REPORT OF THE MEETING ON THE DEVELOPMENT OF GUIDELINES FOR TESTING AND EVALUATION OF LONG-LASTING INSECTICIDAL MOSQUITO NETS

WHO-HQ, Geneva
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COMMUNICABLE DISEASE CONTROL, PREVENTION AND ERADICATION
WHO PESTICIDE EVALUATION SCHEME
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## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. INTRODUCTION</td>
<td>4</td>
</tr>
<tr>
<td>2. DEFINITION OF A LONG-LASTING INSECTICIDAL MOSQUITO NET</td>
<td>5</td>
</tr>
<tr>
<td>3. LABORATORY STUDIES (PHASE I)</td>
<td>6</td>
</tr>
<tr>
<td>3.1 Regeneration time</td>
<td>6</td>
</tr>
<tr>
<td>3.2 Washing procedure</td>
<td>7</td>
</tr>
<tr>
<td>3.3 Bioassays</td>
<td>7</td>
</tr>
<tr>
<td>3.4 Tunnel test</td>
<td>8</td>
</tr>
<tr>
<td>3.5 Rubbing, ultraviolet protection and colour of nets</td>
<td>9</td>
</tr>
<tr>
<td>3.6 Chemical assays</td>
<td>10</td>
</tr>
<tr>
<td>3.7 Criteria for efficacy</td>
<td>10</td>
</tr>
<tr>
<td>4. SMALL-SCALE FIELD TRIALS (PHASE II)</td>
<td>10</td>
</tr>
<tr>
<td>4.1 Study design</td>
<td>10</td>
</tr>
<tr>
<td>4.2 Hut design</td>
<td>11</td>
</tr>
<tr>
<td>4.3 Treatments</td>
<td>11</td>
</tr>
<tr>
<td>4.4 Size, colour and fabric of nets</td>
<td>12</td>
</tr>
<tr>
<td>4.5 Washing procedure</td>
<td>12</td>
</tr>
<tr>
<td>4.6 Criteria for efficacy</td>
<td>13</td>
</tr>
<tr>
<td>4.7 Interim recommendations</td>
<td>13</td>
</tr>
<tr>
<td>5. LARGE-SCALE FIELD TRIALS (PHASE III)</td>
<td>13</td>
</tr>
<tr>
<td>5.1 Selection of study sites</td>
<td>13</td>
</tr>
<tr>
<td>5.2 Design</td>
<td>14</td>
</tr>
<tr>
<td>5.3 Criteria for efficacy</td>
<td>15</td>
</tr>
<tr>
<td>6. RECOMMENDATIONS</td>
<td>15</td>
</tr>
<tr>
<td>ANNEX 1. LIST OF PARTICIPANTS</td>
<td>16</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

A meeting of the Global Collaboration for Development of Pesticides for Public Health (GCDPP)\(^1\) on the development of guidelines for testing and evaluation of long-lasting insecticidal mosquito nets (LNs) was convened at WHO headquarters, Geneva, 4–7 April 2005. The objective of the meeting was to develop specific and standardized criteria, procedures and guidelines for testing LNs for personal protection and malaria control. The guidelines are intended to harmonize the testing procedures carried out by national authorities to generate data for the registration and labelling of LNs. The meeting was attended by nine representatives of industry, six participants from academic or government institutions and four WHO staff members (see list of participants, Annex 1). Professor Marc Coosemans was appointed Chair of the meeting and Dr John Gimnig was appointed Rapporteur. Industry representatives attended the first two days of the meeting to discuss general issues and present their views regarding the evaluation of LNs. The remaining participants then finalized the guidelines over the next two days.

The meeting was opened by Dr Hiroyoshi Endo, Director of the WHO Communicable Diseases Control, Prevention and Eradication Department. In his opening remarks he stated that 90% of the population of sub-Saharan Africa live in areas at risk of endemic or epidemic malaria. As the increase in drug resistance makes treatment more difficult, it has become necessary to focus on and reinforce prevention. To meet this challenge, the Roll Back Malaria (RBM) partnership includes insecticide-treated nets (ITNs) as part of its “prevention intervention strategy”. Dr Endo said that ITNs are effective in preventing malaria, but have several drawbacks. They require regular re-impregnation with insecticide to maintain their efficacy. He noted that the best hope for malaria prevention lies with LNs, which are expected to retain their insecticidal properties for the life of the net. He also highlighted promising new technology for the long-lasting treatment of mosquito nets that are already in use.

