Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions

Guidelines

World Health Organization

Cover Design: Tissot Patrick WHO/NTD
Photo credit: Vestergaard Frandsen
Illustrations (blood cells and mosquito): Meissner Denis WHO/GRA

Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions

Control of Neglected Tropical Diseases
WHO Pesticide Evaluation Scheme
and
Global Malaria Programme
Vector Control Unit

WHO Library Cataloguing-in-Publication Data

Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions.

1.Mosquito control - methods. 2.Bedding and linens. 3.Insecticides - administration and dosage. 4.Clinical trials, Phase II. 5.Clinical trials, Phase III. 6.Guidelines. I.World Health Organization.

ISBN 978 92 4 150170 5 (NLM Classification: QX 600)

Web only.

WHO/HTM/NTD/WHOPES/2011.5

CONTENTS

ACKNOWLEDGEMENTS

The first draft of these guidelines was written by the following experts: Dr Adeline Chan, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Dr John Gimnig, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Dr Albert Kilian, Malaria Consortium, Montagut, Spain; Dr Jo Lines, World Health Organization (WHO), Geneva, Switzerland; Dr Michael MacDonald, United States Agency for International Development, Washington DC, USA; Dr Stephen C. Smith, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Dr Robert A. Wirtz, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Dr Rajpal S. Yadav, WHO, Geneva, Switzerland; and Dr Morteza Zaim, WHO, Geneva, Switzerland.

The first draft was then peer-reviewed by individuals and institutions known for their expertise in the subject. With the technical support of Dr John Gimnig, the WHO secretariat considered all the comments and prepared a second draft, which was reviewed and discussed at a consultation held at WHO headquarters in Geneva, Switzerland, on 22–25 February 2011. Representatives of industry were invited to attend the first 2 days of the meeting to exchange information and share views, after which the second draft and comments were further reviewed by a group of WHO-appointed experts, who finalized the guidelines by consensus. These experts were Dr Daniel Boakye, Noguchi Memorial Institute for Medical Research, Accra, Ghana; Dr Marc Coosemans, Institute of Tropical Medicine, Antwerp, Belgium; Dr John Gimnig, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; Ms Sarah Hoibak, Basel, Switzerland; and Dr Evan Mathenge, Kenya Medical Research Institute, Nairobi, Kenya. The five experts who participated in the consultation declared that they had no conflict of interest in the subject matter of the guidelines.

The following WHO secretariat also attended the meeting: Dr Birkinesh Ameneshewa, Regional Office for Africa; Ms Aurélie Bottelin, Global Malaria Programme, Geneva; Dr Jeffrey Hii, WHO Country Office, Manila, Philippines; Dr Abraham Mnzava, Regional Office for the Eastern Mediterranean; Dr Jo Lines, Global Malaria Programme, Geneva; Dr Raman Velayudhan, Department of Control of Neglected Tropical Diseases, WHO, Geneva; Dr Rajpal Yadav, Department of Control of Neglected Tropical Diseases, WHO, Geneva; and Dr Morteza Zaim, Department of Control of Neglected Tropical Diseases, Geneva.

The WHO Department of Control of Neglected Tropical Diseases and the Global Malaria Programme acknowledge all the individuals and institutions listed above for their important contributions to this work. They also express their sincere thanks to Dr John Gimnig and to the United States Centers for Disease Control and Prevention for their valuable support in preparation of this document. The financial support of the Global Collaboration for Development of Pesticides for Public Health is also gratefully acknowledged. WHO will welcome feedback and suggestions from national programmes, research institutions and industry on the guidelines in order to improve future editions.

1. INTRODUCTION

The main purpose of these guidelines is to assist national vector-borne disease control programmes, and other relevant agencies, in monitoring the durability of long-lasting insecticidal mosquito nets (LN)[1] under operational conditions. The information derived by monitoring will be useful in planning the replacement of worn-out nets in an LN programme, making decisions to procure the most suitable LNs for the setting and understanding the factors associated with the durability of LN products. The guidelines do not cover procedures for evaluating the efficacy for prevention and control of malaria and other vector-borne diseases[2] or any potential risks associated with use of LNs, which are described elsewhere (*1*, *2*). The principles and methods outlined in this document can, however, contribute to such evaluations.

These guidelines are designed for monitoring various LN products. An LN is a factory-treated mosquito net that is expected to retain its biological activity for a minimum number of standard washes as defined by WHO and a minimum time under field conditions. Currently, an LN is expected to retain its biological activity for at least 20 standard WHO washes under laboratory conditions and 3 years of recommended use under field conditions, as defined in the WHO guidelines (*1*). In addition, the methods described here can be used in monitoring the performance of mosquito nets treated with 'do-it-yourself' kits intended for converting mosquito nets into LNs.[3]

LNs play a significant role in the prevention and control of vector-borne diseases, especially malaria. They provide personal protection, and, in settings with sustained high levels of coverage and anthropophilic vectors, they can reduce transmission and protect an entire community (i.e. a mass effect). For malaria control with LNs, WHO now recommends universal coverage.

In many current LN distribution programmes, it is assumed that LNs have a relatively uniform lifespan of about 3 years. Thus, it is often assumed that mass distribution campaigns at 3-year intervals are enough to maintain adequate levels of net coverage throughout the 3-year interval. Although distribution of additional nets through routine immunization and antenatal services is also recommended, this has sometimes been regarded as an option, requiring small quantities of nets. Recent longitudinal studies and surveys have revealed, however, that in this assumption of a uniform 3-year lifespan may be over-optimistic and that the rate at which net coverage declines after a campaign may be substantially more variable, and more rapid, than was previously assumed (*3*). WHO therefore now recommends that delivery of LNs by immunization and antenatal services be given as much priority as delivery through periodic campaigns.

Role of the WHO Pesticide Evaluation Scheme

One activity of the WHO Pesticide Evaluation Scheme (WHOPES) is to evaluate pesticides for public health use. Within its mandate, WHOPES has prepared guidelines for evaluating LNs for safety (*2*) and efficacy (*1*). The recommendations are intended to facilitate the registration and use of these and other public health pesticide products by Member States for the prevention and control of malaria and other vector-borne disease.

As long-term studies are required for full evaluation of candidate LN products, interim recommendations on their use for malaria prevention and control may be given, if they are

[1] 'Long-lasting insecticidal nets' is often abbreviated as LLNs, LLINs or LLITNs. In this document, the standard two-letter code for pesticide formulations is used (see *Manual on development and use of FAO and WHO specifications for pesticides*, available at http://www.who.int/whopes/quality.

[2] While the main focus of these guidelines is the use of LNs for malaria control, the same principles and procedures apply to the use of LNs for other vector-borne diseases.

[3] http://www.who.int/whopes/Insecticides_ITN_Malaria_ok3.pdf.

made with a WHO-recommended insecticide; they have satisfactorily completed laboratory and small-scale field testing for efficacy; and, after at least 20 standard WHO washes, they perform as well as or better than a conventionally treated net washed until just before exhaustion (*1*). LN products may be given a full recommendation if they meet WHOPES criteria after 3 years in large scale field trials. An interim or full WHO recommendation for use of an LN product implies that WHO has evaluated that product for safety and efficacy and that it met the criteria and requirements of the Organization.

A list of WHOPES-recommended LNs and WHO specifications for their quality control and international trade are available at http://www.who.int/whopes. Some information on the performance of different LN products can be derived from data on individual products published in WHOPES working group reports[1]. It should be noted, however, that the primary mandate of WHOPES is to assess individual products against objective standards and usually not to make direct comparisons between products to assess their relative performance for a specific purpose in a specific context.

While WHOPES guidelines are available to assess the risk associated with use of insecticide-treated nets and to evaluate the efficacy of LNs, guidelines are needed to assist national programmes and other agencies to monitor the survivorship, the fabric integrity and the persistence of the bio-efficacy and insecticide content of LNs under operational conditions in various settings.

Procurement

WHOPES-recommended products are expected to meet or exceed defined minimum standards of quality and performance. That does not imply, however, that all such products are technically and functionally identical. There is clear evidence that the fabric integrity of LNs varies widely, not only among different products but also among different locations and settings, due perhaps to local variations in sources of wear and tear (e.g. snagging, rodents, washing and burns) and differences in the vulnerability of products to these factors (*4*, *5*).

