40 and 80 mg AI/m^2 dosages in paddy fields provided effective control ($\geq$80% larval reduction) of *An. sinensis* 1 day post-treatment and lasted until days 17 and 28, respectively. In the same habitat, effective control of *Cx. tritaeniorhynchus* was seen 1 day post-treatment and lasted until day 21 and day 31 for the two dosages, respectively. The 10 and 20 mg AI/m^2 dosages were effective for 1–7 days against both species found breeding in paddy fields.

In small-scale trials in Kenya, spinosad extended release GR applied at 50 and 100 mg AI/m^2 dosages in paddy fields with breeding of *An. arabiensis* were effective from 7 day post-treatment lasting until day 28, whereas, at 25 mg AI/m^2, it gave effective control at day 14 post-treatment until day 35.

In the large-scale trials in paddy fields treated at 40 mg AI/m^2 dose of spinosad extended release GR in China, effective control of late-instar larvae of *An. sinensis* (82–100%) was achieved 1 day post-treatment and lasted until day 7. Similarly, 84–97% reduction of *Cx. tritaeniorhynchus* was achieved 1 day post-treatment and lasted until day 8. In Kenya, application of spinosad GR in paddy fields at 25 mg AI/m^2 produced 88–100% reduction of late-instar larvae of *An. arabiensis* on day 7 post-treatment and lasted until day 31.

In small-scale trials carried out in China in polluted water bodies such as pools with aquatic plants, sewage water pools and wastewater

pools with breeding of *Cx. quinquefasciatus*, application of spinosad extended release GR at 10, 20, 40 and 80 mg AI/m^2 dosages produced effective control (81–100%) usually within 1–3 days after treatment and lasted until about 2–4 weeks. In small-scale trials carried out in Kenya in septic pits, application of spinosad extended release GR at 25, 50 and 100 mg AI/m^2 gave effective control (81–97%) of late-instar larvae of *Cx. quinquefasciatus* after 7–21 days of treatment and lasted until 28–35 days.

In small-scale trials in wastewater drains in India, spinosad extended release GR was applied at 25, 50, 100 mg AI/m^2 dosages. Effective control (81–99%) of late larval instars of *Cx. quinquefasciatus* was observed from day 1–3 post-treatment and lasted until day 7. Moreover, the 150 mg AI/m^2 dose gave 100% larval control from day 1 until day 21 post-treatment. In disused wells with breeding of *Cx. tritaeniorhynchus*, 100 and 150 mg AI/m^2 dosages gave 85–100% larval reduction from day 1–3 until day 7 post-treatment.

In large-scale trials in pools with aquatic plants in China, spinosad extended release GR applied at 40 mg AI/m^2 was effective in controlling the breeding of *Cx. quinquefasciatus* from day 1 until day 21 post-treatment, whereas in sewage- and wastewater pools, the duration of effective control was observed from day 7 or 8 post-treatment and lasted for only 1 or 2 days thereafter. In large-scale trial trials in septic pits in Kenya, 25 mg AI/m^2 dose of spinosad GR gave 87–100% reduction in late-instar larval density from day 14 until day 31 post-treatment. In India, 150 mg AI/m^2 dose gave effective control of *Cx. tritaeniorhynchus* in disused wells, having organic matter and more depth of water, from day 2 until day 28 post-treatment and that of *Cx. quinquefasciatus* in drains from day 1 until day 14 post-treatment.

Considering the above, and noting the safety and efficacy of Spinosad extended release GR, the meeting recommended:

- the use of spinosad 25 extended release GR in open bodies of clean water such as paddy fields as well as in polluted water bodies (e.g. septic pits, sewage pools, pools with aquatic plants and wastewater pools) at 25–40 mg AI/m^2 (250–400 g AI/ha) dosage with an expected duration of efficacy up to about 4 weeks; and

- the use of spinosad extended release GR at higher dosage of 100–150 mg AI/m^2 (1000–1500 g AI/ha), with an expected

duration of efficacy up to about 1–2 weeks, for the control of *Culex quinquefasciatus* in open bodies of water with high organic pollution provided that the water bodies are confined and are not accessible to animals.

