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GUIDELINES *for* TESTING

MOSQUITO ADULTICIDES FOR INDOOR RESIDUAL SPRAYING AND TREATMENT OF MOSQUITO NETS

World Health Organization

Control of Neglected Tropical Diseases
WHO Pesticide Evaluation Scheme

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GUIDELINES FOR TESTING MOSQUITO ADULTICIDES FOR INDOOR RESIDUAL SPRAYING AND TREATMENT OF MOSQUITO NETS

CONTROL OF NEGLECTED TROPICAL DISEASES
WHO PESTICIDE EVALUATION SCHEME

CONTENTS

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1. INTRODUCTION

The purpose of this document is to provide specific and standardized procedures and guidelines for testing mosquito adulticides for indoor residual spraying (IRS) and for treatment of mosquito nets (ITNs). Its aim is to harmonize the testing procedures carried out in different laboratories and institutions to generate data for the registration and labelling of such products by national authorities.

This document is an expanded and updated version of the guidelines recommended by the WHO Pesticide Evaluation Scheme (WHOPES) Informal Consultation on the evaluation and testing of insecticides, held at WHO headquarters (HQ) in Geneva, Switzerland, on 7–11 October 1996 (WHO, 1996). The present guidelines were reviewed and recommended by the Ninth WHOPES Working Group Meeting, held at WHO-HQ in Geneva, Switzerland, on 5–9 December 2005 (WHO, 2006).

The document provides guidance and procedures on laboratory testing and small and large-scale field trials to determine the efficacy, field application rates and operational feasibility and acceptability of an insecticide intended for IRS and/or ITNs for mosquito control. Table 1 summarizes the sequence and objectives of the studies and trials. The procedures provide some information on the safety and toxicity of the insecticide product for non-target organisms, but it is presumed that preliminary eco-toxicity and human safety assessments have been undertaken before any field study is carried out – detailed treatment and analysis of these extra data are beyond the scope of this document.

This document is basically developed for testing insecticide against malaria vectors. However, the basic principles may also be applied for testing and evaluation of insecticides for controlling leishmaniasis vectors.

These guidelines do not address the laboratory and field testing of long-lasting insecticidal mosquito nets, which are the subject of separate WHO guidelines (WHO, 2005).

Table 1. **Sequence of the stages of evaluation of insecticides for indoor residual spraying and/or treatment of mosquito nets for mosquito control**

Phase	Type of study	Aim
Phase I	Laboratory studies	• Intrinsic insecticidal activity • Diagnostic concentration • Irritant or excito-repellent properties • Cross-resistance to other insecticides • Efficacy and residual activity on relevant substrates
Phase II	Small-scale field trials	• Efficacy and persistence under different ecological settings • Dosage of application • Handling and application • Perceived side-effects
Phase III	Large-scale field trials	• Efficacy and residual activity • Operational and community acceptance

2. LABORATORY STUDIES (PHASE I)

The objectives of laboratory studies are to determine the intrinsic activity of the insecticide, its potential irritant or excito-repellent properties and to assess cross-resistance with commonly used insecticides. Laboratory studies also include the determination of the efficacy, including residual activity, of the formulated product on relevant surfaces.

The specific aims of the tests are:

- to establish dose–response line(s) and determine the lethal dosage (LD) of the insecticide for 50% and 90% mortality (LD_{50} and LD_{90}) that allow assessment of the intrinsic activity of the insecticide against susceptible adult mosquito species, using topical applications;

- to determine the lethal concentration (LC) of the insecticide for 50% and 90% mortality (LC_{50} and LC_{90}), as determined by tarsal contact to treated papers;

- to establish a diagnostic concentration for monitoring resistance to the insecticide in the field;

- to determine the "time to first take-off" (FT) for the 50% and 90% of the mosquitoes to take off (FT_{50} and FT_{90}) after exposure to treated substrates;

- to assess cross-resistance with commonly used insecticides;

- to determine the efficacy and residual action of deposits on different substrates.

Standardized mosquito rearing and testing conditions are essential to ensure the reliability and reproducibility of data.

2.1 Intrinsic insecticidal activity

The objective is to determine the intrinsic activity of an insecticide to a target species. This is done by topical application to isolate toxicity from confounding effects resulting from insect behaviour.

Topical solutions are prepared by dissolving technical grade insecticide in acetone, a highly volatile organic solvent which has the advantage of remaining on the insect cuticle for only a short time. The doses used in topical application are expressed in nanograms of active ingredient per mg of body weight of live mosquito. Usually, 50 mosquitoes are weighed to determine their average live weight. To deliver small and constant volumes of insecticidal solutions, a device can be used made from a disposable pipette, 32 mm long (e.g. Drummond Microcaps®) shortened to a length of 6.4 mm so as to emit a volume of 0.1 µl (larger volumes may cause higher mortality caused by solvent toxicity). The pipette is then connected to a rubber bulb via a Pasteur pipette. Whatever the concentration, a constant volume of 0.1 µl is placed on the pronotum of the mosquito (Figure 1). Alternatively, appropriate automatic applicators are now commercially available and may be used for topical application.

A total of 50 susceptible, non-blood-fed, 2–5-day old *Anopheles* mosquitoes are used at each concentration, with at least five concentrations per test covering a range of mortality from 5% to 99%. Mosquitoes are anaesthetized with carbon dioxide for 30 seconds, then placed on a plate cooled to 4 °C to maintain anaesthesia during the manipulations. Two samples of 25 females are used for each concentration of insecticide. A volume of 0.1 µl of insecticide solution of the required concentration is then deposited on the pronotum of females as described above. Two batches of 25 females treated with 0.1 µl of pure acetone serve as controls. After each test, females are transferred into plastic cups and provided with 10% honey-in-water on cotton wool and held for 24 hours at 27 °C ± 2 °C and

80% ± 10% relative humidity (RH). Mortality is recorded 24 hours after the topical applications. Three replicates from separately reared batches are tested and the results pooled for statistical analysis.

Figure 1. Topical application of insecticide to the pronotum of anaesthetized mosquitoes (courtesy of Institut de Recherche pour le Développement, Montpellier, France).

The relationship between dose and mortality is analysed using log-dose probit regression (Finney, 1971). Ideally, five doses giving responses between 0% and 100% are needed for this analysis. Commercial software is now available to compute estimates of the LD_{50} and other LD values and their 95% confidence limits. If mortality exceeds 20% in the control batch, the whole test should be rejected. If mortality in the controls is above 5%, results with the treated samples should be corrected using Abbott's formula:

$$\text{Mortality (\%)} = \frac{X - Y}{100 - Y} \times 100$$

where X = the percentage mortality in the treated sample and Y = the percentage mortality in the control.

It is possible to compare the probit mortality per log dose regressions for two insecticides by a parallelism test (Annex 1). Results of two series of assays are considered as not significantly different if slopes of their log-probit lines are identical (i.e. null hypothesis of the parallelism test is not rejected) and the confidence intervals of their LC_{50} or LD_{50} overlap.

2.2 Diagnostic concentration

Diagnostic concentrations of insecticide are used to detect or monitor the presence of resistance in a vector population and are determined by exposing mosquitoes (tarsal contact) to insecticide deposits on filter-paper.

The WHO-recommended diagnostic concentrations for each group of vectors are chosen so that exposure for a standard period of time (usually 1 hour) followed by 24 hours, holding can be relied upon to cause 100% mortality of individuals of susceptible strains. To avoid spurious reporting of resistance in the field where none may exist, WHO sets the diagnostic concentration at twice the minimum concentration that kills 100%.

The determination of diagnostic concentrations is done with a graded series of dosages of insecticide (technical grade) applied to sheets of filter-paper. Rectangular pieces of filter-paper measuring 12 x 15 cm (Whatman® No. 1 or equivalent) are impregnated with 2 ml of solvent, generally acetone, and mixed with a non-volatile carrier such as silicon oil (e.g. BDH Dow Corning® 556) or Risella® (Shell) or olive oil according to the insecticide tested (the manufacturer should be consulted for both solvent and carrier selection). Oil allows the production of a

stable, thin and homogeneous layer of the active ingredient on the paper and prevents crystallization of active ingredients that are solid at room temperature. The concentrations are generally expressed as the percentage of active ingredient per unit volume of silicon on the filter-paper (the acetone being volatile). Papers are impregnated with 3.6 mg/cm² of the carrier, i.e. 648 mg/paper or 0.66 ml/paper for silicon oil (taking into account that silicon oil has a density of 0.98). A filter-paper impregnated at 1% contains 6.6 mg of technical insecticide, or 367 mg/m².

The impregnation is done by pipetting evenly onto the paper while it is supported on several pins pushed vertically through a piece of cardboard. The paper is then air dried for 24 hours and inserted into a WHO plastic cylinder for exposure to the insects. It should not be used more than five times (WHO, 1998). The WHO tubes for testing susceptibility of adult mosquitoes and the testing method are described in Annex 2.

Batches of 25 non-blood-fed female mosquitoes, aged 2–5 days, are introduced into the holding tube (marked with a green dot) and held for one hour at 25 °C ± 2 °C and 80% ± 10% RH to acclimatize. They are then transferred by gentle blowing in the exposure tube (marked with a red dot), and the kit is held vertically for one hour under subdued light. At the end of the exposure time, mosquitoes are gently blown back into the holding tube, which is placed vertically in a dark place for 24 hours with sucrose solution at 25 °C ± 2 °C and 80% ± 10% RH. Dead mosquitoes are counted after 24 hours.