Dr Endo also noted that LNs are rather complex insecticide products, so testing and evaluation, as well as the development of quality control specifications, face many challenges. He stated that the main objective of the meeting is to standardize the requirements, criteria and testing procedures for their evaluation. The guidelines developed by this meeting will not only assist WHOPES to better streamline the testing and evaluation of such products, but they will also assist industry, registration authorities and national control programmes.

Dr Lorenzo Savioli, Coordinator of the Strategy Development and Monitoring for Parasitic Diseases and Vector Control Unit at WHO, also welcomed participants and noted the need for further standardization of procedures and criteria for the testing and

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\(^1\) Reports of previous meetings of the GCDPP are available from the Information Resource Centre, Communicable Diseases, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland. Fax: +41 22 791 4285; e-mail: cdsdoc@who.int (also at http://www.who.int/whopes/gcdpp).
evaluation of LNs. He noted that the close collaboration of WHO with industry has been crucial to the development of such technologies for public health use.

Dr Morteza Zaim of the WHO Pesticide Evaluation Scheme (WHOPES) reviewed the objectives of the meeting. He noted that the guidelines will not include the testing or evaluation of products for long-lasting post-factory treatment of mosquito nets (these will be subject to separate WHO guidelines), or the testing or evaluation of LNs that use insecticides not currently recommended by WHO for such application. Although there will be observations on the safety of LNs in the field, Dr Zaim added that, before any field study, a preliminary safety assessment must be undertaken (following the generic risk assessment model developed by WHO for this purpose). He also mentioned that the physical properties of the net’s fabric and factors relating to its structural integrity should conform to WHO specifications for netting materials. Generally, the guidelines should be as specific as possible to allow better comparison among the different institutions evaluating these products, but they should also contain some flexibility for local adaptation. Finally, any major issues or recommendations should be noted at the end of the guidelines. It was noted that the guidelines will be published for wide distribution separately from the report of the meeting.

2. DEFINITION OF A LONG-LASTING INSECTICIDAL MOSQUITO NET

The meeting was concerned that the guidelines should clearly define an LN and express in specific terms its longevity. It was noted that the longevity of the net and the longevity of insecticidal activity may not be equal under field conditions and it was suggested that the insecticide treatment should last as long as the net, so the user is never subject to sub-optimal protection from mosquitoes or malaria.

It was also noted that insecticidal longevity would vary according to numerous uncontrollable factors, including washing frequency, washing conditions, physical contact or rubbing of the net, smoke and dust. Such factors vary considerably among different regions and cultures and are difficult, if not impossible, to quantify.

It was suggested that an LN should retain biological activity for at least 20 washes according to the standard WHO recommended protocol and should retain biological activity for at least three years of field use. Representatives of industry were concerned that it would be difficult to meet these requirements in the field. They were particularly concerned that testing might be carried out in an area where LNs were washed frequently and that 20 washes would have been done in less than three years. It was therefore agreed to keep the definition as the retention of biological activity for at least 20 washes under

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3 See http://www.who.int/malaria/vectorcontrol.html.
standard laboratory conditions and the retention of biological activity for at least three years of recommended use under field conditions.

Participants at the meeting recognized that industry cannot guarantee three years’ longevity under varying programme conditions. However, it was agreed that WHOPES and partner organizations could apply this criterion to carefully controlled field evaluations. Therefore, before the start of any Phase III study, local washing conditions should be assessed and areas where washing is deemed excessively harsh should not be eligible for inclusion. The frequency and method of washing under field conditions would also be carefully monitored during the study. If washing conditions prove excessively harsh during the trial, the results should be weighed accordingly.

Finally, the participants agreed that these criteria should not be considered static and may be made more stringent as LN manufacturers develop new products proven to exceed the current minimum standards.

3. LABORATORY STUDIES (PHASE I)

The objectives of the laboratory tests are to determine the efficacy and wash resistance of an LN and to study the dynamics of the insecticide on the fibre. The aim of these experiments is not to simulate washing that would be experienced under field conditions, but rather to provide a consistent, repeatable protocol that allows comparison among different laboratories and different LN products.