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

Table 9.1 Efficacy of spinosad extended release GR as tested against mosquito larvae in small-scale trials against various species in different habitats. The duration of efficacy is from the day when effective control of late instars (>80% reduction in density) was first observed post-treatment until the day it lasted.

Habitat	Dose (active ingredient in mg/m^2)	Species	Post-treatment day		Percent reduction in late-instar larvae	Country and location
			Effective control first observed	Effective control lasted		
Rice fields	10	*An.*	1	3	95–100	China,
	20	*sinensis*	1	3	89–100	Haikou
	40		1	17	82–100	
	80		1	28	89–100	
	10	*Cx.*	1	7	90–100	China,
	20	*tritaeniorhynchus*	1	7	99–100	Haikou
	40		1	21	97–100	
	80		1	31	93–100	
	25	*An.*	14	35	87–100	Kenya,
	50	*arabiensis*	7	28	98–100	Mwea
	100		7	28	89–100	

Table 9.1 contd Efficacy of spinosad extended release GR as tested against mosquito larvae in small-scale trials against various species in different habitats. The duration of efficacy is from the day when effective control of late instars (>80% reduction in density) was first observed post-treatment until the day it lasted.

Habitat	Dose (active ingredient in mg/m^2)	Species	Post-treatment day		Percent reduction in late-instar larvae	Country and location
			Effective control first observed	Effective control lasted		
Pools with aquatic plants	10	*Cx. quinquefasciatus*	7	17	98–100	China, Haikou
	20		2	31	96–100	
	40		2	31	87–100	
	80		1	31	81–100	
Sewage pools	10	*Cx. quinquefasciatus*	3	14	100	China, Haikou
	20		2	14	86–100	
	40		2	31	98–100	
	80		1	28	82–100	
Wastewater pools	10	*Cx. quinquefasciatus*	2	10	87–100	China, Haikou
	20		1	14	85–100	
	40		1	17	90–100	
	80		2	28	95–100	

Table 9.1 contd Efficacy of spinosad extended release GR as tested against mosquito larvae in small-scale trials against various species in different habitats. The duration of efficacy is from the day when effective control of late instars (>80% reduction in density) was first observed post-treatment until the day it lasted.

Habitat	Dose (active ingredient in mg/m^2)	Species	Post-treatment day		Percent reduction in late-instar larvae	Country and location
			Effective control first observed	Effective control lasted		
Septic pits	25	*Cx.*	14	28	86–88	Kenya,
	50	*quinquefasciatus*	7	28	88–97	Mwea
	100		21	35	81–88	
Wastewater drains	25	*Cx.*	3	3	81	India,
	50	*quinquefasciatus*	1	7	80–99	Pondicherry
	100		2	7	83–95	
	150		1	21	100	
Disused wells	25	*Cx. tritaeniorhynchus*	0	_	27–57	India,
	50		0	_	5–36	Pondicherry
	100		3	7	87	
	150		1	7	85–100	

Table 9.2 Efficacy of spinosad extended release GR as tested against mosquito larvae in large-scale trials against various species in different habitats. The duration of efficacy is from the day when effective control of late instars (>80% reduction in density) was first observed post-treatment until the day it lasted.

Habitat	Dose (active ingredient in mg/m$^{2)}$	Species	Post-treatment day		% reduction in late instar larval density	Country and location
			Effective control first observed	Effective control lasted		
Rice fields	40	*An. sinensis*	1	7	82–100	China, Haikou
	40	*Cx. tritaeniorhynchus*	1	8	84–97	China, Haikou
	25	*An. arabiensis*	7	31	88–100	Kenya, Mwea
Pools with aquatic plants	40	*Cx. quinquefasciatus*	1	21	99–100	China, Haikou
Sewage pools	40	*Cx. quinquefasciatus*	7	10	84–86	China, Haikou

Table 9.2 contd Efficacy of spinosad extended release GR as tested against mosquito larvae in large-scale trials against various species in different habitats. The duration of efficacy is from the day when effective control of late instars (>80% reduction in density) was first observed post-treatment until the day it lasted.