A total of 100 mosquitoes (four replicates containing 25 mosquitoes each) are used for each test concentration and for the control. Results are expressed as percentage mortality after 24 hours and corrected for any control mortality. Concentrations should be chosen so that at least one concentration gives 100% mortality, at least two concentrations give between 50% and 99% mortality, and at least two give between 5% and 50% mortality. The concentration/mortality relationship is

determined on three replicate batches. The concentration/mortality results are then pooled to produce a log dose/probit mortality regression line from which the LD_{99} can be estimated. As mentioned above, the diagnostic concentration corresponds to twice the minimum concentration that kills 100%.

2.3 Irritant or excito-repellent properties

The irritant effect of an insecticide is an important characteristic to be considered, as it modifies the tarsal contact time with the treated substrate. It is studied by placing a WHO polyvinyl chloride (PVC) cone to an insecticide-treated substrate, inserting mosquitoes through the hole at the top of the cone and closing this with a polyethylene plug[1] (mosquitoes do not normally rest on the plastic cone or polyethylene plug and most therefore remain in contact with the treated substrate). The irritant properties should first be determined using a technical grade of insecticide on filter-paper at the diagnostic concentration, as described in section 2.2. When significant irritancy is observed with treated filter-papers as compared with a control (i.e. papers impregnated with acetone and silicon oil only), tests may be conducted with relevant formulations of insecticides on various substrates commonly used as building materials (e.g. mud, cement, plywood) and netting materials (polyester, polyethylene, etc.). Substrates, including netting materials, should be sprayed/impregnated at the "recommended dosage", i.e. the lowest concentration that causes mortality >80% and/or knock-down (KD) >95% and which induces the longer residual activity (see section 2.4).

[1] *Supplies for monitoring insecticide resistance and procurement of WHO test kits*. Geneva, World Health Organization 2001 (WHO/CDS/CPE/PVC/2001.2; available at http://www.who.int/whopes/resistance/en/WHO_CDS_CPE_PVC_2001.2.pdf.).

For each test, susceptible non-blood-fed female mosquitoes, aged 2–5 days, are individually introduced into plastic cones. After a settling period of 60 seconds, the time elapsed between the first landing and the next take off of the mosquito is recorded as the FT. For each test, 50 mosquitoes are tested individually. Mosquitoes are then grouped by classes of first take-off time (0–1 s, >1–2 s, >2–4 s, >4–8 s …>128–256 s), and cumulative frequencies are used to calculate the time before 50% and 95% of the mosquitoes' take off (FT_{50} and FT_{95}) using probit analysis. Mosquitoes that do not take off at least once during the test period of 256 seconds are discarded. Where possible, an insecticide with well-known irritant properties (e.g. permethrin, which shows strongly irritant properties) should be used as a positive control when new chemicals are being studied.

The relationship between dose and percentage taking off due to irritability is analysed using log-dose probit regression.

2.4 Insecticide residual activity

Before testing a formulated compound under Phase II, an indication of the minimum dosage required should be provided in order to limit the number of dosages that need to be tested against wild mosquito populations. Such laboratory studies include the study of the efficacy and residual activity of different dosages of the formulated product on different substrates. For insecticide-treated netting material, the studies would also include resistance to washing.

2.4.1 Residual action on mosquito nets

2.4.1.1 Identification of the target doses
A pre-selection of the target dose of candidate insecticides can be done under laboratory conditions by carrying out bioassays with a range of dosages. The number of months during which treated nets provide mortality and/or a KD effect above the cut-off point (80% mortality and/or 95% KD) should be measured.

2.4.1.2 Insecticide treatment of nets
Netting material (white polyester, multifilament, 100 deniers, unless otherwise specified), measuring 30 x 30 cm, washed once to remove finishing products that might otherwise interfere with insecticide uptake, is treated by application of the predetermined volume of insecticide solution onto the surface of the folded net. The volume of insecticide solution should be sufficient to dampen the netting sample (without dripping, i.e. any excess solution left in the dish).

The volume giving saturation can be determined by dipping net samples of known area into a measured volume of water, wringing out the excess water from the netting, measuring the excess volume, and by subtraction determining the volume of water retained by the netting.

The treated sample is left to dry at 30 °C ± 2 °C and then submitted to bioassays. Samples for residual testing are stored, hung-up, under ambient light cycles and temperature.

2.4.1.3 Chemical assays
In order to confirm that the target dose of the insecticide has been achieved, randomly selected samples of treated nets are subjected to chemical analysis. The samples are labelled and kept separately in aluminium foil, and in a cool place, before being dispatched to a pesticide analytical laboratory. The surface area of the netting should be carefully measured to enable the

conversion of the result of chemical assay, i.e. w/w, to weight of the insecticide per unit surface area (e.g. mg/m^2).

2.4.1.4 Bioassays

Non-blood-fed susceptible female mosquitoes aged 2–5 days are introduced into WHO plastic cones for a period of 3 minutes. To minimize the chances of mosquitoes disturbing each other during the short exposures on netting, batches of only 5 females are introduced into each of four cones that are applied to the same net sample (Figure 2). A total of 10 replicates of 5 mosquitoes is used for each sample tested, giving a total of 50 mosquitoes per sample. Results are pooled for analysis.

Figure 2. Cone bioassay on mosquito nets (courtesy of Dr Vincent Corbel, Institut de Recherche pour le Développement (IRD), Montpellier, France).

After exposure, females are placed in 150-ml plastic cups (10 individuals per cup), with sucrose solution provided, and maintained in a climatic chamber for 24 hours at 27 °C ± 2 °C and 80% ± 10% RH. Percentage knock-down after 60 minutes and percentage mortality after 24 hours are recorded.

There are two potential alternatives to the use of WHO cones. These are: (1) the use of WHO test tubes (cylinders) for adult

mosquitoes; and (2) the wire-ball test. However, further calibration with the WHO cone test is required before it can be widely used in testing and evaluation of insecticide for treatment of mosquito nets.

WHO test tubes (cylinders)
The procedure is the same as that described in section 2.2, except that the netting material is attached to a piece of paper of the same strength and size as a WHO test paper (12 x 15 cm), before insertion into the tube. The netting should overlap each edge of the paper by 1 cm (i.e. 14 x 17 cm when double) in order that the 1 cm overlaps can be folded over and attached with sticky tape to the reverse side of the paper. Mosquitoes are introduced from the holding chamber and exposed for 3 minutes before being blown back into the holding chamber.

Wire-ball test
The netting is wrapped around a wire frame (a cubical frame of 15 x 15 x 15 cm or two intersecting circles of about 15 cm in diameter). The netting is held around the frame in such a way that a "sleeve" is left through which 11 mosquitoes can be introduced and removed easily with an aspirator (Figure 3). Mosquitoes are exposed for 3 minutes, after which they are transferred to holding cups for 24-hour post-exposure readings. Where very high mortality rates are found, it is also possible to observe the time for knock-down of each individual mosquito and to determine the median knock-down time, i.e. the time required until the sixth mosquito of a sample of 11 is knocked down.

2.4.1.5 Wash resistance
Net samples (25 cm x 25 cm) are individually introduced into 1-litre beakers containing 0.5 litre deionized water, with 2 g/litre soap[1] (pH 10–11) added just before and fully dissolved. Beakers

[1] Currently, "Savon de Marseille" is recommended as the standard soap. Further standardization, including the use of products recommended by the

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 for 10 minutes in clean, deionized water in the same shaking conditions as stated above. Nets are dried at room temperature and subjected to cone bioassay (see section 2.4.1.4).

Figure 3. Bioassay procedure using wire frame for netting material (courtesy of Dr Mark Rowland, London School of Hygiene and Tropical Medicine, UK).

2.4.2 Residual action on other substrates

2.4.2.1 Identification of the target dosages

The pre-selection of the target dose can be done by carrying out bioassays with a range of dosages on samples of the substrate that are intended to be used for Phase II in experimental huts (mud, concrete, plywood, thatch, bamboo, etc). Tests with WHO cones are the most appropriate technique. Substrates should be

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International Organization for Standardization, or other standard products, is necessary.

prepared, dried, treated and stored as described below. Bioassays are first used to determine the minimum concentration causing 100% mortality. Then they are sprayed at two and four times this concentration. For each substrate, four samples are tested one week after spraying, and then every month until mosquito mortality drops below 80% after 30 minutes' exposure on the treated substrate and 24 hours' holding. The number of months during which mortality is equal to or greater than 80% is reported.

2.4.2.2 Insecticide treatment of substrates

The WHO cone is 12 cm in diameter, the minimum size required for a sample of a substrate to be tested. Blocks of cement, plaster or mud, 5 mm thick, are prepared in Petri dishes, and dried at 27 °C ± 2 °C and 80% ± 10% RH). These and the substrates not requiring pre-preparation, such as wood or thatch, are sprayed with insecticide to make a homogeneous residual deposit of the desired concentration of active ingredient per unit area. Spraying is done using a Potter Spray Tower®, which is internationally recognized as the most precise method of chemical spraying in the laboratory. All substrate samples are then stored unsealed under controlled temperature conditions (30 °C ± 2 °C), humidity (80%), air circulation and ambient light cycles until ready for testing. A minimum of seven replicate blocks per dosage are prepared, at least three for bioassay and four for initial chemical analysis, selected at random.

2.4.2.3 Chemical analysis

The samples of treated substrates to be subjected to chemical assay are placed individually in labelled, aluminium foil before dispatch to a pesticide analytical laboratory. Packaging should be suitable and resist transportation.

2.4.2.4 Bioassays

At least 40 mosquitoes per block need to be tested, in four replicates of 10 mosquitoes. The non-blood-fed susceptible

female mosquitoes aged 2–5 days are introduced into plastic cones for an exposure period of 30 minutes. Substrates are maintained at 30 °C between each bioassay. After exposure, females are placed in 150 ml cups (10 individuals per cup), with sugar solution provided, and maintained in a climatic chamber for 24 hours at 27 °C ± 2 °C and 80% ± 10% RH. The percentage mortality after 24 hours is recorded.