The procedures used for large-scale LN procurement by some agencies and donors are based on the implicit assumption that any technical differences between WHOPES-recommended products are negligible, and procurement decisions are based on delivery deadlines and differences in price, which are often rather narrow. Such policies have important consequences for the LN market. LN technology is still relatively young, and through further research and development may result in LNs that are much more durable. This would greatly improve their cost-effectiveness and operational efficiency. This will occur in practice, however, only if there is a system allowing the market to recognize and reward such improvements in performance.

On the basis of currently available information, it cannot be assumed that a product that is the most durable in one setting will also be the most durable in other settings. Therefore, ranking of the available products is not appropriate at global level. Instead, country-level data on LN durability are needed as a basis for national procurement decisions.

There is evidence that users do distinguish between LN products and that their preferences affect use of these products. A system is needed in which preferences can be taken into account in procurement. Guidelines are needed, and the study design outlined here might be useful for the purpose (see section 9, Operational research).

Durability, user preference, cost, delivery time and registration in the country are all factors that are important in making procurement decisions.

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

Why should we measure the durability of LN products?

Country programmes planning for long-term coverage with insecticide-treated nets need information on the durability of different LN products in local settings, for the following reasons:

- Programmes and agencies must choose products to procure and determine whether particular products are likely to perform better over time than others in their setting.
- Knowledge about the durability of LNs after distribution is needed in order to estimate the necessary rate of replacement in continuous distribution systems, the appropriate interval between campaigns and, when necessary, to plan for disposal or recycling of old nets.

Monitoring of the durability of nets may lead to better understanding of the factors that determine LN durability and laboratory indicators that correlate with these net qualities. It can also provide an opportunity to improve behaviour and communication messages so that users take better care of their LNs.

The purpose of this document is to provide guidelines for national malaria control programs to assess the useful life of LNs distributed in their countries. There are 3 main determinants of the useful life of an LN as defined below (Section 2). It should be noted that while assessment of chemical content and biological efficacy requires facilities and resources that may not be available in all countries, this should not preclude programs from monitoring LNs. Estimates of LN attrition/survivorship and fabric integrity can be done by all programs and it is strongly recommended that all programs implementing LNs as part of a national malaria control strategy perform monitoring of the LNs distributed in their countries. Furthermore, it is strongly recommended that programs distribute several LN products in the site (or sites) selected for monitoring. This will maximize the information gained and provide better guidance for the selection of LN products in future procurements.

2. DEFINITION OF ELEMENTS OF DURABILITY

The three elements to be considered in assessing the durability of LNs are net survivorship, fabric integrity and insecticidal activity (bioefficacy). These components of durability are determined partly by factors intrinsic to the manufacture of the net (e.g. material composition, knitting or weaving pattern, quality of finishing, insecticide type and content, additives, LN technology) and partly by extrinsic factors that cause wear and tear.

Variations in the durability of LNs have been observed between locations, between households in the same location and between nets in the same household. These variations apparently reflect the direct and indirect effects of such factors as climate, quality of housing, location (indoors or outdoors) and type of sleeping space (mattress, reed mat), the presence of rodents and other animals, washing frequency and methods, whether the net is taken down during the day, frequency of use, the number of people who sleep under the net, whether it is used by children or adults, and whether net use is a new or a well-established habit in the household.

The following definitions, descriptions and indicators are proposed for the elements of durability:

Survivorship is the proportion of distributed nets still available for use as intended in the households to which they were given after a defined period, e.g. 1, 2, 3 or more years.

Attrition (opposite of survivorship) is the proportion of nets no longer in use as intended after a defined period after their distribution to the households. Attrition can be categorized by the main reasons why a net is no longer used, namely decay (e.g. destroyed, so torn and worn

out that it is considered useless for protection against mosquitoes), absence (e.g. stolen, given away, moved) or used for other purposes.

Physical or fabric integrity reflects the number, location and size of holes in each net. When possible, the assessment can also be categorized by type of hole (burn, tear, seam failure, nibbled or chewed by animals). The physical or fabric integrity of the surviving nets can be assessed as a function of length of use, until deterioration leads to the net being discarded or used for another purpose.

Insecticidal activity (bioefficacy) is the degree of knock-down, mortality or inhibition of blood-feeding induced in susceptible mosquitoes, as determined by standard WHO test procedures and criteria (i.e. cone bioassay, tunnel test) (*1*). Insecticidal activity is associated with the type and content or availability of insecticide. The insecticide content is expressed as g/kg or mg/m^2 of the LN and is determined by the method outlined in WHO specifications for LNs[1]. This information is of value in interpreting data on bioefficacy. Insecticidal activity can be assessed as a function of length of use.

The interaction between insecticide type and content and the location, size and number of holes in ensuring personal protection has not been studied and remains a priority for research (see section 9, Operational research).

3. OBJECTIVES AND APPROACHES FOR MONITORING DURABILITY

3.1 Objectives

Vector control programmes should build or strengthen their capacity to monitor and evaluate the durability of LNs distributed to targeted populations as a routine programme management activity. This should include measurement of three essential outcomes, namely survivorship (or attrition) rate, fabric integrity and insecticidal activity of LNs over time. The specific objectives include:

- to determine the survivorship and fabric integrity of LNs in various environments and cultural settings to assist in decisions on procurement, replacement, disposal or recycling, in product improvement and to further guide programme planning and practices;
- to assess the insecticidal activity of LNs (bioefficacy and insecticide content) over time as they are routinely used by people in various settings; and
- to compare the durability of different LNs as a basis for decisions on procurement and other programme elements.

Assessment and monitoring of insecticide activity are important components but require more resources, including entomological capacity and access to mosquito colonies. Even if these resources are not available, survivorship and fabric integrity should nonetheless be assessed.

3.2 Approaches

Two main approaches can be used to study LN durability: (i) prospective longitudinal studies in which nets are followed from the time of distribution until a defined end-point is reached; and (ii) retrospective, cross-sectional surveys to assess previously distributed nets in a representative sample of households. Each method has advantages and limitations that

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

should be taken into account in planning durability studies and interpreting the results (Table 1).

Table 1. **Advantages and disadvantages of prospective and retrospective studies**

	Prospective studies	Retrospective studies
Advantages	Easy to monitor loss of nets systematically, as nets can be labelled with indelible markers or other identifiers Allow comparison of different LNs on the basis of prior census and random allocation	Provide immediate information about previously distributed nets, as long as the time of distribution and number of nets received is known
Disadvantages	Users' recall and retention of old nets may be altered by the Hawthorne effect (people more inclined to retain their nets and less likely to dispose of them when damaged because they are being observed) Significant but known portions of the population may have moved into or out of the study area.	Survival cannot be estimated in most settings. Nets available for observation may be a biased sample as worn-out nets are no longer present Significant and unknown portions of the population may have moved into or out of the study area. The number of times for follow-up may be limited. Labels on nets tend to fade or get lost over time, making identification of nets difficult. Recall by users of what happened to a net more than 12 months previously is unlikely to be reliable. Difficult to compare different LNs, as nets of different brands are often not randomly allocated to households and they may have been distributed at different times.

3.2.1 Prospective longitudinal studies

Longitudinal studies are those in which nets are identified at the time of distribution and then followed at regular intervals. Prospective follow-up of a cohort of nets is a suitable method for determining attrition and the decline in fabric integrity and insecticidal activity of a product over time. An issue to be considered in prospective studies is the 'Hawthorne effect', i.e. the possibility that the study itself makes people more inclined to retain their nets, and less likely to give them away or to dispose of them when damaged, as they have been told that the investigators will return to inspect the nets.

When a net is found to be no longer present in a household, care must be taken to determine the reason for its loss or absence. It is difficult to rule out the possibility of bias when a high

proportion of nets are reported as having been given away or moved to other locations while still intact.

3.2.2 Retrospective, cross-sectional surveys

Cross-sectional surveys, often carried out for other purposes, can be used to follow-up previously distributed nets. The main advantage of cross-sectional surveys is that they provide immediate information about previously-distributed nets, as long as there is accurate information about the age of those nets.

However, retrospective methods have a major disadvantage, in that attrition (loss of nets) is difficult to estimate unless there is accurate information on number of nets originally distributed to each household. Without this information, we can observe how many nets are present in the household at a given time point, but we cannot know what proportion they represent of the original number that were distributed; thus the proportion lost since distribution remains unknown. Moreover, the physical condition of those that remain is probably a biased sample. If nets in a population vary in the degree to which they are exposed to wear and tear, and if nets are discarded by their owners when there are too many holes, then a count of the holes in the nets remaining after an interval cannot give a representative or reliable estimate of the wear and tear to which the whole population has been exposed during that interval. Thus, cross-sectional studies of only the surviving nets are likely to be biased, and are expected to give an overestimate of the fabric integrity of LNs. Moreover, retrospective studies allow comparison of the relative durability of different LNs only if they were distributed at the same time, and interpretation will be difficult if they were not randomly allocated to households.