Habitat	Dose (active ingredient in mg/m$^{2)}$	Species	Post-treatment day		% reduction in late instar larval density	Country and location
			Effective control first observed	Effective control lasted		
Wastewater pools	40	*Cx. quinquefasciatus*	8	8	90	China, Haikou
Septic pits	25	*Cx. quinquefasciatus*	14	31	87–100	Kenya, Mwea
Wastewater drains	150	*Cx. quinquefasciatus*	1	14	88–100	India, Pondicherry
Disused wells	150	*Cx. tritaeniorhynchus*	2	28	90–99	India, Pondicherry

10. GENERAL RECOMMENDATIONS

The sixteenth WHOPES working group meeting made the following general recommendations:

- WHOPES to develop specific guidance for study design and statistical analysis to support testing and evaluation of insecticides for public health use according to existing WHOPES guidelines with priority given to LNs and IRS. Specific guidelines for reporting, including shell tables for presentation of data should be included as part of these supplementary guidelines;

- WHOPES to facilitate the development of guidelines for the description and characterization of mosquito strains used for product evaluation; and

- WHOPES to review the procedures for determining the quality of IRS applications.

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ANNEX 2. REFERENCES

Balkew M (2010). *Report on wall bioassay of pirimiphos-methyl, lambda-cyhalothrin and bendiocarb from Ethiopia*. Report submitted to Research Triangle International. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Bhatt RM et al (2013). *Randomised village-scale evaluation to compare the efficacy of two different dosages of Chlorfenapyr SC (240g ai/l) used in indoor residual spraying for malaria vector control in Chhattisgarh state, India*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Boakye DA et al (2013a). *Phase III evaluation to compare insecticidal efficacy and community acceptance of long-lasting insecticidal nets (DURANET) with conventional insecticide treated nets in Ghana*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Boakye DA et al. (2013b). *Phase III evaluation to compare insecticidal efficacy and community acceptance of long-lasting insecticidal nets (NETPROTECT) with conventional insecticide treated nets in Ghana*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Bouriama A et al (2012). *Field evaluation of a Chlorfenapyr suspension concentrate (240g/l) from BASF© against natural populations of Anopheles gambiae in experimental huts, Benin*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES); DOC/IRD/ABC/02/2012].

Brooke B et al (2013). *Small-scale field testing and evaluation to compare efficacy and residual action of a long lasting polymer-enhanced deltamethrin formulation against K-Othrine WG and Icon sprayed on local indoor surfaces in Mpumalanga Province, South Africa*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Chanda E et al (2013). *Efficacy of a capsule suspension formulation of pirimiphos-methyl, ACTELLIC® 300 CS, for indoor residual spraying in areas of high vector resistance to pyrethroids and carbamates in Zambia*. National Malaria Control Centre, Ministry of Health, Lusaka, Zambia. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Coetzee M et al (2012). *Small-scale field testing and evaluation to compare efficacy and residual action of Pirimiphos-methyl 30% CS with Pirimiphos-methyl 50% EC sprayed on local indoor surfaces in South Africa*. Malaria Entomology Research Unit, University of the Witwatersrand, Johannesburg, South Africa. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Coosemans M et al (2012). *Small-scale evaluation of the efficacy and residual activity of Pirimiphos-methyl 30%CS for indoor spraying in comparison with Pirimiphos-methyl 500 Emulsifiable Concentrate.* [Unpublished report to WHO Pesticide Evaluation Scheme (WHOPES)].

D'Alessandro U et al (2012). *Randomised village-scale comparison of two different dosages of Chlorfenapyr SC (240g ai/l) with DDT for indoor residual spraying for malaria vector control in The Gambia*. [Unpublished report to WHO Pesticide Evaluation Scheme (WHOPES)].