2.5 Cross-resistance to other insecticides

When a compound is submitted for evaluation under Phase I, it is important to assess whether there is a cross-resistance with known resistance mechanisms, particularly in mosquito species where resistance is multiple and widespread.

A new compound can be tested first against a multi-resistant strain (if available). If a cross-resistance is noted, the compound can be tested against other insect strains carrying one of the component resistance mechanisms to identify which mechanism is responsible. The compound should also be tested against a susceptible reference strain, i.e. a strain which is considered to present the highest susceptibility level to the main classes of insecticides. Such reference-susceptible strains exist for regionally important *Anopheles* species, e.g. *Anopheles gambiae*, *An. stephensi* and *An. albimanus*. Resistant strains of *Anopheles* species to be used in testing should have the resistance confirmed using well-established assay techniques. The strains should preferably be homozygous for the selected resistance mechanism. If homozygosity cannot be achieved, periodic selection is usually necessary to prevent natural selection for susceptibility alleles causing decline of resistance. Reference strains should be monitored at least twice a year by bioassays or biochemical/molecular assays so that any reversion in resistance can be assessed and corrected by selection.

The comparison of the values obtained with a susceptible mosquito strain with those obtained with a small number of distinct resistant strains (particularly the LD_{50}) gives a good estimation of the existence and level of cross-resistance of the new candidate insecticide (resistance ratio RR_{50} and RR_{95}). Cross-resistance is indicated if the LD_{50} or LD_{95} of a strain carrying a particular resistance mechanism is significantly greater than that of the corresponding susceptible strain.

3. SMALL-SCALE FIELD TRIALS (PHASE II)

The objective of Phase II is to measure the efficacy and residual activity of insecticides on free-flying wild mosquitoes, under well-controlled conditions. Efficacy can be assessed in terms of mortality, residual effect, deterrence, blood-feeding inhibition and induced exophily.

The specific aims of the small-scale field studies are:

- to determine the optimum application dosage(s) and the residual activity;
- to determine the efficacy and the impact on the behaviour of mosquitoes;
- to record the ease of use of insecticide and the perceived side-effects during application and use.

Estimation of the residual activity is done on different local surfaces and does not require particular housing adaptations. However, the determination of efficacy can be done only where entry and exit of mosquitoes are monitored. Moreover, ants and other scavengers that might carry off dead mosquitoes must be prevented. These conditions can be achieved only by using experimental huts, as described in Annex 3.

A prerequisite is good knowledge of the resistance status of the local vector populations to the tested compound (Annex 4).

3.1 Study design

Ideally, there should be several huts available to allow comparison of several treatments simultaneously.

A pre-trial assessment of the attractiveness of the huts is necessary to ensure that they are comparable in attractiveness to target species and that the target species is present in adequate numbers in the study area, and to ensure that the experimental huts are not contaminated by insecticides.

Each trial should have an appropriate negative control and, if possible, a positive control. A negative control is one without insecticide treatment or with the formulation minus the active ingredient. This may be particularly relevant where the formulation or substrate may exert an effect by itself. For a positive control, a product used in the country with good historical data for the purpose of the trial at a recommended dosage will be chosen.

For ITNs, five nets/replicates (one net per night for a week) are used per treatment arm (WHO, 2005). However, when information on net rubbing by users is desirable, the use of the same net can be envisaged, leading to the use of additional huts/replicates. It may also be desirable to assess the loss of efficacy of ITNs after one or two washes.[1] This can be achieved by adding the washed nets as an additional treatment arm. The negative control involves a sleeper plus an untreated net. Treated and untreated nets should be deliberately holed to simulate torn nets. Nets may have a total of six holes (each

[1] Nets are washed in 10 litres of soap solution (2 g/litre of "Savon de Marseille" or equivalent) prepared from well-water or de-chlorinated water, with a maximum hardness of 5 degrees of hardness (dH). Each net is agitated for 6 minutes within a total period of 10 minutes' washing/soaking (approximately 20 rotations per minute). Nets are then thoroughly rinsed twice in fresh water and dried horizontally in the shade. The nets are stored indoors at ambient temperature between washes.

measuring 4 cm x 4 cm), two in each side and one at each end (at head height to the sleeper) to simulate the conditions of a torn net and to put the emphasis on testing whether the insecticidal treatment, rather than the net, effectively prevents biting of the sleeper.

For IRS, the negative control involves only a sleeper. Enough replicates are necessary to ensure a proper assessment of the efficacy of the insecticide.

It is highly desirable that all field operatives and supervisors be blinded as to the allocation of treatments to avoid bias during the trial. If double-blinding of senior investigators and implementers is not possible in practice, the minimal requirement is for single-blinding of field operatives and supervisors.

3.1.1 Rotation of treatments and/or sleepers

The study design for IRS differs from that for ITNs. ITN treatments can be rotated between huts, whereas IRS treatments cannot.

The purpose of rotation is to minimize the variation caused by differences in attractiveness of huts (due to position) and sleepers, as these might otherwise affect the interpretation of the effect due to treatment. The sleepers should be rotated between huts so that every sleeper is allocated to each hut-treatment an equal number of times. In practice, sleepers will need to be rotated daily to maintain balance.

For ITNs, the rotation between huts should be in accordance with a Latin square design in which every treatment is tested in every hut an equal number of times (Annex 5). The rotation of treatments can be done each week, with one or two days between rotations to clean and air the huts and to remove any contamination from previous treatments.

For IRS trials, treatments cannot be rotated, and hence it is essential to show just before the trial starts that there is little or no variation in the attractiveness of huts (this also illustrates the importance of optimum positioning of huts during construction).

3.1.2 Ethical considerations

Ethical clearance should be obtained from the appropriate institutions and authorities before starting the study. Informed consent should be obtained from all volunteers participating in the study. An example of an informed consent form is given in Annex 6. Effective chemoprophylaxis should be provided where appropriate, and volunteers should be medically supervised.

3.2 Treatment procedures

3.2.1 Implementation

Safety instructions and protective measures should be observed. The human and environmental safety of the product should have been assessed before any trial is undertaken. Antidote and instructions for treatment of intoxication should be present on site and made available to the responsible officer.

Experimental huts should be completely refurbished before each new trial and carefully cleaned, sprayed surfaces replaced and absence of contamination demonstrated by appropriate bioassay tests.

Operators must ensure that the insecticide formulation is safely and correctly applied.

For ITNs, instructions for their treatment and use are available from WHO (WHO, 2002).

For IRS, a WHO manual for application of residual sprays for vector control has been prepared for field staff working in national vector control programmes (WHO, 2003). All the operational factors mentioned in the manual are applicable for Phase II trials (e.g. safety during spraying, use of compression sprayers, handling and spraying techniques). The treatments are conventionally applied to the walls, ceiling and doors, but this may be amended according to the nature of the treatment and the manufacturer's recommendations. IRS causes a level of "contamination" to the hut that greatly exceeds that of ITNs, and between trials it will be necessary to remove and replace the door, substrates and ceiling material.

For "dip-it-yourself" kits, pictorial leaflets are provided by the manufacturer.

3.2.2 Assessment of the quality of treatment

In order to ensure that the recommended dose has been accurately applied to the substrate, samples of treated surface should be subjected to chemical assay (see section 2.4).

For ITNs, an additional set of nets is prepared specially for quality control, and from these five samples of 10 cm x 10 cm are cut (one from each side and one from the roof).

For IRS, to assess the accuracy of indoor spraying, at least four filter-papers 5 cm x 5 cm on different walls/heights are attached to the selected surface of each experimental hut before spraying, then removed once dry for chemical analysis.

For both ITNs and IRS, samples are placed individually in labelled, aluminium foil before dispatch to a pesticide analytical laboratory. After analysis of each sample, results are combined for each substrate to provide the average target concentration of insecticide (expressed in mg/m^2 or mg/m^2 of net). Packaging should be suitable to resist transportation.

3.3 Evaluation procedures

3.3.1 Determination of the dosage and residual activity

For ITNs, standard bioassays are carried out in situ on the sides and roof of each net at regular intervals with WHO cones (or wire frames – see section 2.4.1.4) using laboratory-reared, susceptible, females of the main local vector species. Batches of 10 non-blood-fed mosquitoes, 2–5 days old, are exposed for 3 minutes. Mortality is assessed 24 hours post exposure.

For IRS, confirmation of the target dose can be carried out by spraying locally-used housing materials and testing the residual effect. Batches of 10 non-blood-fed mosquitoes, 2–5 days old, are put in a WHO cone and exposed for 30 minutes on each of the walls of each hut and on the ceiling.

For fast-acting insecticides, such as pyrethroids, the KD rate may also be observed for both ITN and IRS, as it can be a sensitive indicator of bioavailability.

The number of weeks/months during which there is mortality above the "cut-off point" (80% mortality after 24 hours' holding) is then reported.

Safety considerations have to be taken into account when selecting the dosage to be tested.

3.3.2 Fumigant aspects of insecticide

Some insecticides may have fumigant properties. This can be assessed by estimating, in comparison with an unsprayed hut (control), the mortality of mosquitoes placed in small cages hanging from the ceiling, from 4–8 hours up to a maximum of

12 hours, at different distances from the sprayed surfaces. The mosquitoes are then kept for 24 hours' observation after being transferred to clean cages.

3.3.3 Efficacy and impact on vector behaviour

During the Phase II trials, adult volunteers should carefully follow the instructions of the trial supervisor. Sleepers should enter the huts at a standard time in the evening and remain inside until a standard time in the morning. From time to time, the supervisor should make an unexpected check at night to ensure that instructions are being followed by the volunteers sleeping in the huts.