If there are records of the distribution of nets to each household, it may be possible to estimate the proportion of nets still present after an interval of time.

4. PROSPECTIVE LONGITUDINAL MONITORING

4.1 Selection of study site

Study sites should represent the environments and cultural settings in which LNs will be distributed. The study site should be large enough to allow selection of sufficient numbers of nets during follow-up. The flow of activities is shown in Figure 1.

4.2 National clearance and ethical considerations

Follow-up of LNs will require visits and interviews in households. Therefore, the necessary ethical clearance at national and institutional level must be obtained in each country before the study begins. At completion of the study, the participating communities should be informed about its outcome. All households from which LNs are to be withdrawn for evaluation of insecticide activity (bioefficacy and chemical content) should be given new LNs.

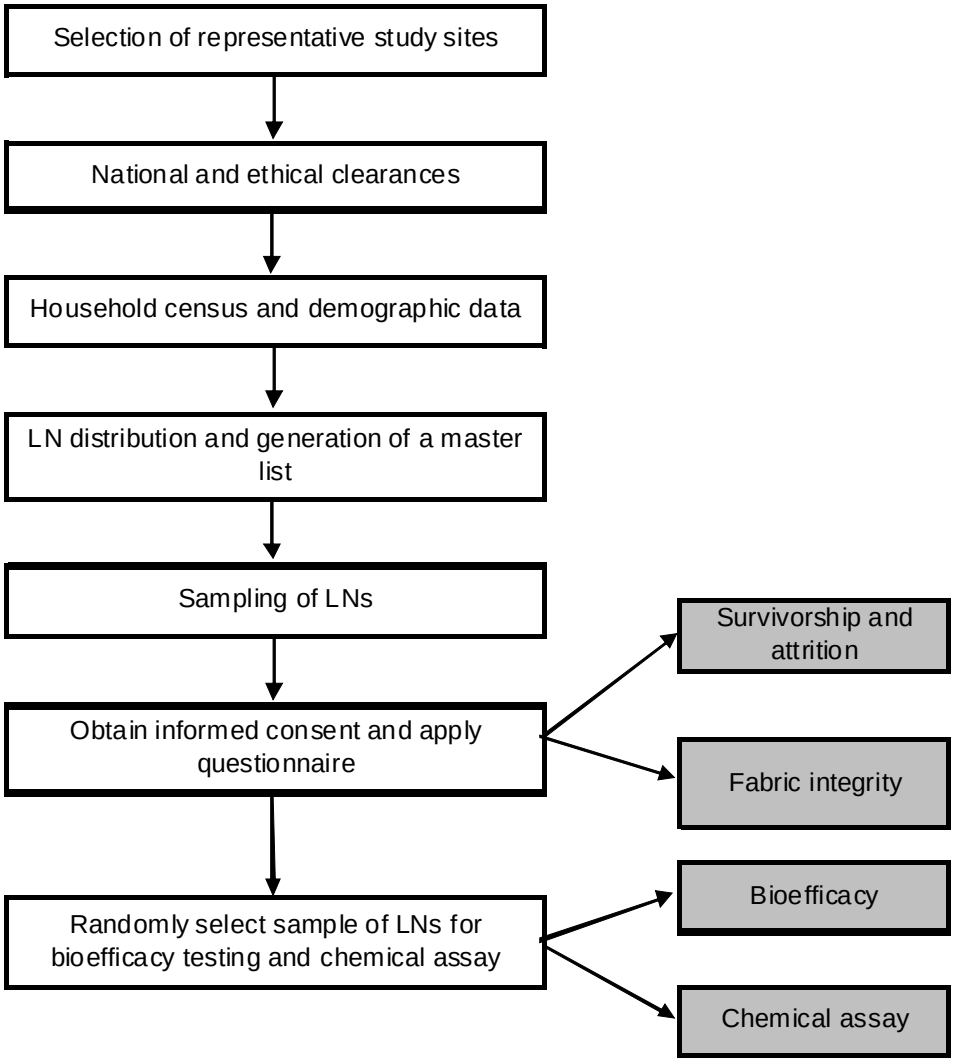
4.3 Community sensitization and informed consent

When entering a selected community, the assistance of opinion leaders should be sought in order to obtain permission to use the community as a study site and to inform the community members of the study's objectives and methods. Informed consent should be obtained from all communities in which nets will be distributed as part of the study. No further consent is needed from households that receive nets but are not selected for follow-up investigations and have no further contact with the investigators.

Informed consent must be obtained from the head or another adult family member of households selected for participation in the follow-up surveys, before each interview. A format for the information sheet and consent form is suggested in Annex I, which should be adapted and translated into the local language. For householders who cannot read the form, the informed consent form should be read out and explained by a member of the investigating team in the local language in the presence of a community witness. After consenting, these people will be asked to mark a thumb impression on the form, and the witness will be asked to sign it. It is important to advise potential participants that they can refuse to participate in the follow-up interview and may keep their LN.

Participants should also be advised to seek medical care at the nearest health facility if they observe any sign or symptom of malaria or other vector-borne disease and any adverse effects of using the nets.

Figure 1. **Flow chart of a prospective longitudinal study design**



4.4 Long-lasting insecticidal nets used in studies

These guidelines can be used to monitor the durability of an LN product distributed in a country or for comparing several LN products. Users of the guidelines are encouraged to include one or more other LNs for comparison, in order to provide information for future procurement. When an LN product is available in another denier and hence might be expected to have a different durability, it could be included for comparison.

All the LNs to be studied must be registered with the national pesticide authority for use in public health (at least for experimental or pilot purposes). Compliance of the LN with the physical and chemical requirements of the WHO specifications[1] or national standards should be verified at the time of procurement. All the LNs in the study should be similar in colour, shape and size, and exactly equal numbers of each LN product should be marked and distributed for follow-up.

4.5 Household census, allocation and tracking of nets

4.5.1 Household census

A census of all the households in the selected study sites should be carried out before LNs are distributed, to provide a framework for random allocation of LNs and for sampling LNs at follow-up. At a minimum, the household census should record the name of the village, the name of the head of the household, the household identification number, the number of adults and children living in the house and the number of nets already in the household. It is also recommended that the number of sleeping places and the global positioning system (GPS) coordinates be recorded to assist in identifying houses during follow-up (Annex II). For quality assurance, the name of the interviewer should also be recorded. The information should be entered into a database to serve as the master list and sampling frame for subsequent LN monitoring. The master list should be updated after each monitoring round, with a record of households that are no longer present.

4.5.2 Sample size and net allocation

In order to estimate the number of LNs for the study, the sample size necessary for a given degree of precision or to detect differences in durability between products should be calculated separately for each main outcome variable (e.g. attrition rate, physical integrity; see section 4.6). The sample size depends on the type of sampling: LNs that are collected and taken away (after replacements are given) for bioassay and chemical residue analysis are lost to follow-up; therefore, the pool of nets at the start must be accordingly larger in order to have the same number of LNs available for assessment for the core outcome variables; if nets are left in place after each assessment, the number needed will be smaller. The sample size should also be adjusted for the effect of a cluster design (between-cluster variation) of net assessment and for the expected attrition. If possible, the net products to be studied should be distributed to a fairly large population, in numbers in excess of that needed on the basis of the initial sample size calculation. A subset of households will be selected from these for the follow-up study. Initial distribution to a large population and selection of a sample at each follow-up should help to ensure that the results are representative and free of covert attempts to influence the attitudes or behaviour of the selected households.

In studies in which more than one LN product is to be monitored, the different products can be allocated either to households or to individuals or sleeping places. When randomization is at household level, each household should be randomly assigned to receive one type of LN.

[1] http://www.who.int/whopes/quality/newspecif/en/.

The number of LNs that each household receives should be based on the national policy for universal coverage. Equal numbers of households should be assigned to receive each LN product to be monitored, although slightly different numbers of each product may distributed because of differences in the numbers of nets required for households in each treatment arm.

Random allocation may be possible if a census is conducted well in advance and a master list of eligible households has been drawn up. Pre-printed lists with each household and LN product they are to receive should be provided at the time of distribution. If a list of households is not available at the time of distribution, LN products can be allocated to consecutive households in a systematic fashion, alternating the different products and providing sufficient nets to each household to cover all family members.

