

REPORT OF THE ELEVENTH
WHOPES
WORKING GROUP MEETING

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WHO/HQ, GENEVA
10—13 DECEMBER 2007

Review of:
SPINOSAD 7,48% DT
NETPROTECT®
DURANET®
DAWAPLUS®
ICON® MAXX



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ICON® MAXX**



**CONTROL OF NEGLECTED TROPICAL DISEASES
WHO PESTICIDE EVALUATION SCHEME**

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CONTENTS

| | Page |
|---|-----------|
| 1. INTRODUCTION | 1 |
| 2. REVIEW OF SPINOSAD 7.48% DT | 2 |
| 2.1 Efficacy – background and supporting documents | 3 |
| 2.2 Efficacy – WHOPES supervised trials | 4 |
| 2.3 Conclusions and recommendations | 7 |
| 3. REVIEW OF NETPROTECT® | 9 |
| 3.1 Safety assessment | 9 |
| 3.2 Efficacy – WHOPES supervised trials | 11 |
| 3.2.1 Laboratory studies | 11 |
| 3.2.2 Experimental hut studies | 13 |
| 3.3 Conclusions and recommendations | 19 |
| 4. REVIEW OF DURANET® | 21 |
| 4.1 Safety assessment | 21 |
| 4.2 Efficacy – WHOPES supervised trials | 22 |
| 4.2.1 Laboratory studies | 22 |
| 4.2.2 Experimental hut studies | 23 |
| 4.3 Conclusions and recommendations | 27 |
| 5. REVIEW OF DAWAPLUS® | 29 |
| 5.1 Safety assessment | 29 |
| 5.2 Efficacy – background and supporting documents | 30 |
| 5.2.1 Laboratory studies | 30 |
| 5.2.2 Field studies | 32 |
| 5.3 Efficacy – WHOPES supervised trials | 37 |
| 5.3.1 Laboratory studies | 37 |
| 5.3.2 Experimental hut studies | 38 |
| 5.4 Conclusions and recommendations | 42 |
| 6. REVIEW OF ICON® MAXX | 44 |
| 6.1 Safety assessment | 44 |
| 6.2 Efficacy – background and supporting documents | 46 |

| | | |
|-----------------|--|------------|
| 6.3 | Efficacy – WHOPES supervised trials | 50 |
| 6.3.1 | Laboratory studies | 50 |
| 6.3.2 | Experimental hut studies | 52 |
| 6.4 | Conclusions and recommendations | 54 |
| 7. | REVIEW OF WHOPES LABORATORY AND SMALL-SCALE FIELD TESTING, EVALUATION OF LONG-LASTING INSECTICIDAL MOSQUITO NETS AND THE WAY FORWARD | 57 |
| ANNEX 1: | CHARACTERIZING THE ACTIVE INGREDIENT RELEASE CHARACTERISTICS OF LONG-LASTING INSECTICIDAL MOSQUITO NETS SUBJECTED TO REPEATED WASHING | 61 |
| Annex 2: | LIST OF PARTICIPANTS | 99 |
| Annex 3: | REFERENCES | 101 |

1. INTRODUCTION

The eleventh meeting of the WHOPES Working Group, an advisory group to the WHO Pesticide Evaluation Scheme (WHOPES), was convened at WHO headquarters in Geneva, Switzerland, from 10 to 13 December 2007. The objective of the meeting was to review the reports of testing and evaluation of spinosad 7.48% DT (tablet for direct application) of Dow AgroSciences, France, for mosquito larviciding, and three long-lasting insecticidal mosquito nets (LNs) for malaria prevention and control, namely: (i) Netprotect[®], deltamethrin (incorporated into filaments) LN of Intelligent Insect Control, France; (ii) DuraNet[®], alpha-cypermethrin (incorporated into filaments) LN of Clarke Mosquito Control, USA; and (iii) DawaPlus[®], deltamethrin (coated) LN of Tana Netting, Thailand. The objective also included the review of reports of Icon[®] MAXX (an insecticide treatment kit) of Syngenta, Switzerland, for treatment of mosquito nets for malaria prevention and control.

The meeting was attended by 15 scientists (see Annex 1: List of participants). Dr Marc Coosemans was appointed as Chairman and Dr Purushothaman Jambulingam as Rapporteur. The meeting was convened in plenary and group sessions, in which the reports of the WHOPES supervised trials and relevant published literature and unpublished reports were reviewed and discussed (see Annex 2: References). Recommendations on the use of the above-mentioned products were made.

The meeting also reviewed the results of WHOPES testing and evaluation of LNs to identify the information on and data gaps for future development and evaluation of such products, and made recommendations for further action.

2. REVIEW OF SPINOSAD 7.48% DT

Spinosad is a natural product produced by fermentation technology that employs the bacterium *Saccharopolyspora spinosa* (Actinomycetales) from which it is obtained by extraction and purification of the whole broth. Spinosad 0.5% GR and 12% SC have previously been evaluated by WHOPES for mosquito larviciding. A WHO safety assessment of spinosad and recommendations¹ for its use, as well as WHO specifications² for quality control of the named products, have previously been published. The WHO Programme on Chemical Safety considers spinosad to be a mosquito larvicide that poses no undue threat to the health of users or to the environment. However, it notes that this assessment relates to spinosad, with the equivalent impurity profile of that used for development of WHO specifications.

Spinosad DT is a tablet for direct application for control of container-breeding mosquitoes. Each tablet weighs approximately 1.34 g and is 12 mm in diameter. The nominal content of the active ingredient is 75 g/kg, equal to approximately 100 mg of active ingredient (AI) per tablet. Each tablet is intended for application to 200 L of water per container for mosquito larval control, i.e. 0.5 mg/L AI.

Each tablet consists of two homogenous horizontal layers of technical spinosad: an outer layer consisting of technical spinosad in an effervescent system providing fast release of the active ingredient upon application to water; and an inner layer formulated to dissolve in water gradually over time.

¹ *Report of the tenth WHOPES Working Group Meeting, WHO/HQ, Geneva, 11–14 December 2006.* Geneva, World Health Organization, 2006 (WHO/CDS/NTD/WHOPES/2007.1; available at <http://www.who.int/whopes/recommendations/wgm/en/>).

² <http://www.who.int/whopes/quality/newspecif/en/>.

The following are extracts from the material safety data sheet of the manufacturer for spinosad 7.48% DT.

| | |
|---|--|
| Acute oral LD ₅₀ (rat) | >5000 mg/kg |
| Acute inhalation LC ₅₀ (rat) | Vapours are unlikely owing to physical properties. Single exposure to any trace dust is not likely to be hazardous |
| Acute dermal LD ₅₀ (rat) | >5000 mg/kg |
| Skin irritation (rabbit) | Essentially non-irritating to skin |
| Eye irritation | May cause slight transient (temporary) eye irritation |
| Sensitization (guinea-pig) | No allergic reaction |

The current review assesses the efficacy of spinosad DT against container-breeding mosquitoes in comparison with the GR formulation for which WHO recommendations have previously been published.

2.1 Efficacy – background and supporting documents

Martinique, France

Marcombe et al (2007) carried out a simulated field trial to evaluate, in plastic containers (175-L capacity), the residual efficacy of spinosad 7.48% DT, in comparison with spinosad 0.5% GR formulation against *Aedes aegypti* in Fort de France, Martinique (French West Indies). Efficacy and persistence were compared over a period of 60 days in blue plastic containers filled with 145-L of domestic water and covered with a mosquito net to prevent oviposition by wild mosquitoes and deposits of debris. The containers were placed under a shelter to protect them from direct sunshine and from rain. The GR formulation was used at a dosage of 0.1 mg/L and 0.5 mg/L; the DT formulation was used at 0.67mg/L AI (1 tablet/145 L). Each dosage was tested with three replicates and three control (untreated) jars.

A total of 100 third-instar larvae of the F1 generation of field-caught *Ae. aegypti* (Vauclin) and 1 g of food (dry cat food) were added to each container on the first day of treatment and thereafter every 10 days. At each cohort, the containers were refilled to maintain the initial level of water. Emerging adults

from each container were collected with electric aspirators and counted. For each treatment and each cohort, rates of inhibition of emergence (%IE) were calculated as an average of the three replicates. In each container, the water temperature and pH were monitored every 10 days; the ambient temperature and hygrometry were also recorded.

The water temperature and pH did not vary between the treatments. The GR formulation produced 98–100% IE for up to 60 days at the two dosages tested. The residual efficacy was not significantly different between the two dosages of spinosad 0.5% GR during the 60-day observation period. The %IE in the containers treated with spinosad DT exceeded 90% for 60 days, except on day 20 post-treatment when an IE of 83% was reported. The investigators considered that this may have been caused by variation in the release rate of the AI in the formulation.

Overall, both the formulations showed good residual efficacy (90%) in plastic containers against *Ae. aegypti* for 60 days. The granules of GR formulation floated at the water surfaces for several weeks, whereas the spinosad DT rapidly sank at the bottom of the containers.

2.2 Efficacy – WHOPES supervised trials

Penang, Malaysia

Jaal et al (2007) carried out simulated field studies in earthen and plastic jars filled with 200 L water to test the efficacy and residual activity of spinosad 7.48% DT against *Ae. aegypti* in comparison with spinosad 0.5% GR. The jars were set around residential houses outdoors, in the shade, protected from rain, and covered with a fine mesh net (mesh size less than 0.5 mm) to prevent wild mosquitoes from breeding. The water was allowed to stand for at least 24 hours before the experiment. Two water regimens were used for each type of jar: full jars at all times (water level replenished weekly before introduction of larvae) and full jars from which half of the water was removed and replenished weekly.

In the earthen jars, the DT and GR formulations were applied at three dosages (0.25, 0.5 and 1.0 mg/L AI) and compared with a control. The granular formulation was weighed and manually

applied onto the water surface in the jars. In the plastic jars only, the DT formulation was used at the dosage of 0.5 mg/L AI (1 tablet/200 L). Each treatment and control was replicated four times. Since a substantial amount of granules remained floating on the water surface, on day 6 post-treatment the granules were mixed in the surface water with a spoon and all granules sank to the bottom.

For assessment purposes, in each of the treated and untreated jars, 25 third-instar larvae of laboratory-cultured *Ae. aegypti* were introduced every week. In each jar, 1.0 g of ground-up larval food was added initially, and 0.5 g was added every week before adding the larvae. Larval mortality was recorded at 72 hours post-treatment after each introduction of larvae. Adult emergence was also observed by counting pupal skins until the next cohort of larvae was introduced. A day before each introduction of larvae, water was added to the jars up to the water-level mark for all treated and untreated jars to replace losses caused by evaporation. The mouth of the jars was covered at all times except during filling, emptying, adding larvae and food, and counting larvae and pupal skins.

In the earthen jars, spinosad 7.48% DT gave 97–100% mortality at all the concentrations tested up to 16 weeks in both water regimens. The granular formulation showed similar results, yielding 96–100% mortality at the three dosages tested up to 16 weeks in the full jars. However, under the water removal and refill regimen, the duration of efficacy of the GR formulation was reduced, with 99–100% mortality at all test concentrations for up to 12 weeks; thereafter, mortality declined to 57–78% at 0.25mg/L, and 78–83% at 0.5mg/L.

In the plastic jars, spinosad 7.48% DT gave 98–100% mortality at 0.5mg/L up to 16 weeks post-treatment in the full jar regimen. Under the water removal/refill regimen, the DT formulation gave 100% mortality up to 12 weeks. While mortality exceeded 80% up to 14 weeks, it dropped below 80% thereafter.

Spinosad DT and GR formulations gave 100% IE for up to 16 weeks under both the water regimens and in both the earthen and the plastic jars at all the dosages tested.

Nonthaburi, Thailand

Mulla MS (2007) carried out field studies in earthen water storage jars and plastic drums filled with tap water to evaluate

spinosad 7.48% DT and spinosad 0.5% GR in Bang Bua Thong, Nonthaburi District, Thailand. The jars and drums were placed on a concrete slab covered with a roof but exposed on all sides. The jars were kept covered with aluminium-fabricated lids at all times except during assessment for about 3–4 hours per week in order to preclude light entry, deposit of debris and oviposition by wild mosquitoes, as well as invasion by predacious macro-invertebrates. The lids for the plastic drums were provided with a fine mesh screen (15 cm in diameter) in the centre to prevent condensation. Water loss caused by evaporation was replenished on a monthly basis. Two water regimens were used in the jars: full jars all the time and jars from which half the volume of water was removed and replaced at each assessment interval. For removal of water, a hose was lowered to mid-depth and the water was pumped out. The drums were full with water at all times.

In the earthen jars, the 7.48% DT and the 0.5% GR formulations were used at three dosages (0.25, 0.5 and 1.0 mg/L AI; corresponding to ½, 1 and 2 tablets per 200 L water). Only two concentrations of DT (0.25 and 0.5 mg/L) and one concentration of GR 0.5 (0.5 mg/L) were used in plastic drums. The granules were spread directly over the water surface. Appropriate controls were also run. All the treatments and control were replicated four times.

After the treatment, 0.5 g of ground-up larval food and then 25 third-instar larvae from a laboratory colony of *Ae. aegypti* were added to the jars or drums at weekly intervals. Live larvae, pupae and pupal skins, and adult emergence were assessed 3 days and 7 days post-addition. By day 7 post-addition, all surviving larvae had pupated and emerged as adults.

The mean number of larvae, pupae or pupal skins was recorded and the level of reduction (%) or inhibition of emergence (IE%) calculated on the basis of successful surviving larvae or emergence (based on pupal skins) in the treated and control regimens based on the original number of larvae released. For the 3-day post-addition reading, surviving larvae, pupal and pupal skins (very few) were the basis for calculating reduction (%); for the 7-day post-addition reading, pupal skins indicating successful emergence were used to calculate %IE. The assessment of efficacy was made at weekly intervals until the level of control dropped below 80%.

The DT formulation at all three dosages produced $\geq 90\%$ IE for 20 days in earthen jars without water exchange, and 20–62 days in earthen jars with the water removal/refill regimen. The GR formulation at the same dosages gave $\geq 90\%$ IE for 34–55 days in earthen jars without water exchange, and 34–48 days in earthen jars with the water removal/refill regimen.

In plastic jars with no water exchange, the DT formulation at 0.25 and 0.5mg/L AI gave $>90\%$ IE for 27–34 days, whereas the GR formulation at 0.5mg/L AI gave $>90\%$ IE for 48 days.

2.3 Conclusions and recommendations

Spinosad is a natural product produced by fermentation technology that employs the bacterium *Saccharopolyspora spinosa* (Actinomycetales) from which it is obtained by extraction and purification of the whole broth. Spinosad 0.5% GR and 12% SC have previously been evaluated by WHOPES for mosquito larviciding. A WHO safety assessment of spinosad and recommendations¹ for its use, as well as WHO specifications² for quality control of the named products, have previously been published. The current "fast track" testing and evaluation assessed the efficacy of spinosad 7.48% DT against container-breeding mosquitoes in comparison with the GR formulation. Spinosad DT is a tablet for direct application for control of container-breeding mosquitoes. Each tablet weighs approximately 1.34 g and contains approximately 100 mg of AI.

In the simulated field trial in Martinique, spinosad 7.48% DT at a dosage of 0.67mg/L AI and 0.5% GR at dosages of 0.1 mg/L AI and 0.5 mg/L AI in plastic drums all gave at least 96% IE of *Ae. aegypti* for the 60-day study period. In Malaysia, spinosad 7.48% DT and 0.5% GR at dosages of 0.25mg, 0.5mg and 1.0mg/L AI in earthen jars gave 100% IE of *Ae. aegypti* for 112 days of the study. In plastic jars only, the DT formulation was tested at a dosage of 0.5mg/L AI, yielding 100% IE over the same period.

¹ Report of the tenth WHOPES Working Group Meeting, WHO/HQ, Geneva, 11–14 December 2006. Geneva, World Health Organization, 2006 (WHO/CDS/NTD/WHOPES/2007.1; available at <http://www.who.int/whopes/recommendations/wgm/en/>).

² <http://www.who.int/whopes/quality/newspecific/en/>.

In Thailand, the DT formulation at dosages of 0.25, 0.5 and 1.0mg/L AI gave $\geq 90\%$ IE for 20–62 days in earthen jars and 27–34 days in plastic jars at 0.25, 0.5mg/L AI. For the GR formulation at dosages of 0.25, 0.5 and 1.0mg/L AI, the %IE exceeded 90% for 34–55 days in earthen jars and 48 days at 0.5mg/L AI in plastic jars. The duration of efficacy of the GR formulation in this study was much shorter (83–111 days) than that observed previously¹ under the same simulated field conditions.

Noting the safety and efficacy of spinosad 7.48% DT, the meeting recommended:

- the use of spinosad 7.48% DT at 0.25–0.5 mg/L AI for the control of container-breeding *Aedes*, with an expected duration of efficacy under field conditions of 4–6 weeks;
- that industry be requested to provide tablets with grooves, facilitating more accurate dosing of containers of different capacities in the field and helping to avoid overdosing in smaller containers;
- that WHO should carry out further risk assessment for the potential use of spinosad in potable water as a mosquito larvicide, noting the relative safety profile of the product.

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.²

¹ *Report of the tenth WHOPES Working Group Meeting, WHO/HQ, Geneva, 11–14 December 2006.* Geneva, World Health Organization, 2006 (WHO/CDS/NTD/WHOPES/2007.1; available at <http://www.who.int/whopes/recommendations/wgm/en/>).

² WHO specifications for public health pesticides are available on the WHO home page on the Internet at <http://www.who.int/whopes/quality/en/>.

3. REVIEW OF NETPROTECT®

Netprotect®¹ is manufactured by Intelligent Insect Control (France) as a deltamethrin (incorporated into filaments) LN. Deltamethrin is incorporated into a mix of high- and medium-density polyethylene net consisting of 0.13 mm, 110 denier monofilaments, with the target dose of 1.8 g/kg AI, corresponding to 63 mg of deltamethrin per LN m². Nets are available in mesh sizes of 20 or 30 complete holes per cm².

Deltamethrin has previously been evaluated by WHOPES² for conventional treatment of mosquito nets, at target dose of 15–25 mg/m².

3.1 Safety assessment

The assessment of the risk to humans of washing and sleeping under the LN, provided by the manufacturer, was assessed by the Finnish Institute of Occupational Health (FIOH, 2006a) on behalf of the WHO Programme on Chemical Safety. The WHO *Generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*³ was used as a guiding document.

¹ In a letter dated 8 August 2007 jointly signed by Intelligent Insect Control and Syngenta (Basel, Switzerland), WHOPES has been informed that the product is being marketed under two trade names: ICON® Life (distributed by Syngenta) and Netprotect® (distributed by Bestnet Europe Ltd).

² Najera JA, Zaim M. *Malaria vector control: decision-making criteria and procedures for judicious use of insecticides*. Geneva, World Health Organization, 2002 (WHO/CDS/WHOPES/2002.5 Rev. 1; available at http://whqlibdoc.who.int/hq/2003/WHO_CDS_WHOPES_2002.5_Rev.1.pdf).

³ *A generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*. Geneva, World Health Organization, 2004 (WHO/CDS/WHOPES/GCDPP/2004.6; available at http://whqlibdoc.who.int/hq/2004/WHO_PCS_04.1.pdf).

The following assumptions were used by the proposer in drafting the assessment:

1. deltamethrin used in making the LN is from a source supported by WHO specifications;¹
2. a systemic acceptable exposure level (AEL) of 0.01 mg/kg body weight (identical to the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) 2000 acceptable daily intake (ADI) of 0–0.01 mg/kg for dietary exposure);
3. a default value of 10% was used for dermal absorption of deltamethrin (for which no actual dermal absorption or relative toxicity data are available) during sleeping under the net;
4. inhalation exposure while sleeping under the net is negligible given the low vapour pressure of deltamethrin;
5. the default values of the generic model were used for the assessment of the dermal exposure and oral exposure from hand-to-mouth transfer during use or from sleeping under the LN;
6. rather than the default value, an experimentally-derived 7.5% removal of AI by sucking has been used for the assessment of oral exposure from sucking and chewing the net;
7. total dermal exposure calculated for an adult, assuming washing of three nets, and for a child, assuming the washing of one net, was estimated using a release rate of 6.7% deltamethrin content to the washing water. This non-default value is supported by experimental data.

FIOH concluded that the assessment of risk to health of the maintenance and use of Netprotect® LN by the manufacturer are performed in compliance with the WHO generic model; that the conclusions of the safety assessment are justified; and, when used as instructed, washing or sleeping under the LN does not pose undue risk to the exposed adults, children or newborns.

¹ <http://www.who.int/whopes/quality/newspecif/en/>.

3.2 Efficacy – WHOPES supervised trials

3.2.1 Laboratory studies

Montpellier, France

Laboratory studies were conducted to evaluate the regeneration time, resistance to washing and efficacy of Netprotect® (Bonnet et al, 2006a) according to WHO guidelines for the laboratory testing of LNs. Four white nets were provided for the test and 10 pieces (25 cm x 25 cm) were cut from each net. A total of 28 samples were used to estimate the regeneration time and wash resistance before washing and after 1, 5, 10, 20 or 25 times.

Washes were done by introducing net samples separately into 1-L beakers with 0.5 L of soap solution (2 g/L of Savon de Marseille). The beakers were shaken in a water bath at 155 movements per minute for 10 minutes. The samples were then rinsed twice by shaking them in 0.5 L of deionized water for 10 minutes in the same shaking conditions as stated above.

Bioassays were conducted in WHO cone tests fixed to the net samples. Five female mosquitoes (*Anopheles gambiae*, Kisumu strain, 2–5 days old) were introduced into the cones for 3 minutes and then transferred to cages where they were held with access to sugar solution for 24 hours. The bioassays were replicated 10 times for a total of 50 mosquitoes tested on samples of each net. Knock-down (KD) was measured at 60 minutes post-exposure; mortality was recorded 24 hours post-exposure.

In addition to the standard WHOPES testing requirements, bioassays analogous to the wire-ball test for measuring median time to KD were done by introducing 11 mosquitoes into a circular chamber (10 cm in diameter and 1 cm high) cut into a glass plate. The net was suspended between the thick Plexiglas® plate and a thinner plate with a hole of similar size. A glass plate covered the top of the chamber. Mosquitoes were introduced into the chamber through a small hole in the glass plate. Within the chamber, mosquitoes were forced into contact with a piece of the treated netting and were removed as they were knocked down. The time required to KD the sixth mosquito was recorded as the median time to KD.

Regeneration time was measured by cone tests performed on nets that had been washed once or three times. Bioassays were conducted +1, +3 and +5 days after the final wash. KD was 100% regardless of the number of washes or number of days post-wash. Mortality was 97% on day 1 after three washes. All other tests resulted in 100% mortality. Using the time to KD test, median time to KD on an unwashed net was 354 seconds. After three washes, median time to KD rose to 687 seconds; it fell to <400 seconds after 3 days. Based on all the data, the regeneration time was considered to be 3 days. Therefore, the time interval between consecutive washings in the wash resistance study was 3 days.

In a wash resistance study, the mortality of mosquitoes exposed to Netprotect[®] was 100% before and after one wash. At 5 and 10 washes, mortality was 97% and 99% respectively. Mortality declined to 78% after 15 washes and 76% after 20 washes. However, KD was 100% to 20 washes. Because Netprotect[®] met one of the two WHOPES criteria for Phase I testing of an LN in the cone test ($\geq 80\%$ mortality or $\geq 95\%$ KD after 20 washes), further studies using the tunnel test were not required.