3.1 Regeneration time

In order to determine the time period required for the regeneration of an LN after standard washing and holding at 30°C, bioassays (as outlined below) are carried out at 24-hour intervals on net samples washed and dried once, or three times consecutively, until initial biological activity is restored. Surface insecticide is not expected to be depleted on LNs washed once, but it is on those washed three times. Insecticide bioavailability (efficacy) curves will be established and compared for these groups of nets. The time required (in days) to reach the plateau of bioavailability is the period required for regeneration of the net. If the two curves differ, the longer period will be adopted as the washing interval in Phase I and Phase II studies to ensure that wash resistance is not overestimated.

Determination of regeneration time is of great importance for LNs, particularly for those where the insecticide is incorporated within the net fibre. It was agreed that WHOPES would raise the requirements by insisting on the need for a short regeneration time.

Additional holding temperatures were suggested but it was felt that they would require significantly more work and would cloud the interpretation of results, and it was agreed that 30°C was a reasonable regeneration temperature.
3.2 Washing procedure

Washing procedure is a critical component of wash resistance testing. Participants agreed that there is too little data on the effect on insecticide retention of different soaps and detergents, of how long nets are soaked, and of how aggressively nets are agitated.

Industry representatives presented data on the effect of washing using different African soaps and detergents in comparison with using savon de Marseille, the soap currently recommended by WHO. All had similar pH values of around 9.5 (one had a pH of around 10) and washing with most of the soaps removed deltamethrin from polyester nets at similar rates as washing with savon de Marseille. Only two soaps were less aggressive.

It was also shown that the concentration of the soap strongly affects the pH of the water, which may affect the rate of degradation of pyrethroids. Water quality (particularly hardness) may also affect the rate of insecticide loss. Since these factors vary among regions and cultures, it was agreed that the primary goal of the washing procedure should be to have a standardized method that could be reliably replicated in different laboratories. The current method of washing requires that net samples (25 cm x 25 cm) be individually introduced into a 1-litre glass bottle containing 0.5 l deionized water and 2 g/l fully dissolved soap (pH 10–11). Bottles are immediately introduced into a water bath at 30°C and shaken for 10 minutes at 155 movements per minute. The samples are then removed and rinsed twice in clean, deionized water for 10 minutes and shaken as stated above. Nets are dried at room temperature and stored in the dark at 30°C between washes.

The soap currently used in laboratory washing procedures is savon de Marseille. Since formulations of this commercial soap may vary in different areas and over time, it was agreed that this should eventually be replaced by a standard International Organization for Standardization (ISO) soap or by a well-defined soap designed specifically for this testing process. It should be representative of the soaps most commonly used for washing nets in sub-Saharan Africa. This is why it was decided that a soap rather than a detergent should be used. Representatives from Vestergaard-Frandsen and Intelligent Insect Control are currently testing a standard soap and will submit the results to WHOPES as soon as they are available.

3.3 Bioassays

WHO cone bioassays should be performed on nets washed 0, 1, 5, 10, 15 and 20 times (or more as necessary) to measure the efficacy of the insecticide treatment after repeated washing. WHO cone tests have been used to measure the efficacy of insecticide-treated nets for many years. However, many participants felt the WHO cone test has some drawbacks and suggested that an alternative testing method be developed. The main concern was that insecticides with strong excito-repellant properties, such as permethrin and etofenprox, would be at a disadvantage in cone tests as exposed mosquitoes might spend more time resting on the cone (therefore less time on the treated net) than they
would if exposed to a less repellant insecticide. An additional concern was that the cone test requires a certain amount of skill, as testers must rapidly introduce and remove mosquitoes from the cone within a short time without damaging them. Results from cone tests vary even when the same net is tested repeatedly in the same laboratory. Potential alternatives include the use of WHO test tubes (cylinders) for adult mosquitoes and the wire-ball test. In the tube test, netting material is rolled in a double layer and the upper part of the tube is also covered with the netting. Mosquitoes are introduced from the holding chamber and exposed for three minutes before being blown back into the holding chamber. The advantage of this test is that it is very easy and can be done by people with minimal skill in handling mosquitoes. Also, it has been found to generate very consistent results. The two concerns regarding this method are that it cannot be performed in situ in the field and that more data are needed to calibrate it with the WHO cone test. It was recommended that a multi-centre test be conducted to calibrate the tube test with the cone test, with the aim of eventually establishing the WHO tube test as the standard bioassay for LN and ITN evaluations.