Alternatively, different LNs could be randomly assigned within the same household. This allows direct comparisons of net durability and preference and equal exposure of nets in different types of households (e.g. rich and poor). If, however, there is a strong preference for one type of LN in a household, which affects the use of different LNs, this assignment could bias the estimate of LN durability. Allocation by this method should be random, if a roster of households with the number of nets required is generated during the census, or systematic, as described above for household-level allocation.

The LNs to be distributed in the evaluation should be marked to distinguish them from those that will be (or have been) distributed during a campaign or bought or received from other sources. At a minimum, a label should be sewn into the net at the factory, showing (perhaps with a machine-readable bar-code[1]) date of manufacture and batch number. Additional labels with a unique study code might be applied by the study staff when the nets are opened to identify them as study nets. The study code should be recorded on the master list. In some projects, it was found that labels tended to be lost over time; therefore, the use of permanent ink, car paint or coloured, tear-proof thread knotted into the netting is recommended for long-term labelling.

Owners should be asked to begin using their nets immediately and to store any existing nets for use when the LNs provided are worn out. It might be beneficial to conduct a ‘hang-up’ campaign within 1 month of distribution to ensure that recipients are using their new nets.

4.6 Sampling

If sampling is done at net level, a list of randomly selected nets with their unique code numbers and information on the household to which they were distributed (e.g. household identification, name of head of household, GPS coordinates) should be given to the staff who are sampling the nets in the field, who should sample all the nets on the list.

If sampling is done at the household level, the number of nets distributed to each household should be estimated, and then the number of households required to reach the estimated sample size if all the nets in each selected household are sampled should be determined. Sampling should be simple random sampling, so that there is an equal probability for selection of each household on the list.

Determination of the sample size should take into account the precision and the difference necessary to detect each outcome measure for the different LN products. The estimated sample size should also take into account variations between nets and between households. It should be based on the outcome that requires the largest sample, although subsamples can be taken for some measures (e.g. bioefficacy). It may be simplest to estimate the sample

[1] Applied to the net label by the study organizers or the manufacturer, allowing easy reading of stored information (product, batch, manufacturing data, distribution date), as long as it has not faded or the label been lost.

size on the basis of attrition, as, for outcomes such as bioefficacy, it may not be feasible to test large numbers of nets. If sampling is done at household level and all nets in the household are sampled, the sample size should be adjusted for design effect and the questionnaire adjusted accordingly.

A sample of 250 LNs per product will allow detection of a 10% point difference in LN attrition rate if the best-performing product has an attrition rate of 10%. This sample size will also allow detection of a 12% point difference in LN attrition rate if the best-performing product has an attrition rate of 20%. A list of additional households should be generated from the master list as alternatives for refusals and absence of a person to interview.

The number of nets available for monitoring fabric integrity will decrease over time; however, as observed in previous studies, the fabric integrity of surviving nets is not expected to change much after a certain time if torn nets are thrown away (and hence lost to follow-up). With an attrition rate of 40% after 2 years, a sample of 250 LNs per product will provide at least 150 nets for fabric integrity measurement, which is considered sufficient to detect major differences between products in a given setting.

A subsample of nets that have been assessed for fabric integrity should be randomly selected and withdrawn (after replacement) for measuring insecticidal activity. In previous studies, 30 nets per LN product at each time was found sufficient for bioefficacy testing, but a larger sample will provide more precision. As mentioned above, the overall number of nets needed for the study should be increased by the number withdrawn. Ideally, nets for bioefficacy testing should be selected randomly from a roster of nets in the study.

4.7 Follow-up and questionnaire on durability of nets

Households selected for monitoring should be located by the name of the head of household and by GPS coordinates, when available, for follow-up. Families that have moved (and taken their nets with them), refused to participate or refused to allow inspection of their LNs should be marked as censored on the master list of households and be replaced by another household from the list. Households should be visited up to three times until they are recoded as unresponsive and a replacement is used.

A standard questionnaire should be used to collect data from an adult household member at each follow-up. The information collected should include the status of each study LN distributed to the household and the patterns of LN use and handling. Observations on fabric integrity and the condition of the LN can also be included.

The use of mobile technology (e.g. ‘personal digitial assistants’) for recording responses to the questionnaire is recommended, as it may allow automated data checking, GPS readings and photographic records of nets in the field. A sample questionnaire is shown in Annex IV.

Follow ups should be conducted at 6, 12, 24 and 36 months post-distribution. A fresh random sample is selected at each time point and the survey methodology should be repeated at each time point.

4.8 Measurement of expected outcomes

4.8.1 Net survivorship and attrition

To measure survivorship, households should be visited and the physical presence of the LN recorded (Annex IV, section 2). If the net is still present in the household, the investigator should record whether the net is being used for its intended purpose. Nets that have never been used should also be recorded but should be excluded from the analysis. If the net is no longer in the house, the investigator should determine how it was lost.

4.8.2 Fabric integrity

Fabric integrity is assessed from the questionnaire by counting the number of holes (including tears in the netting and split seams) by their location on the net and their size (Annex VI, section 4). Holes can be classified into the following categories:

- smaller than a thumb (0.5–2 cm),
- larger than a thumb but smaller than a fist (2–10 cm),
- larger than a fist but smaller than a head (10–25 cm) and
- larger than a head (> 25 cm).

Holes less than 0.5 cm can be ignored. Evidence of repairs to the net fabric and the type of repair should also be recorded on the form.

In rapid field surveys, holes are usually counted in nets that are still hanging over a bed. A more accurate count can be made by removing each net and arranging it over a frame; however, this takes more time, and fewer nets can be counted for the same effort.

In some cases, the nature of the hole or the user may give clear information about the main cause of the holes, and this should also be recorded.

4.8.3 Insecticidal activity

The recommended tests for bioefficacy are the WHO cone test and, when necessary, tunnel test, as these are direct measures of the amount of insecticide available to contact and kill mosquitoes (1). Chemical assays of the insecticide content of nets provide useful supporting information, but the results may be misleading by themselves, particularly for nets with incorporated insecticide, in which much of the insecticide is inside the fibres and not available to contact and kill mosquitoes.

If whole nets are transported from the field to the laboratory, care should be taken to ensure that the nets are kept separate and they are not exposed to excessive heat. Nets should be cut immediately, wrapped in aluminium foil and stored at 4 ^{o}C.

Samples for bioefficacy testing and determination of insecticide content should be prepared according to the scheme shown in Figure 2, as recommended in the FAO/WHO Manual (*8*).[1]

[1] Combination nets (made of different types of fabrics with different treatment techniques) may require a different sampling scheme. WHO specifications for such products should be consulted for the recommended sampling scheme.

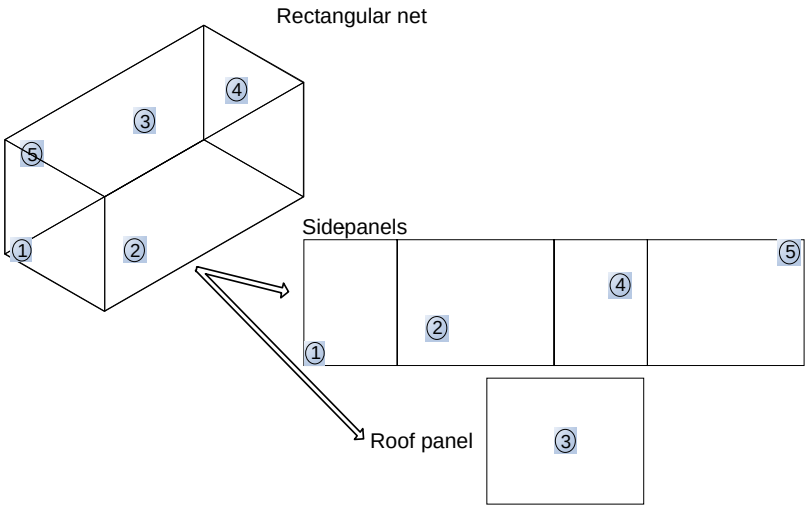


Figure 2. **Sampling pattern of the LNs**

Bioefficacy

Insecticidal activity, or bioefficacy is determined in WHO cone bioassays (for mortality and knock-down) and, when necessary, in tunnel tests (for mortality and blood-feeding inhibition). These procedures should be conducted according to WHO guidelines, as described below.