Each early morning, mosquito collection must be made separately, from the veranda, room and net (if present), with reliable records of location. Resting and dead mosquitoes are collected using an aspirator, from inside the net, and from the room, exit and veranda traps. Mosquitoes from each of these collection sites are identified to genus and, as far as this can be done in the field, to species, and scored as dead or alive and as fed or unfed. Physiological status (fed / unfed / gravid / semi-gravid) of the mosquitoes should be recorded. Live mosquitoes are placed in cups and given access to sugar solution for 24 hours to assess any delayed mortality. It may not be possible to control the conditions during holding as strictly as in Phase I studies. However, humidity and temperature should be controlled within tolerable limits by use of insulated containers or wet towels wrapped around the holding cages. Data must be carefully reported on the daily record sheets (Annex 7) by the local supervisor.

The compilation of data for each treatment allows determination of the four indicators of efficacy and mosquito behaviour (see section 3.5.1. and Annex 8).

3.3.4 Safety and operational issues

Spraymen and other handlers of insecticides or treated nets should be questioned about any perceived adverse effects. This can provide a useful indicator of whether it is acceptable to progress a given insecticide to testing at Phase III. Ease of application by the spraying operators should be reported (mixing, dilution of insecticide, spraying, impregnation). The sleepers are questioned regularly during the study period about side-effects, and the responsible officer is expected to pay special attention to any spontaneous complaints.

3.4 Data analysis

3.4.1 Indicators

Four indicators are used to assess the efficacy of a formulated insecticide sprayed on walls or applied to nets: deterrence, induced exophily, inhibition of blood-feeding and mortality. These indicators are calculated relative to the untreated control hut with respect to four criteria:

- The entry rate, which is the total number of female mosquitoes found in the hut and exit traps. A reduction of entry rate (deterrence) is observed with certain types of repellent insecticide, presumably because the insecticide vapour or dust is detectable by mosquitoes before they enter a treated hut.

- The exit rate, which is the proportion of female mosquitoes found in the exit traps compared with the total number found in the hut and traps. The reduction of exit rate allows estimation of induced exophily or excito-repellency.

- The blood-feeding rate, which is the proportion of blood-fed female mosquitoes compared with the total number found in the hut. The reduction in the number of blood-fed mosquitoes between a treated hut and a control hut allows an assessment of the blood-feeding inhibition caused by the insecticide.

- The mortality rate, which is the proportion of female mosquitoes found dead in the hut immediately after and 24 hours later. The difference in mortality between a control hut (natural mortality) and a treated hut allows assessment of the insecticide-induced mortality rate.

If a treatment deters a significant number of mosquitoes from entering the hut, the values given by proportions blood-feeding or killed in the treatment hut may underestimate the full personal protective effect. The personal protective effect of a treatment in an experimental hut study is determined by the reduction in the number of blood-fed mosquitoes in the treatment hut relative to the number blood fed in the control hut. It may be estimated using the following formula and expressed as a percentage: $100 \times (B_c - B_t)/B_c$, where B_c is the total number blood-fed in the control huts and B_t is the total number blood-fed in the treatment huts. The overall insecticidal effect of a treatment needs to take into account that significant numbers were deterred and not killed by the treatment. It can be estimated by the following formula and expressed as a percentage: $100 \times (D_t - D_c)/E_c$, where D_t is the total number of mosquitoes dying in the treatment hut, D_c is the total number dying in the control hut and E_c is the total number entering the control hut.

3.4.2 Statistical analysis

Prior to treatment, a statistical test should be applied to ensure that there is no appreciable difference between huts in attractiveness to mosquitoes. The number of female mosquitoes

entering each hut is tabulated by species and day. It is likely that the distributions from day to day will be found to be over-dispersed and fit a Poisson distribution with variance equal to the mean. Therefore, Poisson regression analysis or a non-parametric test such as Kruskal-Wallis or Wilcoxon rank-sum test should be used.

After the interventions have begun, the number of mosquitoes of each species entering the huts, the proportion of mosquitoes that exit early, the proportion that are killed within the hut and the proportion that successfully blood-feed may be compared by species and analysed using Poisson regression for numeric data and logistic regression for proportional data (e.g. Stata 6 software). The clustering of observations made in one hut-night, and controlling for any variation between huts and sleepers, needs to be controlled for. Comparisons between treatments are made by successively dropping treatments from the overall comparison. This process allows each treatment to be compared with every other one. As a less powerful but valid alternative, the numbers of blood-fed and dead mosquitoes and overall totals collected from each hut may be compared using the non-parametric Kruskal-Wallis test.

4. LARGE-SCALE FIELD TRIALS (PHASE III)

The efficacy of insecticide formulations found to be suitable for IRS or ITNs in experimental hut or small-scale field trials (Phase II) should be evaluated in large-scale field trials under optimal field conditions against mosquito populations at the community level.

The specific aims of the large-scale field trials are:

- to establish the efficacy of insecticide formulations at the selected application rates against the target vector species, when applied to all or most households in the community;

- to confirm residual activity and application intervals;

- to observe the ease of application and handling of the insecticide product, and to record any perceived side-effects on operators and households;

- to observe community acceptance of the new insecticides or formulations.

Study site selection will usually be done where IRS or ITNs has already been shown to be effective against malaria. In areas where this is not known, epidemiological evaluation of IRS/ITNs may be necessary but is outside the scope of these guidelines.

4.1 Study design

Phase III trials are designed as cluster-randomized trials. For the purposes of these guidelines, the unit of intervention is the

village because the effect of the intervention is to act upon the entire community and population of mosquitoes within it, even though not all households may accept or use the control measure. The usual effect of insecticide, with respect to community protection, is to reduce the longevity, density and infectivity rate of the vectors. Where the objective of the study is to determine personal protection only, this is best achieved through epidemiological study. This subject is not addressed in this document.

It is essential that treatment and comparison areas are eco-epidemiologically homogenous. Also, there should be as little infiltration as possible of adult mosquitoes into the treated area from outside. In practice, an isolated village with its human population, breeding habitats and environment should provide an acceptable site, provided that the village is away from the influence of mosquito populations from other untreated areas. If known, the flight range of the vector species should be taken into account. Where such ideal conditions are not feasible, it may be possible to increase the size of the area to several villages and use its central part for evaluation, thus achieving a barrier of treated villages. Such a barrier should be wider than the known or expected flight range of the vectors.

The communities should be allocated to intervention or control arms at random in order to minimize the bias attributable to other risk factors and to permit a clear demonstration of the effect of the intervention (with causal interpretation). Owing to heterogeneity between communities, it may be desirable to stratify the communities in terms of size, location, environment (types of breeding site), transmission rates, coverage of household protection measures and entomological parameters. Collection of such baseline data in order to have matching intervention and control groups may require a preparatory phase of a few months to a year, depending on the entomological and transmission patterns of the area. Within each stratum, communities are randomly allocated to the intervention or

control arms. It is recognized that it is increasingly difficult to identify communities where ITNs are not already used by some households. Provided communities are stratified according to the proportion of households using nets, this will not confound the effect of any IRS intervention being tested. For ITN trials, already-existing ITNs should be substituted with test ITNs.

Given the unquestionable effectiveness of ITNs and IRS, it may no longer be acceptable to run ITN or IRS trials with negative controls. A positive control, such as deltamethrin- or permethrin-treated nets, would be an acceptable alternative, but it would then be difficult or impossible to demonstrate a difference in efficacy between treatment and control arms regardless of the number of communities recruited. An alternative to a positive control would be to apply an equivalent form of protection that has no effect on vector populations: chemoprophylaxis, for example.

Matched pair designs are a special case in which the communities are stratified in pairs and one member is then randomly assigned to the treatment arm and the other to the control arm. Stratified designs are usually preferable to matched pair designs. Cluster-randomized trials with fewer than five clusters per arm are inadvisable, because parametric tests may be unreliable with such small numbers and because non-parametric tests require at least four clusters per arm to achieve statistical significance (Anonymous, 2002). The number of entomological monitoring sites should be equal in each community, and will depend on the number of communities in each arm, the power of the study to detect an expected or minimum percentage impact and the available resources. Because houses may vary greatly in their attractiveness to mosquitoes, for practical reasons and consistency, the same entomological monitoring sites and sentinel houses should be maintained throughout the study.

For ITN trials, a history of ITN usage in the community is desirable but not essential, provided all reasonable means (e.g. good public relations and health information) are applied to maximize appropriate usage. Pre-intervention social science research may be necessary to find out about the community's understanding of malaria and vector control and to identify factors that may influence acceptance. Formative research is an opportunity to collect information on sleeping habits and sleeping surfaces, net preferences and net size – factors that might otherwise affect the acceptability of the intervention. The guidance of a social scientist or anthropologist should be sought.

4.2 Ethical considerations

Ethical clearance should be obtained from the appropriate institutions and authorities. This should include the examination of the study protocol, the informed consent form and the trial's information sheet, which will be provided to the study communities. In general, the following ethical rules must be applied:

- The benefits of research should be equitable among the communities and individuals involved. Communal consent must be obtained from community leaders.

- The participants should be informed in clear, comprehensible terms in the local language about the objectives, study protocol, and advantages and inconveniences. Participants should be told they have complete liberty to participate or refuse to participate. The content of an information sheet cleared by the ethics committee should be made available to every community member.

- Confidentiality of information must be maintained.

- Assurances should be given that the community and local, regional and national health officials will be informed about the trial's findings.

For interventions applied to the entire community, the community must decide collectively, although individuals do have the option to refuse at a household level. A village committee or mechanism that can represent the interests of the community is required.

A checklist and an example of an informed consent form are provided in Annex 6 for guidance.