The wire-ball test is performed by wrapping netting material around a wire frame and introducing 11 mosquitoes into the ball, then recording the time required for six mosquitoes to be knocked down. There were concerns that this test was even more difficult to perform than the cone test and that it introduced subjectivity. Knockdown has been observed in nets where high performance liquid chromatography indicated essentially no insecticide on the net, leaving the interpretation of results open to question. Furthermore, knockdown effect is an attribute of insecticide and this test may not be useful when comparing nets treated with different insecticides, especially those with slow knockdown. However, it was felt that this test could provide additional information on bioavailability for the manufacturer during product development. It was therefore recommended that manufacturers consider this method during development, but that it is not appropriate for measuring efficacy in LN evaluations.

A susceptible strain of *Anopheles gambiae* was recommended as the standard species for use in the bioassays. However, this species cannot be used in laboratories in South and Central America or in South and South-East Asia. It was decided that alternative species may be used in these regions but that the results should be calibrated with those obtained using *Anopheles gambiae*. *Aedes aegypti* was also suggested as the test mosquito as it is much easier to rear in the laboratory, is circumtropical in its distribution and there is no major concern about accidental release. However, since these products are to be used primarily for malaria prevention, it was felt that *Anopheles* species should be preferred for testing. To ensure that there is no contamination of the test strain, six-monthly testing of the strain’s insecticide susceptibility was recommended.

### 3.4 Tunnel test

Insecticide-treated nets function by repelling, preventing blood feeding and killing mosquitoes. The WHO cone test only measures the killing effect of nets. It was therefore decided that an additional test is necessary to ensure that nets with strong excito-repellant properties are not eliminated from consideration based on a test of mortality after
three minutes’ exposure under WHO cones. The efficacy (mortality and blood-feeding inhibition) of LNs washed 20 times or more that no longer meet the criteria of standard cone bioassays will therefore also be tested in the laboratory by releasing non-blood-fed female anopheline mosquitoes, aged 5–8 days, in a glass tunnel 60 cm length (square section 25 cm x 25 cm). A 25-cm square cage is fitted (extension) at each end of the tunnel and covered with polyester netting. A disposable cardboard frame, holding the treated netting sample, is placed at one third of the tunnel’s length. The surface of netting “available” to mosquitoes is 400 cm² (20 cm x 20 cm), with nine holes, each of 1 cm in diameter: one hole is located at the centre of the square; the other eight are equidistant and located at 5 cm from the edge.

In the shorter section of the tunnel, bait (e.g. guinea-pig for Anopheles gambiae) is placed, unable to move. One hundred female mosquitoes are introduced in the cage at the end of the longer section of the tunnel at 18:00. The females can fly freely in the tunnel but must make contact with the piece of netting and locate the holes in it before passing through to reach the bait.

The following morning, at 09:00, the mosquitoes are removed from the two sections of the tunnel and counted separately, and the immediate mortality is recorded. Live females are placed in plastic cups containing honey solution; any delayed mortality is recorded after 24 hours. During the tests, cages are maintained under subdued lighting at 27°C ± 2°C and 80% ± 10% relative humidity.

Several tunnels are to be used simultaneously, one tunnel with untreated netting always being used as a negative control. Blood-feeding inhibition is assessed by comparing the proportion of blood-fed females (alive or dead) in treated and control tunnels. Overall mortality is measured by pooling the immediate and delayed (24-hour) mortalities of mosquitoes from the two sections of the tunnel.

Nets washed at least 20 times that cause ≥80% mortality and/or ≥90% blood-feeding inhibition in tunnel tests meet the criteria to undergo Phase II testing.

The major objection to the tunnel test was that it might be de facto a way of lowering the cut-off criteria for an LN. However, it was decided that the cut-off criteria for the tunnel test are in fact very stringent and it was generally agreed that nets with high excito-repellent properties may provide good protection from malaria even if they do not effectively kill mosquitoes.