WHO cone bioassays: WHO cone assays should be conducted on 25 cm x 25 cm pieces cut from positions 2, 3, 4 and 5 of each sampled net, which should be adjacent to the places from which the netting for chemical assay was collected. Position 1 should be excluded, as it may be exposed to excessive abrasion in routine use, as this portion of the net is frequently handled when it is being tucked under the bed or mattress. Two standard WHO cones are fixed with a plastic manifold onto each of the four netting pieces (Figure 3).

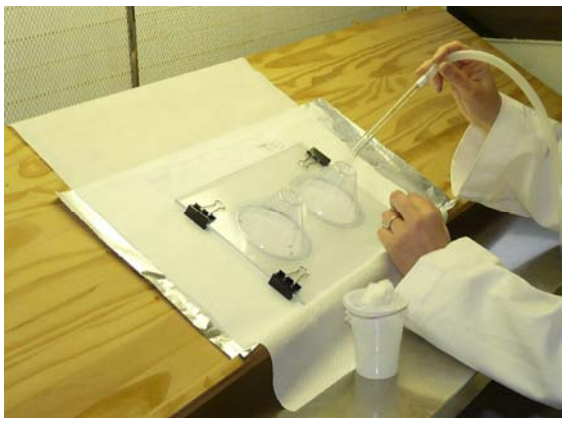


Figure 3. **Cone bioassay on long-lasting insecticidal mosquito net (courtesy of Dr Vincent Corbel, Institut de Recherche pour le Développement (IRD), Montpellier, France).**

Five susceptible, non-blood-fed, 2–5-day-old female *Anopheles* (species to be stated in the test report) are exposed for 3 min in each cone and then held for 24 h with access to sugar solution. Two replicates should be placed at each position. Knock-down is measured 60 min

after exposure, and mortality is measured after 24 h. A negative control, from an untreated net, should be included in each round of cone bioassay testing.

If the mortality in the control is between 5% and 20%, the data should be adjusted with Abbott's formula.[1] If the mortality in the control is > 20%, all the tests should be discarded for that day. Bioassays should be carried out at 27 ± 2 °C and 80 ± 10% relative humidity.

The bioassay results for the netting pieces from each sampled LN should be pooled to determine if the net meets the WHO efficacy requirement, i.e. ≥ 80% mortality or ≥ 95% knock-down. If the net fails these criteria, a tunnel test should be conducted on one of the four net samples that caused mortality closest to the average mortality in the cone bioassay.

Tunnel test:. The tunnel test is used to measure the mortality and blood-feeding success of host-seeking mosquitoes in an experimental chamber (Figure 4). The assay is carried out in a laboratory by releasing non-blood-fed female anopheline mosquitoes, aged 5–8 days, into a 60-cm tunnel (25 cm x 25 cm square section) made of glass. At each end of the tunnel, a 25-cm square cage covered with polyester netting is fitted (extension). At one third of the length of the glass tunnel, a disposable cardboard frame is placed with the LN netting sample. The surface of netting available to the mosquitoes is 400 cm^2 (20 cm x 20 cm), with nine holes each of 1 cm in diameter: one hole is located at the centre of the square, and the other eight are equidistant and located 5 cm from the border. In the shorter section of the tunnel, a bait (e.g. guinea-pig for *An. gambiae*) is placed, which is unable to move but is available for biting. In the cage at the end of the longer section of the tunnel (Figure 4, area C1), 100 female mosquitoes are introduced at 18:00. They are free to fly in the tunnel but have to make contact with the piece of netting and locate the holes in it before passing through to reach the bait. After taking a blood meal, the females can fly back to the cage at the end of this compartment and rest.

The following morning, at 09:00, the mosquitoes are removed with a glass suction tube and counted separately from each section of the tunnel; mortality and blood-feeding rates are recorded. During the tests, the cages are maintained at 27 ± 2 °C and 80% ± 10% relative humidity under subdued light. A tunnel with untreated netting is always 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 mortality rates of mosquitoes from the two sections of the tunnel.

As blood-feeding in controls has a considerable effect on mortality in the presence of treated samples (i.e. the host-seeking behaviour increases the chance of contact with treated fabric), a minimum cut-off value of the blood-feeding rate in controls should be established for tunnel tests, i.e. the test will be valid only if blood-feeding in controls is above a certain limit. In one WHO study, a cut-off of 36% was used (*5*); however, higher rates such as 50% might be more appropriate.

[1] Abbott's formula: Adjusted mortality (%) = 100 x (X – Y) / (100 – Y), where X is the percentage mortality in the treated sample and Y is the percentage mortality in the untreated control sample

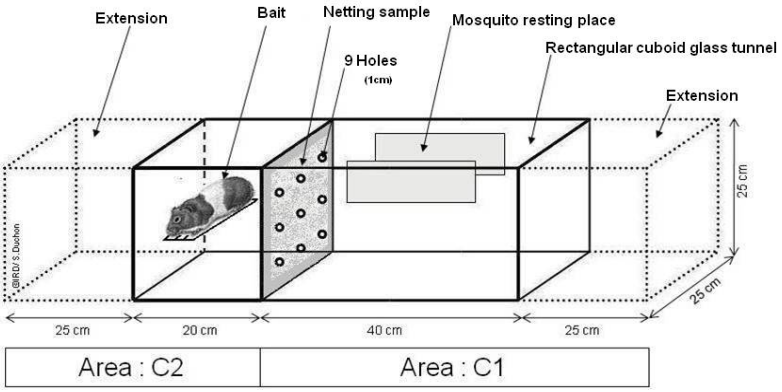


Figure 4. **A diagrammatic sketch of the tunnel used for the study of efficacy of long-lasting insecticidal mosquito nets with extensions on either sides to fix mosquito cages (courtesy of Dr Stéphane Duchon, Institut de Recherche pour le Développement (IRD), Montpellier, France).**

Insecticide content

Insecticide content is the amount of active ingredient per gram of the LN as determined by chemical assay. Four pieces of 30 cm x 30 cm netting should be cut from positions 2, 3, 4 and 5 in Figure 2 for determination of insecticide content. A sample from position 1 of the net can be included in baseline assays, but it should be analysed separately in subsequent assays. The four subsamples (five in baseline sampling) are then pooled for chemical analysis and estimation of the total active ingredient content of the LN. This combined sample is rolled up and placed in clean labelled aluminium foil for storage in a refrigerator (+ 4 ^{o}C) before dispatch to a quality control laboratory for chemical assay. A WHO collaborating centre or ISO/GLP certified laboratory should be used. The analytical methods (Collaborative International Pesticides Analytical Council) recommended in the WHO specifications[1] for quality control must be used for determining the total content of the active ingredient.

5. RETROSPECTIVE CROSS-SECTIONAL SURVEY METHODS

When the performance of LNs that have already been distributed in the communities is to be measured in national programmes, retrospective cross-sectional studies (section 3.2.2) can be conducted. The sampling of nets may differ from that for prospective studies, but the same methods for assessing durability can be used. Retrospective surveys of LNs can be combined with other cross-sectional surveys (e.g. malaria indicator surveys).

[1] WHO specifications are given at www.who.int/whopes/quality/newspecif.

A key element of any retrospective survey is identification of the age of previously distributed nets. Frequently, the batch numbers of the distributions can be identified; ideally, rosters of households, with the number of nets distributed to each house, are available, as these allow estimation of net survivorship. If the date of distribution cannot be determined by examination of the batch number or other identifying mark, retrospective surveys of net durability should not be undertaken.

As for prospective studies, the target area should be identified, ethical clearance should be obtained and sample sizes estimated. Households should be randomly selected from a household roster, if one is available. If a roster is not available, methods such as cluster sampling can be used. The questionnaire in Annex IV can be adapted for this purpose, and the methods for measuring fabric integrity and insecticidal activity described above should be used. Often, it is not possible to estimate net survivorship or attrition in retrospective surveys; however, if the number of nets distributed to the household is known, survivorship can be estimated from the remaining number.

6. DATA QUALITY

Standard operating procedures should be developed for data collection, entry and management. The data collected will be kept in both hard copy and digital format for analyses and future reference.

If the study results are to be used as a basis for procurement, it is important that 10% of the households be revisited by an independent person to verify that the survey was not biased. This task may be contracted to an independent consultant or agency, which should not, however, have access to the data from the first collection. Some cases of discordance between the two data sets is to be expected, but there should not be significant differences between products in the frequency of discordance

7. DATA ANALYSIS

7.1 Survivorship and attrition

The analysis should include data on all nets recorded during the exercise at each time interval, but stratified by LN product. The number of nets in the sample, the proportion of the indicator and 95% confidence interval should be reported (taking account of the sampling design, i.e. cluster sampling, if applicable).