4.3 Treatment procedures

4.3.1 Implementation

IRS requires well-trained technicians able to ensure the safe and correct application of the insecticide formulation, as specified in the WHO Manual for IRS (WHO, 2003), which describes all the operational issues. Coverage of IRS should be (i) total – all dwellings except sentinel houses (see below) are sprayed (members of sentinel families should be provided with untreated bednets), (ii) complete – all sprayable surfaces are treated, (iii) sufficient – ensuring the uniform application of the target dosage, and (iv) repeated – if the duration of the trial is longer than the duration of effective action of the insecticide treatment. All the houses in the treated villages are numbered and entered in the trial database (see section 4.5). A number of houses (around six houses per village) should be left untreated in order to serve as sentinel houses for monitoring mosquito population density pre and post intervention.

In ITN trials, high coverage and usage of ITNs are essential to show mass effect on mosquito populations. For communities new to nets, this will require an intensive education campaign

with further encouragement during the implementation phase. The treatment of mosquito nets is carried out by well-trained technicians, according to the WHO guidelines (2002), which describe all the operational issues are described. For dip-it-yourself kits, instruction leaflets are provided by the manufacturer. Each net is numbered with an indelible marker. Several nets are removed at random at the beginning and end of the intervention period for bioassay and chemical assays using the methods described in section 3.2.2. In contrast to Phase II trials, nets are not artificially holed since the extent to which they remain intact or become torn is a matter of importance and should be monitored.

Spraymen and supervisors should strictly follow the insecticide label recommendations and the safety instructions provided by the principal investigator. Safety should also be ensured during transport, storage and disposal of pesticides. Pesticide workers should be informed of the adverse health effects of pesticides, including signs and symptoms of poisoning of the pesticide they are using.

Any large-scale trial should be overseen by an experienced physician, who should monitor the workers, respond to any adverse health event, and recognize the signs and symptoms of different types of pesticide poisoning. Parents should be warned about risky situations involving children.

Close supervision of the various activities is needed, according to a regular schedule and also with unannounced checks by the project supervisors. Such checks should include whether the activities accord with the project plan and process indicators.

4.3.2 Quality control

4.3.2.1 Verification of target dose

Samples of ITNs are subject to chemical assays at the beginning of the trial to determine the applied dose. At least 30 samples

from as many nets are desirable to account for the higher variability in insecticide content expected on nets used under field conditions. Five pieces of netting measuring 10 cm x 10 cm are cut from the middle of each side and roof, labelled, and stored individually in aluminium foil for transport to a pesticide analytical laboratory for chemical assay (see section 2.4). The surface area of the netting should be carefully measured to enable conversion of the result of the chemical assay, i.e. w/w, to weight of the insecticide per unit surface area (e.g. mg/m^2). Samples are pooled to determine the average concentration of the insecticide on each net. Owners of sampled nets are provided with a new net.

For IRS, it is very important that spraymen are properly trained, use well-maintained and calibrated equipment and are closely supervised during the spray campaign. Papers (Whatman® No. 1) attached to the walls of randomly selected houses may be removed after the spray campaign and assayed for pesticide residue. Filter-papers are preferred to scrapings of sprayed mud surfaces because of difficulties of standardization. It is emphasized that chemical assay is no substitute for close supervision of spraymen.

4.4 Assessment

4.4.1 Efficacy

Several entomological parameters are relevant or required to estimate the entomological efficacy of a control intervention.

4.4.1.1 Vector density

Different methods of measuring population abundance may be used, each with advantages and limitations. Monitoring is carried out in untreated "sentinel rooms", which are maintained for this purpose throughout the study. Six sentinel rooms per village are usually sufficient. Monitoring of mosquito resting

density in treated rooms is also necessary for measuring the efficacy and residual activity of the insecticide treatment.

Human landing catches. Vector density is traditionally monitored using human landing catches (HLC), which measure the number of landing mosquitoes captured per person per night. There are two ways of doing this: the collector himself can act as bait, sitting with bare legs on which the mosquitoes are caught with an aspirator as they land, or collectors work in pairs and take turns to catch from each other. HLC should preferably be organized in the treatment arms simultaneously or on successive nights and carried out at regular intervals. The sampling errors caused by variation in catcher efficiency or attractiveness may be reduced by increasing the number of capture sites per cluster. Catchers may be required to work within and outside houses to assess indoor and outdoor biting rates (exo-endophagy). In regions where vectors are mainly zoophilic or present at low densities (e.g. South Asia), HLC results in low capture rates and poor catcher efficiency; other methods are preferred (see below). To establish more accurately the abundance of zoophilic vectors in a sprayed cluster when HLC gives limited data, catches are sometimes made from domestic animals (usually cattle).

Ideally, HLC is conducted all night long to be fully representative of vector density and transmission. If, for practical reasons, this is not possible, HLC can be restricted to the hours of peak infective biting of the target species (some species bite more in the evening, others late at night). HLC should be divided into shifts of no more than 2 hours. Care should be taken to conduct the HLC during the same period each night. The biting cycle of individual species may change according to season, weather or ambient conditions; biting rates are reduced in windy conditions, may be influenced by moon phase (some species are more active on moonlit nights) and temperature (activity restricted to dusk or dawn during cooler months). It is important to be aware that restricting the period of

HLC may introduce bias. Alternative methods to HLC have been developed.

CDC light trap catches. Where a correlation between CDC light trap catches set beside occupied untreated nets and HLC has been established, light trap catches may be used as a surrogate method of collection (e.g. for *An. gambiae* in east Africa). This method is much less labour-intensive than HLC because only a small, day-time working team is needed to collect and re-set several traps per day. In this situation, CDC light traps provide a reliable alternative that overcome the ethical constraints and catcher variability associated with HLC.

Indoor resting density. Indoor resting collections provide information on population density and are the favoured approach for estimating biting rates in regions where vectors are zoophilic and where HLC yields low numbers of mosquitoes per night. Indoor resting density is usually monitored using space spray catches in sentinel rooms which are not exposed to IRS or ITNs. Hand catches or resting mosquitoes are an alternative method but are less accurate. Space spraying is done with a pyrethrum flit gun or non-residual pyrethroid aerosol. All openings to the outside are covered and sheets placed over the floor beforehand. Catches are identified to species and the gonotrophic status is recorded. A reduced proportion of gravid or semi-gravid mosquitoes may indicate insecticide-induced mortality or repellency. Indoor resting collections are indicative only of human biting rates if the proportion feeding on humans is established. Blood-meal identification of individual mosquitoes of a sample is carried out using precipitin or ELISA tests. From the product of indoor resting catch and the human blood index, an estimate of human biting rates may be derived. Assigning six rooms per village for monthly space spray and exit trap catches gives meaningful data on mosquito density, which is expressed as the number of vectors captured per room (or per person in the room) per unit time.

Additional information on exit rates or repellency of the insecticide may be obtained by attaching exit traps to windows of sprayed and unsprayed houses. The exit trap collections from sprayed houses, while revealing information about insecticide-induced repellency and residual activity, are not informative about vector density in the village.

Pit trap collections. Pit traps dug in the ground, if attractive to the vector species, may provide information on outdoor resting behaviour if the vector commonly rests outdoors or is driven outdoors by the repellent activity of the insecticide. Pit traps are not used routinely, and many species will prefer to rest on outdoor vegetation.

4.4.1.2 Vector longevity

The main effect of insecticide is to reduce the life expectancy of mosquitoes and hence the probability of transmitting malaria. The simplest method of estimating mosquito lifespan in the field is to measure the parous rate of a sample of mosquitoes collected in HLC or space spray catches. The ovaries of unfed or freshly fed mosquitoes are dissected to assess whether the tracheoles are coiled or uncoiled. Uncoiled tracheoles indicate that a female has developed and laid eggs at least once in her lifetime. The proportion of such parous females is an indirect measure of the probability of daily survival of mosquitoes in the population. For a good adulticide, a marked reduction in the proportion parous should be observed.

4.4.1.3 Infectivity rate

This is traditionally measured by dissection of salivary glands for sporozoites. Diagnosis of *Plasmodium* species can be carried out using an ELISA test to detect circum-sporozoite protein (CSP) of *Plasmodium falciparum* or *P. vivax* (using species-specific monoclonal antibody) in the crushed heads and thoraxes of specimens, which can be stored dry on silica gel. Because sporozoite rates are often less than 1%, even in hyper-endemic

areas, at least several hundred specimens are needed to make meaningful comparisons between study arms. One advantage of ELISA is that it can distinguish between vivax and falciparum infections in areas of dual transmission. CSP of *P. ovale* and *P. malariae* may pose problems of specificity and sensitivity. The estimation of infection rate as measured by dissection of the salivary glands generally gives an underestimate of the prevalence rate of CSP and is more subject to inter-operator variability. With a successful control intervention, few mosquitoes would survive the time required for sporozoites to mature, and so the sporozoite rate should be greatly reduced. In areas of meso- or hypo-endemicity areas where sporozoite rates may be less than 0.3%, samples of mosquitoes may be pooled into groups of 10 before application of the ELISA test with no loss of sensitivity. The overall numbers of mosquitoes tested may thus feasibly be increased to the several thousands that would be required to detect a significant reduction as a result of an insecticide treatment.

4.4.1.4 Entomological inoculation rate and vectorial capacity

Entomological inoculation rate (EIR) is an important entomological indicator for measuring the epidemiological impact of a vector control intervention. EIR is the number of infective bites per person per night. It is estimated from the product of sporozoite rate multiplied by the human landing rates, or equivalent estimates of human biting rates if this has been established for the vector species (e.g. CDC light traps). Both components of EIR should be reduced by an effective insecticide. Human landing rates and sporozoite rates are arguably the most important parameters to measure during community-randomized insecticide control trials.