In a complementary study, median time to KD was measured daily up to 5 days on nets that had been washed 1, 5, 10, 15 or 20 times. Median time to KD after one day was longest on nets washed 15 (845 seconds) or 20 times (1310 seconds). By day 6 after the last wash, the median time to KD had fallen to below 500 seconds. However, median times to KD were higher than those for nets washed <15 times and held for an equal number of days. In all cases, median time to KD after washing and holding for up to 7 days never returned to the level of an unwashed Netprotect[®] (354 seconds).

Chemical analyses showed an initial deltamethrin concentration of 1.95 g/kg, slightly higher than the target of 1.8 g/kg but within specifications ($\pm 25\%$). After 20 washes, average deltamethrin content was 1.52 g/kg. The overall retention of deltamethrin after 20 washes was 77.6%; the estimated retention per wash ranged from 98.7% to 101.7% (average=99.7%).

3.2.2 Experimental hut studies

Kou Valley, Burkina Faso

Netprotect[®] was tested in experimental huts against free-flying wild *An. gambiae* in the Kou Valley in Burkina Faso (Dabire et al., 2007b) according to WHO guidelines. The huts used for testing were made from concrete bricks with a corrugated iron roof, a ceiling of thick polyethylene sheeting and a concrete base with a water-filled channel to prevent the entry of ants. Mosquitoes were able to enter the huts through slits (1 cm) constructed from pieces of metal. The metal pieces were fixed at an angle to allow easy entry but to impede exiting mosquitoes. A veranda trap (2 m long, 1.5 m wide and 1.5 m high) projected from the back of the hut and was used to estimate the proportion of mosquitoes exiting the hut.

Five different treatment arms were tested as follows: (i) untreated net (polyethylene); (ii) Netprotect[®], unwashed; (iii) Netprotect[®], washed 20 times; (iv) polyester net conventionally treated at 25 mg/m² AI, unwashed; and (v) polyester net conventionally treated at 25 mg/m² AI, washed just before exhaustion (two washes), defined as the last wash that provided mortality exceeding 80% or KD exceeding 90%.

A total of five huts were used along with five sleepers. Different net treatments were rotated among the huts each week; sleepers were rotated among the huts each night in a Latin square design. The trial was run for 5 weeks. Five nets were used for each treatment arm, with a different net used each night. Six holes (4 cm x 4 cm) were made in each net to simulate a torn net.

Nets were washed using a protocol modified from WHO washing procedures. Nets were washed individually in 10 L of well water and 2 g/L of Savon de Marseille. Each net was agitated using a long pole for 3 minutes, left to soak for 4 minutes and then agitated for 3 minutes. The total washing time was therefore 10 minutes. Each net was rinsed twice using the same methods but using only 10 L of well water without the soap. Nets were washed at 3-day intervals according to the regeneration time established in the Phase I study.

WHO cone bioassays were conducted on five nets (one per treatment arm) immediately before washing, just after the completion of washing and at the completion of the study. KD and mortality were recorded 60 minutes and 24 hours after exposure. A sixth net from each treatment arm was retained for chemical analysis. Four pieces each of 50 cm² size were taken before and another four pieces were taken just after washing. At the end of the study, one net from each arm was sampled for chemical analysis using the same methods.

The hut study was conducted with sleepers entering the huts at dusk and remaining inside until dawn. In the morning, dead mosquitoes were collected from the floor of the huts and the exit traps. Resting mosquitoes were collected from walls and roofs. These mosquitoes were provided sugar water and held for 24 hours to assess delayed mortality. The primary outcomes measured included deterrence (reduction in hut entry relative to the control hut); induced exophily (proportion of mosquitoes found in the exit traps); blood-feeding inhibition (proportion of mosquitoes that were blood fed relative to the control); and immediate and delayed mortality (proportion of dead mosquitoes in all entered).

Initial bioassays showed 100% mortality and KD on all net treatments except the control net. After two washes, mortality for the conventionally treated net fell just below WHOPES-defined thresholds ($\geq 80\%$ mortality or $\geq 95\%$ KD). Therefore, two washes were required to reach until just before exhaustion. Bioassays conducted just after washing showed mortality on the conventionally treated net washed to exhaustion to be 70.9%. For the Netprotect[®] washed 20 times, mortality was 54.4%. KD for conventionally treated nets washed just before exhaustion (two washes) and Netprotect[®] washed 20 times was 96.4% and 100% respectively. After the hut trial was completed, mortality was 0% for the untreated net and 100% for the unwashed Netprotect[®] net. Mortality exceeded 80% for all other treatment arms. KD was $>98\%$ for both the unwashed Netprotect[®] and the Netprotect[®] washed 20 times. KD for the unwashed conventional net was 90.4% and 84.6% for the conventionally treated net washed to exhaustion.

In the experimental hut trial, the treated nets caused deterrence of *An. gambiae* ranging from 29.8% to 80.1%. The degree of deterrence was significantly higher for the unwashed

conventionally treated net compared with all other treatments. Deterrence among all the other treatments was not significantly different. Deterrence for the Netprotect[®] was 48.4% for the unwashed net and 44.0% for the net washed 20 times.

Induced exophily of *An. gambiae* was significantly higher in the treated nets compared with the control net. Exophily was 29.3% for the control net compared with 59.8% for the unwashed conventionally treated net and 50.4% for the conventionally treated net washed to exhaustion. Exophily rates were 72.6% for the unwashed Netprotect[®] and 70.6% for the Netprotect[®] washed 20 times. These rates were statistically higher than for the control net and for both of the conventionally treated nets.

The rate of blood-feeding inhibition of *An. gambiae* was highest in the unwashed Netprotect[®] (50.9%) and lowest in the conventionally treated net washed until just before exhaustion (15.3%). Blood-feeding inhibition for the Netprotect[®] washed 20 times was 22.6%. Blood-feeding rates were significantly lower for the unwashed Netprotect[®] than for all other nets. The blood-feeding rate for the Netprotect[®] washed 20 times was not significantly different from either the unwashed conventionally treated net or the conventionally treated net washed to exhaustion.

Similarly, the rate of mortality (corrected for control mortality) of *An. gambiae* was highest for the unwashed Netprotect[®] (58.1%) and lowest for the conventionally treated net washed to exhaustion (19.6%). Mortality for the Netprotect[®] washed 20 times was 24.6% and was not significantly different from the conventionally treated net washed to exhaustion.

Chemical analyses of the nets before washing, after the completion of washing and after the completion of the study showed initial deltamethrin concentrations of 1.41–1.45 g/kg for the Netprotect[®] and 0.70–0.87 g/kg for the conventionally treated nets. Concentrations did not significantly decline on the unwashed Netprotect[®] (deltamethrin concentration at end of study=1.33 g/kg) or the unwashed conventionally treated net (deltamethrin concentration at end of study=0.88 g/kg). Deltamethrin concentrations on the conventionally washed treated nets declined to undetectable levels after washing. For the washed Netprotect[®], the concentration of deltamethrin was

0.78 g/kg after 20 washes (45% loss) and 0.88 g/kg at the end of the study (38% loss). Deltamethrin R-isomer was 19% of deltamethrin content for the unwashed Netprotect[®] and 26.2% for the Netprotect[®] washed 20 times.

No adverse effects were reported by sleepers during the study. However, sleepers were rotated among treatment arms so that the frequency of side-effects among people sleeping under Netprotect[®] for several consecutive nights could not be determined.

Muheza, United Republic of Tanzania

The efficacy of Netprotect[®] was evaluated in experimental huts against free-flying wild mosquitoes in Muheza, United Republic of Tanzania (Tungu et al., 2007b). The huts were constructed with brick walls plastered with mud, a wooden ceiling lined with hessian sack-cloth, an iron sheet roof and open eaves. The huts were fitted with window traps and veranda traps on two sides; open eaves on the other two sides allowed entry of host-seeking mosquitoes into the hut. The huts were set on concrete plinths with a water-filled moat to exclude ants.

Six treatment arms were evaluated as follows: (i) Netprotect[®], unwashed; (ii) Netprotect[®], washed 20 times; (iii) Netprotect[®], washed to exhaustion (23 washes); (iv) polyethylene net, conventionally treated with the K-O TAB and washed until exhaustion (16 washes); (v) polyethylene net, conventionally treated with the K-O TAB and washed 20 times; and (vi) untreated net.

Nets were evaluated in six different huts with six different sleepers rotated in a Latin square design. Three nets were used in each treatment arm; each net was tested twice in each hut. Six holes (4 cm x 4 cm) were cut in each net to simulate a torn net.

Nets were washed in 10 L of soap-water solution (2 g/L of Savon de Marseille). The nets and solution were agitated for 3 minutes using a pole, left to soak for 4 minutes and then agitated for a further 3 minutes. The total immersion time was therefore 10 minutes. The nets were rinsed twice in 10 L of water and agitated as described above.

Bioassays were conducted on the conventionally treated net and the Netprotect® to determine the exhaustion point. Washes were done as described above, and WHO cone bioassays were conducted on the top and all four sides (minimum of 50 mosquitoes tested per net). The number of washes required to exhaust each net was defined as when mortality and KD consistently dropped below WHOPES-defined thresholds ($\geq 80\%$ mortality and $\geq 95\%$ KD). WHO cone bioassays were also conducted on one net from each arm before washing, and after completion of 20 washes.

The experimental hut study was conducted with sleepers entering the huts at dusk. Mosquitoes were collected in the morning. Dead mosquitoes were collected from the floors of the room and veranda and from window traps. Live mosquitoes were collected resting from the walls and roof of the hut and verandas. The number of mosquitoes in the two veranda traps was multiplied by two to adjust for mosquitoes escaping through the eaves on the other two sides of the hut. The primary outcomes measured included deterrence (reduction in hut entry relative to the control hut); induced exophily (proportion of mosquitoes found in the exit traps); blood-feeding inhibition (proportion of mosquitoes that were blood fed relative to the control); and immediate and delayed mortality (proportion of dead mosquitoes).

Initial bioassays on unwashed Netprotect® and conventionally treated nets resulted in $>95\%$ mortality and KD. For the conventionally polyethylene treated net, KD consistently fell below 95% after 15 washes, whereas mortality fell below 80% after 17 washes. The exhaustion point was therefore set at 16 washes. For the Netprotect®, KD fell below 95% after the 13th wash, while mortality remained above 80% until the 23rd wash. The exhaustion point was therefore set at 23 washes. For the nets washed 20 times, cone bioassays after the 20th wash resulted in 74% KD and 70% mortality for the conventionally treated net. For the Netprotect®, KD and mortality after 20 washes were 84% and 88% respectively.

During the experimental hut trial, *An. funestus* was more abundant than *An. gambiae*. A total of 734 *An. funestus* were captured during the trial, averaging 3–4 per hut per night. Fewer *An. funestus* were found in the huts with either the conventionally treated net or the Netprotect®. However, there

were no statistically significant differences in the number of *An. funestus* captured in the huts between any of the treatments.

Mortality of *An. funestus*, corrected for control, was highest with the unwashed Netprotect® (74.7%). This declined to 50.6% after 20 washes and to 51.5% after 23 washes. The mortality rates for *An. funestus* in huts with the Netprotect® washed 20 or 23 times were significantly higher than mortality in huts with the conventionally treated net washed 16 times (20.5%) or 20 times (25.9%).

The blood-feeding rate of *An. funestus* was significantly lower in the Netprotect® treatment arms, regardless of washing, compared with the control huts. Blood-feeding rates were intermediate for the conventionally treated nets and not significantly different from those of either the Netprotect® or the control net. The rate of blood-feeding inhibition ranged from 59% to 74% for the Netprotect® treatment arms and from 38% to 45% for the conventionally treated nets.

Exophily of *An. funestus* was high in all treatments, including the control huts. More than 90% of *An. funestus* exited huts in all treatment groups, with no statistically significant differences among the treatment arms.

The number of *An. gambiae* captured in the huts was lower than the number of *An. funestus*. A total of 120 *An. gambiae* were captured during the trial. Though the numbers were low, and statistical significance was not reached for many of the comparisons, the general trends are similar to those observed for *An. funestus*. In all treatment arms, fewer *An. gambiae* were collected in huts with the Netprotect® or the conventionally treated net compared with the control net. However, given the low numbers, the differences were not statistically significant.

Mortality of *An. gambiae* was zero in the control huts. In huts with the Netprotect®, mortality ranged between 43.8% and 80%. Mortality in huts with the conventionally treated nets ranged between 5% and 33.3%. In all treatment huts, mortality was significantly higher than that observed in the control huts. Mortality in the huts with an unwashed Netprotect® was significantly higher than that observed in all other treatment arms. Mortality in huts with conventionally treated nets washed 16 times was lower than all other treatment arms but was higher

than the control. Mortality in the huts with Netprotect[®] washed 20 times, Netprotect[®] washed 23 times or a conventionally treated net washed 20 times was not statistically different.

The rate of blood-feeding inhibition of *An. gambiae* showed similar trends to that of *An. funestus*. However, the number of *An. gambiae* collected was low, limiting statistical power to detect differences among the treatments.

Exophily of *An. gambiae* was high in all treatment arms, with no statistically significant differences observed between any of the treatment arms.

The authors reported an unusually high cut-off for net exhaustion for the conventionally treated net. This was attributed to the use of polyethylene nets and potential of a higher binding affinity for pyrethroid insecticides. Therefore, results from a previous trial using polyester nets were also presented. In the previous trial, the threshold for the conventionally treated net was determined to be six washes. Deterrence was higher for the conventionally treated polyester net washed 6 times compared to a Netprotect[®] washed 20 times. However, exophily, mortality and blood feeding inhibition were similar and the overall killing effect of the Netprotect[®] washed 20 times was higher than that of the conventionally treated polyester net washed 6 times.

3.3 Conclusions and recommendations

Netprotect[®] is manufactured by Intelligent Insect Control (France) as a deltamethrin (incorporated into filaments) LN, with the target dose of 1.8 g/kg AI corresponding to 63 mg of deltamethrin per m² of the monofilament polyethylene fabric. The net fibres are 110 denier and consist of a mixture of high- and medium-density polyethylene.

A WHO assessment of the manufacturer's compliance with assessment of exposure to and risks of washing and sleeping under a Netprotect[®] was in line with the WHO generic risk assessment model and, although some default values of the guideline were not used but justified, the conclusions were in agreement: washing the LN or sleeping under the net does not pose any undue risk to adults, children or newborns.

Laboratory studies indicated that Netprotect[®] LNs met the WHOPEs Phase I criteria of a KD effect exceeding 95% after 20 washes. Mortality dropped below the cut-off value of 80% after 15 washes. After depleting surface insecticide on washing nets, maximum bioavailability was achieved within 3 days at 30 °C.

Field studies demonstrated a better or equal impact of Netprotect[®] LNs washed 20 times on mortality and blood-feeding inhibition of prominent malaria vectors compared with that of the polyester or polyethylene nets conventionally treated with deltamethrin and washed until just before exhaustion. This confirms that Netprotect[®] fulfils the WHOPEs main efficacy criteria of Phase II studies.

Given the safety, efficacy and wash resistance of Netprotect[®] in laboratory and small-scale field studies, it is recommended:

- that a time-limited *interim recommendation* be given for the use of Netprotect[®] in the prevention and control of malaria;
- that WHOPEs coordinates large-scale field studies (WHOPEs Phase III) of Netprotect[®] LNs to confirm their long-lasting efficacy, longevity and fabric integrity as well as community acceptability, as a requirement for developing *full recommendations* on the use of the product.

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.¹

¹ WHO specifications for public health pesticides are available on the WHO home page on the Internet at <http://www.who.int/whopes/quality/en/>.

4. REVIEW OF DURANET®

DuraNet® net is manufactured by Clarke Mosquito Control (USA) as an alpha-cypermethrin (incorporated into filaments) LN. Alpha-cypermethrin is incorporated into 150-denier, mono-filament, high-density polyethylene fibres, with the target dose of 5.8 g/kg AI, corresponding to 261 mg of alpha-cypermethrin per LN m².

Alpha-cypermethrin has previously been evaluated by WHOPES¹ for conventional treatment of mosquito nets, at the target dose of 20–40 mg/m².

4.1 Safety assessment

The assessment of risk to humans of washing and sleeping under the LN, provided by the manufacturer, was assessed by FIOH (FIOH, 2007a) on behalf of the WHO Programme on Chemical Safety. The WHO *Generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*² was used as a guiding document.

The following assumptions/methodologies were used by the proposer in drafting the assessment:

1. alpha-cypermethrin used in making the LN is from a source supported by WHO specification;³
2. adopts the JMPR⁴ ADI of 0–0.02 mg/kg per day for long-term exposure; the European Union Acute Reference Dose (ARfD) of 0.04 mg/kg for short-term exposure; and the default dermal absorption rate of 10%; the proposer

¹ Najera JA, Zaim M. *Malaria vector control: decision-making criteria and procedures for judicious use of insecticides*. Geneva, World Health Organization, 2002 (WHO/CDS/WHOPES/2002.5 Rev. 1; available at http://whqlibdoc.who.int/hq/2003/WHO_CDS_WHOPES_2002.5_Rev.1.pdf).

² *A generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*. Geneva, World Health Organization, 2004 (WHO/CDS/WHOPES/GCDPP/2004.6; available at http://whqlibdoc.who.int/hq/2004/WHO_PCS_04.1.pdf).

³ <http://www.who.int/whopes/quality/newspecif/en/>.

⁴ <http://www.inchem.org/documents/jecfa/jecmono/v53je05.htm>.

- demonstrates experimentally that this value is an overestimation of true dermal penetration;
3. uses an experimental release rate of 8 mg/m² of alpha-cypermethrin content into the washing solution;
 4. assumes negligible inhalation exposure given the low vapour pressure of alpha-cypermethrin;
 5. uses experimental data on leaching to artificial sweat and artificial saliva, rather than the default values, for the assessment of exposure from sleeping under the net.

FIOH concluded that the proposer's assessment follows WHO generic guidelines. Where the default values of the guidelines are not used, this is justified by experimental data on the actual product. FIOH agrees with the proposer's conclusion that using and washing DuraNet[®] does not pose undue health risks.

4.2 Efficacy – WHOPES supervised trials

4.2.1 Laboratory studies

The efficacy of and resistance to washing of DuraNet[®] were determined according to WHO guidelines for laboratory testing of LNs¹ (Finot et al., 2006). A total of 4 white nets were used, and for each net 10 pieces (25x25 cm) were cut. Chemical analysis revealed an average content of 6.14 g/kg alpha-cypermethrin that remains practically unaltered after 20 washes (94.4% overall retention). The variation of the dose between nets was in the range of 0.5% and 2.0%. Bioassays were carried out using 2- to 5-day-old, non-fed females of a colony strain of *An. gambiae* (Kisumu) having no detectable resistance mechanisms. Untreated and 25mg/m² deltamethrin treated net materials were used as negative and positive controls, respectively. KD was observed after 3 minutes of exposure in cone bioassays and 60 minutes of holding, and mortality after 24 hours. After depleting surface insecticide by washing and drying three times consecutively, bioassays revealed that biological activity was optimal after 1 day (KD: 100%, mortality 98%). This means that no regeneration time was needed after washing. Bioassays were further performed after several cycles

¹ *Guidelines for laboratory and field testing of long-lasting insecticidal nets*. Geneva, World Health Organization, 2005 (WHO/CDS/WHOPES/GCDPP/2005.11).

of washing (following WHO standard wash procedures) and drying (0, 1, 5, 10, 15 and 20 washes – four replicates of bioassays per cycle) with a time period of 1 day between the washes. KD was maximal (100%) up to 20 washes. Mortality was high until 5 washes (98%) and dropped below 80% after 10 washes. After 20 washes, only 45% of the *An. gambiae* were killed. However DuraNet[®] fulfilled the criteria of laboratory tests (WHOPES Phase I) by inducing a KD of 100% after 20 washes.

4.2.2 Experimental hut studies

DuraNet[®]LN nets were evaluated in experimental huts following WHO guidelines¹ in two locations, Burkina Faso and the United Republic of Tanzania, using free-flying wild malaria vectors. Efficacy was evaluated in terms of blood-feeding inhibition, deterrence, induced exophily and mortality. The design of the experimental huts was slightly different between the two localities: in Burkina Faso, the mosquitoes could only escape outside to a single veranda trap, while in the United Republic of Tanzania they could escape into window traps or via the eaves to screened or open verandas. To adjust for unrecorded escapes through the openings, the numbers of mosquitoes collected in screened verandas were multiplied by two.

In each locality, five or six trap-huts were used, corresponding to five or six treatment arms. Each week, after cleaning and ventilating the huts, treatment arms were rotated through the huts according to a Latin square scheme. In the United Republic of Tanzania, three nets per arm were used twice a week, while in Burkina Faso nets were changed daily (five nets per arm). Sleepers were rotated randomly among huts each night of the study. Each net was deliberately pierced with six holes (4 cm x 4 cm) to simulate a torn net. One additional net per treatment arm was used for chemical analyses and bioassays. Standard procedures were used for washing the nets. Cone bioassays were carried out using *An. gambiae* (Kisumu strain). The data of the hut trials were analysed using non-parametric tests for numeric data and logistic regression for proportional data.

¹ *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets*. Geneva, World Health Organization, 2005 (WHO/CDS/WHOPES/GCDPP/2005.11; available at <http://www.who.int/whopes/guidelines/en/>).

According to Phase I experiments (Finot et al., 2006) no regeneration time was required after washing and one-day intervals between successive washes were therefore applied.

Kou Valley, Burkina Faso

The following five treatment arms were tested (Dabire et al., 2007c): (i) untreated net (same fabric and mesh size as DuraNet[®]); (ii) unwashed DuraNet[®] LN; (iii) DuraNet[®] LN washed 20 times; (iv) unwashed polyester net conventionally treated with alpha-cypermethrin (Fendona 10% SC) at 40 mg/m² AI (WHO recommended concentration); (v) polyester net conventionally treated with alpha-cypermethrin (Fendona 10% SC) at 40 mg/m² AI and washed until just before exhaustion, defined as the last wash for which the net still causes $\geq 80\%$ mortality or $\geq 95\%$ KD.

Five nets were used per treatment arm, and each net was tested one night a week (5 nights per week).

Nets were evaluated during 5 consecutive weeks (May–June 2007) on *An. gambiae* s.s. Both molecular M and S forms of *An. gambiae* occur in sympatry in the study village. The M form was normally largely predominant during the testing period (>80%). Based on results from 2005–2006, the allele frequency of the “kdr” mutation responsible for KD resistance was very low in the M form (0 to 0.04) but high in the S form (0.8 to 1).

Before any washing, all treated nets were fully effective (1 hour post-exposure KD and 24 hour post-exposure mortality of 100%) in cone bioassays (3 minute exposure). The cut-off point of a net conventionally treated with alpha-cypermethrin at 40 mg/m² AI was here considered to be three washes (KD: 55%, mortality: 45%). Hence, the number of washes required before exhaustion was taken as two (KD: 100%, mortality: 89%)

The bio-efficacy of the nets used in each treatment arm was studied before and after the field evaluation. The unwashed DuraNet[®] LN and conventionally treated nets (alpha-cypermethrin at 40 mg/m² AI) were fully effective, with 100% KD after 60 minutes post-exposure and 100% mortality following 24 hours post-exposure. The results obtained before the field evaluation for DuraNet[®] LN washed 20 times and for conventionally treated nets washed before exhaustion (two

washes) were almost similar for KD (100%) and mortality (respectively 79% and 89%). No major changes occurred after the trial for these washed nets: KD was still 100% and mortality even increased with the DuraNet[®] LN washed 20 times (94%), while it decreased slightly for the conventionally treated nets washed to just before exhaustion (68%).