3.5 Rubbing, ultraviolet protection and colour of nets

There was brief discussion on whether to assess the impact of rubbing on the longevity of insecticidal activity in the laboratory. There are several tests available in the textile industry to test colour fastness that could be adapted to assess the effect of rubbing. Whether the effect should be measured by biological measures or by chemical assays was not clear.
Participants asked whether specific test procedures should be considered for LNs that include ultraviolet protection. It was concluded that this is not necessary as the efficacy and longevity of the final product is of primary concern and this will be studied by the methodology and procedures outlined in the guidelines.

The impact of coloration on the efficacy and bioavailability of the insecticides in an LN was discussed and it was recommended that if coloured LNs are marketed, their bioavailability curves should be studied in the laboratory and compared with those of white nets and, if significantly different, they should be considered as a separate product, requiring full testing and evaluation.

3.6 Chemical assays

It was recommended that a certificate of chemical analysis should be provided by the manufacturer to ensure that the concentration of the active ingredient is within 25% of the declared concentration. It was also noted that chemical assays of the total insecticide content of the netting (following the methodology recommended by the manufacturer) should be carried out before and after wash resistance studies in order to improve the interpretation of test results. To avoid multiple analyses, samples from the same net may be pooled.

3.7 Criteria for efficacy

It was agreed that a net should be considered to have passed Phase I requirements if, after 20 or more washes, mortality is >80% or knockdown is >95% in the WHO cone test, or if mortality is >80% or blood-feeding inhibition is >90% in the tunnel test. Washing and bioassays should continue until the net falls below these criteria or until the number of washes claimed by the manufacturer is reached.

4. SMALL-SCALE FIELD TRIALS (PHASE II)

The efficacy of LNs that pass Phase I testing will then be determined in experimental huts under semi-natural conditions, in terms of blood-feeding inhibition, deterrence, induced exophily and mortality, and using susceptible, free-flying, wild mosquitoes. The small-scale field testing will also record any perceived side effects among users of the LN.

4.1 Study design

The impact of washed and unwashed LNs on the blood-feeding inhibition of susceptible (confirmed by WHO susceptibility tests), free-flying, wild mosquitoes (anophelines and, where possible, culicines), their tendency to be repelled from or driven out of houses, and their mortality will be assessed using experimental huts fitted with entry slots to prevent escape of mosquitoes, exit and/or screened veranda traps, and mechanisms for excluding
ants and other scavengers that might carry dead mosquitoes from the huts during the night.

In the past, one treatment arm has been assigned to one hut for the duration of the study to avoid contamination. However, thorough cleaning and airing of the huts can eliminate any contamination. It was therefore recommended to rotate nets and sleepers in a Latin square design. One treatment arm would be assigned to one hut each week. A different replicate net would be tested within each treatment arm each night and sleepers would rotate among the huts each night. If treatments are not rotated among the huts, baseline studies should be carried out to verify that there is no bias among the huts in terms of the number of mosquitoes that enter them.

The following treatment arms were recommended (see Section 4.3): (1) unwashed LN; (2) LN washed 20 times; (3) LN washed according to the manufacturer’s claim (or maximum number of washes as determined during Phase I); (4) polyester, conventionally treated net washed under Phase II conditions (see Section 4.5) 20 times; (5) polyester, conventionally treated net washed under Phase II conditions until just before exhaustion; and (6) untreated net (preferably of the same fabric and mesh size as the test LN).

It was recommended that the study continue for 6, 12 or 18 weeks as necessary to achieve adequate numbers of mosquitoes for statistical comparison. The number of weeks should be a multiple of six to allow for the treatment arms to rotate through each hut the same number of times during the course of the study.

It was agreed that six holes (4 cm x 4 cm) would be cut in the net to simulate a torn net. There was concern about the ethical implications of doing this but it was felt that blood-feeding inhibition was an important measure to assess a candidate LN. Volunteers would therefore be provided with chemoprophylaxis, if appropriate, and medical supervision during the study.

Participants recommended chemical assays at the start of the study to ensure that the product meets the specifications provided by the manufacturer (LN) or the target concentration (non-washed conventionally treated net). The insecticide concentration should be within 25% of the target dose.

4.2 Hut design

Hut design was reviewed during the meeting, as several different designs are employed in different settings. However, these differences are unlikely to affect the outcome of the study significantly, provided that appropriate controls are used.