Vectorial capacity (VC), or the number of new infections generated by a vector population, is represented by the equation: $VC = ma^2 p^n/-\log_e p$, where “ma” is the human landing rate, “a” is the proportion feeding on humans, “p” is the mean life expectancy in days of the vector population and “n” is the length

of the gonotrophic cycle. It brings together many of the entomological parameters, mentioned above, in a single formula that describes transmission rates. However, in practice it is difficult to measure accurately, is sensitive to smaller perturbations in life expectancy and estimates have a large standard error. For these reasons, EIR is usually preferred over vectorial capacity.

4.4.2 Residual activity

Bedrooms are selected at random to carry out at the beginning and the end of the intervention period in situ bioassays on treated surfaces using WHO plastic cones to determine insecticide bioavailability and residual activity. Residual activity of insecticide on sprayed surfaces and netting is measured using the procedures described in sections 2.4 (Phase I) and 3.4 (Phase II). The tests are performed at monthly intervals until the end of the transmission season or the end of the trial, or until no further treatment mortality is observed. A variety of local representative sprayed surfaces should be assessed, including cement, plaster and mud walls, thatch and wood. At least 100 mosquitoes should be tested per substrate (10 replicates).

4.5 Operational acceptability and safety

4.5.1 Safety

Spraymen and handlers of insecticides are at higher risk of exposure and should therefore be carefully monitored and questioned about any perceived adverse effect. It is advisable that the health status of workers should be examined pre-employment, and during and after handling pesticides. Brief records of exposure should be kept for each worker, including information on the product used, amount applied (e.g. number of pump charges), main activity (mixing, loading, etc.), total working hours, type and use of protective clothing and any

perceived adverse health effects. A list of mild, moderate and severe signs and symptoms of pesticide poisoning can be kept as reference.

Perceived adverse effects of indoor residual spraying of insecticide, or use of ITNs by communities involved in the large-scale testing of the insecticide, should be questioned and reported. Basic surveys could be carried out with questionnaires that reveal the history of pesticide exposure and use of pesticides in households. Questions should include personal information, information on the products exposed to in indoor and other applications (e.g. domestic use), duration of exposure, any existing medical condition (e.g. asthma, allergy) and perceived adverse health effects.

4.5.2 Acceptability

Acceptability of treatment varies according to the benefits perceived by the population, the degree of inconvenience caused and any unpleasant side-effects caused by the treatment. Perceived risks may lead to refusal. Baseline data are collected from a random sample of households within each treatment arm at the start of the intervention and every 6 months thereafter. A qualified social scientist should be recruited to develop a culturally sensitive questionnaire, which should be pre-tested before use. Focus group discussion may yield valuable qualitative information that would not emerge from predetermined questionnaires. Rumours of treatment effects are common and should be captured. Households should be interviewed to assess perceived adverse or beneficial side-effects, and information on net utilization rates and patterns of use.

Acceptability can also depend on the rate and method of washing, as it is one factor determining the residual activity of the insecticide on the nets. Local methods of washing can be easily reported by simple observation, while the rate of washing

can be measured indirectly by treating the nets with a washable marker a few months into the trial and returning a month later to record the proportion of nets that have been washed.

Whereas user participation is required for effective use of ITNs, IRS requires acceptance of spraying by the community. IRS needs the active cooperation of householders in preparing the houses for spraying and subsequently in maintaining the insecticide residue by refraining from re-plastering of walls. The perceived benefits or acceptability of chemical control may change over time if people forget how bad insect nuisance was in the past. Factors that can limit acceptability include visible insecticide residues on walls, an unpleasant odour, or skin and nasal irritation from some insecticide products.

Ease of application by the spraying operators should also be reported (mixing, dilution of insecticide, spraying, impregnation).

4.6 Data analysis and interpretation

The plan of analysis should form part of the study design to be decided before implementation. Expert statistical advice is essential to ensure the study is sufficiently powered.

The primary unit of replication and analysis is the community. Within each community there will be replication between sentinel entomological monitoring sites. The preferred choice of statistical method will take into account the variation existing between communities and between sentinel sites. Multivariate analysis is therefore the preferred approach since it adjusts for such variation before estimating the effect of the treatment. Proportional data (e.g. parous rates, sporozoite rates, bioassay mortality) should be analysed using logistic regression analysis (which is also used for evaluating experimental hut data). Numeric entomological data (e.g. mosquito resting density,

human landing catches or CDC light trap catches) are likely to be over-dispersed (i.e. not normally distributed between sites) and should be analysed using Poisson regression or transformed using logs to a normal distribution before applying analysis of variance.

Entomological data should be carefully recorded onto appropriately designed forms and double-entered, independently, into a database for analysis.

Analysis of the entomological parameters provides information on the probable epidemiological impact of the treatment on malaria transmission, as indicated by the estimates of EIR or vectorial capacity derived from these parameters, while recognizing that neither EIR nor vectorial capacity may accurately estimate the force of infection. EIR, the product of the number of infective bites per unit period multiplied by the sporozoite rate (often cited as an indicator of the endemicity of an area), is increasingly being used to indicate the impact of vector control interventions. An overall analysis of entomological indicators will provide estimates of the efficacy of the treatment, while an analysis done by period may show changes in residual impact of the intervention over time. The residual activity of an insecticide is also shown by changes in the proportion killed using cone or wire-frame bioassays on sprayed surfaces.

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ANNEX 1
PRINTOUT OF THE COMPUTERIZED PROBIT ANALYSIS

Topical application

Analyzed file: KISPET06 Date: 11/05/98 Insecticide: permethrin control mortality: 4 (2 / 50)

N	Killed	Total	Dose	Obs. mortality	Corrected mort. (1st estimation)
1	3	40	0.6	7.5	3.6
2	5	40	1	12.5	8.8
3	11	40	2	27.5	24.5
4	30	40	4	75.0	74.0
5	33	40	6	82.5	81.8
6	45	45	8	100	100

Iterations: 16 Y = 3.48248 + 3.24284 * X

Natural mortality (last estimation): 5.1 % p(X^2 = 2.14482, df = 3) = 0.5429

The data are well represented by a line

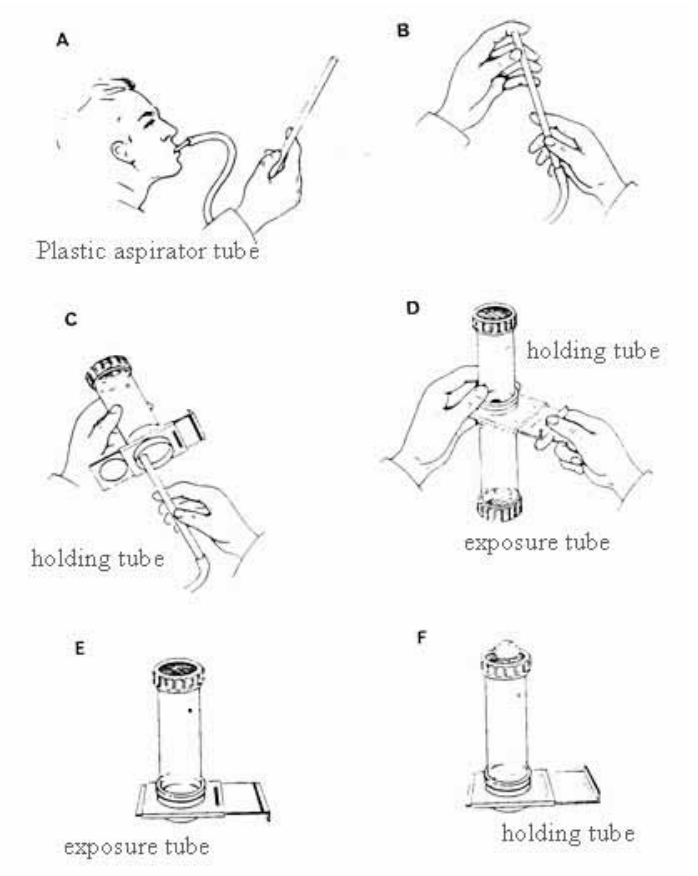
n	dose	corr. mort. (%)	probit	total treated	killed	killed (expected)	X^2 contribution
1	0.60	2.6	3.1936	40	03	2.51*	0.4888
2	1.00	7.8	3.6441	40	05	4.48*	0.1129
3	2.00	23.6	4.3069	40	11	13.20	0.5804
4	4.00	73.7	5.641	40	30	27.40	0.7628
5	6.00	81.6	5.9059	40	33	34.03	0.1999
6	8.00	100.0	-	45	45	41.62*	-

LD	Level of conf.	Range
01 = 0.56295	0.95	0.27234 < LC < 0.84960
02 = 0.68318	0.95	0.35662 < LC < 0.99045
03 = 0.77247	0.95	0.42298 < LC < 1.09221
04 = 0.84723	0.95	0.48075 < LC < 1.17593
05 = 0.91339	0.95	0.53344 < LC < 1.24907
10 = 1.18236	0.95	0.76068 < LC < 1.53973
20 = 1.61620	0.95	1.16065 < LC < 1.99807
30 = 2.02490	0.95	1.55926 < LC < 2.43427
40 = 2.45473	0.95	1.98298 < LC < 2.91522
50 = 2.93758	0.95	2.44468 < LC < 3.50111
60 = 3.51542	0.95	2.96113 < LC < 4.27966
70 = 4.26165	0.95	3.57091 < LC < 5.40493
80 = 5.33931	0.95	4.37212 < LC < 7.22522
90 = 7.29845	0.95	5.69320 < LC < 10.98633
95 = 9.44765	0.95	7.02796 < LC < 15.64424
96 = 10.18546	0.95	7.46722 < LC < 17.35381
97 = 11.17120	0.95	8.04193 < LC < 19.71842
98 = 12.63130	0.95	8.87108 < LC < 23.38010
99 = 15.32887	0.95	10.34572 < LC < 30.60387

Regression line : Y = A + slope * (X - M)
A = 5.06223 (SE : 0.12286) in probit unit - Slope = 3.2428 (SE : 0.4812) - M = 0.4871 in log10 (dose) unit and 3.0701 in dose unit. Variance of the LC50 : 0.00144343 in log10(dose) unit

ANNEX 2
THE WHO TUBES FOR TESTING SUSCEPTIBILITY OF ADULT MOSQUITOES

Description and testing procedure



The WHO tube test kit consists of two plastic tubes (125 mm in length, 44 mm in diameter), with each tube fitted at one end with a 16-mesh screen. One tube (exposure tube) is marked with

a red dot, the other (holding tube) with a green dot. The holding tube is screwed to a slide unit with a 20 mm hole into which an aspirator will fit for introducing mosquitoes into the holding tube. The exposure tube is then screwed to the other side of the slide unit. Sliding the partition in this unit opens an aperture between the tubes so that the mosquitoes can be gently blown into the exposure tube to start the treatment and then blown back to the holding tube after the timed exposure (generally one hour). The filter-papers are held in position against the walls of the tubes by four spring wire clips: two steel clips for attaching the plain paper to the walls of the holding tube and two copper clips for attaching the insecticidal paper inside the exposure tube.