D'Alessandro U et al (2013). *Randomised village-scale evaluation to compare the efficacy of pirimiphos-methyl CS (300 g AI/L) with pirimiphos-methyl EC (500 g AI/L) and with DDT for indoor residual spraying for malaria vector control in The Gambia (SCC number 1295)*. Medical Research Council Laboratories (UK) The Gambia. [Unpublished Report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Duchon S et al (2013). *Field evaluation of a deltamethrin long-lasting insecticidal net (Yahe LN) against natural populations of Anopheles minimus in experimental huts, Thailand*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Finnish Institute of Occupational Health (2010). *Exposures and health risks associated with indoor residual spraying of Deltamethrin SC 62.5*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Finnish Institute of Occupational Health (2011). *Exposures and health risks associated with indoor residual spraying of Actellic® 300 CS.* [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Finnish Institute of Occupational Health (2012). *Exposures and health risks associated with indoor residual spraying of chlorfenapyr (BAS 306 1) assessed using the WHO Generic Model for Indoor Residual Spraying of Insecticides Revision 1.* [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Gimnig J et al (2013a). *Field evaluation of the Duranet in western Kenya*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Gimnig J et al (2013b). *Field evaluation of the Netprotect in western Kenya*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Konate L, Diagne M, Faye O (2013) Determining *the residual effectiveness of pirimiphos-methyl (ACTELLIC® 50 EC and 300 CS) for indoor residual spraying (IRS) in Senegal*. Report of Laboratoire d'Ecologie Vectorielle et Parasitaire (LEVP), UCAD, Dakar, Senegal. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Liu Q, Meng F, Ren D (2013). *Field evaluation (Phase II and Phase III) of two spinosad formulations — Natular G30, an extended release granule formulation and Natular T30, a tablet formulation, against Culex quinquefasciatus, Cx. tritaeniorhynchus and Anopheles sinensis in Haikou, China*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Mathenge E (2013). *Report of Phase II and III field trials to evaluate the efficacy of spinosad tablet for direct application (spinosad T30) and spinosad extended release granules (spinosad G30) against Culex and Anopheles mosquito larvae in Kenya*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Morgan J, Hemingway J (2012). *Assessment of the WHO discriminating dosage and stability test of pirimiphos-methyl impregnated papers*. Liverpool School of Tropical Medicine, UK. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Nabie Bayoh M et al (2013). *Efficacy of extended release formulations of Natular^{R} (spinosad) against Anopheles gambiae larvae (Diptera: Culicidae) in naturalistic habitats in western Kenya*.

[Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

N'Guessan R et al (2007). Chlorfenapyr: A pyrrole insecticide for the control of pyrethroid or DDT resistant *Anopheles gambiae* (Diptera: Culicidae) mosquitoes. *Acta Tropica*, 102:69–78.

N'Guessan R et al (2009). Control of pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes with chlorfenapyr in Benin. *Tropical Medicine and International Health* 14:389–395.

Ngufor C et al (2011). Combining indoor residual spraying with chlorfenapyr and long-lasting insecticidal bed nets for improved control of pyrethroid-resistant *Anopheles gambiae*: an experimental hut trial in Benin. *Malaria Journal*, 10:343.

Odhiambo MTO et al (2013). *Evaluation of polythylene based long-lasting treated bednet Netprotect on population density of Anopheles mosquitoes in western Kenya*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Oliver SV et al (2010). Evaluation of the pyrrole insecticide chlorfenapyr against pyrethroid resistant and susceptible *Anopheles funestus* (Diptera: Culicidae). *Tropical Medicine and International Health*, 15:127–131.

Oxborough RM et al (2010). Evaluation of indoor residual spraying with the pyrrole insecticide chlorfenapyr against pyrethroid-susceptible *Anopheles arabiensis* and pyrethroid-resistant *Culex quinquefasciatus* mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 104:639–645.