Chemical residue analysis showed an average content of 5.04 g/kg for the unwashed DuraNet[®], which complies with the declared content (5.8 g/kg). After 20 washes, the dose fell to 3.40 g/kg, corresponding to an overall retention value of 67.5%. On the same net, variation between samples was low (0.9 to 5.9% relative standard deviation, RSD). The observed dose for conventionally treated nets (44.8 mg/m²) was close to the target dose (40mg/m²). After two washes of the conventionally treated nets, no insecticide could be detected.

During a five-week collecting period, 367 *An. gambiae* were collected in the control huts. Densities of *An. funestus* and *Culex quinquefasciatus* were very low (81 mosquitoes) and were not further considered.

No significant reduction of the entry rates of *An. gambiae* females was observed in the presence of treated nets, except with the unwashed conventionally treated nets (79%). The exit rate in the control arm was 29%, which significantly increased when treated nets were used (DuraNet[®] LN unwashed 82%; DuraNet[®] LN washed 20 times, 75%; unwashed conventionally treated polyester nets, 79%; and exhausted conventionally treated polyester nets, 50%).

Blood-feeding inhibition was very high with unwashed DuraNet[®] LN (84%) but decreased to 56% when washed 20 times, which was similar to unwashed conventionally polyester treated nets (69%). Exhausted conventionally treated polyester nets reduced blood-feeding by only 29%, which was still significant. Similar figures were observed for mortality: 89% with unwashed DuraNet[®] LN, around 50% with DuraNet[®] LN washed 20 times and unwashed conventionally treated polyester nets, and only 20% with exhausted conventionally treated polyester nets.

Personal protection, taking into account the blood-fed females in the control and treated arms, was 70% with DuraNet[®] LN

washed 20 times, compared with only 7% with the exhausted conventionally treated polyester nets (two washes). The overall insecticidal effect of unwashed Duranet[®] LN, taking into account the deterrent effect, dropped from 65% to 31% after 20 washes. The high deterrent effect observed with the unwashed conventionally treated polyester nets induced an overall killing effect of only 7%. This percentage increased after two washes from 20% to 27%, mainly as a result of the higher number of females entering in the treated huts compared with the control.

Muheza, United Republic of Tanzania

The following six treatment arms were tested (Tungu et al., 2007a): (i) untreated net; (ii) unwashed DuraNet[®] LN, (iii) DuraNet[®] LN washed 20 times; (iv) DuraNet[®] LN washed until just before exhaustion; (v) polyester net conventionally treated with alpha-cypermethrin 10% SC at 40 mg/m² AI (WHO recommended concentration) washed 20 times; and (vi) polyester net conventionally treated with alpha-cypermethrin 10% SC at 40 mg/m² AI and washed until just before exhaustion, defined as the first wash for which the net causes $\leq 80\%$ mortality or $\leq 95\%$ KD.

Three nets were used per treatment arm during each 6-night rotation; each net was tested for 2 nights. Nets were evaluated against *An. gambiae* and *An. Funestus* during 6 consecutive weeks. No chemical assay data were available on samples of mosquito nets in this trial.

Before washing, the one-hour KD observations and the 24-hour post-treatment mortality readings from the cone bioassays (3-minute exposure) were 100% on all treated nets. After washing the conventionally treated net 20 times, KD fell to 6% and mortality to 10%, while these values remained high with DuraNet[®] LN washed 20 times (86% and 90% respectively). The number of washes to just before exhaustion was six for the conventionally treated nets (KD: 82%; mortality: 92%).

During the 36 night collections, 143 *An. gambiae* and 529 *An. funestus* were collected in huts with untreated nets. No significant deterrent effect was observed on *An. gambiae* or *An. funestus*. The majority of *An. gambiae* (88%) and *An. funestus* (85%) were collected from the window traps and veranda traps

in huts with untreated nets. This exophily trend was similar in the treated arms.

DuraNet[®] LN induced high mortality of *An. gambiae* that entered the hut. This fell significantly with the number of washes (0 washes: 96%, 20 washes: 83%, 23 washes 82%). Conventionally treated nets washed 6 and 20 times scored significantly lower for mortality (65% and 43% respectively) than the washed DuraNet[®] LN. Mortality rates for *An. funestus* were similar to those of *An. gambiae* with respect to DuraNet[®] LN (0 washes: 93%; 20 washes: 81%, 23 washes: 80%) and conventionally treated nets (6 washes: 68%; 20 washes 40%). The overall killing effect (mortality adjusted for the deterrent effect) of DuraNet[®] LNs after 20 washes was higher than that of conventionally treated nets washed to just before exhaustion, defined here as six washes (for *An. gambiae* 57 and 46% respectively and for *An. funestus* 71 and 67%).

The DuraNet[®] LN outperformed the conventionally treated nets in terms of blood-feeding inhibition. For *An. gambiae*, 20% were blood fed with the untreated nets and only 7% or less were bloodfed with washed or unwashed DuraNet[®] LN. The rate of blood-feeding inhibition with DuraNet[®] LN ranged from 63.5% with the unwashed nets to 72.6% with nets washed 20 times. Compared with the untreated nets, no significant blood-feeding inhibition was observed with the conventionally treated nets washed six times. Similar trends in blood-feeding inhibition between treatments were observed for *An. funestus*.

The overall personal protection was high with unwashed DuraNet[®] LN (*An. gambiae*: 71%, *An. funestus* 71%). At 20 washes, DuraNet[®] LN provided a higher protection than the exhausted conventionally treated nets (*An. gambiae*: 78.6% and 50% respectively; *An. funestus* 65% and 51% respectively).

4.3 Conclusions and recommendations

DuraNet[®] is manufactured by Clarke Mosquito Control (USA) as an alpha-cypermethrin (incorporated into filaments) LN, with the target dose of 5.8 g/kg AI corresponding to 261 mg of alpha-cypermethrin per monofilament polyethylene fabric m².

A WHO assessment of the manufacturer's compliance with assessment of exposure to and risks of washing and sleeping under a DuraNet[®] was in line with the WHO generic risk assessment model and, although some default values of the guideline were not used but justified, their conclusions were in agreement.

Laboratory studies revealed that DuraNet[®] LNs met the WHOPES Phase I criteria of a KD effect exceeding 95% after 20 washes, despite mortality dropping below the cut-off value of 80% after five washes. After depleting surface insecticide on washing the LN, initial bioavailability level was achieved within one day at 30 °C.

Field studies demonstrated a better impact of DuraNet[®] LNs washed 20 times on mortality and blood-feeding inhibition of prominent malaria vectors compared with that of the conventionally treated polyester nets (40 mg/m² AI) washed until just before exhaustion. This confirms that DuraNet[®] fulfils the WHOPES main efficacy criteria for Phase II studies.

Considering the safety, efficacy and resistance to washing of DuraNet[®] in laboratory and small-scale field studies, it is recommended:

- that a time-limited *interim recommendation* be given for the use of DuraNet[®] in the prevention and control of malaria;
- that WHOPES coordinates large-scale field studies (WHOPES Phase III) of DuraNet[®] LNs to confirm their long-lasting efficacy, longevity and fabric integrity as well as community acceptability, as a requirement for developing *full recommendations* on the use of the product;

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.¹

¹ WHO specifications for public health pesticides are available on the WHO home page on the Internet at <http://www.who.int/whopes/quality/en/>.

5. REVIEW OF DAWAPLUS®

DawaPlus® net is manufactured by Tana Netting (Thailand), in collaboration with Bayer Environmental Science, as a deltamethrin (coated) LN. Deltamethrin suspension concentrate (SC) is coated on knitted multi-filament polyester fibres, at the target dose of 1.33 g/kg in 75-denier yarn and 1 g/kg in 100-denier yarn, corresponding to 40 mg of deltamethrin per LN m², using a polymer as a binder.

Deltamethrin has previously been evaluated by WHOPES¹ for conventional treatment of mosquito nets, at target dose of 15–25 mg/m² AI.

The manufacturer has disclosed the nature of the binder used in coating the insecticide, and has confirmed that it is the same as the binder used in making another public health insecticide,² already subject to the WHO safety assessment.

5.1 Safety assessment

The assessment of risk to humans of washing and sleeping under the LN, provided by the manufacturer, was assessed by FIOH (FIOH, 2006b) on behalf of the WHO Programme on Chemical Safety. The WHO *Generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*³ was used as a guiding document.

¹ Najera JA, Zaim M. *Malaria vector control: decision-making criteria and procedures for judicious use of insecticides*. Geneva, World Health Organization, 2002 (WHO/CDS/WHOPES/2002.5 Rev. 1; available at http://whqlibdoc.who.int/hq/2003/WHO_CDS_WHOPES_2002.5_Rev.1.pdf).

² Safety assessment of K-O TAB 1-2-3 in: *Report of the tenth WHOPES Working Group Meeting, WHO/HQ, Geneva, 11–14 December 2006*. Geneva, World Health Organization, 2006 (WHO/CDS/NTD/WHOPES/2007.1, available at: <http://www.who.int/whopes/recommendations/wgm/en/>).

³ *A generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*. Geneva, World Health Organization, 2004 (WHO/CDS/WHOPES/GCDPP/2004.6; available at http://whqlibdoc.who.int/hq/2004/WHO_PCS_04.1.pdf).

DawaPlus[®] is based on deltamethrin AI and copolymer adjuvant that have already been subject to a WHO safety assessment. The only difference between the current and previous product is the target dose of insecticide contained in DawaPlus[®] (40 mg AI/m²).

Based on experimental data, a maximum of 9% removal of deltamethrin content in each wash, rather than the 30% default value used in the generic model, has been used in assessing exposure during the washing of the LN.

FIOH concluded that the assessment of health risks of the maintenance and use of DawaPlus[®] LN by the manufacturer are performed in compliance with the WHO generic model; the conclusions on safety, when used as instructed, are justified; and that washing or sleeping under the LN does not pose undue risk to adults, children or newborns.

5.2 Efficacy – background and supporting documents

5.2.1 Laboratory studies

Supporting data were provided by the manufacturer to demonstrate the variability of AI (deltamethin) within and between nets, to determine the resistance to washing of DawaPlus[®] and to assess a model for insecticide loss applied to the DawaPlus[®] (Tana Netting Co., Ltd., 2007).

Variation in deltamethrin content within and between nets was studied. Three different sampling protocols were used. In the first two, five samples were cut along a diagonal as outlined in the *Manual on development and use of FAO and WHO specifications for pesticides*.¹ The size of each sample was either 30 cm x 30 cm or 60 cm x 60 cm, the five samples representing 3% or 12% of the total size of the net, respectively. The third sampling protocol was similar to that used by the manufacturer in the quality control process, by which five samples (70 cm x 70 cm) are cut from different parts of the net, representing 20% of the total size of the net. The manufacturer

¹

http://whqlibdoc.who.int/publications/2006/9251048576_eng_update2.pdf.

reported intra-net variations in RSD of 16.9–28.5 g/kg for "3% sampling procedure", 5.7–22.5 g/kg for "12% sampling procedure" and 7.6–15.4 g/kg for "20% sampling procedure". The manufacturer reported variability from the declared content of 1.33 g/kg deltamethrin ranging between –16% and +41%.

To assess the wash resistance of several formulations of DawaPlus[®], a protocol was employed, based on current WHOPES guidelines for the evaluation of long-lasting nets but deviating in some aspects of the washing and bioassays. Pieces of netting (90 cm x 90 cm) were prepared and washed 30 times. At 0, 10, 15, 20, 25 and 30 washes, six subsamples (12 cm x 15 cm) were cut from the net. The subsamples were taken from the same net, such that a single strip of approximately 12 cm x 15 cm remained after 30 washes.

Washing was carried out daily using 2 L of soap solution (2 g/L, Savon de Marseille) in a 5-L bucket. Each sample was placed in the soap solution and mixed twice with a glass rod. Samples were then allowed to soak for 4 minutes. Excess water was then squeezed from the samples and the samples dipped six times in the soap solution. They were then soaked for a further 6 minutes. The samples were rinsed in flowing water. The excess water was squeezed from the net samples before they were allowed to dry on aluminum foil for 24 hours before the next washing.

Bioassays were carried out in WHO susceptibility kits. Subsamples cut from the nets were attached to filter paper and placed inside the exposure tube, lining the sides of the tube. A total of 20 mosquitoes (*An. gambiae*) were placed in the holding tube. The holding tube and the exposure tube were connected using the sliding gate and mosquitoes were then transferred by opening the gate and gently blowing them into the exposure tube. Mosquitoes were held in the exposure tube for 3 minutes before being transferred back to the holding tube and monitored for KD and mortality. KD was measured at 5-minute intervals up to 30 minutes, and thereafter at 45 minutes and 60 minutes. Mortality was recorded 24 hours post-exposure.

Five different formulations of the deltamethrin SC were tested, which varied in the wetting agent used and the production process. For all but one formulation, KD and mortality remained equal to or above 95% and 80% respectively until 20 washes.

For the remaining formulation, KD after 20 washes was 94% and mortality was 75%.

Using these bioassay data and chemical analysis of deltamethrin content, a model of insecticide loss was applied to the DawaPlus[®]. Chemical analyses were done pre-wash and after 20, 25 or 30 washes. Assuming a model of exponential loss, a retention index was calculated by taking the “nth” root of post-washing deltamethrin content divided by the pre-washing content. N is defined as the number of washes. Exponential curves were then fitted to estimate the retention index at each wash. Based on these data, the average retention index was calculated as 94%. Total concentration at n washes was calculated by multiplying the initial deltamethrin concentration by the retention index raised to the nth power. Assuming that the surface concentration was equal to the amount of deltamethrin lost between washes, the surface concentration could be calculated by subtracting the total concentrations at subsequent washes. Using this model and comparing it with the bioassay data, it was estimated that a minimum surface concentration of 10 mg AI/kg of net would result in $\geq 95\%$ KD and that 15 mg AI/kg would result in $\geq 80\%$ mortality. It was further estimated that these minimum surface concentrations corresponded to a total concentration of 200 mg AI/kg. Deltamethrin concentrations after 20 washes were near to or below these thresholds.

Using a second set of nets, the retention index was calculated as 97%; after 20 washes, the total concentration was estimated as 360 mg/kg with a corresponding estimated surface concentration of 14.5 mg/kg. The manufacturer, however, concluded that it is essential to apply the above model to more bioassay trials done on DawaPlus[®] in order to confirm its applicability. It also noted the need to confirm whether surface concentration or total concentration is the driver of the technology's efficacy.

5.2.2 Field Studies

Kyenjojo District, Uganda

A three-year field study of DawaPlus[®] was initiated in five villages in western Uganda. Nets were distributed to village residents in April 2006. Surveys of net use were conducted at

6-monthly intervals and nets sampled after 12 months in the field for bioassays and chemical analysis. The results from year 1 have been reported (Kilian, 2007).

Bioassays were conducted using WHO cone tests. Five female *An. gambiae* (Kisumu strain, 2–5 days old) were introduced into four cones applied simultaneously to 30 cm x 30 cm net samples. The mosquitoes were exposed for 3 minutes and then transferred to paper cups with access to sugar solution. The process was repeated so that a total of 40 mosquitoes were tested against each net sample. KD was measured 60 minutes post-exposure and mortality was measured 24 hours post-exposure. Chemical analysis was done using gas chromatography with electron capture detection.

After 6 months, 27.7% of the nets had been washed; by 12 months, 50.4% of nets had been washed. At 6 months, 15.4% of all nets had at least one hole. By 12 months, 33.6% of nets had at least one hole.

The average deltamethrin concentration among DawaPlus[®] nets at baseline was 35.9 mg/m². After 12 months, average deltamethrin concentration on DawaPlus[®] nets was 26.4 mg/m². At baseline, KD and mortality of *An. gambiae* exposed for 3 minutes to DawaPlus[®] nets were 96.9% and 78.5%. After 12 months of field use, KD was 98.7% and mortality was 99.7%. Within and between net variations in deltamethrin concentrations at baseline were high for a factory-treated net and were similar to those observed for a field-treated technology. The coefficient of variation (the standard deviation expressed as a percentage of the mean) between nets was 45.7% for DawaPlus[®] and ranged from 16.0% to 79.7% for the KO-Tab 123. For within-net variations, the average percentage difference from the mean was 22.4% for the DawaPlus[®] and ranged from 19.6% to 28.6% for the KO-Tab 123.

In comparison, conventionally treated nets from a previous study conducted in the same area had initial deltamethrin concentrations of 42.8 mg/m² at baseline and 1.4 mg/m² after 12 months of use. Average KD of *An. gambiae* fell from 100% at baseline to 59.5%, while mortality fell from 99.4% to 44.8%.

The authors concluded that the DawaPlus[®] LN performed significantly better than a conventionally treated net after 12 months of use, but further follow up is planned to assess the duration of longevity of the DawaPlus[®] LNs.

Malanville, Benin

The DawaPlus[®] LN was evaluated in experimental huts in Malanville, Benin (Hougard et al., 2006) according to WHO guidelines. The huts used for testing were made from concrete bricks with a corrugated iron roof, a ceiling of thick polyethylene sheeting and a concrete base with a water-filled channel to prevent the entry of ants. Mosquitoes were able to enter the huts through slits (1 cm) constructed from pieces of metal. The metal pieces were fixed at an angle to allow easy entry but to impede exiting mosquitoes. A veranda trap (2 m long, 1.5 m wide and 1.5 m high) projected from the back of the hut and was used to record the proportion of mosquitoes exiting the hut.

A total of 10 different huts were used in the study with the following 10 treatment arms: (i) untreated net, 100 denier; (ii) polyester net, 100 denier, conventionally treated at 25 mg/m², unwashed; (iii) DawaPlus[®], 100 denier, unwashed; (iv) DawaPlus[®], 100 denier, washed 20 times; (v) polyester net, 100 denier, conventionally treated at 40 mg/m², washed 20 times; (vi) polyester net, 100 denier, conventionally treated at 25 mg/m², washed just before exhaustion (two washes); (vii) DawaPlus[®], 75 denier, unwashed; (viii) DawaPlus[®], 75 denier, washed 20 times; (ix) polyester net, 75 denier, conventionally treated at 40 mg/m², washed 20 times; (x) polyester net, 75 denier, conventionally treated at 25 mg/m², washed to just before exhaustion (two washes).

Five nets were used in each arm, along with one net for chemical analysis. The nets were rotated within the huts each night for one week. The net treatments were rotated through a different hut each week. Sleepers were rotated among huts on a daily basis. Six holes (25 cm x 25 cm) were cut in each net to simulate a torn net.

Bioassays were conducted on each net before washing, just after washing and after the completion of the experimental hut trial. Five cones were placed on the five sections of the net (roof and four walls). Five mosquitoes were introduced into

each cone for 3 minutes and then held in small cages to record KD at 60 minutes and mortality at 24 hours. This was repeated so that a total of 50 mosquitoes were tested per net. For the conventionally treated nets washed until exhaustion, bioassays were conducted after each wash until mortality fell below 80% and KD below 95%. For both the 75-denier and the 100-denier net, mortality and KD dropped below these thresholds after two washes.

Adult volunteers slept under the nets, and mosquitoes were collected in the morning. Dead mosquitoes were collected from the floor and resting mosquitoes from the net, walls and roof using aspirators. Live mosquitoes were placed in small cups with access to sugar solution and held for 24 hours to assess delayed mortality. The primary outcomes measured included deterrence (reduction in hut entry relative to the control hut); induced exophily (proportion of mosquitoes found in the exit traps); blood-feeding inhibition (proportion of mosquitoes that were blood fed relative to the control); and immediate and delayed mortality (proportion of dead mosquitoes).

The bioefficacy of the nets in cone tests was 100% before washing. After washing 20 times, KD and mortality of *An. gambiae* exposed to conventionally treated nets fell to <10%, regardless of the denier. For the 75- and 100-denier DawaPlus[®] washed 20 times, mortality dropped to 9.8% and 10% respectively and KD to 29.5% and 31.8%, respectively. For the conventionally treated net washed to exhaustion, mortality was 73.9% and 77.6% for the 75- and 100-denier nets; KD was 88.4% and 94.0%. After 60 nights of field testing, KD and mortality as measured in WHO cone tests had changed little.

For the 100-denier nets, the unwashed DawaPlus[®] LN and the unwashed conventionally treated net reduced hut entry by *An. gambiae* by 41.5% and 25% respectively. The conventionally treated net washed to exhaustion reduced hut entry by 22.3%. The DawaPlus[®] LN washed 20 times and the conventionally treated net (40 mg/m²) washed 20 times did not significantly affect hut entry by *An. gambiae*. There was no difference in the number of *An. gambiae* entering huts with a DawaPlus[®] LN washed 20 times compared with a conventionally treated net washed to exhaustion.

Induced exophily of *An. gambiae* was increased by the presence of a 100-denier treated net, regardless of type or number of washes. The number of females in the veranda traps increased by 1.2 to 1.4 fold. There was no significant difference in the number of females exiting the huts with the DawaPlus[®] LN washed 20 times compared with the conventionally treated net washed to exhaustion.

Blood-feeding rates of *An. gambiae* were significantly reduced in all huts with 100-denier treated nets compared with the control hut, except for the huts with a conventionally treated net (40 mg/m²) washed 20 times. There was no significant difference in the number of blood-fed mosquitoes in huts with the DawaPlus[®] LN washed 20 times compared with a conventionally treated net washed to exhaustion. No significant blood-feeding inhibition was observed for the conventionally treated net washed 20 times. Blood-feeding inhibition among the other treatments ranged from 64.0% to 88.7%.

Mortality of *An. gambiae* was significantly higher in huts with treated nets compared with the control huts. For the 100-denier treated nets, mortality was lowest in the conventional net treated at 40 mg/m² and washed 20 times (26.7% after correcting for control mortality) and highest in the unwashed conventional net treated at 25 mg/m² (95.3%). Mortality in the DawaPlus[®] LN washed 20 times was 55.1% and was significantly lower than mortality for the conventionally treated net washed to exhaustion (67.6%).

Similar trends were observed for the 75-denier nets as well as against mosquitoes other than *An. gambiae*.

Bayer Environmental Sciences provided analytical data confirming the the DawaPlus[®] LNs used in this study complied with acceptable limits for declared deltamethrin content ($\pm 25\%$ target dose).

5.3 Efficacy – WHOPES supervised trials

5.3.1 Laboratory studies

Montpellier, France

A laboratory study was conducted to assess the efficacy, resistance to washing and regeneration of the DawaPlus[®] LN (Duchon et al., 2006). Four DawaPlus[®] nets and four untreated nets were provided by the manufacturer. Eight pieces of netting (25 cm x 25 cm) were cut from each net. Four net pieces were used for the regeneration study; the remaining 28 were used for the wash-resistance study.

To determine the regeneration time of the DawaPlus[®] LN, four net samples were washed and dried three times consecutively. WHO cone bioassays were conducted at 1, 3, 5, 7, 10 and 14 days after the third wash. The time required to reach the initial level of biological activity is defined as the regeneration time. To determine the wash resistance of the DawaPlus[®] LN, net samples were washed using standard WHO procedures. Four net samples were removed from the washing cycle before washing and after 5, 10, 15, 20 and 25 washes for bioassays. Samples were washed five times per week and held at 30 °C over the weekend.