ANNEX 3
DESIGN OF THE INDIVIDUAL EXPERIMENTAL HUTS

An experimental hut is a simulated house in which all entering, exiting and dead and blood-fed mosquitoes can be recorded. It is made of local material and is characterized by the presence of a gutter or moat around the hut to protect against ingress of scavenging ants which would otherwise eat the mosquitoes killed by the treatment. It is also characterized by the presence of veranda and exit traps to catch mosquitoes which may exit during the night.

The presence of permanent or semi-permanent water bodies close to the experimental huts is desirable to ensure the availability of larval mosquito breeding sites. Volunteers sleeping inside the hut should be available for the entire trial and should maintain the same behavioural patterns throughout, as these could constitute an important source of variation that must be controlled for.

Two designs of standardized experimental huts, both fitted with veranda traps, have been used extensively for testing adulticides under Phase II.

One originated in the United Republic of Tanzania (Figures 5 and 6) and is still used there. The floor is about 3 x 3 m so that there is room for one bed. There are 1 cm gaps all around the eaves. The modern huts are designed to be multipurpose for testing of ITNs and IRS, and are constructed of brick walls plastered with mud on the inside, a wooden ceiling lined with sackcloth made of natural fibre and a roof made of galvanized iron. Window traps and verandas screened with netting on two sides of the huts capture mosquitoes leaving via the 1-cm eave gaps. The other two sides are left open so that mosquitoes can

enter through the eave gaps. Sheets are laid over the floor every night to ease the collection of knocked-down mosquitoes in the morning. Each morning's collection inside a hut, plus its window traps, is added to the collection in the two screened veranda traps, multiplied by two. This multiplication is to allow for the inevitable unrecorded escapes on the other two sides whose verandas are left unscreened to allow routes for entry of wild mosquitoes via the eave gaps. At the end of each week the screening is moved from the north and south verandas to the east and west ones, or vice versa, so as to compensate in the long run for possible biased sampling caused by any tendency of mosquitoes to exit selectively in one compass direction (e.g. towards the rising sun).

The other design of experimental hut, commonly used in west Africa (Figures 5 and 6), is similar in principle to that used in east Africa. However, the west African hut has cement walls and consists of a single room with entry window slits on three sides and a large screened veranda on the fourth side. Entry of mosquitoes is only possible through the four window slits, which are specifically designed to inhibit mosquitoes from exiting. Mosquitoes can exit only into the veranda, which can be shut at dawn by lowering a curtain separating the sleeping room from the veranda.

Both types of huts are built on concrete slabs and are surrounded by a water-filled moat or gutter to exclude ants and other scavengers that might otherwise carry off dead mosquitoes from the huts during the night. The gap between huts is equidistant. If possible, huts are constructed in a row (with equal spacing between huts) in front of the main breeding sites to reduce any variation in hut attractiveness.

In some regions of the world (e.g. South Asia), it is so hot in the summer that people sleep under ITNs outdoors. It is desirable to have a method of Phase II testing that simulates these conditions. Experimental huts are less appropriate as they do not

attract exophagic or exophilic vectors such as *An. nigerrimus* or *An. pulcherrimus*. This problem has been solved by use of outdoor entomological platforms (Figure 4) measuring 5 x 5 m, which are surrounded by a "moat" to prevent ingress of scavenging ants, and on which the ITN or other vector control tools can be erected, and over which a giant trap net, measuring 4 x 4 m and 2.5 m high, is suspended during the night. The trap net is oriented over the platform in such a way as to allow a 5-cm gap between the lower edge of the trap net and the platform. Mosquitoes enter the trap net through this gap and approach the ITN, which contains human volunteers. Mosquitoes are either killed or may be caught inside the trap net. Some mosquitoes will escape through the gap, but losses are reduced by lowering the bottom edge of the trap net to the floor one hour before dawn. The proportion of mosquitoes that are killed or are inhibited from blood-feeding can be calculated in the same way as for experimental huts.

Figure 4. Design of an outdoor entomological platform in a field site in Pakistan (courtesy of Dr Mark Rowland, London School of Hygiene and Tropical Medicine).

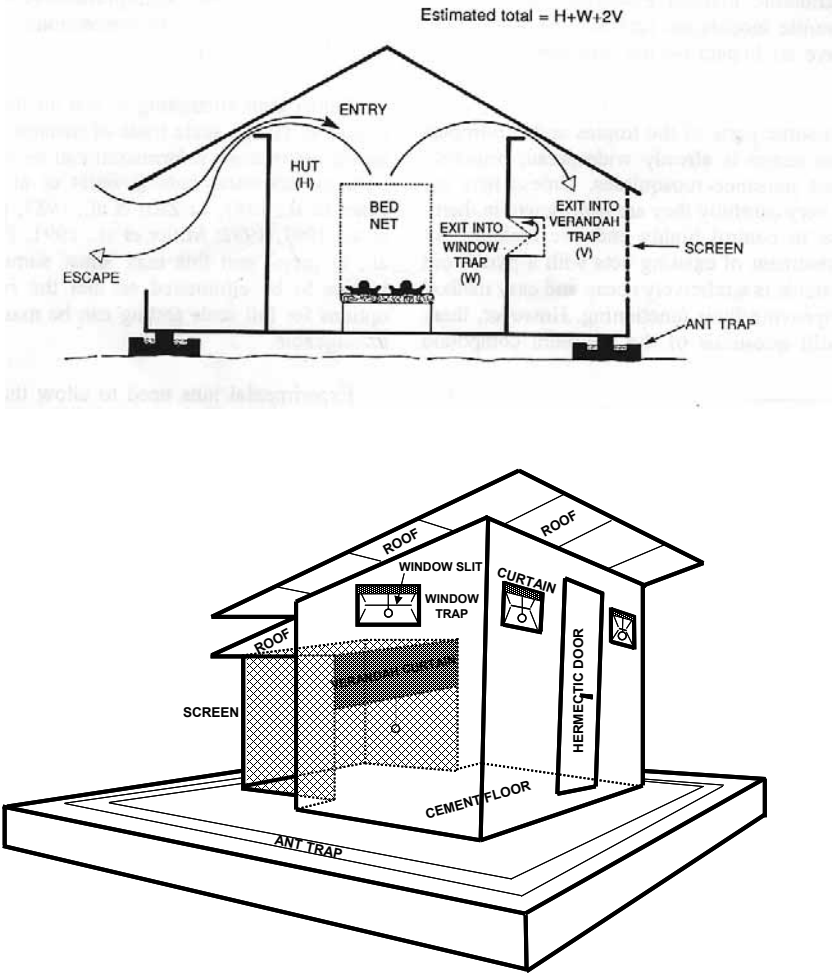


Figure 5. Design of two experimental huts commonly used in west and east Africa (top: United Republic of Tanzania, courtesy of Professor C.F. Curtis, London School of Hygiene and Tropical Medicine; bottom: west Africa, courtesy of Dr J.M. Hougard, Institut de Recherche pour le Développement (IRD), Benin).

Figure 6. General view of two types of experimental huts commonly used in west and east Africa (top: United Republic of Tanzania, courtesy of Dr Mark Rowland, London School of Hygiene and Tropical Medicine; bottom: west Africa, courtesy of Dr J.M. Hougard, Institut de Recherche pour le Développement (IRD), Benin,

ANNEX 4
MONITORING SUSCEPTIBILITY STATUS OF TARGET SPECIES TO INSECTICIDES

Mosquitoes of known adult age can be obtained using larval collection, or the F1 progeny from wild-caught females (a minimum of 50 female mosquitoes is considered sufficient to ensure enough genetic variability in the larval progeny). Where adults derived from larval collections are used, the type of breeding sites concerned (e.g. rice fields, rain water collections, irrigation channels, river beds, wells) should be specified since exposure to pesticides can differ with the type of water body. At the same time, a comparative test on a corresponding susceptible strain should be undertaken to check the quality of the papers, whenever possible. A minimum of 100 mosquitoes should be tested for any insecticide at the diagnostic concentration, with 4–5 replicates of 25 mosquitoes per test kit. Additional information on the test procedure for insecticide resistance monitoring of malaria vectors and the format for recording results of susceptibility tests on adult mosquitoes is available from WHO (1998).

A mortality rate between 98% and 100% is considered to indicate susceptibility; 80–97% mortality suggests the possibility of resistance that needs to be confirmed. Mortality <80% indicates resistance. For the two last categories, the identification of mechanisms by biochemical analysis or PCR tests, when and where possible, helps to confirm bioassay results and to provide more information about resistance status, leading to a better interpretation of the results.