Oxborough R (2013). *Laboratory and simple hut bioassay evaluation of Syngenta microencapsulated pirimiphos-methyl (Actellic® 300 CS) for IRS at KCMUCo, Tanzania*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2009). *Determination of alpha-cypermethrin in Duranet and in nets conventionally treated with alpha-cypermethrin (60 samples for the WHOPES large-scale Phase III testing and evaluation of Duranet in Sundargarh District, Orissa, India).* Test report N° 22058. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2010a). *Determination of alpha-cypermethrin in Duranet and Conventional ITN (63 baseline samples for the WHOPES Phase III testing and evaluation of Duranet in Ghana).* Test report N° 22234. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2010b). *Determination of deltamethrin in Netprotect and conventionally treated nets (60 baseline samples for the WHOPES Phase III testing and evaluation of Netprotect in Cambodia).* Test report N° 22255-A. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2010c). *Determination of deltamethrin in PermaNet 2.0 (31 baseline samples for the WHOPES Phase III testing and evaluation of Netprotect in Cambodia).* Test report N° 22255-B. [Unpublished report to the Institute of Tropical Medicine, Antwerp, Belgium].

Pigeon O (2010d). *Determination of deltamethrin in Netprotect and Conventional ITN (60 baseline samples for the WHOPES Phase III testing and evaluation of Netprotect in Ghana).* Test report N° 22242. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2011a). *Determination of chlorfenapyr, deltamethrin and bendiocarb in filter papers (25 samples for the WHOPES testing of products in Benin).* Test report N° 22642. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2011b). *Determination of deltamethrin in filter papers (84 samples for the WHOPES testing and evaluation of deltamethrin 62.5 g/L SC in India).* Test report N° 22690. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2011c). *Determination of alpha-cypermethrin in conventionally treated nets (ITN) (30 samples collected after 1 year of field use for the WHOPES Phase III testing and evaluation of Duranet in Orissa State, India).* Test report N° 22337/1. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2011d). *Determination of alpha-cypermethrin in conventionally treated nets (ITN) (30 samples collected after 1 year of field use for the WHOPES Phase III testing and evaluation of Duranet in Ghana).* Test report N° 22337/2. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2011e). *Determination of deltamethrin in Netprotect, PermaNet 2.0 and conventionally treated nets (93 samples collected after 6 months for the WHOPES Phase III testing and evaluation of Netprotect in Cambodia).* Test report N° 22421. [Unpublished report to the Institute of Tropical Medicine, Antwerp, Belgium].

Pigeon O (2011f). *Determination of deltamethrin in Netprotect and conventionally treated nets (60 samples collected after 1 year of household use for the WHOPES Phase III testing and evaluation of Netprotect in Cambodia).* Test report N° 22614-A. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2011g). *Determination of deltamethrin in PermaNet 2.0 (30 samples collected after 1 year of household use for the WHOPES Phase III testing and evaluation of Netprotect in Cambodia).* Test report N° 22614-B. [Unpublished report to the Institute of Tropical Medicine, Antwerp, Belgium].

Pigeon O (2012a). *Determination of pirimiphos-methyl in filter papers (96 samples for the WHOPES IRS testing and evaluation of Pirimiphos-methyl 30% CS compared to Pirimiphos-methyl 50% EC in South Africa).* Test report N° 22930. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2012b). *Determination of pirimiphos-methyl in filter papers (72 samples for the WHOPES IRS testing and evaluation of Pirimiphos-methyl 30% CS compared to Pirimiphos-methyl 50% EC in India).* Test report N° 22671. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2012c). *Determination of pirimiphos-methyl in filter papers (144 samples for the WHOPES IRS testing and evaluation of Pirimiphos-methyl 30% CS compared to Pirimiphos-methyl 50% EC in Vietnam).* Test report N° 22671. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2012d). *Determination of pirimiphos-methyl in filter papers (24 samples for the WHOPES IRS testing and evaluation of Pirimiphos-methyl 30% CS in Vietnam).* Test report N° 23031/A1. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2012e). *Determination of pirimiphos-methyl and p,p'-DDT in filter papers (57 samples for the WHOPES IRS testing and*