Bioassays were conducted in WHO cone tests fixed to the net samples. Five female mosquitoes (*An. gambiae*, Kisumu strain, 2–5 days old) were introduced into the cones for 3 minutes and then transferred to cages where they were held with access to sugar solution for 24 hours. A total of 50 mosquitoes were tested on each net sample (200 mosquitoes were exposed on four net samples at each wash interval). KD was measured at 60 minutes post-exposure and mortality recorded at 24 hours post-exposure.

In the regeneration study, mortality on the DawaPlus[®] LNs before washing was low (39%). One day after three washes, mortality dropped to 26%. After 3 days, mortality was 34%. There was no significant difference observed between 1 and 3 days. KD was 93% on an unwashed net. One day after the net samples had been washed three times, KD was 89%. After 3 days, knockdown was 93%. Therefore, the regeneration time

was defined as 1 day and the washing interval for the wash-resistance study was set at 1 day.

In the wash resistance study, unwashed DawaPlus[®] LNs showed low mortality (39%) but KD was 93%, close to the WHOPES-defined threshold of 95%. Average mortality of *An. gambiae* exposed to nets that had been washed up to 25 times ranged from 29% to 77%. Mortality increased after one wash to 77% but declined thereafter. For washes 5–25, mortality ranged from 29% to 52%. However, KD on nets that had been washed up to 25 times was $\geq 95\%$ for all washing intervals, except for 10 washes where KD was 92%. It was concluded, based upon the cone bioassays, that the DawaPlus[®] LNs met the criteria for WHOPES Phase I testing; no tunnel test was employed.

Chemical analyses showed an initial deltamethrin concentration of 1.21 g/kg, slightly below the target of 1.33 g/kg but within specifications ($\pm 25\%$). Between-net RSD was 23% and AI content of one of the four net samples was 0.86 g/kg, i.e. outside the specifications for deltamethrin content. After 20 washes, average deltamethrin content was 0.65 g/kg. The overall deltamethrin retention after 20 washes was 53.8% and estimated retention per wash ranged from 90.8% to 97.3% (average=95.2%).

5.3.2 Experimental hut studies

Muheza, United Republic of Tanzania

The DawaPlus[®] LN was tested in experimental huts in Muheza, United Republic of Tanzania (Tungu, et al., 2007c) according to guidelines recommended by WHOPES. The huts were constructed with brick walls plastered with mud, a wooden ceiling lined with hessian sack cloth, an iron sheet roof and open eaves. The huts were fitted with window traps and veranda traps on two sides. The other two sides had open eaves to allow entry of host-seeking mosquitoes into the hut. The huts were set on concrete plinths with a water-filled moat to exclude ants.

Six treatment arms were evaluated as follows: (i) DawaPlus[®], unwashed; (ii) DawaPlus[®], washed 20 times; (iii) DawaPlus[®], washed to exhaustion (22 washes); (iv) polyester net,

conventionally treated with the KO-Tab (deltamethrin water dispersible tablet) and washed until exhaustion (six washes); (v) polyester net, conventionally treated with the KO-Tab and washed 20 times; and (vi) untreated polyester net.

Nets were evaluated in six different huts with six different sleepers rotated in a Latin square design. Three nets were used in each treatment arm, and each net was tested twice in each hut. Six holes (4 cm x 4 cm) were cut into each net to simulate a torn net.

Nets were washed in 10 L of soap solution (2 g/L of Savon de Marseille). The nets and solution were agitated using a pole for three minutes, left to soak for 4 minutes and then agitated for a further 3 minutes. The total immersion time was therefore 10 minutes. The nets were rinsed in water twice and dried between washes.

Bioassays were conducted on the conventionally treated net and the DawaPlus[®] LN to determine the exhaustion point. Washes were done as described above, and WHO cone bioassays were conducted on the top and all four sides (minimum of 50 mosquitoes tested per net). WHO cone bioassays were also conducted on one net from each arm before washing, and after completion of 20 washes.

Chemical analyses were conducted on 20 pieces cut from one net from each treatment arm before washing. The nets retained for chemical analysis were then washed and, upon completion of washing, 20 pieces were cut from the net for chemical analysis. At the end of the hut study, one net from each arm was retained for chemical analysis. All chemical analyses were done using high performance liquid chromatography.

The experimental hut study was run with sleepers entering the huts at dusk. Mosquitoes were collected in the morning. Dead mosquitoes were collected from the floors of the room and veranda and from window traps. Live mosquitoes were collected resting from the walls and roof of the hut and the two verandas. The number of mosquitoes in the veranda traps was multiplied by two to adjust for mosquitoes escaping through the eaves on the other two sides of the house. The primary outcomes measured included deterrence (reduction in hut entry relative to the control hut); induced exophily (proportion of

mosquitoes found in the exit traps); blood-feeding inhibition (proportion of mosquitoes that were blood fed relative to the control); and immediate and delayed mortality (proportion of dead mosquitoes).

Initial bioassays on unwashed DawaPlus[®] LNs and conventionally treated nets resulted in >95% mortality and KD. For the conventionally treated net, KD fell below 95% after three washes, whereas mortality fell below 80% after seven washes. The exhaustion point was therefore set at six washes. For the DawaPlus[®] LN, KD fluctuated between 80% and 90% until the 20th wash. Mortality remained above 80% until 21 washes. The exhaustion point was therefore set at 22 washes. For the nets washed 20 times, cone bioassays after the 20th wash resulted in 50% KD and 48% mortality for the conventionally treated net. For the DawaPlus[®] LN, KD and mortality after 20 washes were 68% and 90% respectively.

Average initial deltamethrin concentrations were 50.5 mg/m² for the DawaPlus[®] LN. Initial concentrations for two conventionally treated nets were 35.8 mg/m² for one and 28.8 mg/m² for the other. Deltamethrin concentrations for the DawaPlus[®] LN were 35.7 mg/m² after 20 washes and 44.8 mg/m² after 22 washes. Deltamethrin concentrations on the washed, conventionally treated nets were unexpectedly high (6.6 mg/m² after 6 washes and 7.4 mg/m² after 20 washes).

The hut trial was run for 6 weeks (36 nights of collection). *An. funestus* and *An. gambiae* were the predominant mosquito species captured during the hut trials, with much higher numbers of *An. funestus* (3063 collected) than *An. gambiae* (545 collected). For *An. funestus*, there was a trend for the DawaPlus[®] treatments to show higher deterrence, although the differences in the number of females captured per night were not statistically different among any of the treatment groups.

All treatments showed high rates of *An. funestus* exiting from the huts. All treated nets had significantly higher rates of exophily compared with the control net, but there were no differences among the treated nets, regardless of type of net (DawaPlus[®] or conventionally treated) or number of washes.

Mortality of *An. funestus* was highest (>55% after correcting for control mortality) in the DawaPlus[®] treatments regardless of the number of washes. In contrast, mortality for the conventionally treated nets was 45.7% after 6 washes and 44.1% after 20 washes. Mortality rates of *An. funestus* in the huts with DawaPlus[®] LNs washed 0, 20 or 22 times were significantly higher than those in huts with conventionally treated nets washed 6 or 20 times. Overall killing effect was calculated as $100 \times (K_t - K_u) / T_u$, where K_t is the number killed in the treated huts, K_u is the number killed in the control huts and T_u is the total number collected from the control huts. Because mortality rates and deterrence rates were both higher in the DawaPlus[®] treatments, the overall killing effect was no different from that of the conventionally treated nets.

The rate of blood-feeding by *An. funestus* was significantly higher in the control huts compared with all other treatment arms. It was 61% to 64% in the unwashed DawaPlus[®] unwashed and the DawaPlus[®] washed 20 times, respectively. This was not significantly different from the 73% blood-feeding inhibition rate for the conventionally treated net washed to exhaustion. Personal protection was calculated as $100 \times (B_u - B_t) / B_u$, where B_u is the number bloodfed in the control huts and B_t is the number blood fed in the treated huts. Personal protection ranged from 71.7% to 78.8% among the three DawaPlus[®] treatment arms and the conventionally treated net washed to exhaustion. For huts with a conventionally treated net washed 20 times, personal protection was 53%.

For *An. gambiae*, there was no significant difference in hut entry among any of the treatment groups. The rate of exiting was higher in huts with treated nets compared with the control huts. However, there was no difference in exiting rates among the treated nets regardless of type of net (DawaPlus[®] or conventionally treated net) or number of washes.

Mortality of *An. gambiae* in the control huts was 4.7%; mortality in all other treatments was >45% (after correcting for control mortality). Mortality for the DawaPlus[®] washed 20 times was 60.5%, while mortality for the conventionally treated net washed to exhaustion was 50.9%. The difference was not statistically significant. The difference in mortality between a DawaPlus[®] washed 20 times and a conventionally treated net washed 20

times was significantly different. Overall killing effect was lowest for the conventionally treated net washed to exhaustion (33.6%) and highest for the unwashed DawaPlus[®] LN (54.2%).

Blood-feeding rates of *An. gambiae* were significantly higher in the control huts compared with all other treatments except the conventionally treated net washed 20 times, where the differences were not statistically significant. Blood-feeding rates among the treated net arms were not significantly different, regardless of type of net or number of washes. Blood-feeding inhibition ranged between 63% and 77% for the DawaPlus[®] treatment arms and was 67% for the conventionally treated net washed to exhaustion.

5.4 Conclusions and recommendations

DawaPlus[®] net is manufactured by Tana Netting Co. Ltd. (Thailand), in collaboration with Bayer Environmental Science, as a deltamethrin (coated) LN. Deltamethrin SC is coated on knitted multi-filament polyester fibres, at the target dose of 1.33 g/kg in 75-denier yarn and 1 g/kg in 100-denier yarn, corresponding to 40 mg of deltamethrin per LN m², using a polymer as a binder.

The WHO assessment of the compliance of the manufacturer's assessment of exposure to and risks of washing and sleeping under a DawaPlus[®] LN was in line with the WHO generic risk assessment model. Although some default values of the guideline were not used but justified, the conclusions were in agreement; and that the washing of the LN or sleeping under the net do not pose undue risk to adults, children or newborns.

The within-net variation of deltamethrin content on DawaPlus[®] nets is high. The manufacturer's proposed 20% area sampling of a net for quality control resulted in an RSD of 7.7–15.4 g/kg for within net variation, higher than that currently proposed by WHO for development of specifications for LNs (RSD ≤5%), despite the analysis of unusually large samples.

The manufacturer reported that variability between nets from the declared content of 1.33 g/kg deltamethrin on DawaPlus[®] LNs ranged between –16% and +41%, which is outside the

limits of $\pm 25\%$ proposed for development of WHO specifications for "heterogeneous" pesticide formulations (including LNs) with declared content of active ingredient of up to 25 g/kg.

Laboratory studies revealed that DawaPlus[®] LNs met the WHOPES Phase I criteria of a KD effect exceeding 95% after 20 washes. The mortality, however, was consistently lower than the 80% WHO criteria and demonstrated unexpected variability, which may have been due to variability in initial deltamethrin content. After depleting surface concentrations caused by washing, the initial level of bioavailability was achieved within one day.

Experimental hut studies in Benin demonstrated a lesser impact of DawaPlus[®] LNs washed 20 times on mortality and blood-feeding inhibition of prominent malaria vectors compared with that of the conventionally treated polyester nets (25 mg/m² deltamethrin) washed until just before exhaustion. Blood-feeding inhibition of DawaPlus[®] LNs washed 20 times was not significantly different from that of a conventionally treated net washed until just before exhaustion.

In experimental hut studies carried out in the United Republic of Tanzania, there were no significant differences in blood-feeding inhibition and mortality between DawaPlus[®] LNs washed 20 times or conventionally treated nets washed until just before exhaustion.

Noting the above, the meeting concluded:

- that the marginal efficacy and performance of the product do not fulfil the requirements of WHO for a long-lasting insecticidal mosquito net;
- that the high within- and between-net variability in deltamethrin content may have had a significant impact on the observed efficacy. This will also impact the development of standards and methods for quality control of this product. The manufacturer is urged to reduce the variability of deltamethrin content in conformity with limits recommended by WHO.

6. REVIEW OF ICON[®] MAXX

ICON[®] MAXX is manufactured by Syngenta, Switzerland, as a "dip-it-yourself" treatment kit for converting mosquito nets into long-lasting insecticide-treated nets. The kit is based on the slow-release capsule suspension (CS) of lambda-cyhalothrin that has previously been evaluated by WHOPES and has been recommended for treatment of mosquito nets¹. WHO specifications for quality control of lambda-cyhalothrin CS are available on the WHO home page on the Internet.²

ICON[®] MAXX is presented as a twin sachet pack, containing 6 ml of lambda-cyhalothrin 10% CS and 6 ml of binding agent, sufficient for the treatment of an individual polyester mosquito net. The target dose of ICON[®] MAXX on a family size (130 x 180 x 150 cm) polyester mosquito bed net is 50 mg/m².

The company has disclosed the nature of the binder to WHOPES, and this information will be treated as confidential. The company has certified that it will inform WHOPES of any change to the binder, as this may require the re-assessment of the safety and efficacy of ICON[®] MAXX and therefore of WHO's recommendation on the product.

6.1 Safety assessment

The assessment of risk to humans of mosquito nets impregnated with ICON[®] MAXX and the subsequent use of such nets, provided by the manufacturer, was assessed by FIOH (FIOH, 2007b) on behalf of the WHO Programme on Chemical Safety. The WHO *Generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*³ was used as a guiding document.

¹ Najera JA, Zaim M. *Malaria vector control: decision-making criteria and procedures for judicious use of insecticides*. Geneva, World Health Organization, 2002 (WHO/CDS/WHOPES/2002.5 Rev. 1; available at http://whqlibdoc.who.int/hq/2003/WHO_CDS_WHOPES_2002.5_Rev.1.pdf).

² <http://www.who.int/whopes/quality/newspecif/en/>.

³ *A generic risk assessment model for insecticide treatment and subsequent use of mosquito nets*. Geneva, World Health Organization,

The following assumptions/methodologies were used by the proposer in drafting the assessment:

1. inhalation exposure during sleeping under the net is negligible given the low vapour pressure of lambda-cyhalothrin;
2. adopts 0.0038 and 0.0025 mg/kg body weight as AELs for "acute" and "chronic" systemic exposure (values identical to those set by JECFA for long-term exposure to cyhalothrin¹ and to that of the European Union for short-term and long-term exposures to lambda-cyhalothrin²);
3. uses 0.1% and 1% as the dermal absorption rates for the concentrated preparations and the dilutions of the insecticide. The values differ greatly from the default assumptions of the generic model, but the proposer justifies this by experimental data in vivo and in vitro from studies in animals and in humans.
4. considers dipping and washing the nets to be an acute exposure scenario, which is in line with the generic model; uses 0.01 mL per operation for assessing dermal exposure during preparation of the dipping solution; uses a 5% release of insecticide content into washing solution for assessing dermal exposure during washing; and that dermal exposure is independent of the number of nets washed by the same individual during the same day, as a larger number of nets requires use of a larger volume of washing water, and the dermal absorption is mainly dependent on the concentration of the product in the washing liquid, and the surface area exposed.

FIOH concluded that the characterization of the risks performed by the proposer closely follows the WHO generic model; where default assumptions are not accepted, justification mostly in the form of actual experimental data, are presented. The conclusion,

2004 (WHO/CDS/WHOPES/GCDPP/2004.6; available at http://whqlibdoc.who.int/hq/2004/WHO_PCS_04.1.pdf).

¹ WHO Food Additives Series 53: Cyhalothrin (addendum) 2004; available at

<http://www.inchem.org/documents/jecfa/jecmono/v53je04.htm>.

² http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1-24_en.pdf.

in line with the generic model, is that no unacceptable exposures were found in the preparation, maintenance and use of the nets. *However, accidental ingestion of a whole content of the sachet may be hazardous to a child.*

6.2 Efficacy – background and supporting documents

Moshi and Muheza, United Republic of Tanzania

ICONET[®] MAXX (= Icon MAXX) LNs were evaluated in experimental huts in the United Republic of Tanzania, using free-flying wild malaria vectors. Efficacy was evaluated in terms of blood-feeding inhibition, deterrence, induced exophily and mortality. Mosquitoes could escape via the eaves to screened or open verandas. To adjust unrecorded escapes in the latter case, the numbers of mosquitoes collected in screened verandas were multiplied by two.

Sleepers were rotated randomly among huts each night of the study. Each net was deliberately pierced with six holes (4 cm x 4 cm) to simulate a torn net. One additional net per treatment arm was used for chemical analyses and bioassays. Standard WHO procedures were used for washing the nets. Cone bioassays were carried out using a colony strain of *An. gambiae* (Kisumu strain). The data of the hut trials were analysed using non-parametric tests for numeric data and logistic regression for proportional data. ICONET[®] MAXX treatments were executed by the company; conventional treatments were done by the London School of Hygiene and Tropical Medicine.

To establish the number of washes just before exhaustion, polyester nets were conventionally treated with lambda-cyhalothrin 2.5CS (ICONET) at the dose of 15mg/m² (WHO recommended dose) (Rowland et al., 2007). At four washes, KD and mortality fell below the critical thresholds, meaning that lambda-cyhalothrin conventionally treated nets washed three times should be the standard reference.

Three experimental hut trials were performed.

The first trial was carried out in Moshi in three huts comparing (i) untreated polyester net; (ii) ICONET[®] MAXX treated polyester nets washed 20 times; and (iii) polyester net conventionally

treated at 15 mg/m² AI of lambda-cyhalothrin 2.5CS (ICONET) and washed 20 times. Each treatment was tested in each hut for 4 consecutive nights on two separate occasions (24 test nights).

During the 24 nights, 195 *An. arabiensis* and 184 *C. quinquefasciatus* were collected in the control huts. A significant deterrent effect was observed on *An. arabiensis* with the conventionally treated nets washed 20 times (31%) but not with ICONET[®] MAXX treated nets washed 20 times (8%). The ICON[®] MAXX washed 20 times induced a deterrent effect only on *Culex* mosquitoes (25%).

Exophily is naturally high with *An. arabiensis* and low with *C. quinquefasciatus*. In control huts, exit rates were respectively 73% and 56% for these species. In huts with treated nets, most mosquitoes were found in the window traps and verandas (95–98% *An. arabiensis*, and 89–98% *C. quinquefasciatus*).

Few mosquitoes penetrated the holed-treated netting compared with the untreated netting. Less than 1% of the mosquitoes entered ICONET[®] MAXX treated nets washed 20 times, compared with 5% in conventionally treated nets washed 20 times and 29% in untreated nets.

The mortality of *An. arabiensis* was high (70%) with the ICONET[®] MAXX treated nets washed 20 times compared with the conventionally treated nets washed 20 times (40%). These figures contrast with the low mortality (13%, 5% and 1% respectively) observed for *Culex* mosquitoes, known to be highly resistant to pyrethroids. The overall killing effect on *An. arabiensis* was 66% with the ICONET[®] MAXX treated nets washed 20 times and 29% with the conventionally treated nets washed 20 times. The killing effect on the pyrethroid-resistant *C. quinquefasciatus* was much lower (9% and 4% respectively).

Blood-feeding inhibition was higher with the washed ICONET[®] MAXX treated nets compared with the washed conventionally treated nets, and was significant for *C. quinquefasciatus* (94% and 73% respectively) but not for *An. arabiensis* (89% and 66% respectively). The personal protection from *An. arabiensis* biting was 90% with the washed ICONET[®] MAXX treated nets and

76% with the washed conventionally treated nets. Protection from *C. quinquefasciatus* biting was 96% and 73% respectively.

The second trial was also conducted in 2006 in Moshi. The following six treatment arms were tested: (i) untreated polyester net; (ii) unwashed ICONET[®]MAXX polyester treated nets; (iii) ICONET[®]MAXX polyester treated nets washed 20 times (WHO criteria for an LN); (iv) ICONET[®]MAXX polyester treated nets washed to the cut-off point (27 washes); (v) polyester net conventionally treated with lambda-cyhalothrin 2.5CS (ICONET) at 15 mg/m² AI (WHO-recommended concentration) and washed to the cut-off point (four washes); (vi) polyester net conventionally treated with lambda-cyhalothrin 2.5CS (ICONET) at 15 mg/m² AI (WHO-recommended concentration) and washed 20 times.

Each treatment was tested for four consecutive nights in each of the six huts. Two nets were used per treatment arm.

During the 24 nights, 483 *An. arabiensis* and only 35 *C. quinquefasciatus* were collected in the control huts. No significant deterrent effect was observed for any treatment arm. The majority of *An. arabiensis* (81%) exited the control huts during the night, and no insecticide-induced exophily was apparent.

Mortality with ICONET[®]MAXX treated nets (42–48%) was significantly higher than the mortality observed with conventionally treated nets (32–38%). No significant difference in mortality was observed between unwashed and washed nets of either treatment. As no significant deterrent effect was observed, overall killing effect and mortality are almost the same.

All insecticide treatments provided significant blood-feeding inhibition (ranging from 53% to 70%). Blood-feeding inhibition for ICONET[®]MAXX treated nets washed 20 times was similar to that of the conventionally treated nets washed to the cut-off point (70% and 67% respectively).

The third trial was conducted in 2007 in Muheza. The following six treatment arms were tested: (i) untreated polyester net; (ii) unwashed ICONET[®]MAXX polyester treated nets; (iii)

ICONET[®]MAXX polyester treated nets washed 20 times (WHO criteria for an LN); (iv) ICONET[®]MAXX polyester treated nets washed to cut off (27 washes); (v) polyester net conventionally treated with lambda-cyhalothrin 2.5CS (ICONET) at 15 mg/m² AI (WHO-recommended concentration) and washed to cut off (four washes); (vi) polyester net conventionally treated with lambda-cyhalothrin 2.5CS (ICONET) at 15 mg/m² AI (WHO-recommended concentration) and washed 20 times. The six treatments were rotated through the six huts over 6 weeks with each treatment arm tested in each hut for 6 nights. Three nets were tested in each treatment arm and were rotated such that each net was tested for two nights in each hut.

At the end of the trial, one net of each arm was taken and four 10 x 10cm pieces of netting (one from each side) were cut and subjected to chemical analysis. The HPLC analysis showed that the target dose of 15 mg/m² AI lambda-cyhalothrin for conventionally treated nets was reached (13.2 ± 6.1 mg/m² AI). For the ICONET[®]MAXX polyester treated net, the observed dose (59.7 ± 29.1 mg/m² AI) was higher than that announced by the manufacturer (50 mg/m² AI). Washing the conventionally treated nets clearly removed the insecticide (after 4 washes: 0.5 ± 0.1 mg/m² AI; after 20 washes 0.2 ± 0.1 mg/m² AI), while about 50% (29.0 ± 18.3 mg/m² AI) of the insecticide remained on the ICONET[®]MAXX treated net after washing 20 times and about 10% after 27 washes.

During the 36 nights, 87 *An. gambiae* and 207 *An. funestus* were collected in the control huts. Deterrence on *An. gambiae* was the same with ICONET[®]MAXX unwashed or washed 20 times (61%) and was similar (45%) to conventionally treated nets washed to the cut-off point. With *An. funestus*, the deterrent effect was significantly higher with ICONET[®]MAXX washed 20 times compared with the conventionally treated nets washed to cut off (71% and 25% respectively).