The following indicators should be used and disaggregated by survey time (e.g. 6, 12, 24 or 36 months):

Survivorship:

Numerator: Total number of each LN product present in surveyed households (and available for sleeping under) x 100

Denominator: Total number of each LN product distributed to surveyed households

Attrition rate-1 for nets that have been destroyed or disposed of:

Numerator: Total number of each LN product reported as lost due to wear and tear (poor condition) in surveyed households x 100

Denominator: Total number of each LN product distributed to surveyed households

Attrition rate-2 for nets not available for sleeping under:

Numerator: Total number of each LN product reported as lost for reasons other than poor fabric integrity (given away, stolen, sold or used in another location) in surveyed households x 100

Denominator: Total number of each LN product distributed to surveyed households

Attrition rate-3 for nets used for other purposes:

Numerator: Total number of each LN product reported as being used for another purpose in surveyed households x 100

Denominator: Total number of each LN product distributed to surveyed households

For each LN product, the survivorship rate plus attrition rate-1, attrition rate-2 and attrition rate-3 should add up to 100%.

Two products can be reported to show significantly different survivorship at a given time if the 95% confidence limits for survivorship do not overlap. Additional statistical analysis may be necessary if the confidence limits overlap.

7.2 Assessment of fabric integrity

Fabric integrity is analysed for all the LNs found in the households (and used for sleeping under), and all the LNs are assessed for holes at each monitoring round, disaggregated by LN product. Two indicators should be calculated at each survey time: the proportion of LNs with holes and a hole index.

Proportion of LN with any holes (with 95% confidence interval):

Numerator: Total number of each LN product with at least one hole of size 1–4

Denominator: Total number of each LN product found and assessed in surveyed households

The hole index is calculated by weighting each hole by size and summing for each net. If the weight of hole sizes 1, 2, 3 and 4 was A, B, C and D, respectively, the hole index would be calculated as:

Hole index = (A x no. of size-1 holes) + (B x no. of size-2 holes) + (C x no. of size-3 holes) + (D x no. size-4 holes).

The holes should be weighted according to the average area of each hole category. For the hole size categories described above, the weights would be 1, 23, 196 and 578, which correspond to the areas estimated on the assumption that the hole sizes in each category are equal to the mid-points.

For each product type, the mean (and standard deviation) as well as the median (and interquartile range) hole index should be determined. The hole index for different products can be compared by analysis of variance for normally distributed data or the Kruskal-Wallis test or Poisson regression for non-parametric data.

7.3 Insecticidal activity

The results of the cone and tunnel tests should be considered together in judging net performance. A candidate net will be deemed to meet the requirements for an LN if, at the

end of 3 years, at least 80% of the sampled nets retain bioefficacy in the WHO cone bioassay or the tunnel test as described in WHO guidelines.[1]

Information on insecticide content can be used to support interpretation of the results of bioefficacy tests. The mean (and standard deviation) insecticide content should be reported at each survey time in order to estimate the average rate of insecticide loss from the original loading dose.

7.4 Factors related to durability of nets

Factors that contribute to durability, as measured by survivorship, fabric integrity and insecticidal activity, can be assessed by multivariate regression analysis. The contributing factors include socioeconomic status, the house environment and behaviour related to net use, handling and washing, which can be derived from answers to the questionnaire (Annex IV). A statistician should be consulted for such an analysis.

8. REPORTING

Outcomes should be communicated to the community, to relevant national programmes and to other stakeholders. Investigators are also encouraged to publish the results of these studies in peer-reviewed journals.

9. OPERATIONAL RESEARCH

To better understand the issues, assumptions and factors associated with the field performance of LNs and to fill knowledge gaps, operational research is needed in various eco-epidemiological and sociocultural settings where LNs have been chosen as the main intervention. Some important questions and issues for consideration by researchers are:

- How should a 'worn-out' net be defined for various programme decisions (e.g. quantification, net replacement at household level, estimation of epidemiological impact from coverage data)? How does the protection given by an LN decline as it loses insecticide and gains holes, and what is the interaction between insecticide loss and hole acquisition?
- What is the community attitude towards net repair, and at what point do users dispose of their nets or use them for other purposes? What are the user preferences, and how do they affect use and selection of a product for national procurement? What methods are suitable for measuring preferences?
- How do environmental (e.g. housing type, bed construction) and human factors (e.g. washing, handling and use) affect the durability of nets in routine use?
- A chemical assay is needed to determine the concentration of insecticide on the surface of nets, rather than total insecticide content. The results of such a chemical assay should correlate to those of a bioassay. The assay might be designed for determining the surface concentration in situ, rather than cutting and shipping net

[1] Bioefficacy criteria:
Cone bioassay: criteria for acceptance: mortality of mosquitoes ≥ 80% or knock-down ≥ 95%
Tunnel test: mortality of mosquitoes ≥ 80% or blood-feeding inhibition ≥ 90%

samples to a laboratory for chemical assay, as storage and shipping conditions can affect the surface concentration.

- Methods are needed to test the resistance of LN netting to the physical deterioration and hole formation observed in the field (e.g. tensile breaking, tearing, snagging, puncturing and abrasion). Such tests would be useful for manufacturers of LNs to improve the durability of LN netting and would be used to upgrade the specifications for LNs.
- What, if anything, should LN programmes do with nets that are time-expired or 'worn out'? Is this netting used, and useful, for other purposes? What are the environmental risks associated with discarded nets that are still loaded with insecticide, and what are the options for recycling or safely disposing of them?

10. REFERENCES

1. World Health Organization (2005). *Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets.* Geneva, World Health Organization (WHO/CDS/WHOPES/GCDPP/2005.11). Available at http://whqlibdoc.who.int/hq/2005/WHO_CDS_WHOPES_GCDPP_2005.11.pdf (accessed March 2011).
2. World Health Organization (2004). *A generic risk assessment model for insecticide treatment and subsequent use of mosquito nets.* Geneva, World Health Organization (WHO/CDS/WHOPES/GCDPP/2004.6). Available at http://whqlibdoc.who.int/hq/2004/WHO_PCS_04.1.pdf (accessed March 2011).
3. World Health Organization (2009). *World malaria report 2009*. Geneva, World Health Organization.
4. World Health Organization (2009). *Report of the twelfth WHOPES working group meeting*. Geneva, World Health Organization. Available at http://whqlibdoc.who.int/hq/2009/WHO_HTM_NTD_WHOPES_2009_1_eng.pdf (accessed March 2011).
5. World Health Organization (2009). *Report of the thirteenth WHOPES working group meeting.* Geneva, World Health Organization. Available at http://whqlibdoc.who.int/publications/2009/9789241598712_eng.pdf (accessed March 2011).
6. FAO/WHO (2010). *Manual on development and use of FAO and WHO specifications for pesticides.* Second revision of the first edition. Available at: http://www.who.int/whopes/quality/en/ (accessed March 2011).

ANNEX I. INFORMED CONSENT FORM (SUGGESTED TEMPLATE)

Name of project: Monitoring durability of long-lasting insecticidal mosquito nets under operational conditions

Name of principal investigator: _____

Name of organization: _____

Household identification No. _____

Part 1. Information sheet

Note: This is a proposed template, which can be modified and adapted according to national ethical guidelines.

Introduction

My name is <name of investigator> and I work for <name of institution>. I invite you to carefully read this document <or understand its contents as read by a literate witness> before accepting to participate in this study. The aim of this study is to check or compare the action of various factory-treated insecticidal mosquito nets that are expected to retain their power to kill malaria mosquitoes after several washes and 3 or more years of use.

This study has been cleared by the ethical committee of <give name>.

Purpose and Background of the study

First some background information. As you probably know, malaria is a major disease in <name of study area or country> and is transmitted from one person to another through the bites of certain mosquitoes. These mosquitoes usually bite after dark. Sleeping under a mosquito net protects against mosquitoes that bite in the night. If the net has been treated with a chemical that kills insects (insecticides), it gives better protection against mosquito bites. Some kinds of nets are given a special treatment in the factory and do not require re-treatment until the end of their useful life; these are called long-lasting insecticidal mosquito nets (LNs or LLINs).