evaluation of Pirimiphos-methyl 30% CS in The Gambia). Test report N° 23031/A2. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2012f). *Determination of chlorfenapyr and deltamethrin in filter papers (96 samples for the WHOPES IRS testing and evaluation of Chlorfenapyr 24% SC compared to Deltamethrin 25% WG in Vietnam).* Test report N° 23031/B1. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2012g). *Determination of chlorfenapyr and p,p'-DDT in filter papers (67 samples for the WHOPES IRS testing and evaluation of Chlorfenapyr 240 g/l SC in The Gambia).* Test report N° 22765. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2012h). *Determination of deltamethrin in filter papers (120 samples for the WHOPES IRS testing and evaluation of Deltamethrin 62.5 g/L SC compared to Deltamethrin 25% WG in Vietnam).* Test report N° 23031/B2. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2013a). *Chemical analysis of 54 samples of filter papers treated with pirimiphos-methyl from the WHOPES Phase III IRS study on Pirimiphos-methyl 30% CS compared to Pirimiphos-methyl 50% EC in India.* Test report N° 23184. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2013b). *Chemical analysis of 180 samples of filter papers treated with chlorfenapyr and deltamethrin from the WHOPES Phase III IRS study on Chlorfenapyr 24% SC compared to Deltamethrin 25% WG in India.* Test report N° 23212. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2013c). *Chemical analysis of 135 samples of filter papers treated with deltamethrin from the WHOPES Phase III IRS study on Deltamethrin 62.5 g/L SC compared to Deltamethrin 25% WG in Mexico.* Test report N° 23237. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2013d). *Chemical analysis of 50 samples of Duranet collected after 3 years of household use from the WHOPES Phase III study of Duranet in India.* Test report N° 22185/1. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2013e). *Chemical analysis of 50 samples of Duranet collected after 3 years of household use from the WHOPES Phase III study of Duranet in Ghana.* Test report N° 22185/2. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2013f). *Chemical analysis of 26 samples of Netprotect collected after 3 years of household use from the WHOPES Phase III study of Netprotect in Cambodia.* Test report N° 23178/A1. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2013g). *Chemical analysis of 30 samples of PermaNet 2.0 collected after 3 years of household use from the WHOPES Phase III study of Netprotect in Cambodia.* Test report N° 23178/B. [Unpublished report to the Institute of Tropical Medicine, Antwerp, Belgium].

Pigeon O (2013h). *Chemical analysis of 27 and 29 samples of Netprotect collected after 1 and 2 years of household use from the WHOPES Phase III study of Netprotect in Ghana.* Test report N° 23364. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2013i). *Chemical analysis of 50 samples of Netprotect collected after 3 years of household use from the WHOPES Phase III study of Netprotect in Ghana.* Test report N° 23178/A2. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2013j). *Chemical analysis of 90 samples of Yahe LN, PermaNet 2.0, conventionally treated nets and untreated nets for the WHOPES Phase II testing and evaluation of Yahe LN in Thailand.* Test report N° 23317. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Pigeon O (2013k). *Chemical analysis of 75 samples of Yahe LN, PermaNet 2.0 and conventionally treated nets for the WHOPES Phase II testing and evaluation of Yahe LN in Tanzania.* Test report N° 23284. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Prakash A (2012). *Cluster-randomized village-scale (Phase III) evaluation of deltamethrin SC 62.5 g AI/L for indoor residual spraying against Anopheles minimus s.l., the major malaria vector in north-east*

India. Regional Medical Research Centre, Indian Council of Medical Research, Assam, India. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Raghavendra K et al (2011a). Chlorfenapyr: a new insecticide with novel mode of action can control pyrethroid resistant malaria vectors. *Malaria Journal*, 10:16.