Exophily rates of *An. gambiae* and *An. funestus* were high with untreated nets (79% and 89% respectively). A significant insecticide-induced exophily occurred only with nets washed to the cut-off point (ICONET[®]MAXX washed 27 times and conventionally treated nets washed 4 times) for both species.

With *An. gambiae*, mortality with ICONET[®]MAXX treated nets was similar over 0, 20, or 27 washes (around 70%) and was twice as high as mortality observed with conventionally treated nets washed to cut-off point (36%). Unwashed ICONET[®]MAXX treated nets induced mortality of *An. funestus* of 78%. The mortality of *An. funestus* was significantly higher with ICONET[®]MAXX washed 20 times compared with the conventionally treated nets washed to the cut-off point (61% and 52% respectively).

Significant blood-feeding inhibition was observed for both species with ICONET[®]MAXX treated nets washed or unwashed but was lower with *An. funestus* (around 40%) than with *An. gambiae* (80–54%). Blood-feeding inhibition of the ICONET[®]MAXX treated nets washed 20 times was similar for *An. funestus* and significantly higher for *An. gambiae* compared with the conventionally treated nets washed to cut off (38% vs. 31% and 54% vs. no inhibition respectively). The personal protective effect against biting *An. gambiae* was of 82% with ICONET[®]MAXX treated nets washed 20 times and 58% with conventionally treated nets washed to cut off. For *An. funestus*, these values were 82% and 48% respectively.

Chemical analysis showed that average (n=20) lambda-delta-cyhalothrin content of ICON[®] MAXX samples was 59.7±29 mg/m² (mean ± 95% CI) while the ICONET treated nets had 13.2±6.1 mg/m², close to the target of 15 mg/m².

6.3 Efficacy – WHOPES supervised trials

6.3.1 Laboratory studies

Bonnet et al. (2006b) conducted laboratory tests to measure the efficacy and resistance to washing of nets treated with ICONET[®]MAXX (= Icon MAXX) according to WHO guidelines for laboratory testing of LNs¹. Four white and four blue polyester nets were each cut in two to provide two different batches of samples for estimating the regeneration time and resistance to

¹ *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets*. Geneva, World Health Organization, 2005 (WHO/CDS/WHOPES/GCDPP/2005.11; available at <http://www.who.int/whopes/guidelines/en/>).

washing. These half-nets were individually treated by manual dipping in a solution of 250 ml containing 3 ml ICON 10 CS and 3.5 ml MAXX binding agent to reach the target dose of 50 mg/m² lambda-cyhalothrin. From each half-net, eight pieces (25 x 25 cm) were cut. Bioassays were carried out using 2–5 day old, non blood-fed females of the colony strain of *An. gambiae* (Kisumu).

To determine the time period for regeneration of the nets after washing, cone bioassay tests were carried out at different intervals of time (+1, +3, +5 and +7 days) on white net samples washed (WHO standard washes) and dried once and three times consecutively and stored at 30 °C. Mortality for unwashed nets was low (13%) but increased after one and three consecutive washes (31% and 35% respectively). The KD effect was close to 100% whatever the number of washes performed. Similar results were obtained with blue net samples.

Performances on KD and mortality did not improve after keeping the washed white or blue net samples for 3, 5 or 7 days at 30 °C, suggesting a regeneration time of one day.

Bioassays were further performed to establish the wash resistance of white net samples before and after 1, 5, 10, 15 and 20 WHO standard washes. Mortality was very low (13%) before washing and significantly increased after a few cycles of wash–dry–wash (73% and 89% at one and five washes respectively). The mortality decreased under the cut-off value of 80% after 10 washes and was only 32% after 20 washes. The KD effect remained above 95% after 20 washes, which fulfils the WHO requirement for Phase I.

Chemical analysis of net samples after treatment and before washing indicated a dose of 34.4–45.6 and 34.1–58.4 mg/m² AI for the white and blue net samples respectively compared with the target dose of 50 mg/m² AI. The between-net relative standard deviation was 13% for the unwashed white net and 23% for the unwashed blue net. After 20 washes of the white net samples, retention of insecticide was only 29%, which was still enough to induce a KD of 100% but insufficient for inducing a high mortality. The calculated retention per wash ranged from 92.7% to 97.6%, with an average of 94.6%.

6.3.2 Experimental hut studies

Kou Valley, Burkina Faso

ICON[®]MAXX treated nets were evaluated in experimental huts in Burkina Faso, using free-flying wild malaria vectors. Efficacy was evaluated in terms of blood-feeding inhibition, deterrence, induced exophily and mortality. The mosquitoes could only escape outside to a single veranda trap.

The number of huts used corresponded to the number of treatment arms. After ventilation and cleaning, the treatment arms were rotated daily but huts were thoroughly cleaned each day and control mortality indicated no evidence of contamination of the huts. Sleepers were rotated randomly among huts each night of the study. Each net was deliberately pierced with six holes (4 cm x 4 cm) to simulate a torn net. One additional net per treatment arm was used for chemical analyses and bioassays. Standard procedures were used for washing the nets. Cone bioassays were carried out using a colony of Kisumu strain of *An. gambiae*. The data of the hut trials were analysed using non-parametric tests for numeric data and logistic regression for proportional data.

The efficacy of nets in experimental huts was evaluated in from August to September 2007 (Dabire et al., 2007a). Both molecular M and S forms of *An. gambiae* occur in sympatry in the study village. The M form was largely predominant (84.7%). In previous years, the frequency of the *kdr* allele was <5% in the M form and was >80% in the S form. After the hut trial, the allele frequency of the “*kdr*” mutation responsible for KD resistance was 0.72 and 0.91 in the M and S form respectively. Given this unexpected increase of *kdr* allele frequency in the M form, the current trial does not completely fit with the WHOPES requirements where products are tested on a susceptible vector population.

With bioassays on ICON[®] MAXX treated nets, KD dropped below 95% after three washes and mortality below 80% after two washes. The number of washes to before exhaustion is then two. However, the authors decided to take one wash as a cut-off point just before exhaustion, considering the low mortality (60.3%) at wash two. At wash one, KD was 96% and mortality 85%.

The following five treatment arms were tested: (i) untreated polyester net; (ii) unwashed ICON[®]MAXX polyester treated nets; (iii) ICON[®]MAXX polyester treated nets washed 20 times; (iv) polyester net conventionally treated with lambda-cyhalothrin (ICON CS 10) at 15 mg/m² AI (WHO-recommended concentration) and unwashed; (v) polyester net conventionally treated with lambda-cyhalothrin (ICON 10% CS) at 15 mg/m² AI and washed just before exhaustion (one wash).

Five nets were used per treatment arm and each net was tested once a week during 7 weeks on the basis of five collecting nights a week. The treatment arms were rotated daily among the huts according to a Latin square scheme. Huts were ventilated, cleaned and washed every day.

Before washing, all treated nets were fully effective in terms of KD (100%). Mortality exceeded 90% with the conventionally treated nets, while only 70% mortality was observed with the ICON[®]MAXX treated nets. After washing the ICON[®]MAXX treated nets 20 times, mortality fell to 49%. No significant change in mortality was observed with conventionally treated nets after one wash. The results of bioassays were similar on nets collected at the end of the trial.

Chemical residue analysis showed an average (n=4) content of 1.22 g/kg for the unwashed ICON[®]MAXX treated nets, which complies with the expected dose (1.25 g/kg corresponding to 50 mg/m² for 100-denier net) but with a high variation between the nets (range: 1.49–0.65 g/kg). Moreover, the within-net variation of ICON[®] MAXX was very high, ranging from 15% to 97% (RSD). This was higher than the within-net variation for the conventionally treated nets, where the RSD values on two unwashed nets were 22% and 43%. After 20 washes, the dose fell to 0.42 g/kg, corresponding to an overall retention value of 28.2%. The observed dose (14.4 mg/m²) for conventionally treated nets was close to the target dose of 15 mg/m². After one wash, the AI content of the conventionally treated nets was 4 mg/m².

During the 35 nights, 365 *An. gambiae* were collected in the huts with the control nets.

A significant deterrent effect was noted with the unwashed conventionally treated nets and, to a lesser extent, with the unwashed ICON[®]MAXX treated nets (63% and 24% respectively). Deterrence did not occur with conventionally treated nets washed once. The entry rate was significantly increased by 40% with ICON[®]MAXX treated nets washed 20 times.

Exophily was doubled (around 70%) with ICON[®]MAXX treated nets (unwashed and 20 times washed) compared with unwashed conventionally treated nets. The induced exophily with the conventionally treated nets washed just before exhaustion was less pronounced (60%) but still significant compared with the control nets (35%).

Mortality was relatively low for the ICON[®]MAXX treated nets (unwashed and 20 times washed) and unwashed conventionally treated nets (between 23 and 30%) but significantly higher than the mortality recorded with the conventionally treated nets washed to just before exhaustion (16%).

Blood-feeding inhibition with all treated nets was relatively low (between 42% and 48%), and no difference was observed between the treatments.

On *An. gambiae* population presenting a high allele frequency of *kdr* gene, the ICON[®]MAXX treated net washed 20 times was equally effective for blood-feeding inhibition and significantly more effective as far as the mortality is concerned than a conventionally treated net washed to just before exhaustion.

6.4 Conclusions and recommendations

ICON[®] MAXX is manufactured by Syngenta, Switzerland, as a "dip-it-yourself" treatment kit for converting polyester mosquito nets into long-lasting insecticide-treated nets. The kit is based on the slow-release CS of lambda-cyhalothrin that has previously been evaluated by WHOPES and has been recommended for treatment of mosquito nets at the target dose of 50mg/m² (corresponding to 1.25 g/kg for a 100-denier fabric).

ICON[®] MAXX is presented as a twin sachet pack, containing 6 ml of lambda-cyhalothrin 10% CS and 6 ml of binding agent, sufficient for the treatment of an individual polyester mosquito net. The target dose of ICON[®] MAXX on a family size (130 x 180 x 150 cm) polyester mosquito bed net is 50 mg/m².

The company has disclosed the nature of the binder to WHOPES, and this information will be treated as confidential. The company has certified that it will inform WHOPES of any change to the binder, as this may require the re-assessment of the safety and efficacy of ICON[®] MAXX and therefore of WHO's recommendation on the product.

The WHO assessment of the compliance of the manufacturer's assessment of exposure to and risks of treatment, washing and sleeping under ICON[®] MAXX treated nets is in line with the WHO generic risk assessment model. *However, accidental ingestion of the contents of a whole sachet may be hazardous to a child.*

Laboratory studies supervised by WHOPES showed that ICON[®] MAXX treated nets met the WHOPES requirements for KD ($\geq 95\%$ after 20 washes). Mortality demonstrated unexpected variation, ranging from 13% on an unwashed net to 89% on a net washed 5 times. This variation may have been the result of variation in initial lambda-cyhalothrin concentrations. After washing, maximum KD and mortality of ICON[®] MAXX treated nets was reached in one day at 30 °C, and this was true for both blue and white nets. The treatment dose was around the target dose of 50mg/m², but with a high variation between the nets (up to 30%). The variation in initial concentration of the ICON[®] MAXX treated nets was higher than that of ICONET treated nets. The dose fell to around 10mg/m² after 20 washes, which was still enough to induce a high KD but not a high mortality (32%).

The WHOPES supervised small-scale field study (experimental huts) in Burkina Faso was carried out in the presence of populations of *An. gambiae* resistant to kdr. Despite the insecticide resistance, ICON[®] MAXX treated nets washed 20 times were more effective in causing mortality of *An. gambiae* than conventionally treated nets washed to just before

exhaustion and were equally effective for blood-feeding inhibition.

The trials performed in the United Republic of Tanzania on insecticide-susceptible anopheline populations (*An. funestus*, *An. arabiensis*, *An. gambiae*) demonstrated that ICON[®] MAXX had significantly higher mortality and blood-feeding inhibition of both *An. gambiae* and *An. funestus* compared with a conventionally treated net washed to the cut-off point.

Considering the safety, efficacy and resistance to washing of nets treated with ICON[®] MAXX in laboratory and small-scale field studies, it is recommended:

- that a time-limited *interim recommendation* be given to ICON[®] MAXX as an LN;
- that WHOPEs coordinates large-scale field studies (WHOPEs Phase III studies) to confirm the long-lasting efficacy of the treatment kit, and as a requirement for developing *full recommendations* on the use of the product;
- that given the heterogeneity in AI concentration observed on nets during trials, nets treated with ICON[®] MAXX cannot be recognized as equivalent to WHOPEs-recommended, factory-produced LNs.

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control.¹

¹ WHO specifications for public health pesticides are available on the WHO home page on the Internet at <http://www.who.int/whopes/quality/en/>.

7. REVIEW OF WHOPES LABORATORY AND SMALL-SCALE FIELD TESTING AND EVALUATION OF LONG-LASTING INSECTICIDE-TREATED MOSQUITO NETS AND THE WAY FORWARD

Over the past two years, 11 new long-lasting candidate technologies (9 LNs and 2 treatment kits) have been or are being tested by WHOPES. Through this testing, data have been generated that have contributed to a better understanding of these technologies. It has also provided WHOPES with an opportunity to review test procedures and criteria to assess LN products and to make recommendations on their use.

Annex 1 presents a detailed analysis of the laboratory results obtained with a number of these products. A precise understanding of retention/release of insecticide from LNs through successive washes is necessary to develop representative models for individual products. Also, it is important to assess the relationship between the measured or estimated surface concentration (bio-available fraction of the insecticide) and the biological efficacy of the insecticidal netting. The overall objective is to refine test procedures and specifications in order to reliably differentiate substandard from high-quality LNs and extend specification to equivalent products.

Considering the report in Annex 1 and the other data/information provided on WHOPES testing and evaluation of LNs, the working group discussed the information gap and made the following recommendations:

1. Industry

- Manufacturers should define the wash characteristics of their LN, based on chemical assays, and follow WHO standard washing procedures. This should be based on determination of the total content of AI before washing and at a minimum of seven wash points, i.e, 1, 3, 5, 10, 15, 20 and 25, to show if these are consistent in batches produced over time.
- Manufacturers should show whether simple measurements (e.g. based on two wash points) can be

used reliably to predict "surface concentrations" after 20 WHO standard washes.

- Manufacturers should ensure that typical variations in the manufacturing process (changes in yarn source, colour, heat settings, knitting, etc.) do not affect the efficacy of their LN.
- Manufacturers should ensure acceptable homogeneity of the AI in their LN products, recognizing that heterogeneity in distribution of AI compromises efficacy, quality control and possibly safety. Manufacturers should minimize the within-net heterogeneity of AI distribution so that the RSD does not exceed 5%, when five pieces of 30 cm x 30 cm are analysed as a single sample according to the scheme recommended in the Manual on development of FAO and WHO specifications for pesticides¹. Moreover the average AI content between nets should not exceed $\pm 25\%$ of the declared AI content, as specified in the same manual.
- Manufacturers, where practicable, should calibrate their bioassays against the bioassays used for WHOPES phase 1 testing (test methods, mosquito species and strain), for internal quality control.

2. *WHOPES and research institutions*

- Further standardize the WHO washing procedure by recommending a detergent approved by the International Standards Organization. The new wash method should then be used by manufacturers, CIPAC and testing laboratories in routine quality control and testing for compliance.
- Further standardize the WHO washing procedure for the Phase II studies, noting the variable results obtained in previous field trials.

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http://whqlibdoc.who.int/publications/2006/9251048576_eng_update2.pdf.

- In WHOPES Phase I testing and evaluation of LNs, bioassay and chemical analysis should be performed on unwashed LNs and after washes 1, 3, 5, 10, 15, 20 and 25, following WHO standard washing procedures, to better define the retention curves and to better describe the product.
- Further explore the potential use of median time to KD in WHOPES Phase I "regeneration" studies to assess the bioavailability of the insecticide after washing.
- Further standardize the WHOPES Phase II (experimental hut) studies by using the following research arms: (i) unwashed LN; (ii) LN washed 20 times; (iii) *polyester* conventionally-treated net washed to just before exhaustion; (iv) unwashed *polyester* net conventionally treated with the same insecticide as that of the LN and at the highest dose recommended by WHO (positive control); (v) untreated *polyester* net (negative control). Additional arms may be included as required.
- Three concurrent WHOPES Phase II studies should be carried out, where possible, and no fewer than two, to minimize the variability in results and to ensure timely availability of study outcomes for WHOPES consideration.
- WHOPES should keep under review the correlation between phase I and II data, to optimize the testing requirements.
- WHO specifications for LNs and their quality control should be based on chemical assay only, realizing the limitations in standardizing bioassays throughout the world.
- Until it can be demonstrated that more sophisticated measurements will provide meaningful results for quality control purposes, WHO specifications for wash resistance should be based on a minimum of 90% retention of AI per wash.

- Reiterate that WHOPES Phase I efficacy studies constitute an essential part of the determination of equivalence of LN products for extension of WHO specifications.

ANNEX 1: CHARACTERIZING THE ACTIVE INGREDIENT RELEASE CHARACTERISTICS OF LONG-LASTING INSECTICIDAL MOSQUITO NETS SUBJECTED TO REPEATED WASHING

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Summary

An overview of active ingredient behaviour in insecticidal netting in response to washing is presented. Mathematical models of active ingredient retention/release with washing are considered but, in cases where transition between models occurs, prediction of retention/release performance may be unreliable. Acceptable performance of LN is defined by WHO as retention of biological activity (e.g. mosquito mortality $\geq 80\%$) through 20 standard washes but there is no simple physico-chemical measurement corresponding to this definition. Mosquitoes which land on the netting are exposed only to active ingredient on the surface but surface concentrations are conceptually and practically difficult to define and measure. Control of mosquitoes (measured as mortality or knock-down, KD) appears to decline from good to poor within very narrow ranges of surface concentration, with the critical ranges for change evidently differing between, and possibly within, products. These differences may be due to variations in spatial presentation and/or distribution of active ingredient, and possibly other factors, but any particular value for surface concentration (however this is expressed) does not correspond to a specific level of biological response. Consequently, there is no point in trying to develop highly accurate and precise analytical test methods to measure/estimate surface concentrations after 20 washes, nor to use them to develop limits for WHO specifications. WHO specifications should provide limits and methods giving an indication of whether or not some retention/release actually occurs but they cannot be used to prove that a particular net will provide acceptable efficacy, before or after 20 standard washes. Retention of $\geq 50\%$ active ingredient over an interval of 5 standard washes is proposed as a simple rule-of-thumb limit but, in cases of doubt and especially if this value is $>95\%$, retention of efficacy after 20 washes can only be checked by bioassay. Expression of surface concentration as mg/kg is proposed. Mortality and KD in WHO laboratory bioassays are shown to be non-equivalent, so mortality is proposed as the criterion of choice for acceptability of efficacy.

A scheme is proposed for harmonization of sampling and washing procedures for use in chemical and biological assays of insecticidal netting. Efficacy data should be recognized as part of the WHO determination of equivalence for LN and guidelines should be developed to ensure transparency and consistency of decision-making.

Introduction

In various forms, insecticidal netting plays an important role in the control of certain vector-borne diseases, particularly when made into bed nets for prevention of malaria by exclusion of mosquitoes.

Insecticidal netting may be manufactured by (i) incorporation of insecticide within the yarn before knitting; (ii) coating the knitted yarn with a polymer containing insecticide; or (iii) binding a pesticide formulation to the knitted yarn. Alternatively, (iv) untreated bed nets may be treated in the field with an insecticide formulation. Category (iv) products may be sold in the form of a kit consisting of an untreated net and the insecticide formulation, which is dispersed in a small volume of water for dipping. Various technologies may be involved in each of the above categories and, consequently, the range of commercial products available presents a wide range of performance characteristics. Insecticidal nets are intended to provide a barrier to mosquitoes for an extended period, during which they are expected to be washed intermittently. Manufactured products in categories (i), (ii) and (iii) are usually designated as long-lasting insecticidal netting (LN), reflecting the longer period and greater number of washes through which efficacy should be retained.

Currently, the polymers used to form the filaments and yarn, from which insecticidal netting is woven, are mostly polyester or polyethylene. These are semi-crystalline polymers, containing both crystalline and amorphous regions. The chemical and physical nature of polymers used to coat the weave in LN, or to encapsulate active ingredient in CS, or to bind formulated active ingredient to netting, is generally regarded as confidential information by manufacturers.

All currently available LN products contain a pyrethroid insecticide as the active ingredient. Pyrethroids have physico-

chemical and biological properties which, when incorporated into suitable netting, provide products with a combination of physical and chemical barriers that is highly effective in preventing penetration by mosquitoes over an extended period and after repeated washing.

The terms “release” and “retention” of active ingredient are used to describe the washing behaviour of insecticidal netting. The percentage of active ingredient removed from the netting surface by a standard wash (WHO 2005) is considered to have been released to the surface of the netting, while the percentage remaining within the netting is retained (released% + retained% = 100%). After washing, a proportion of the retained active ingredient is then released to the surface. Release or retention are usually determined indirectly, by analysis of the total active ingredient content of two separate pieces of netting, one of which is washed and the other is not. Depending upon the analytical method used, the proportion of total active ingredient content extracted during analysis of each piece of netting may be $\approx 100\%$ or $< 100\%$. It is generally assumed that the proportion of total active ingredient extracted by analysis is constant, whether or not the netting has been washed, so the percentage change in active ingredient content produced by washing represents released active ingredient, irrespective of the efficiency of extraction of the method used. Nonetheless, some confusion can arise from the use of analytical methods which extract $<< 100\%$ of the active ingredient. In principle, the confusion could be eliminated if released active ingredient (i.e. active ingredient in the wash liquor) could be determined directly but this is technically challenging. Irrespective of how the measurements are made, some retention and release are essential if insecticidal netting is to work properly, because 100% retention (0% release) represents an inactive product (active ingredient which is inaccessible to aqueous surfactant is unlikely to be accessible to mosquitoes walking on the surface) and 0% retention (100% release) represents a product that is rendered inactive by the first wash.

Based on standardized laboratory tests, the World Health Organization's (WHO) initial ³¹ minimum criterion for

³¹ Criteria based on other mosquito bioassays may also be taken into account.

maintenance of efficacy by LN is that the netting must produce $\geq 80\%$ mortality and/or $\geq 95\%$ knock-down (KD) of mosquitoes in cone test bioassays after 20 washes (WHO 2005). Clauses and limits for active ingredient retention/release in WHO specifications for LN products are intended to address this minimum criterion. To comply with the minimum criterion requires careful product design, to ensure the correct balance between initial active ingredient content and the release of active ingredient. WHO standard bioassays cannot be used throughout the world for quality control purposes, so physico-chemical tests must be used instead but the use of such tests to define efficacy is problematic. As a minimum for the development of a meaningful specification, it is essential to have a detailed knowledge of the release/retention characteristics of the product and confidence that manufacturing parameters will be under strict control, to avoid subtle/unmeasured but potentially important changes which could affect efficacy of the product.

WHO has evaluated and published specifications for two types of LN (WHO 2006, 2007a). One is manufactured from mono-filament yarn, within which the active ingredient is incorporated. The other utilizes warp-knitted fabric of polyester multi-filament fibres, coated with a carrier-polymer containing the active ingredient. At the time of developing these WHO specifications, no other products were available for comparison but, since then, many new products have emerged. Recent trials of newer LN products (WHO 2007b) exposed problems in determining their equivalence (as defined by FAO/WHO 2006) with products on which the existing WHO specifications are based. These problems prompted the present reconsideration of various aspects of active ingredient retention and release.