4.3 Treatments

As most experimental hut field sites have a limited number of huts, it was decided to include (1) an unwashed LN; (2) an LN washed 20 times; (3) an LN washed the number
of times claimed by the manufacturer (or as determined from Phase I); (4) a conventionally treated net (using the same insecticide at WHOPES’ recommended dose) washed 20 times; (5) a conventionally treated net washed until just before exhaustion; and (6) an untreated net. For a conventionally treated net, the number of washes the net can withstand before its insecticidal activity falls below the WHO cut-off point (>80% mortality or >95% knockdown) should be determined on site with daily washing followed by WHO cone tests. The “20 washes” LN would be the net used to confirm that the product meets the criteria required for definition as an LN, while the LN washed at least the number of times claimed by the manufacturer would simply verify the claims.

A washed LN should perform at least as well as a net conventionally treated with the same insecticide at the WHO recommended concentration. An unwashed conventional ITN was first considered for comparison but no existing LN could meet this criteria. It was therefore agreed to replace this treatment arm with a conventional net washed until just before exhaustion. A conventional net washed 20 times is added as a control to assist in the interpretation and publication of results.

4.4 Size, colour and fabric of nets

It was agreed that the size of the net could differ according to hut design and that this is unlikely to affect the outcome. However, it is recommended that an appropriate size be selected for the hut being used in the study.

If it is determined in Phase I that net products with different colours do not differ in performance, then it does not matter what colour is used in Phase II studies.

Conventionally treated and untreated nets used in Phase II should normally be of the same net fabric as the LNs being tested. However, since most conventional nets currently available are polyester, and since wash-off of insecticide may be much higher on other fabrics, it was recommended that the conventional nets tested should be polyester nets.

4.5 Washing procedure

Various washing procedures have been used for Phase II studies in the past. It was agreed that the washing procedure should mimic, as closely as possible, that used for Phase I washing. This is to avoid adding more variables to the study and to link the Phase I and Phase II studies better. It was recommended that individual nets be washed for 10 minutes in 10 l of water (2 g/l of savon de Marseille), stirring at approximately 20 rotations per minute. However, the procedure would differ somewhat from that of Phase I. First, although the concentration of soap in the water is similar, the total amount of soap per unit area of net is significantly lower when washing the whole net. Second, nets would be rinsed twice, soaking and stirring the nets in clean water for 1–2 minutes each time. This was recommended because 10-minute rinses were considered difficult to achieve under field conditions. The water used in washing should be well water or de-chlorinated tap water, preferably at 30°C with a hardness lower than 5 dh (degrees of hardness), as hardness may affect insecticide degradation. The time between washes
should be the regeneration time as measured during Phase I. To ensure the nets are not affected by light, rain or handling, they should be dried in the shade (preferably in a covered area to avoid rainfall) and then wrapped in paper and stored inside.

4.6 Criteria for efficacy

It was initially proposed that the washed LN’s performance should equal or surpass that of a non-washed conventionally treated net. However, it was recognized that this was too stringent and would eliminate most LNs, including those currently holding WHOPES recommendations. After careful consideration, it was agreed that a candidate LN washed 20 times or more would pass Phase II and receive provisional recommendation as an LN if it performed as well as or better than a conventionally treated net that has been washed until just before exhaustion as determined using WHO cone tests (24-hour mortality at least 80% and 60-minute post-exposure knockdown at least 95%).

4.7 Interim recommendations

In view of the long-term studies that may be required to fully evaluate an LN product, interim recommendations for its use for malaria prevention and control may be given, subject to the following conditions: the LN has been treated with one of the insecticides recommended by WHO for the treatment of nets; satisfactory completion of laboratory and small-scale field testing; and confirmation that after at least 20 WHO standard washes the LN’s performance in experimental huts equals or surpasses that of a conventionally treated net washed until just before exhaustion. It is assumed that in such circumstances the information available on the performance of the conventionally treated nets will help to anticipate the performance of the LN product in operational settings.

5. LARGE-SCALE FIELD TRIALS (PHASE III)

Phase III studies are large-scale studies to determine efficacy, longevity and fabric integrity in real-life situations, as well as community acceptance of an LN.