Raghavendra K et al (2011b). Evaluation of the pyrrole insecticide chlorfenapyr for the control of *Culex quinquefasciatus* Say. *Acta Tropica*, 118:50–55.

Rodríguez A (2013). *Testing and evaluation of Deltamethrin SC (62.5 g a.i./L) for indoor residual spraying against malaria vectors in a cluster-randomized (Phase III) trial in Mexico*. Centro Regional de Investigación en Salud Pública, Instituto Nacional de Salud Pública, Chiapas, Mexico. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Rossignol M et al (2011). *Intrinsic toxicity and irritant properties of chlorfenapyr against Anopheles gambiae according to WHOPES phase I protocols*. [Unpublished report to WHO Pesticide Evaluation Scheme (WHOPES); DOC/LIN/IRD/07/11].

Rowland M (2010a). *Residual activity of Bayer deltamethrin SC 62.5 compared to DDT under ambient household condition using bioassays in simple experimental huts at KCMC, Moshi, Tanzania*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Rowland M (2010b). *Efficacy of Bayer deltamethrin SC 62.5 compared to DDT WP against free flying Anopheles arabiensis in experimental hut trial, KCMC, Moshi, Tanzania*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Rowland M et al (2013). A new long-lasting indoor residual formulation of the organophosphate insecticide pirimiphos-methyl for prolonged control of pyrethroid-resistant mosquitoes: an experimental hut trial in Benin. *PLoS ONE*, 8:e69516.

Sadanandane C, Gunasekaran K, Jambulingam P (2013). *Small- and large-scale evaluation of Natular™ G30 and T30 formulations against immatures of Culex species in polluted water habitats in India*.

[Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Sharma SK et al (2013). *Phase III evaluation to compare insecticidal efficacy and community acceptance of long-lasting insecticidal net Duranet with conventional insecticide treated nets in India*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Srivastava HC, Haq S, Pant CS (2012). *Small-scale field testing and evaluation to compare residual efficacy of primiphos-methyl CS (Actellic 30% CS) with primiphos-methyl 50%EC by indoor residual spraying on different local indoor surfaces in Gujarat, India. National Institute of Malaria Research, Delhi, India*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Srivastava HC, Pant CS, Oza PJ (2013). *A randomized village-scale evaluation to compare the efficacy of pirimiphos-methyl CS (300 g AI/L) with pirimiphos-methyl EC (500gAI/L) by indoor residual spraying for malaria vector control in Gujarat state, India. National Institute of Malaria Research, Gujarat, India*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Tungu K et al (2013). *Evaluation of Yahe LN against Anopheles gambiae s.l. and Culex quinquefasciatus in experimental huts in Tanzania*. National Institute for Medical Research, Amani Research Centre, Muheza, Tanzania and London School of Hygiene and Tropical Medicine, London, UK. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Van Roey K et al (2013a). *Small-scale evaluation of the efficacy and residual activity of pyrimiphos-methyl 30%CS for indoor spraying in comparison with pyrimiphos-methyl 500 emulsifiable concentrate in Vietnam (Trial 2 & 3)*. Institute of Tropical Medicine, Antwerp, Belgium. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Van Roey K et al (2013b). *Small-scale evaluation of the efficacy and residual activity of Chlorfenapyr 24% SC for indoor spraying in comparison with Deltamethrin 25% water dispersible granules. Final report of 19 March 2013*. NIMPE-Vietnam - ITMA-Belgium. [Unpublished report to WHO Pesticide Evaluation Scheme (WHOPES)].

Van Roey K et al (2013c). *Small-scale evaluation of the efficacy and residual activity of deltamethrin 62.5% SC for indoor spraying in comparison with Deltamethrin 25% water dispersible granules*. Institute of Tropical Medicine, Antwerp, Belgium. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

Van Roey K et al (2013d). *Large-scale field trial (Phase III) to study the efficacy, longevity and community acceptance of Netprotect*. [Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)].