Mechanisms and patterns of active ingredient release and retention³²

The pattern of active ingredient retention/release from insecticidal netting, over a series of washes, is a key characteristic for maintenance of acceptable efficacy. Unless

³² Possible losses by volatilization from the surface are not considered but release/retention behaviour, as described here, should not be affected by the way in which active ingredient is removed from the surface of insecticidal netting.

the pattern is both well-characterized and predictable, a truly meaningful specification cannot be developed for a product.

Some information on active ingredient retention and release was provided from WHO trials (WHO 2007b, 2008³³). although not sufficient for the development of reliable specification limits. Only active ingredient present on the polymer surface is likely to be transferable to mosquitoes and so the critical requirement is to determine the surface concentration corresponding to the WHO minimum criterion for efficacy.

Published evaluations (WHO 2006, 2007a) imply that LN products behave rather like solid solutions with respect to active ingredient release and retention. That is, active ingredient on the surface of netting is partitioned into aqueous surfactant, according to the relative affinities for active ingredient of solid and liquid phases. After washing, active ingredient deeper within the netting is slowly redistributed by diffusion to equilibrium, replenishing the surface with active ingredient, typically after about 1 day at room temperature. The rate of equilibration may be increased by gentle heating but, at least in the case of coated-filament LN (WHO 2007a), heating to higher temperatures may permanently damage the product.

In principle, the pattern of active ingredient release/retention from polyester and polyethylene fibres in insecticidal netting is expected to be broadly consistent with their presumed semi-crystalline state (regions of crystalline polymer within a matrix of amorphous polymer). Close alignment and packing of polymer chains in crystalline regions make them relatively impervious to small molecules although, when penetrated (for example by heating the fibres with powerful solvents), swelling and loss of the crystal structure occurs. The disordered arrangement of polymer chains in amorphous regions creates flexibility and space within which small molecules may readily interact with the polymer. Both polyester and polyethylene are of low polarity and their amorphous regions are penetrated most easily by small molecules of low polarity. Water, being highly polar, presumably does not penetrate the polymer significantly during washing. On the other hand, the non-polar regions of surfactants (soaps, detergents) may interact strongly with

³³ Working documents presented to the 11th WHOPES Working group Meeting.

amorphous polymer close to the surface of fibres, in addition to solubilizing active ingredient from the surface.

Small molecules capable of interacting with filament-, coating- or binder-polymers can be expected to diffuse readily through amorphous regions. In addition to the active ingredient, many other chemicals involved at various stages of manufacture may be present within amorphous regions of the filament, coating, binder or encapsulating polymers. Manufacturers of yarn and/or netting may or may not add such chemicals deliberately and may endeavour to remove them. For example, efforts may be made to remove residual monomer/oligomers and spinning oils, whereas plasticizers or anti-oxidants may be added. Complete removal of any compound from the polymer is, however, very difficult.

When amorphous polymer regions are saturated with small molecules, the latter can diffuse (migrate) and equilibrate relatively freely. Diffusion will also occur across polymer boundaries, for example between a coating and its supporting filament, with the distribution being determined by the relative affinity of the two polymers for the small molecule. However, as small molecules are progressively removed from the surface of netting, their concentration within the polymer decreases and interactions between the polymer chains increase, restricting both the movement of the remaining small molecules within amorphous regions and the ability of external small molecules to penetrate the polymer. This is an important process in insecticidal netting, because the number of washes at which the restrictive effects become significant depends on the total content of small molecules, not the content of active ingredient alone.

Hypothetically, retention/release from a polymer may occur in two or three stages, with more or less gradual transitions between them (Figure 1).

The **reservoir** stage should be restricted to products such as CS-treated netting and perhaps some insecticide-incorporated filament LN, which contain particulate/droplet inclusions of free active ingredient and/or other small molecules³⁴. As long as

³⁴ At the time of incorporation into insecticidal netting products, the active ingredient may be dispersed in emulsion, particulate or

free solid/liquid remains within the inclusions, the amorphous polymer regions remain saturated, so that the amount of active ingredient on the surface remains constant at equilibrium. Because the same quantity of active ingredient is lost at each wash, the decline in total content is linear. When the free solid/liquid approaches exhaustion there is a transition through the free-migration stage, ultimately to the closing stage. The total content, t_n , of active ingredient in washed netting with reservoir stage behaviour can be expressed as:

$$t_n = t_0 - t_0 n(1 - r) \quad (i)$$

where

t_n = active ingredient total content after wash n , g/kg

t_0 = active ingredient total content at wash 0 (pre-washing), g/kg

r = retention index (a constant), expressed as a fraction

n = number of washes.

The retention index, r , in the reservoir stage is calculated as:

$$r = 1 - ((1 - t_n/t_0)/n) \quad (ii)$$

At equilibrium, the active ingredient surface concentration, s , in the reservoir stage is not changed by the number of washes.

$$s = (t_0 - t_n)/n \quad (iii)$$

All products that do not contain particulate/droplet inclusions of free active ingredient and/or other small molecules at the time of first use will show no reservoir stage behaviour and presumably start with **free-migration** stage behaviour. Where the total content of small molecules is sufficient to saturate the polymer but there are no free solid/liquid inclusions, the product behaves rather like a solid solution. A proportion of the total content of small molecules (including a proportion of active ingredient) is removed from the surface at each wash. After equilibration, the same proportion (but a smaller quantity) is removed by the next wash, so the decline in total content is non-linear.

In the free-migration stage, the active ingredient total content of washed netting can be expressed as:

encapsulated form. If all low molecular weight species (including active ingredient) initially incorporated in this form dissipate by migration throughout the polymer before the product is used, its wash performance will start at the free-migration stage.

$$t_n = t_0(r^n) \quad (\text{iv})$$

In the free-migration stage, the retention index, r , may be calculated as:

$$r = \sqrt[n]{(t_n/t_0)} \quad (\text{v})$$

At equilibrium, the active ingredient surface concentration, s , of washed netting in the free-migration stage can be expressed as:

$$s = t_0(r_2^n) - t_0(r_2^{(n+1)}) \quad (\text{vi})$$

The **closing** stage begins when the content of small molecules within the polymer declines to levels at which interaction between amorphous polymer chains increasingly inhibits migration. Surface concentrations decline proportionately more with each wash than in the free-migration stage, ultimately to undetectable levels, and the retention index approaches 1.00 (or 100%).

Transitions between stages are marked by increasing retention index but it may be very difficult to differentiate the stages and transitions occurring during repeated washing of a particular product, especially in relatively heterogeneous products. Figures 1 and 2 indicate that subtle increases in retention index may lead to changes in surface concentration which would have a major impact on mortality/KD. So, although the above equations permit the surface concentration at 20 washes to be estimated, rather than measured directly, they are not applicable to any product showing signs of transition before 20 washes. It may be possible to develop appropriate equations for such products but it would be necessary to demonstrate that the transition is consistent within and between production batches.

An ideal LN product would therefore retain either reservoir stage or free-migration stage behaviour throughout 0-20 washes, making it relatively easy to develop reliable methods to predict surface concentrations throughout. Where a transition occurs before 20 washes, it may be necessary to measure actual surface concentration after wash 20 for good manufacturing quality control. This is technically more challenging but, unless the transition is both consistent and can be described by an appropriate equation, the surface concentration after wash 20 may be seriously over-estimated. Figure 3 shows how a test method based on interpolation

(washes 6 and 20 are used as examples only) or extrapolation of wash data could provide a misleading impression of the surface concentration after wash 20. Curve A implies a surface concentration of about 300 mg/kg after wash 20, whereas curve B (fitted to the same data) indicates that the surface concentration is effectively zero after wash 12.

If the active ingredient is not the most abundant small molecule present within the polymer, active ingredient retention/release will be determined by the retention/release pattern of other chemicals present. Pyrethroid insecticides have exceptionally low solubility in water, so other low molecular weight chemicals present in the polymer may be removed more efficiently by washing than the active ingredient. The decline in their concentration may therefore induce a transition to the next retention/release stage even if the total active ingredient content of the polymer appears to decline only slightly. Consequently, if retention/release of other small molecules occurs undetected, it may be more difficult to understand and predict retention/release of the active ingredient.

It is conceivable that other small molecules present on the surface of insecticidal netting could also alter mosquito behaviour, or the efficiency of active ingredient transfer between polymer surface and insect. If such small molecules are surfactants, present only on the surface and easily removed by washing, their effects could disappear quickly. Other kinds of small molecule may have longer-lasting effects on both active ingredient migration within the polymer and transfer from the surface to mosquitoes.

Retention/release patterns of eight LN products (WHO 2007b, WHO 2008³⁵) are summarized in table 1 and figures 4 and 5. Free-migration stage (equation iv) curves were fitted for comparison in the figures, except in the case of the Icon MAXX-treated net (figure 5) which was presumed to have reservoir stage behaviour. The scatter in data for six of the products, which was presumably due to sampling error (i.e. variation in active ingredient distribution across the netting), was such that either of equations (i) or (iv) could be fitted to them. The deviations from equation (iv) curves fitted to data from Hiking

³⁵ Working documents presented to the 11th WHOPES Working group Meeting.

and Netto products appear more consistent with a gradual transition³⁶ from free-migration stage to closing stage behaviour, occurring over the 20 washes. The transition is also reflected in the general increase in running average values for retention index observed with increased washing of these two products (table 1). If transitions occurred in the other products, they were obscured by sampling error. Running average values produced from washes 15 and 20 could mean that surface concentrations after wash 20 were over-estimated, although presumably not to the extent implied by curve B in figure 3 (where interpolation was between washes 6 and 20). Additional data are required for all products, to enable the true patterns of release/retention to be established beyond doubt.

Washing, surface concentration and spatial aspects of efficacy

When insecticidal netting is washed, a complex partition process is involved, in which a truly stable equilibrium is not reached. Emulsified surfactant in the aqueous wash liquid solubilizes active ingredient from the surface, competing with the polymer for solvation of the active ingredient (and competing with the active ingredient for solvation of the polymer). The nature, concentration and relative volume of surfactant, the temperature, the agitation, and the washing time may all affect the position of the apparent equilibrium at the end of a wash. Hence all these characteristics must be carefully standardized to produce a reliable and reproducible test of washing characteristics. A standardized test cannot represent all or any of the wide range of washing processes which may be utilized throughout the world but it is essential for reliable and meaningful assessment of insecticidal netting products.

At one extreme, washing in cold distilled water will remove virtually no active ingredient. At the other extreme, heating in a suitable organic solvent will remove virtually all the active ingredient. Real-life washing procedures represent something between these two extremes but the polymer "surface" from which active ingredient is removed (and hence the "surface concentration") is defined by the wash conditions. In the

³⁶ Deviation of Hiking and Netto data from the fitted curves appears too uniform to be due to either losses of coating polymer during washing or random sampling error.

context of washing insecticidal netting, “surface” and “surface concentration” are therefore unlikely to correspond to any view of the surface topography at microscopic or molecular levels. Ignoring the possibility that washing may physically remove some of the carrier polymer, differences in release/retention produced by different washing regimes may represent differences in competition for active ingredient to the same depth into the surface, or differences in the extent of surfactant penetration into the surface. The underlying reasons for the differences are not particularly important in the context of developing specifications for insecticidal netting but it is essential to recognize that any measurement of surface concentration is unlikely to represent the concentration experienced by mosquitoes which contact the surface.

Nonetheless, if carefully standardized procedures are used throughout, measurements of the **decline** in surface concentration should be correlated with the biological response of mosquitoes, because the proportion of decline should be similar. Correlation of measured surface concentration with biological effect is complicated by the non-linearity of dose-response curves and the inevitable absence of any change in response at concentrations above the minimum producing 100% response or below the maximum producing 0% response.

The mechanisms by which active ingredient is transferred from the surface of netting to the site(s) of activity within mosquitoes do not appear to be well established. Irrespective of the relative importance of vapour- and solid-phase transfer³⁷, the concentration of active ingredient on the surface of netting must influence the amount transferred to mosquito legs, as will the relative affinity of the active ingredient for polymer and insect surfaces. Contact time and the total surface area contacted must be other important factors, with the latter presumably related to the topographies³⁸ of the filament and yarn surfaces and of the netting weave. Two insecticidal netting products with

³⁷ Given the very low volatility of pyrethroids and their affinity for the polymers used, their “surface concentrations” must be much higher than those in the immediately surrounding air

³⁸ Topography differs according to scale, from the molecular to the macroscopic, and it may be impossible to define which aspects of topography make the most important contribution to active ingredient transfer.

identical measured surface concentrations of active ingredient would produce very different bioassay results if mosquitoes make more, and/or more effective, contact with the surface of one product than the other. Such spatial (3-dimensional) aspects of efficacy are impossible to quantify and it is possible that even apparently trivial changes in manufacturing (e.g. yarn source, weave tension, etc.) could lead to significant shifts along the concentration axis of bioassay dose-response curves.

The relationships between estimated surface concentration and mortality and KD of mosquitoes were assessed from WHO trials data on eight products (WHO 2007b, 2008³⁹) and the results are presented in table 2 and figures 7 and 8. The content of active ingredient was measured at intervals of 0, 1, 3, 5, 10, 15 and 20 washes so, as explained in the previous section, surface concentration at 20 washes may have been lower⁴⁰ than those shown.

The LN products differed considerably in surface concentration, irrespective of whether these values were expressed as mg/kg, mg/m² netting or mg/m² filament surface area (only mg/kg data are presented in table 2). This could reflect differences in actual surface concentration, or differences in the ability of the wash liquid to remove active ingredient from the surface, or both. Ignoring surface those concentrations which produced 100% mortality or KD, the relatively few data for six products containing deltamethrin as the active ingredient appear to show that surface concentrations (however expressed) producing a similar biological response may be widely different. These differences perhaps reflect differences in the spatial presentation of active ingredient on the surface which, as mentioned above, is an aspect of active ingredient transfer that is not amenable to measurement but is potentially very important.

Presumably the spatial presentation of active ingredient was consistent within each product represented in figures 7 and 8. If so, despite the inevitable variability of the bioassay data

³⁹ Working documents presented to the 11th WHOPES Working group Meeting.

⁴⁰ This is less likely for the DawaPlus LN, because analysis after 25 washes indicated that "surface concentrations" did not decline markedly before 20 washes.

(presumably exacerbated by variability of active ingredient distribution within the LN products), figures 7 and 8 indicate that, within the critical ranges where response is <100% but >0%, big changes in mortality and KD occur as a result of small changes in surface concentration. The data are too few to draw firm conclusions but, within the critical ranges, declines in surface concentration of approximately 1-20 mg/kg appear to be associated with serious declines in mortality. A broadly similar picture is evident in the cases where KD is <100%. Such small declines in surface concentration imply that the efficacy of nets may change rather suddenly from good to poor at some stage with repeated washing.

Criteria for acceptable efficacy in laboratory tests

In laboratory trials, WHO currently uses two primary laboratory tests for efficacy of LN products against mosquitoes: 24 h mortality and 60 min KD after a “cone test” with 3 minutes exposure (WHO 2005). Follow-up measurements may also be made of 24 h mortality and blood-feeding inhibition (BFI) in a 15-h “tunnel test”. Limits for acceptability of performance in the cone test are $\geq 80\%$ mortality and/or $\geq 95\%$ KD, and in the tunnel test $\geq 80\%$ mortality and/or $\geq 90\%$ BFI, after 20 standard washes of the LN. Additional bioassay tests are used in subsequent field trials by WHO.

The “and/or” options provided in the cone and tunnel tests give the impression that 80% mortality and 95% KD (or 90% BFI) are equivalent and should be produced by similar doses. Of course, where both tests produce results of $\approx 100\%$ or $\approx 0\%$, the two end points are indistinguishable. However, as shown in Figure 9, most of the data from WHO cone tests of netting containing alpha-cypermethrin, deltamethrin, lambda-cyhalothrin or permethrin indicate that 95% KD corresponds to something in the region of 20-30% mortality, not 80% mortality. There were insufficient data from tunnel tests to make a similar comparison.

The differences between the dose-response curves for mortality and KD in the cone test appear to be dose-related, not product-related, because WHO trials of very different products produced similar relationships. Hypothetical dose-response curves are shown in figure 10, superimposed upon a free-migration curve of active ingredient surface concentration. Although figure 10 is

not derived from real data, it is consistent with the experimental data.

A consequence of the narrow dose ranges over which biological response changes dramatically is that response cut-off values for decision-making are inevitably set within a region in which small errors in measurement can have a disproportionately large impact. This problem is compounded by the high sampling error associated with the very variable active ingredient distribution in many types of insecticidal netting, especially netting treated by dipping. The influence of sampling error on the setting cut-off values, or specification limits, based on repeated washing of insecticidal netting is the opposite of that on estimating the toxicity of chemicals (see figure 12). So, the closer a cut-off or specification limit is to 50% biological response, the greater number of bioassay data required to demonstrate that performance remains acceptable than if the limit is set at or about 100% (or 0%). Naturally, setting specification or other cut-off limits based on retention of 100% biological effect (mortality, KD, etc.) has the drawback that it may be unclear how close the product/limit is to the critical range of surface concentration, where a big change in efficacy may occur within few additional washes. Cut-off values based upon 95% biological effect therefore probably represent the optimum and are currently used as the criterion for KD in WHO cone tests.

As the two existing WHO criteria for biological effect in the cone test correspond to different surface concentrations of active ingredient, they are not equivalent and one them should be designated as the basis for WHO specifications. Possibly the criterion could be chosen on a case-by-case basis but mortality is clearly more stringent than KD and therefore appears to be the criterion of choice. If the current 80% mortality limit was to be replaced by a 95% limit, this would also be more stringent – perhaps corresponding to 1-2 fewer washes than the number required to reach 80% mortality.

Correlations between other WHO measures of efficacy were also considered. Figure 11 shows that an approximately 1:1 relationship exists between mortality and BFI in WHO tests

conducted in experimental huts (WHO 2008⁴¹). As in the case of the tunnel test, considered above, there were too few data to make a meaningful comparisons of other parameters. Comparison of paired bioassay results (e.g. mortality and KD) produced by a single test is straightforward because each pair of corresponding data relate to exactly the same piece of netting, group of mosquitoes, etc. Comparisons between different types of test are more problematic because additional uncontrolled test variables are introduced. Presumably for this reason, correlations were not apparent between data from WHO laboratory bioassays and field tests of efficacy, conducted on the same insecticidal netting product but using different nets.

Measurement of retention/release and surface concentration of active ingredient

Because small changes in surface concentration can lead to a big change in biological effect, it might seem reasonable to conclude that high precision methods are required for setting, and for checking compliance with, specification limits based upon biological effect after 20 standard washes. However, if changes in spatial presentation of the active ingredient can also significantly influence efficacy, exact measurement of surface concentration could be misleading (in addition to being costly). For example, if an apparently trivial change in the yarn or weaving leads to a significant change in the efficiency of active ingredient transfer to mosquitoes, the corresponding specification limits would also have to be changed. The presence, absence or relative concentration of other small molecules in/on the polymer may also affect active ingredient transfer. Therefore, counter-intuitively, it may not be sensible to base specifications for LN upon highly accurate and precise measurement of surface concentrations after 20 washes. The use of a cruder, if apparently less definitive, approach may be more appropriate.

The use of bioassays for worldwide testing of compliance with a WHO specification for LN presents essentially insuperable obstacles, because universal circulation of standard mosquito strains creates unacceptable bio-security risks and because it is difficult to maintain and standardize test conditions and operator

⁴¹ Working documents presented to the 11th WHOPES Working group Meeting.

skill levels. Nonetheless, cross-calibration of manufacturers' bioassay methods against the WHO standard bioassay would be worthwhile, though even in this case it would be necessary to consider very carefully any subsequent modification of either method.

Even if exact estimates/measurements of the surface concentration after wash 20 could actually be meaningful (first paragraph, this section), the production of robust test methods would be technically challenging, because of the very small amount of active ingredient per unit mass of netting at that point and because of the variability of active ingredient distribution in LN products. Supposing that these challenges could be overcome, it would remain impossible to determine the surface concentration experienced by a mosquito walking on the netting. Therefore, although determination of surface concentrations after 20 washes may be important for research or manufacturing control purposes, such measurements should not be used to set quality limits in WHO specifications.

However, some control of retention/release characteristics is better than none and crude measurements, similar to those currently defined by existing WHO specifications for LN, could continue to be employed. The wash procedures utilized in test methods supporting the existing specifications require improvement and standardization but, for the reasons already given, it is neither necessary nor worth attempting to produce high precision methods. The method required, and the corresponding limits in WHO specifications for LN, would be intended only to indicate that retention/release is within the appropriate order of magnitude.

For example, a WHO specification limit for retention of $\geq 50\%$ of the active ingredient after 5 standard washes (e.g. over a range of either 0-5, 1-6 or 15-20 washes) would indicate a retention index of $\geq 90\%$ for reservoir behaviour or $\geq 87\%$ for free-migration behaviour. Compliance with such a limit would not prove that efficacy remains acceptable after 20 standard washes of the LN, because this can only be achieved using standard bioassays.

Retention of $\geq 95\%$ of the active ingredient after 5 standard washes would indicate that retention is very high (retention index $\geq 99\%$ for either reservoir or free-migration behaviour).

Using a crude test, it would be difficult to distinguish between good and bad products having such a high retention index but this would be true even if the test provided very high accuracy and precision. Retention cannot actually reach 100% in practice but it seems clear that some LN products can provide good efficacy with a retention index of about 99%. In those cases where the crude test of retention index produces a very high value, and especially if there is reason to believe that efficacy is no longer being maintained after 20 standard washes, bioassay tests would be required to resolve the issue. As far as practicable, manufacturers' routine quality control should incorporate bioassays, to minimize the potential for this problem to occur and to ensure that manufacturers are alert and responsive to changes in "hidden" characteristics (such as spatial presentation).

Expression of surface concentration

As explained above, surface and surface concentration are concepts defined by the measurement process which, for specifications purposes, must involve washing. If data for surface concentration of active ingredient are to have any meaning at all, the washing procedure must therefore be completely consistent, otherwise the definition of surface will vary in an uncontrolled manner. Surface concentration results may also be calculated and expressed in various ways.

The simplest expression is in the form of mass of active ingredient per unit mass of netting (e.g. mg/kg). This makes no attempt to define the surface, as such, although surface concentration remains defined by the washing method. The main benefit of this form of expression is that it should provide values of definable accuracy and precision.

Alternatively, surface concentration may be expressed as mass of active ingredient per unit area of netting (e.g. mg/m² of netting). Measurement of netting area is somewhat more error-prone than measurement of the mass but this form of expression is widely used.

A third possibility is that surface concentration could be expressed as mg/m² of filament surface area. In principle, these values should be closer to the concentrations experienced by mosquitoes landing on the surface but, as

explained above, they would not represent the actual concentrations available to mosquitoes. Additionally, measurement of filament surface area per unit weight/area of netting is technically challenging and it would be necessary to define filament surface. To avoid these problems, filament surface area (per g or m² of netting) could be calculated from the filament count of the yarn, its denier/decitex and density. However, values calculated in this way may not be very accurate if the filaments are not uniformly circular in cross-section and/or the density is either not known or is variable across the netting. In addition, if exposure of mosquitoes to active ingredient is influenced as much by the 3-dimensional presentation of netting surfaces and/or the presence of other small molecules on the surface as it is by surface concentration, results expressed in this way may be no more indicative of the potential exposure of mosquitoes to active ingredient than results expressed as mg/kg.