National malaria programmes are now distributing LNs for malaria prevention and control in areas targeted for this intervention. The community you live in has been targeted to receive LNs. We want to measure how long LNs actually last in routine use in the households in your community.

Your area, <name of area>, has been selected for this study. As you may be aware, we first asked your community leaders <name of community leaders> to give permission for this study. Then, various LNs were distributed, such that every household has at least one study LN to sleep under. To know how durable these nets are, my team has now come to your village, in consultation with your community leader or village headperson, to investigate these issues.

Information on study nets

The study nets given to the selected households are factory-treated LNs. In this study, the insecticides used are: <give name of each LN product, its manufacturer and the insecticide used>. Note that these products are not new: they are all well-established. They have been extensively tested by the World Health Organisation and are recommended as being safe and effective. Therefore, the question in this study is not "are they effective?"; rather we are asking "how long do they last?"

Type of study

In this study, we are following the nets over time, to see how quickly they get holes and wear out. Nets of the different brands have been given to many/all the families in the area. A smaller number of these households, chosen at random, are being re-visited every few months, to see whether the nets are still in use and still in good condition.

Participant selection

After this area was selected for the study, we gave each household a number. In order to pick households for the follow-up visits, we used a computer to choose numbers at random, and that is how we chose the nets to check. We are asking you for an interview because your net is one of the selected nets, or because you are the <parent / guardian / head of household> of a child who uses one of the selected nets.

Procedures

I would therefore like to have your consent to be interviewed; this will last about < approximate time > minutes.

During the interview, I will ask you some questions about your household, the status of the LN given to you or your child and how you use and handle your net. I will ask you to show the net to me, so I can see how much it has worn.

<NOTE to interviewer: If the net has been selected for bioassay testing, read 'A' below; alternatively, if this net has not been selected for bioassay testing, then read 'B'.>

A – We would like to take some nets away with us, and measure how much of the insecticide is still there and how much has worn off. Your net has been selected at random for this testing. If you agree to give us permission to take away this net, then I will immediately give you a replacement net, which will be yours to keep and you can put it on the bed today.

B – I will not damage the net, and after the interview, I will return it.

At the completion of the study, all villagers will be told the main outcomes of the study in a community meeting in the village.

Confidentiality

All information related to your participation will be kept confidential and will not be revealed to anyone, except if required by law, such as in a legal request for the list of beneficiaries. Your identity will not be revealed in any reports or publications resulting from the study. The results of the interview will be put into a computer, but with the code numbers of the household, but without the names of the people interviewed.

The data collected will be kept for analysis. It will be stored for some time on paper and in the computer, but may eventually be destroyed.

Voluntary participation: right to refuse or withdraw consent

Your participation in the interview is entirely voluntary. You are not under any obligation to participate, and you have the right to refuse this invitation.

If at any time during the interview, you decide not to participate further, you are free to withdraw immediately, with no further discussion; this will have no adverse consequences for you. Whether you choose to participate or not, you will still receive all the public services you usually do. The study nets that have been given to your household belong to you and are yours to keep. In a few cases, we may ask you to give an old net back to us in exchange for a replacement new one, but you may refuse this request if you wish.

Who to contact

If you have any questions, please ask them, either now or later. If you wish to ask questions later, you may contact any of the following:

<name, address and telephone number of the principal investigator >

Any important new information concerning the results of our study will be made known to you. This proposal has been reviewed and approved by <name of the ethics committee>, whose task it is to make sure that study participants are protected from harm. If you wish to find out more about this committee, please contact <name, address and telephone number>.

We are leaving a copy of this informed consent form with you for your information and future reference.

Part 2. Certificate of consent

(This is an integral part of the information sheet and not a stand-alone document)

I have read this information in <name local language>, *or it has been read to me in my native language. I have had the opportunity to ask questions about it, and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this study, and I understand that I have the right to withdraw from the study at any time without in any way affecting my rights. I also understand that the principal investigator of the study can exclude my household from the study without my consent. I have been given a copy of this consent form.*

Print name of participant _____

Date and signature of participant

__/__/__ (*dd/mm/yy*)

If illiterate

I have witnessed the interviewer reading the consent form to the potential participant. The reading was careful and accurate and the individual had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of independent literate witness: _____

(If possible, this person should be selected by the participant and should have no connection to the research team.)

Signature of the witness and date _____ ___/___/___ (dd/mm/yy)

I have read or witnessed the reading of the consent form to the potential participant. The reading was careful and accurate and the individual had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of researcher

_____

Date and signature of researcher

___/___/___ (dd/mm/yy)

ANNEX II. HOUSEHOLD CENSUS AND DEMOGRAPHIC DATA FORM

Village:	District:
Date:	Enumerator

Household identification	Name of head of household	GPS coordinates	No. of children < 5 years	No. of children 5–15 years	No. of adults (> 15 years)	No. of existing LNs	No. of existing other nets	No. of sleeping places	No. of LNs to be allocated
|___|___|___|___|			|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|
|___|___|___|___|			|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|
|___|___|___|___|			|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|
|___|___|___|___|			|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|
|___|___|___|___|			|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|
|___|___|___|___|			|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|
|___|___|___|___|			|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|
|___|___|___|___|			|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|
|___|___|___|___|			|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|	|____|____|

ANNEX III. NET MASTER LIST

Village code	Head of household	Household identification	Study arm (type of LN)	Net identification	Date of distribution	Date of withdrawal of net

ANNEX IV. SAMPLE QUESTIONNAIRE FOR MONITORING DURABILITY OF NETS UNDER OPERATIONAL CONDITIONS

Questions should be adapted to local settings

To be filled in before the interview

Identification number I____I____I____I (to be filled in by supervisor)

0.1 Code of interviewer I____I____I

Day / month / year

0.2 Date I____I____I / I____I____I / I____I____I____I____I

0.3 Name of village I_____I

0.4 GPS coordinates of household I_____I

0.5 Household identification number I____I____I____I_____I

0.6 Long-lasting insecticidal net number I____I____I____I____I

To be filled in by the supervisor at the end of the day

0.7 Code of supervisor I____I____I

Comments_____
_____

I confirm that the questionnaire is complete.

Date : I____I____I / I____I____I / I____I____I____I____I

Name I_____I

SignatureI_____I

To be filled in by data entry clerks during data entry

Data entry clerk 1	Data entry clerk 2
Date I___I___I / I___I___I / I___I___I___I___I	Date I___I___I / I___I___I / I___I___I___I___I
Signature_____	Signature_____

Section 1: *I would like to ask you (head of household or adult > 18 years) some questions about your household*			
Q #	Questions and filters	Coding category	Answer (enter coding categories)
1.1	Who is responding to the questions?	1….Yes 0….No	Head of household I____I User of net I____I Parent or guardian of user(s) of net I____I Other adult in household I____I
1.2	What is the highest level of education of the head of the household?	1….None 2….Religious school 3….Primary school 4....Secondary school 5….Higher education 6….Other, specify	I____I Other _____
1.3	Does your household have electricity?	1….Yes 0….No	I____I
1.4	What is the principal type of toilet facility used by members of the household?	1….Own flush toilet 2….Shared flush toilet 3….Own pit latrine 4….Shared pit latrine 5….Bush or field 6….Other	I____I Other _____
1.5	What is the principal household source of drinking-water?	1….Piped water into home 2….Protected well in home 3….Unprotected well in yard 4….Open well in yard 5….Protected well in yard 6….Unprotected public well 7….Protected public well 8….Tap in yard 9….Tanker truck 10...Bottled water 11...Public tap 12…Rainwater	I____I Other _____

		13…Surface water 14…Spring 15…Other	
1.6	How many people slept in your household last night?		Adults > 15 years I____I____I 5–15 years I____I____I < 5 years I____I____I
1.7	How many sleeping places were used last night in your household? *(including sleeping places outside and temporary spaces)*		I____I____I
1.8	How many mosquito nets that can be used for sleeping does your household have? *(Probe for any nets currently not in use: stored, saved, still in packaging)*		I____I____I
1.9	Of the total number of mosquito nets, how many are LNs? *(Observe)*		I____I____I
Section 2: LN status (for selected net identification I____I____I____I____I____I)			
Q #	Questions and filters	Coding category	Answers (Enter coding category)
2.1	Is this net still in the household, and can it be used for sleeping under? *(Look for the net in the household, including those still in package or being used for other purpose)*	1….Yes → Skip to section 3 0….No	I____I
2.2	If No, why is this net is no longer available for sleeping under in the household?	1….Net was damaged and thrown away 2….Net was given away to others 3….Net was stolen 4….Net was sold 5….Net is being used in another location 6….Net is being used for another purpose 7…Other, specify	I____I Other _____