5.1 Selection of study sites

It is important to select study sites that will be representative of most areas where LNs will be used, given the wide diversity of cultural practices surrounding nets. It was agreed that preliminary surveys should be conducted before initiating any Phase III study, and that areas with excessively harsh washing practices should be avoided, as there are areas where nets are washed frequently or where particularly strong soaps or detergents are used. The preliminary surveys should assess previous ownership of ITNs, frequency of use, washing and care, and preferences for net shape, size or colour. Responses to those surveys should guide the selection of net shape, size and colour for use in the actual trial. Although white nets may be washed more frequently, it was agreed that the net size and
shape (and possibly colour) were unlikely to significantly affect the outcome of the trial. However, control nets should be of the same type as test nets.

5.2 Design

The candidate LN and conventionally treated nets should be randomly distributed among several villages. It was agreed that nets of the same colour, conventionally treated with the same insecticide at the WHO recommended concentration should be compared. These nets should be studied for at least one year or until they fail to meet the cut-off criteria. The study should be limited to three years to provide industry with a clear timescale. However, if possible, monitoring of nets should continue until the LN fails to meet the cut-off criteria.

Destructive sampling of nets should be done at six-month intervals thereafter and enough nets should be distributed to account for loss of nets due to sampling as well as unexpected losses over the six-month period. Although it was recommended that sample size calculations be made prior to initiating any Phase III study, it was felt that sampling 30 nets at each round should be adequate. Every six months, 30 LNs and 30 conventionally treated nets are randomly sampled. The nets are removed from each household sampled and replaced with new nets. Once sampled, a household is no longer eligible for sampling in subsequent rounds. Two side-by-side pieces of netting (25 cm x 25 cm) are removed from the middle of one of the longer sides of the net, placed in aluminium foil and stored in a cool, dark place until eventual chemical analysis and bioassays are performed.

During the sampling of nets, the owner should be interviewed to assess frequency of use and washing. Interviewers should at least confirm that the net is hanging. Since interview assessment of washing frequency is unreliable, it is recommended that another net in the household, or a net in a neighbouring household, be marked with a water-soluble marker and revisited one month later to obtain a more accurate picture of washing frequency in the community.

It was decided to conduct similar bioassays to those carried out in Phase I. Standard WHO cones should be fitted to the netting samples and five mosquitoes introduced into each cone. This should be replicated 10 times, resulting in a total of 50 mosquitoes exposed per netting. If 80% of the nets do not meet WHO criteria (>80% mortality or >95% knockdown), then the six nets with the lowest mortality and/or knockdown should be exposed in a tunnel test. If all six fail to meet the WHO criteria for the tunnel test (>80% mortality or >90% blood-feeding inhibition), then the net does not meet WHO criteria as an LN. It was decided to test only the six net samples with the lowest biological activity in the cone test as the tunnel test cannot easily be performed on large numbers of samples. This assumes that nets determined to have higher biological activity in the cone test will also exhibit higher biological activity in the tunnel test. According to this assumption, if at least one of the nets tested in the tunnel test meets the WHO criteria, then at least 80% of the nets will meet WHO criteria.
5.3 Criteria for efficacy

Rather than base the criteria for efficacy on average percentage mortality (or knockdown) it was agreed that at least 80% of nets must meet the WHO cut-off criteria (>80% mortality or >95% knockdown in a cone test or >80% mortality and/or >90% blood-feeding inhibition in a tunnel test). These criteria were selected to avoid cases where outliers with low insecticidal activity could significantly lower average bioassay results to below the threshold despite the majority of LNs meeting WHO criteria for long-lasting insecticidal activity. A net that meets WHO criteria for at least three years of field use will be considered an LN.

6. RECOMMENDATIONS

The meeting reviewed and adopted the Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets and recommended its publication and wide distribution by WHOPES. The meeting also made the following recommendations.

- WHOPES should develop and evaluate, in a multi-centre study, a simple, reproducible laboratory test method to assess the efficacy of LNs more reliably than WHO cones, particularly for LNs treated with insecticides with strong excito-repellent properties.

- Test methods should be calibrated with alternative vector species, particularly those in regions outside sub-Saharan Africa.

- In collaboration with industry, WHOPES should develop and calibrate a standard soap for Phase I and Phase II washing protocols.

- Efforts should be made to strengthen the capacity of local national malaria control programmes and other institutions for the testing and evaluation of LNs.
ANNEX 1. LIST OF PARTICIPANTS

Industry

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