Sampling, washing, testing and the problem of heterogeneity

Variation in active ingredient distribution is inevitable in all insecticidal netting products but the degree of heterogeneity found depends upon the size of sample tested. Measured heterogeneity is increased by testing smaller samples and decreased by testing larger samples, which tend to provide results closer to the average but conceal heterogeneity occurring on a smaller scale. Sample size is particularly important in testing insecticidal netting because it is fairly difficult (or impossible for some tests) to mix the material thoroughly before removing test portions.

Nets treated by dipping tend to have highly heterogeneous distributions within nets, although between-net variations may be relatively small. At the other extreme, LN products in which the active ingredient is incorporated into filaments at spinning may show rather uniform distributions, within- and between-nets. Coated-filament LN products are intermediate, tending to one extreme or the other according to the manufacturing process and the physical form of the active ingredient applied.

Although mosquitoes tend to move around on mosquito nets in their efforts to gain entry for blood-feeding, and thus experience a range of surface concentrations, it seems unlikely that a

highly heterogeneous product would provide better control than a more uniform, but otherwise similar, product. Moreover, high heterogeneity is likely to make interpretation of biological test data more difficult, because it is impossible to know whether or not variations in biological test data are associated with the heterogeneity or some other uncontrolled factor in the tests. Setting of limits for active ingredient content and retention, and monitoring for compliance with those limits, are also likely to be compromised by high levels of heterogeneity. Thus heterogeneity of active ingredient distribution should be minimized in insecticidal netting products.

Heterogeneity of active ingredient distribution makes the measurement of retention/release and surface concentration (and also total active ingredient concentration) very dependent upon the size and method of preparation of the samples tested. As mentioned earlier, many aspects of the washing method can also influence results. To ensure consistency, comparability and reliability of test results, methods of sampling, sample preparation and washing should be harmonized and carefully standardized.

It seems logical that certain long-established sampling and testing parameters should form the core of future moves towards harmonization and standardization. So, for all tests, sampling should be performed according to the scheme recommended in the FAO/WHO manual (FAO/WHO 2006) and the sample size and wash method should be identical to that used for WHO laboratory bioassays (WHO 2005). This would establish clear links between the data produced by chemical and biological assays and greatly enhance the comparability of data. The current recommendation for sampling of a net is to remove 5 pieces systematically (FAO/WHO 2006) and the current protocol for phase 1 bioassays is to test 25 x 25 cm pieces (WHO 2005). Sampling for chemical and biological assay could be harmonized if five 12.5 x 12.5 cm pieces could be taken from each net as the sample. The samples for bioassay would then be slightly different from those currently used but should be more representative of the net. The samples used for chemical analysis in phase 1 WHO trials (WHO 2005) would then be identical to those required for testing for compliance with WHO specifications. Repeated washing of large numbers of samples is very time-consuming and labour-intensive but this could be minimized if the five

pieces are washed as a single sample for each test. Again, this represents a slight deviation from the existing WHO phase 1 washing procedure but it would enable the same procedure to be used for both efficacy trials and testing for compliance with the WHO specification.

Although the harmonized scheme proposed above involves slight changes to existing protocols, it has the additional benefit that it would harmonize the approach to applying WHO specification tolerances for active ingredient content ($\pm 25\%$), and within-net variation ($\pm 5\%$) for determination of storage stability and retention/release of active ingredient, in LN products (FAO/WHO 2006).

Equivalence

For the extension of existing WHO specifications for LN to the products of other manufacturers, equivalence (FAO/WHO 2006) is based on compliance with the existing specification and satisfactory performance in WHO efficacy trials (WHO 2005, 2007b). To ensure transparency and consistency in decision-making, it may be helpful to develop simple guidelines for the determination of equivalence of efficacy.

References

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|-----------------|---|
| FAO/WHO 2006 | Manual on development and use of FAO and WHO specifications for pesticides. March 2006 revision of the 1 st edition. Available only on the internet at http://www.fao.org/ag/agp/agpp/pesticid/ and http://www.who.int/whopes/en/ . Accessed 13 March 2007. |
| WHO 2005 | Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets. Document WHO/CDS/WHOPES/GCDPP/2005.11. World Health Organization, Geneva, 2005. |
| WHO 2006 | WHO specifications and evaluations for public health pesticides. Permethrin long-lasting (incorporated into filaments) insecticidal net. Evaluation report 331LN/200 at http://www.who.int/whopes/quality/permethrin_LN_July_2006.pdf . |
| WHO 2007a | WHO specifications for public health pesticides. Deltamethrin, FAO/WHO evaluation report 333/2006.1 at http://www.who.int/whopes/quality/deltamethrin_eval_may_2007_in.pdf . |
| WHO 2007b | Report of the tenth WHOPES working group meeting. World Health Organization, Geneva, 2007 (WHO/CDS/NTD/WHOPES/2007.1). |

Table 2. Comparison of calculated surface concentrations of active ingredient and bioassay data from 7 LN and 1 user-treated products

| | Wash No. | Surface active ingredient (mg/kg*) | KD (%) | Mortality (%) |
|----------|----------|------------------------------------|--------|---------------|
| PermaNet | 0 | 73.2 | 100 | 100 |
| | 1 | 70.9 | 100 | 100 |
| | 3 | 66.6 | 100 | 100 |
| | 5 | 62.5 | 100 | 100 |
| | 10 | 53.4 | 100 | 97 |
| | 15 | 45.7 | 100 | 78 |
| | 20 | 39.0 | 87 | 23 |
| Hiking | 0 | 41.6 | 100 | 95 |
| | 1 | 40.5 | 98 | 57 |
| | 3 | 38.5 | 54 | 8 |
| | 5 | 36.7 | 35 | 2 |
| | 10 | 32.3 | 51 | 6 |
| | 15 | 28.5 | 12 | 5 |
| | 20 | 25.1 | 1 | 1 |
| Yorkool | 0 | 25.5 | 83 | 16 |
| | 1 | 25.2 | 100 | 16 |
| | 3 | 24.5 | 87 | 11 |
| | 5 | 23.8 | 100 | 49 |
| | 10 | 22.2 | 100 | 65 |
| | 15 | 20.8 | 100 | 34 |
| | 20 | 19.4 | 62 | 6 |
| Netto | 0 | 69.3 | 100 | 100 |
| | 1 | 66.7 | 100 | 100 |
| | 3 | 61.8 | 98 | 78 |
| | 5 | 57.2 | 100 | 71 |
| | 10 | 47.3 | 86 | 28 |
| | 15 | 39.0 | 21 | 5 |
| | 20 | 32.2 | 18 | 3 |
| Duranet | 0 | 14.0 | 100 | 100 |
| | 1 | 13.9 | 100 | 100 |
| | 3 | 13.9 | | |
| | 5 | 13.8 | 100 | 99 |
| | 10 | 13.7 | 100 | 68 |
| | 15 | 13.5 | 100 | 76 |
| | 20 | 13.4 | 100 | 45 |

* Calculated from exponential curves fitted to the g/kg data in Table 1, except for Icon MAXX, to which a straight line was fitted (an exponential curve provided a very slightly better fit to the data but the difference in calculated surface concentrations was small).

Table 2 continued. Comparison of calculated surface concentrations of active ingredient and bioassay data from 7 LN and 1 user-treated products

| | Wash No. | Surface active ingredient (mg/kg) | KD (%) | Mortality (%) |
|------------|----------|-----------------------------------|--------|---------------|
| Netprotect | 0 | 26.3 | 100 | 100 |
| | 1 | 25.9 | 100 | 100 |
| | 3 | 25.3 | 100 | |
| | 5 | 24.6 | 100 | 97 |
| | 10 | 23.4 | 100 | 99 |
| | 15 | 21.9 | 100 | 78 |
| | 20 | 20.2 | 100 | 76 |
| Icon Maxx | 0 | 46.2 | 83 | 5 |
| | 1 | 46.2 | 100 | 31 |
| | 3 | 46.2 | 100 | 42 |
| | 5 | 46.2 | | |
| | 10 | 46.2 | | |
| | 15 | 46.2 | | |
| | 20 | 46.2 | | |
| DawaPlus | 0 | 34.9 | 93 | 39 |
| | 1 | 34.0 | 98 | 77 |
| | 3 | 32.0 | | |
| | 5 | 30.1 | 100 | 57 |
| | 10 | 25.8 | 92 | 33 |
| | 15 | 22.2 | 96 | 29 |
| | 20 | 19.0 | 95 | 42 |
| | 25 | 16.3 | 99 | 52 |

* Calculated from exponential curves fitted to the g/kg data in Table 1, except for Icon MAXX, to which a straight line was fitted (an exponential curve provided a very slightly better fit to the data but the difference in calculated surface concentrations was small). This assumes that the product displayed reservoir stage behaviour and consequently there is no decline in surface concentration with washing.

Figure 1. Hypothetical retention patters, with and without transitions, with repeated washing

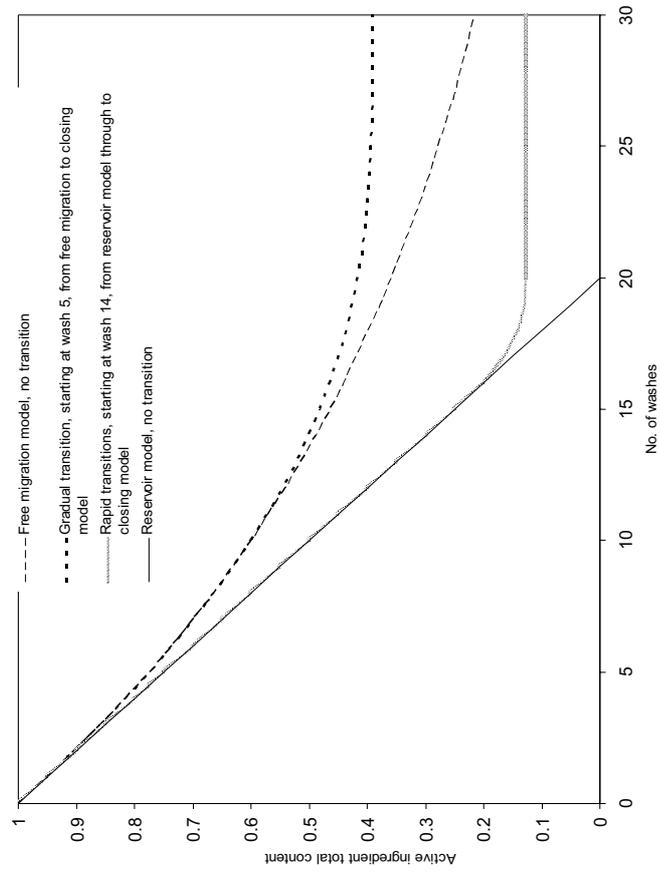


Figure 2. Hypothetical active ingredient surface decline patterns, corresponding to the total active ingredient declines in Figure 1

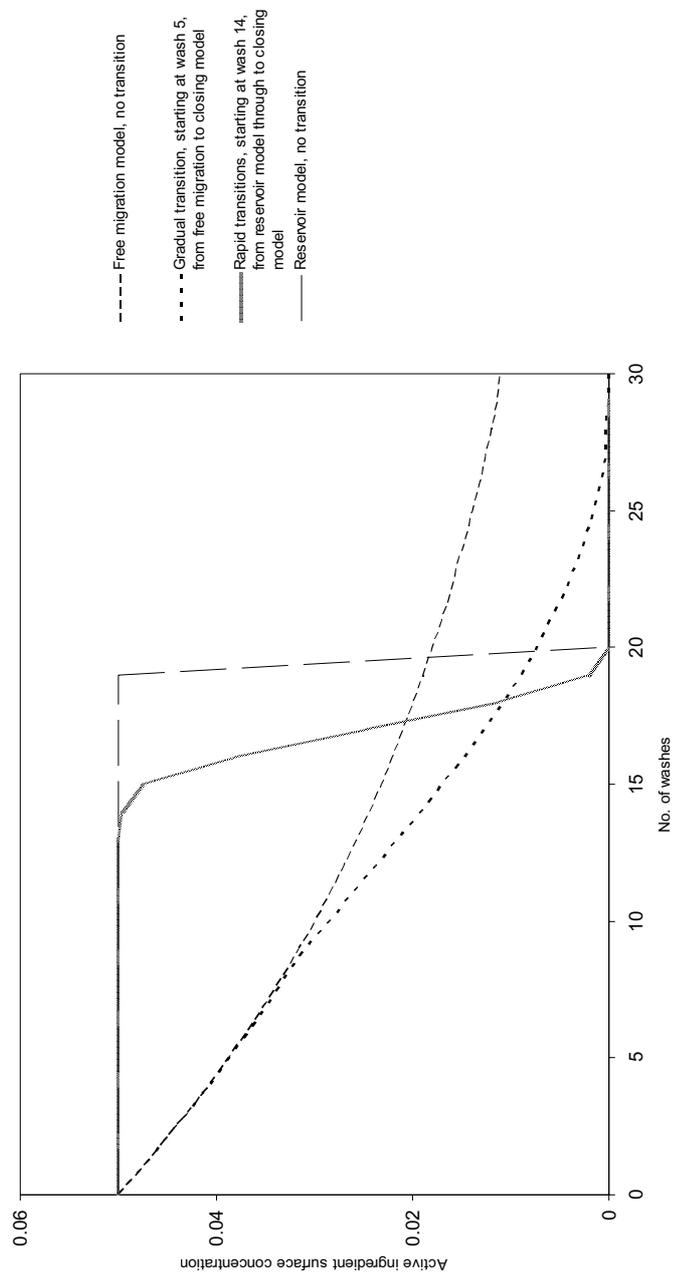


Figure 3. Potential errors from interpolation of retention data

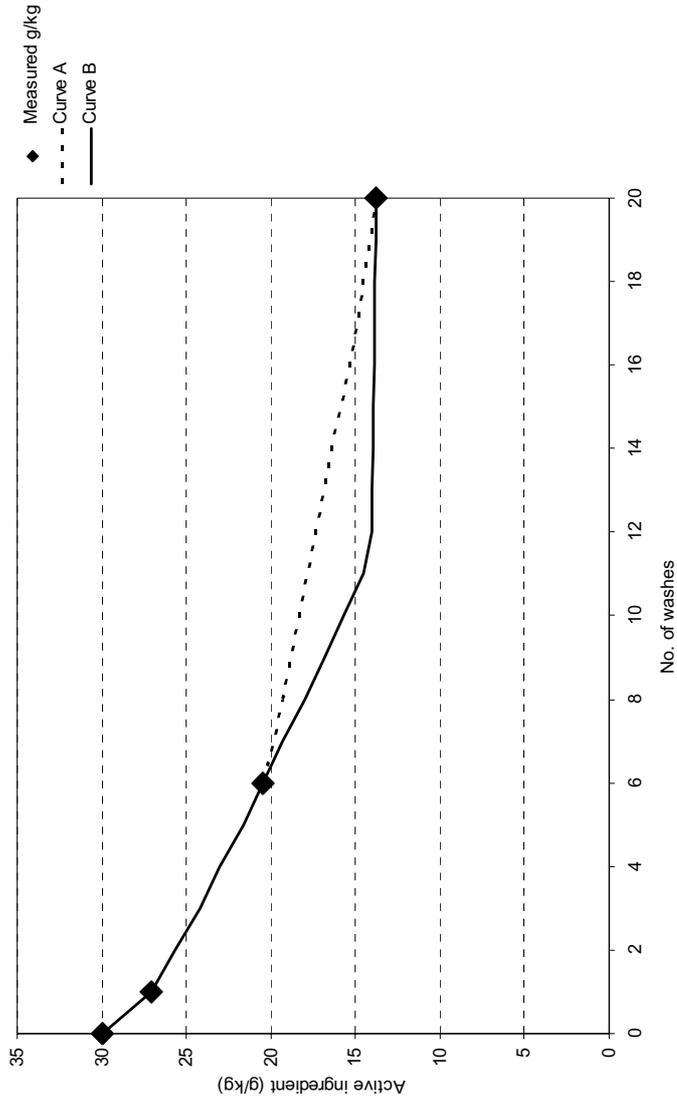


Figure 4. Effect of washing on deltamethrin total content of 4 LN products: actual values and curves fitted to the data

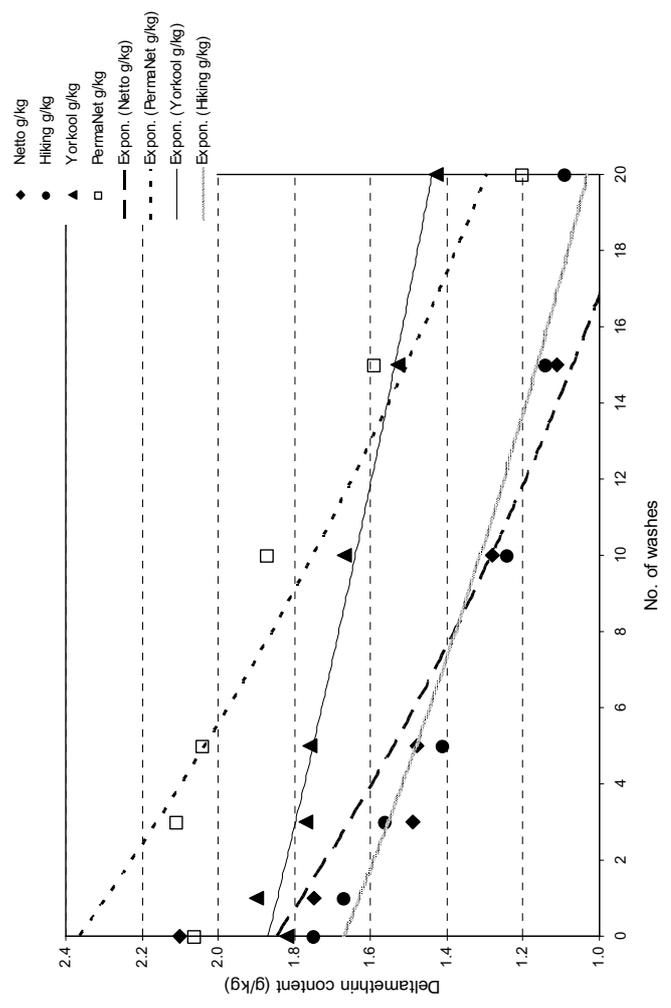


Figure 5. Effect of washing on active ingredient total content of 2 LN and 1 user-treated products: actual values and curves fitted to the data

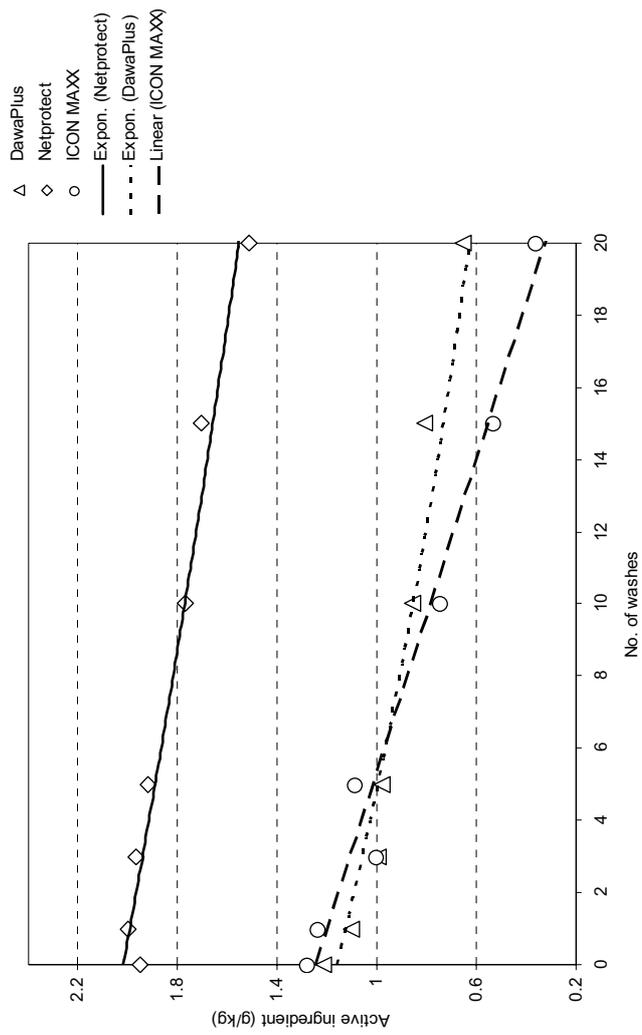


Figure 6. Effect of washing on active ingredient total content of an LN product: actual values and curve fitted to the data

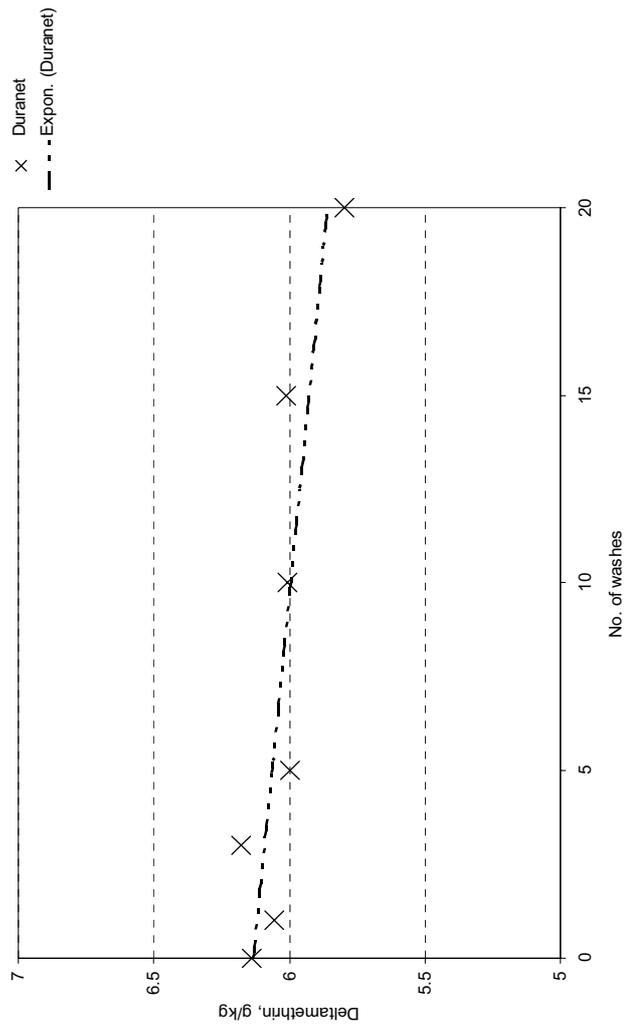
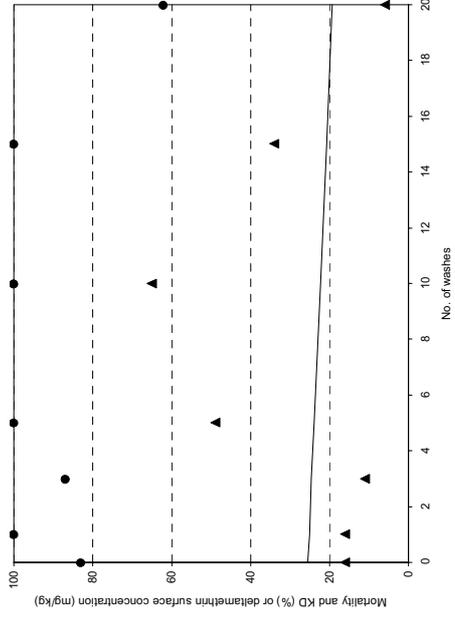
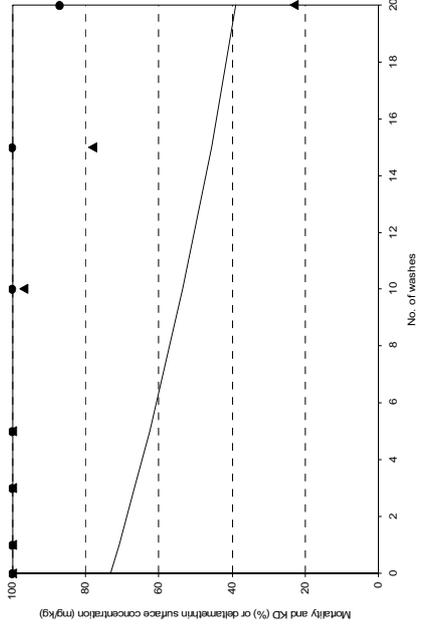