ANNEX 3. CHEMICAL ASSAY OF FILTER-PAPERS USED IN THE QUALITY CONTROL OF IRS APPLICATIONS

The filter-papers collected for chemical analysis were wrapped in aluminium foil and sent to the Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium (WHO Collaborating Centre for Quality Control of Pesticides). Upon receipt they were stored into a deep freezer at –18°C until the chemical analysis. Each filter-paper was weighed, the surface was accurately measured and the filter-paper was cut into small pieces before chemical analysis.

The chemical analysis of filter-papers sprayed with pirimiphos-methyl was performed using an analytical method based on the CIPAC method for pirimiphos-methyl in technical and formulated products. Pirimiphos-methyl was extracted by ultra-sonication in acetone for 15 min with 4,4'-dimethoxybenzophenone as internal standard. The pirimiphos-methyl content was measured by gas chromatography with flame ionization detection (GC-FID) using internal standard calibration.

The chemical analysis of filter-papers sprayed with chlorfenapyr was performed using an analytical method based on the CIPAC method for chlorfenapyr in technical and formulated products. Chlorfenapyr was extracted by ultra sonication in acetonitrile for 10 min. The chlorfenapyr content was measured by high performance liquid chromatography with UV diode array detection (HPLC-DAD) using external standard calibration.

The chemical analysis of filter-papers sprayed with deltamethrin was performed using an analytical method based on the CIPAC method for deltamethrin in technical and formulated products. Deltamethrin was extracted in isooctane/dioxane (80/20, v/v) by ultra-sonication for 15 min and then by shaking for 30 min with dipropyl phthalate as internal standard. The deltamethrin content was measured by high performance liquid chromatography with UV diode array detection (HPLC-DAD) using internal standard calibration.

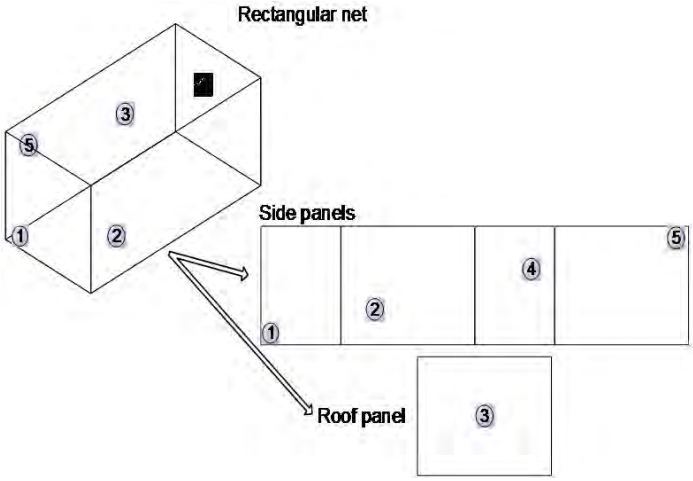
The chemical analysis of filter-papers sprayed with bendiocarb was performed using an analytical method based on the CIPAC method for bendiocarb in technical and formulated products. Bendiocarb was extracted in acetonitrile by ultra-sonication for 20 min with propiophenone as internal standard. The bendiocarb content was measured by ultra-high performance liquid chromatography with UV

diode array detection (UHPLC-DAD) using internal standard calibration.

The chemical analysis of filter-papers sprayed with DDT was performed using an analytical method based on the CIPAC method for DDT in technical and formulated products. DDT was extracted by ultra-sonication in acetone for 15 min. The p,p'-DDT content was measured by gas chromatography with flame ionization detection (GC-FID) using external standard calibration.

All these analytical methods were validated for their specificity, linearity of chromatographic response, repeatability, reproducibility, accuracy and limit of quantification. The methods performances were checked concurrently with the analysis of filter-papers samples by analyzing quality control samples (spiked samples) in order to validate the analytical results.

ANNEX 4. POSITIONS FROM WHICH NET PIECES WERE CUT FOR CHEMICAL AND BIOLOGICAL ASSAYS



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