2.3	How many months ago did this net become unavailable for sleeping under in the household?	1…0–6 months → End questionnaire 2…> 6 months → End questionnaire 9…Don't know→ End questionnaire	I____I
Section 3: LN use and handling (for selected net identification I____I____I____I____I____I)			
3.1	Has this net ever been used for sleeping under?	Yes No → End questionnaire	I____I
3.2	Was this net used last night to sleep under?	1….Yes → Skip to 3.4 0….No	I____I
3.3	If No, why was this net not used last night?	1….Yes 0….No	Too hot I____I Don't like the smell I____I Feel "closed in" I____I No malaria now I____I No mosquitoes I____I The net is too torn or old I____I Net not available I____I Used another net I____I User did not sleep here I____I Other I____I _____ Don't know I____I
3.4	In the past week, how often was the net used?	1….Every night (7 nights) 2….Most nights (5–6 nights) 3….Some nights (1–4) 4….Not used at all (0 nights) 9….Don't know	I____I
3.5	How many adults (> 15 years) slept under this net last night?		I____I

3.6	How many children 5–15 years slept under this net last night?		I___I
3.7	How many children < 5 years slept under this net last night?		I___I
3.8	During which periods of the year is this net used to sleep under?	1… All year 2….Only the rainy seasons 3….Only the dry season 9….Don't know	I___I
3.9	Is this net ever used for sleeping under away from the main house? If yes, where?	1….Taken to the fields 2….Taken to the beach 3….Taken to the forest 4….Taken to the farm hut 5….Other, specify 6….Not used away →Skip to Q.3.11 9….Don't know →Skip to Q. 3.11	I___I Other _____
3.10	During which periods of the year is this net used to sleep under away from the main house?	1… All year 2….Only the rainy seasons 3….Only the dry season 9….Don't know	I___I
3.11	Has this net ever been used over the following types of sleeping places?	1…Yes 0…No	Reed mat I___I Cut bamboo I___I Grass I___I Foam mattress I___I Wooden bed frame (finished) I___I Wooden bed frame (sticks) I___I Metal bed frame I___I Bare floor or ground I___I Other, specify I___I _____

3.12	Do you tuck the net in at night?	1….Yes 0….No 9….Don't know	I___I
3.13	Has the net ever been washed?	1….Yes 0….No →Skip to Q.4.1 9….Don't know →Skip to Q.4.1	I___I
3.14	When was the last time you washed the net?	1…1 week ago 2….1 week to 1 month ago 3….1–3 months ago 4….3–6 months ago 5….> 6 months ago 9….Don't know	I___I
3.15	What type of soap was used?	1….None 2….Local bar soap 3….Detergent powder 4….Mix (bar and detergent) 5….Bleach 9….Don't know	I___I
3.16	How long did the net soak for?	1….Did not soak the net 2….< 1 h 3…..> 1 h 9….Don't know	I___I
3.17	Was the net scrubbed hard or beaten on a hard surface (e.g. rocks, with sticks)?	1….Yes 0….No 9….Don't know	I___I
3.18	Where was the net dried?	1….Outside in the sun 2….Outside in the shade 3….Inside 9….Don't know	I___I
Section 4: LN condition (for selected net identification I___I___I___I___I)			
4.1	In the past month, have any new holes appeared in the net that you	1….Yes 0….No	I___I

	are aware of?	9….Don't know	
4.2	What caused these new holes?	1….Yes 0….No	Tore or split when caught on an object I____I Was burned I____I Was caused by animals I____I Children I____I In another way I_____I, specify _____ Don't know I____I
4.3	How is the net found? (Observe)	1…. Hanging loose over sleeping place 2….Hanging tied in knot 3….Hanging folded 4….Visible but not hung up 5….Stored away	I____I
4.4	What type of sleeping place is the net hanging over? (Observe)	1….Reed mat 2….Cut bamboo 3….Grass 4….Foam mattress 5….Wooden bed frame (finished) 6….Wooden bed frame (sticks) 7….Metal bed frame 8….Nothing 9….Other, specify	I____I Other _____
4.5	Where is it found? (Observe)	1….Inside 2….Outside → Skip to Q.4.9	I____I
4.6	What is the principal type of flooring in the room where the net is found? (Observe)	1….Soil or sand 2….Wood, palm, bamboo 3….Cement (inlcluding vinyl) 4….Cement 5….Carpet 6….Other	I____I Other _____
4.7	What are the walls of the room in which the net is found in made of? (Observe)	1….Mud brick 2….Mud with wood frame 2….Concrete 3….Twigs 4….Wood 5….Straw 6….Bamboo	I____I____I Other _____

		7….Corrugated iron 8….Lime-plastered 9….No walls (used outside) 10… Other, specify	
4.8	What is the roof or ceiling of the room in which the net is found in made of? (Observe)	1….Grass thatch 2….Corrugated iron 3….Concrete 4….Reed mats 5….Wood 6….Tiles 7….Other, specify	I____I Other _____
4.9	Do you use an open flame for cooking, heating or lighting where the net is found?	1….Yes 0….No	Wood fire I____I Charcoal fire I____I Wax candle I____I Oil lamp with a glass I____I Oil lamp without glass I____I Other, specify I____I _____
4.10	What type of holes are observed?	1….Yes 0….No	Horizontal tears at bottom I____I Holes at hanging points I____I Open seams I____I Burn holes I____I Holes from rodents I____I Whole section missing I____I
4.11	Number of holes of size 1	Less than size of thumb (0.5–2 cm)	Roof I____I____I Upper I____I____I Lower I____I____I Seams I____I____I
4.12	Number of holes of size 2	Larger than thumb, smaller than fist (2–10 cm)	Roof I____I____I Upper I____I____I Lower I____I____I Seams I____I____I
4.13	Number of holes of size 3	Larger than fist, smaller than head (10–25 cm)	Roof I____I____I Upper I____I____I Lower I____I____I Seams I____I____I

4.14	Number of holes of size 4	Larger than head (> 25 cm)	Roof I____I____I Upper I____I____I Lower I____I____I Seams I____I____I
4.15	Number of holes repaired		Stitched I____I____I Knotted I____I____I Patched I____I____I

ANNEX V. CONE BIOASSAY OF NETS COLLECTED IN HOUSEHOLDS

Name of person performing bioassays: ……..

1. Date of test (dd/mm/yyyy): |__|__|/|__|__|/|__|__|__|__|
2. LN code: |__|__|__|__|__|__|__|__|
3. Temperature: |__|__|°C Relative humidity: |__|__|__| %
4. Test mosquito species:
5. Age of mosquitoes: |___| days
6. Test start time (h/min): End time (h/min):

Net position	Replicates[a]	No. of mosquitoes exposed[b]	No. knocked down after 1 h	No. dead after 24 h	No. alive after 24 h	% knocked down	% mortality
1[c]	1						
	2						
2	1						
	2						
3	1						
	2						
4	1						
	2						
5	1						
	2						
Control	1						
	2						

[a] Two cones on each net sample (replicates 1 and 2)

[b] Usually, five mosquitoes per cone; exposure time, 3 min

[c] Net position 1 should be tested only in baseline bioassay

ANNEX VI. TUNNEL BIOASSAY OF NETS COLLECTED IN HOUSEHOLDS

Name of person performing bioassays: ……...

1. Date of test (dd/mm/yyyy): |__|__|/|__|__|/|__|__|__|__|
2. LN code: |__|__|__|__|__|__|__|__|
3. Temperature: |__|__|°C Relative humidity: |__|__|__| %
4. Test mosquito species and strain:
5. Age of mosquitoes: |___| days
6. Test start time* (h/min): End time (h/min):

   * Females are introduced at 18.00 h and collected at 09:00 h.

		Blood-fed females		Unfed females		Total	
		Alive	Dead	Alive	Dead	Alive	Dead
Control	Compartment 1						
	Compartment 2						
	Total						
Treatment (LN)[a]	Compartment 1						
	Compartment 2						
	Total						

Compartment 1, long section of tunnel into which mosquitoes are released (area C1, Figure 4); compartment 2, section between test netting and animal bait

[a] Add additional treatment rows when more than one subsample of the same net or samples of other nets are run in parallel.

Control of Neglected Tropical Diseases
WHO Pesticide Evaluation Scheme
and
Global Malaria Programme
Vector Control Unit