Figure 7. Mosquito mortality (triangle) and KD (circle) and estimated surface concentrations of deltamethrin in 4 LN products

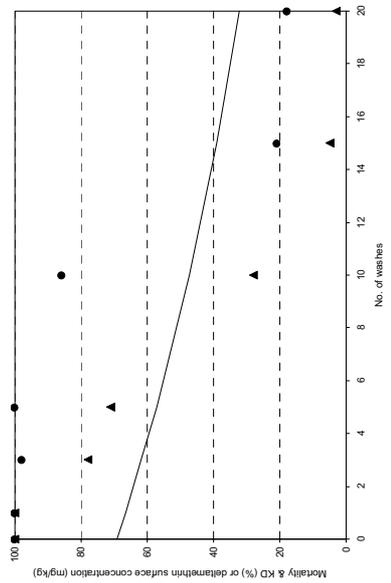


Yorkool LN

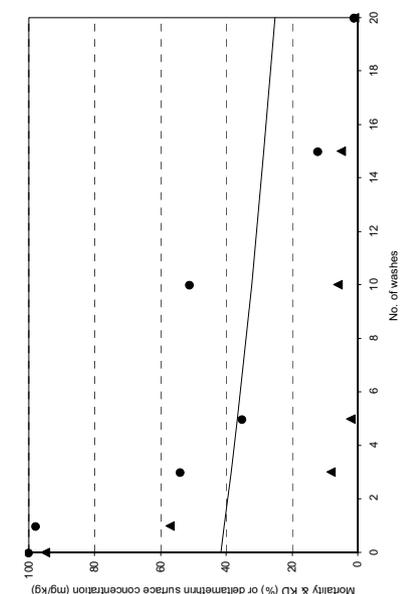


PermaNet LN

Figure 7 continued. Mosquito mortality (triangle) and KD (circle) and estimated surface concentrations of deltamethrin in 4 LN products

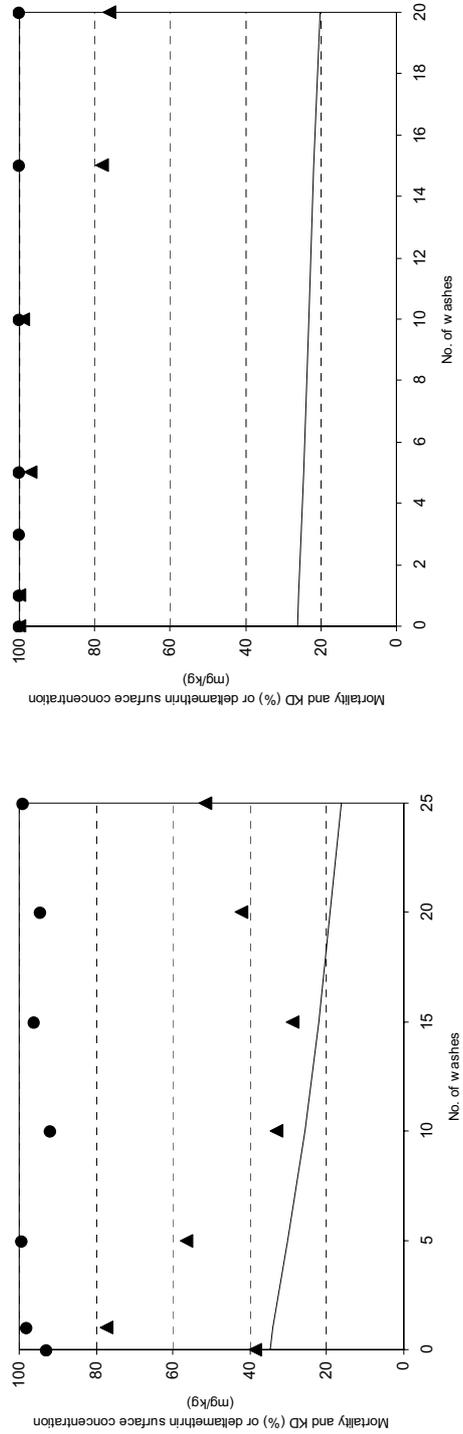


Netto LN



Hiking LN

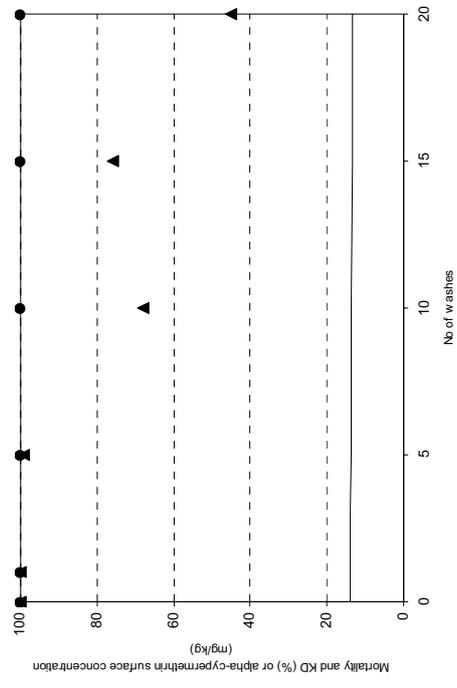
Figure 8. Mosquito mortality (triangle) and KD (circle) and estimated surface concentrations of active ingredient in 3 LN and 1 user-treated products



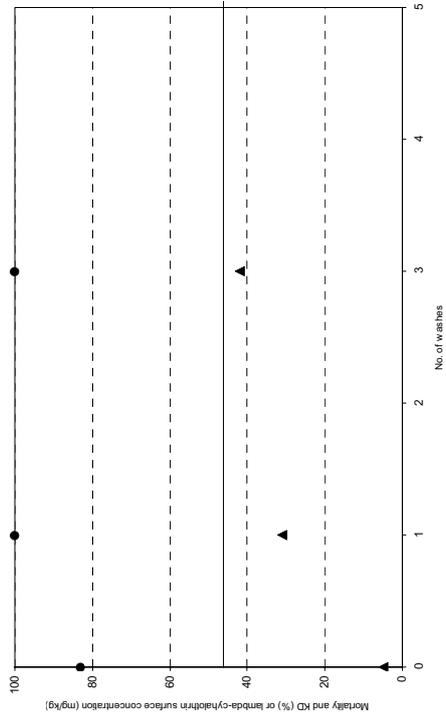
DawaPlus (deltamethrin)

Netprotect (deltamethrin)

Figure 8 continued. Mosquito mortality (triangle) and KD (circle) and estimated surface concentrations of active ingredient in 3 LN and 1 user-treated products

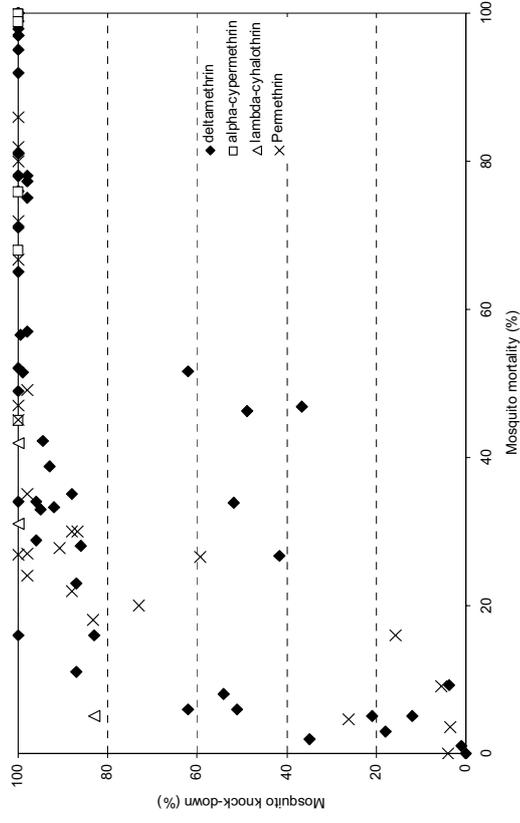


DuraNet (alpha-cypermethrin)



Icon MAXX (treatment kit, lambda-cyhalothrin)

Figure 9. Correlation between mortality and knock-down of *Anopheles gambiae* in WHO cone tests, produced by 7 LN and 3 user-treated products, each containing one of four pyrethroids



In general, 95% knock-down corresponds to approximately 20-30% mortality (not 80% mortality) with deltamethrin. The small proportion of data for permethrin (3/24) and deltamethrin (6/65) which appear inconsistent with the general pattern were produced from dipped nets in 2 laboratories which tested only the dipped nets. Other data from the same laboratories, together with the few results available for alpha-cypermethrin and lambda-cyhalothrin, were consistent with the pattern of the majority of data for permethrin and deltamethrin.

Figure 10. Hypothetical dose-response curves for mosquito mortality and KD in WHO cone tests, together with a free migration release curve for active ingredient surface concentration on insecticidal netting (vertical lines at 15 and 19 washes correspond to about 80% mortality and 95% KD, respectively)

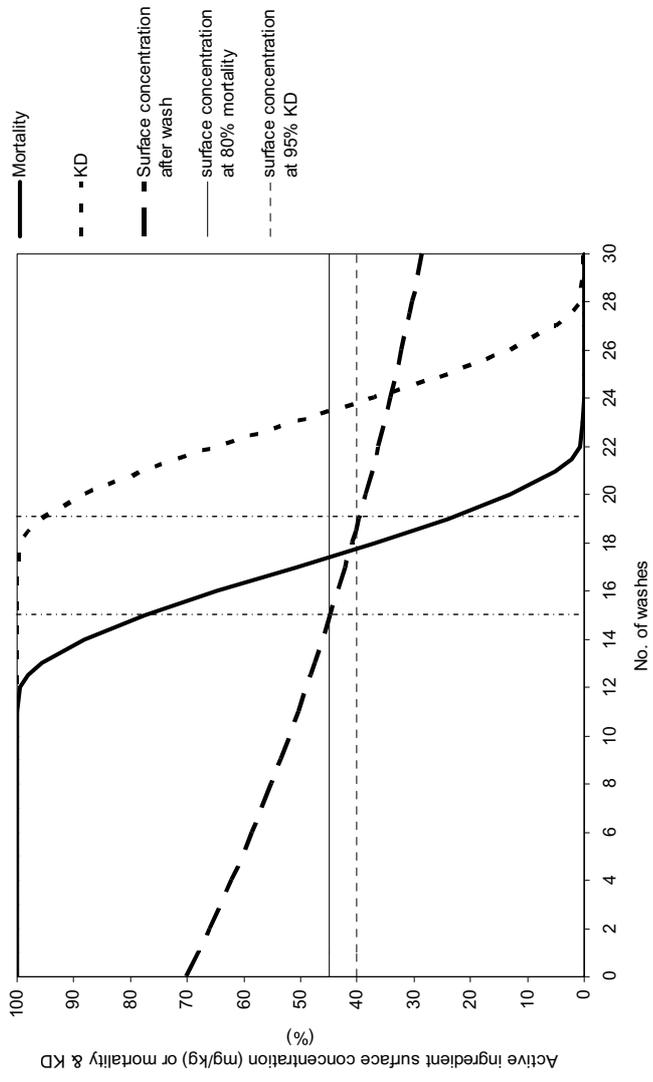


Figure 11. Correlation between mortality and blood-feeding inhibition of *Anopheles gambiae* and *An. funestus* in WHO experimental hut studies, produced by 7 LN and 2 user-treated (1 washed almost to exhaustion as a positive control) products, each containing one of four pyrethroids

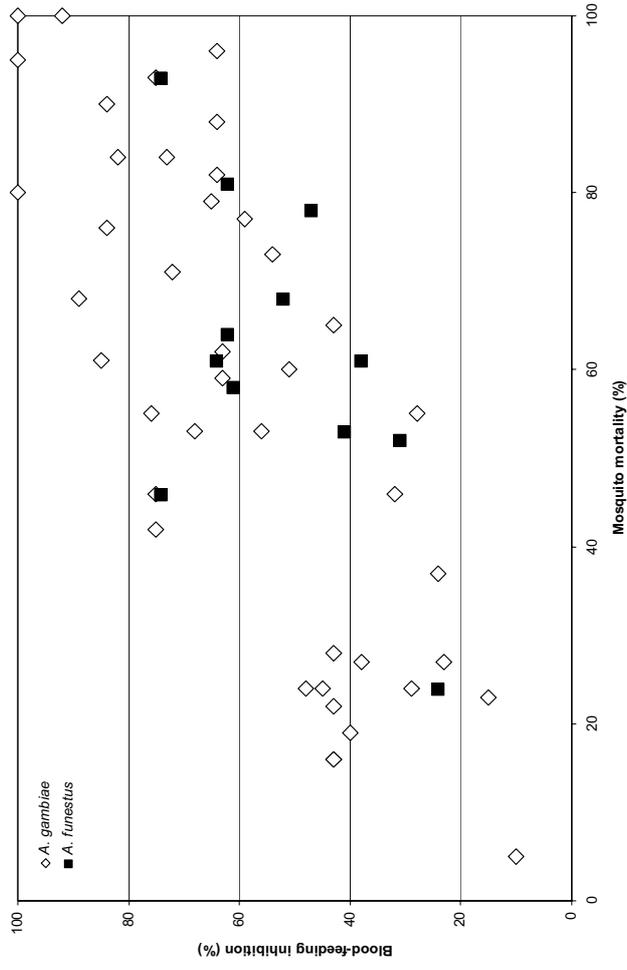
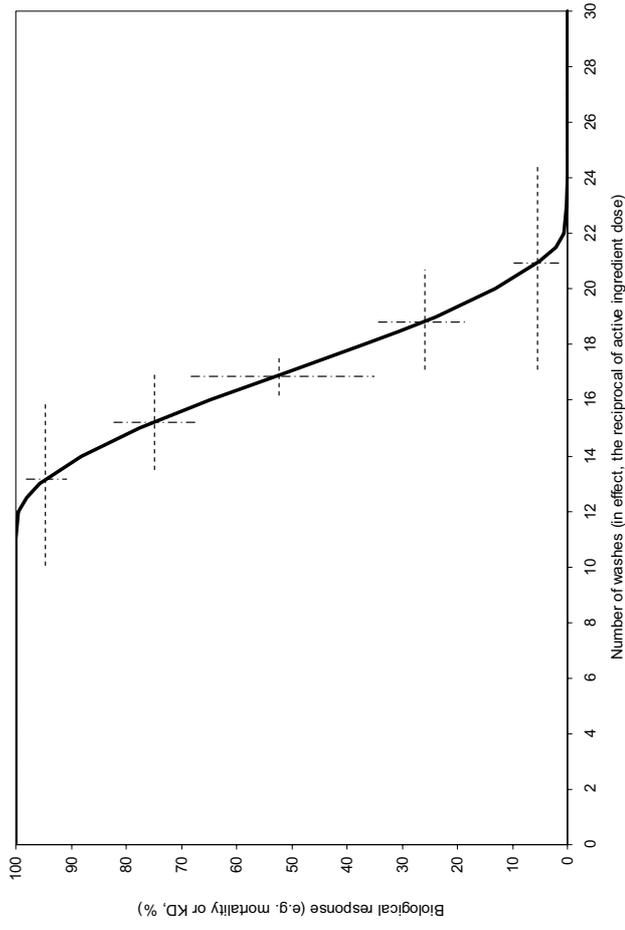


Figure 12. Magnitude of experimental error in determining concentration- and response-based parameters in bioassays



The x-axis scale is reversed from that of a typical dose-response plot, to illustrate how the influence of experimental error on setting limits based on repeated washing of insecticidal netting is the opposite of that on estimating toxicity, etc. Errors in determining the concentration at which a level of response occurs (horizontal error bars) are **lowest** at 50% response, whereas errors in determining the level of response at a concentration (vertical error bars) are **highest** at this point. Thus estimates of LD₅₀ are much more precise than LD₉₅ (or LD₅), whereas cut-off points for efficacy of washed insecticidal netting are much more precise when based on KD₉₅ (or KD₅) than KD₅₀.

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ANNEX 3: REFERENCES

Bonnet J, Duchon S, Corbel V (2006a). *Regeneration, wash resistance and efficacy of long-lasting insecticidal mosquito net (NetProtect®) against susceptible Anopheles gambiae* (DOC/LIN/06/01; unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)).

Bonnet J, Duchon S, Corbel V (2006b). *Regeneration, wash resistance and efficacy of long-lasting treatment technology of Syngenta ICONET® MAXX against susceptible Anopheles gambiae*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Clarke Mosquito Control (2006). *Duranet® LLIN – material safety data sheet* (Revision 04.07.2006).

Clarke Mosquito Control (2007). *Duranet – release of active substance – tentative for understanding of diffusion's mechanism*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Dabire R et al (2007a). *Efficacy of a mosquito treatment kit of Syngenta (Icon MAXX) for impregnation of polyester mosquito nets against Anopheles gambiae in experimental huts in Burkina Faso*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Dabire R et al (2007b). *Efficacy of a deltamethrin long-lasting insecticidal net (NetProtect) against Anopheles gambiae in experimental huts in Burkina Faso*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Dabire R et al (2007c). *Efficacy of alpha-cypermethrin long-lasting insecticidal mosquito net (Duranet) from Clarke Mosquito Control against Anopheles gambiae in experimental huts in Burkina Faso* (DOC/IRD/CREC/07/07; unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)).

Dow AgroSciences (2006). *Spinosad direct application tablet (DT) – product concept* (Revised April 11, 2006 and July 2006).

Confidential report to the WHO Pesticide Evaluation Scheme (WHOPES).

Dow AgroSciences (2006). *Spinosad DT – draft label*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Dow AgroSciences (2006). *Spinosad DT – material safety data sheet* (effective date: April 11, 2006).

Dow AgroSciences (2006). Determination of spinosad concentration in water over time after application of a single spinosad DT tablet. *Product development report PR-R0001*. Confidential report to the WHO Pesticide Evaluation Scheme (WHOPES).

Duchon S, Bonnet J, Corbel V (2006). *Regeneration, wash resistance and efficacy of long-lasting insecticidal mosquito net (Dawa Plus nets) from SiamDutch against susceptible mosquitoes of Anopheles gambiae* (DOC/LIN/06/04; unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Finnish Institute of Occupational Health (2006a). *Exposures and health risks from the deltamethrin-impregnated mosquito net use and washing, 8 September 2006*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES) on Netprotect®.

Finnish Institute of Occupational Health (2006b). *Exposures and health risks from the use and maintenance of Tana Netting mosquito nets, September 8, 2006*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Finnish Institute of Occupational Health (2007a). *Assessment of exposures and health risks from the use of alpha-cypermethrin-treated DuraNet® extra long-lasting insecticidal mosquito nets (LLINs), February 13, 2007*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Finnish Institute of Occupational Health (2007b). *Evaluation, revision 18 July 2007 of Syngenta assessment "exposures and health risks from the treatment and subsequent use of long*

lasting lambda-cyhalothrin-treated mosquito nets". Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Finot L, Bonnet J, Corbel V (2006). *Regeneration, wash resistance and efficacy of alpha-cypermethrin long-lasting insecticidal mosquito net (DuraNet) from Clarke Mosquito Control against susceptible mosquitoes of Anopheles gambiae* (DOC/LIN/05/18; unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)).

Hougard JM et al (2006). *Efficacy of a long-lasting insecticidal mosquito net (Dawa Plus nets) from SiamDutch Mosquito Netting Co., Ltd against Anopheles gambiae in experimental huts in Benin* (DOC/IRD/CREC/14/06; unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)).

Intelligent Insect Control (2007). *Deltamethrin long-lasting insecticidal net or netting: Net Protect* (Draft specification November 20, 2007). Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Jaal Z (2007). *Medium-scale evaluation of spinosad DT (ready to use tablet) and GR against dengue vector Aedes aegypti in a tropical environment*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Kilian A (2007). *Field testing of DAWA-Plus, a factory treated long-lasting insecticidal mosquito net based on the KO-Tab 123 technology. Interim report after 12 months of follow up*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Marcombe S et al (2007). *Field evaluation of spinosad GR 0.5% and DT 7% in plastic water containers for the control of Aedes aegypti in Martinique (France)* (DOC/LIN/IRD/68/07; unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES)).

Mulla MS, Thavara U, Tawatsin A (2007). *Field evaluation of spinosad direct application tablet (DT) in comparison to GR for the control of Aedes aegypti larvae in Thailand*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

PSB Corporation (2006). *Study of washing effect on total deltamethrin content for "DAWAPlus®" pre-treated LLIN mosquito net sample (57S061730_corr 1)*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Rowland M et al (2007). *Evaluation of the Syngenta ICON® MAXX long-lasting insecticide net treatment against Anopheles gambiae, An. arabiensis and An. funestus in experimental huts in Muheza (Tanga) and Moshi (Kilimanjaro), Tanzania*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Syngenta (2006). *Iconet® MAXX – WHOPES evaluation of Iconet® MAXX a long lasting treatment for mosquito nets based on lambda-cyhalothrin. Information sheet*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Syngenta Crop Protection AG (2006). *Iconet® MAXX. Draft label text*. Unpublished confidential report to the WHO Pesticide Evaluation Scheme (WHOPES).

Syngenta (2007). *ICON® MAXX insecticide net treatment – the new and innovative long lasting treatment for mosquito bednets and curtains (MB/BEA/5201/01.07)*.

Tana Netting Co. Ltd. (2006). *DawaPlus® product specifications sheet*.

Tana Netting (2007). *DawaPlus® – supplementary data for WHOPES phase II evaluation*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Tungu PK et al (2007a). *Evaluation of a Duranet™ LN, developed by Clarke Mosquito Control against Anopheles gambiae and Anopheles funestus in laboratory and experimental huts in Tanzania*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Tungu PK et al (2007b). *Evaluation of NetProtect® long lasting insecticide treated net (LN) against Anopheles funestus and Anopheles gambiae in experimental huts in Muheza, Tanzania*. Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).

Tungu PK et al (2007c). *Evaluation of DawaPlus® long lasting insecticidal net (LN) against Anopheles gambiae and An. funestus in experimental huts in Muheza, Tanzania.* Unpublished report to the WHO Pesticide Evaluation Scheme (WHOPES).