ANNEX 5
LATIN SQUARE ROTATION SCHEME

Testing six different treatment arms in experimental huts

		ITNs option 1						IRS or ITN option 2					
		Treatment rotation (1 to 6)						Volunteer rotation (A to F)					
Week	Day	Hut 1	Hut 2	Hut 3	Hut 4	Hut 5	Hut 6	Hut 1	Hut 2	Hut 3	Hut 4	Hut 5	Hut 6
1	1	1	2	3	4	5	6	A	F	E	D	C	B
	2	1	2	3	4	5	6	B	A	F	E	D	C
	3	1	2	3	4	5	6	C	B	A	F	E	D
	4	1	2	3	4	5	6	D	C	B	A	F	E
	5	1	2	3	4	5	6	E	D	C	B	A	F
	6												
	7	Ventilating, cleaning and washing the hut						No volunteer resting inside the hut					
2	8	2	3	4	5	6	1	A	F	E	D	C	B
	9	2	3	4	5	6	1	B	A	F	E	D	C
	10	2	3	4	5	6	1	C	B	A	F	E	D
	11	2	3	4	5	6	1	D	C	B	A	F	E
	12	2	3	4	5	6	1	E	D	C	B	A	F
	13												
	14	Ventilating, cleaning and washing the hut						No volunteer resting inside the hut					
3	15	3	4	5	6	1	2	A	F	E	D	C	B
	16	3	4	5	6	1	2	B	A	F	E	D	C
	17	3	4	5	6	1	2	C	B	A	F	E	D
	18	3	4	5	6	1	2	D	C	B	A	F	E
	19	3	4	5	6	1	2	E	D	C	B	A	F
	20												
	21	Ventilating, cleaning and washing the hut						No volunteer resting inside the hut					
4	22	4	5	6	1	2	3	A	F	E	D	C	B
	23	4	5	6	1	2	3	B	A	F	E	D	C
	24	4	5	6	1	2	3	C	B	A	F	E	D
	25	4	5	6	1	2	3	D	C	B	A	F	E
	26	4	5	6	1	2	3	E	D	C	B	A	F
	27												
	28	Ventilating, cleaning and washing the hut						No volunteer resting inside the hut					
5	29	5	6	1	2	3	4	A	F	E	D	C	B
	30	5	6	1	2	3	4	B	A	F	E	D	C
	31	5	6	1	2	3	4	C	B	A	F	E	D
	32	5	6	1	2	3	4	D	C	B	A	F	E
	33	5	6	1	2	3	4	E	D	C	B	A	F
	34												
	35	Ventilating, cleaning and washing the hut						No volunteer resting inside the hut					
6	36	6	1	2	3	4	5	A	F	E	D	C	B
	37	6	1	2	3	4	5	B	A	F	E	D	C
	38	6	1	2	3	4	5	C	B	A	F	E	D
	39	6	1	2	3	4	5	D	C	B	A	F	E
	40	6	1	2	3	4	5	E	D	C	B	A	F

ANNEX 6
GUIDELINES FOR DEVELOPMENT OF INFORMED CONSENT FORM

For: [name the group of individuals for whom this consent is written]

Name of principal investigator:
Name of organization:
Name of sponsor:
Name of proposal:

PART I: Information sheet

This sheet is a suggestion/example that can be modified according to the national rules and guidelines

1. Introduction
Briefly state who you are and explain that you are inviting them to participate in research that you are carrying out.

2. Purpose of the research
Explain in lay terms why you are doing the research.

3. Type of research intervention
Briefly state the type of intervention that will be undertaken.

4. Participant selection
State why this participant has been chosen for this research (adult males and females will be preferably recruited among the inhabitants of the study site, after having announced in the district, through oral advertisements, that the project is looking for volunteers). The selection will ensure that equal opportunities are provided to everybody.

5. Voluntary participation
Indicate clearly that volunteers can choose to participate or not. State that they will still receive all the services they usually do whether they choose to participate or not.

6. Information on the insecticide formulation [name of the insecticide formulation]

Explain to the participant why you are testing the insecticide formulation.
Provide as much information as is appropriate and understandable about the insecticide formulation, such as its manufacturer or location of manufacture, and the reason for its development.
Explain the known experience with this insecticide formulation.
Explain comprehensively, if any, all of the known side-effects/toxicity of this insecticide formulation.

7. Participant protection against malaria

Explain to each participant the safeguards that will be provided (e.g. chemoprophylaxis, where relevant) to protect them from malaria infection, and, if necessary, their treatment.

8. Description of the process, procedures and protocol

Describe or explain to the participant the exact procedures that will be followed, on a step-by-step basis, and the tests that will be done.

9. Duration

Include a statement about the time commitments of the research for the participant, including both the duration of the research and follow-up.

10. Side-effects

Potential participants should be told if there are any known or anticipated side-effects caused by the insecticide formulation (dermal irritation, sneezing, headache, burning sensation in the eyes, lacrimation, etc.) and what will happen in the event of a side-effect or an unexpected event.

11. Risks

Explain and describe any possible or anticipated risks. Describe the level of care that will be available in the event that harm does occur, who will provide it and who will pay for it. For example, any possible allergies to the insecticide formulation pointed out by the volunteers will be referred to the closest sanitary body.

12. Discomforts

Explain and describe the type and source of any anticipated discomforts that are in addition to the side-effects and risks discussed above. It could be scratching caused by mosquito bites and/or blisters, redness or allergic dermatitis caused by the insecticide itself.

13. Benefits

Mention only those activities that will be actual benefits (as an additional protection from mosquito bites) and not those to which they are entitled regardless of participation.

14. Incentives

State clearly what you will provide the participants with as a result of their participation. WHO does not encourage incentives. However, it recommends that reimbursements for expenses incurred as a result of participation in the research be provided.

15. Confidentiality

Explain how the research team will maintain the confidentiality of data, especially with respect to the information about the participant which would otherwise be known only to the physician but would now be available to the entire research team.

16. Sharing the results

Where relevant, your plan for sharing the findings with the participants should be provided.

17. Right to refuse or withdraw

This is a reconfirmation that participation is voluntary and includes the right to withdraw.

18. Who to contact

Provide the name and contact information of someone who is involved, informed and accessible (a local person who can actually be contacted). State also that the proposal has been approved, and how.

This proposal has been reviewed and approved by [name of the local ethical committee], whose task is to make sure that research participants are protected from harm. If you wish to find about more the Local Ethical Committee, please contact [name, address, and telephone number].

PART II: Certificate of consent

This section can be written in the first person. It should include a few brief statements about the research and be followed by a statement similar to the one given in bold below. If the participant is illiterate but gives oral consent, a witness must sign. A researcher or the person reviewing the informed consent must sign each consent.

I have read the foregoing information, or it has been read to me. 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 as a participant in this research and understand that I have the right to withdraw from the research at any time without in any way affecting my medical cure.

Print name of participant: _____

Signature of participant: _____

Date: _____
Day / month / year

If illiterate
A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team).

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

Print name of witness: _____ **AND**
Thumb print of participant

Signature of witness: _____

Date: _____
Day / month / year

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

Print name of researcher: _____

Signature of researcher: _____

Date: _____
Day / month / year

A copy of this Informed Consent Form has been provided to participant _____ (initialled by the researcher/assistant).

ANNEX 7
PHASE II EVALUATION DATA SHEET – SAMPLE

Name of Proposal: **Name of Principal Investigator:**

Name of Local Supervisor: **Study area:** **Starting date:**

IRS ☐ **ITN** ☐ **Hut n°:** **Treatment condition:**

Page n°: **Species:**

Immediate observation		**Week n°**	**1**					**2**					**3**					**4**					**5**			
		Night n°	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
		Date																								
Veranda	N° Live	Unfed																								
		Blood Fed																								
	N° Dead	Unfed																								
		Blood Fed																								
Into Hut	N° Live	Unfed																								
		Blood Fed																								
	N° Dead	Unfed																								
		Blood Fed																								
Under Net	N° Live	Unfed																								
		Blood Fed																								
	N° Dead	Unfed																								
		Blood Fed																								
Additional mort. after 24 hrs		Night n°	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
		Date																								
Veranda	N° Dead	Unfed																								
		Blood Fed																								
Into hut	N° Dead	Unfed																								
		Blood Fed																								
Under Net	N° Dead	Unfed																								
		Blood Fed																								

ANNEX 8
PHASE II – ANALYSIS OF DATA

Example of a computer-generated analysis

		Control	T1	T2	T3	T4	T5
Deterrency	**females catches**	**1041**	**1174**	**631**	**787**	**591**	**1097**
	deterrency (%)	-	-12.78	39.39	24.40	43.23	-5.38
	inside hut	752	306	137	254	136	771
Exophily	**inside veranda**	**289**	**868**	**494**	**533**	**455**	**326**
	inside net	0	0	0	0	0	0
	inside net (%)	0.00	0.00	0.00	0.00	0.00	0.00
	exophily (%)	27.76	73.94	78.29	67.73	76.99	29.72
	95% Conf. limits	25,04-30,48	71,42-76,45	75,07-81,51	64,46-70,99	73,59-80,38	27,01-32,42
	P	-	0.00	0.00	0.00	0.00	0.32
	induced exophily (%)	-	63.92	69.94	55.32	68.14	NS
	multiplied by	-	2.66	2.82	2.44	2.77	1.07
Blood feeding	**blood fed**	**1,009**	**1,100**	**494**	**690**	**417**	**1,055**
	mean number / night	14.21	15.49	6.96	9.72	5.87	14.86
	blood fed (%)	96.93	93.70	78.29	87.67	70.56	96.17
	95% Conf. limits	95,88-97,97	92,31-95,09	75,07-81,51	85,38-89,97	66,88-74,23	95,04-97,31
	P	-	0.00	0.00	0.00	0.00	0.32
	blood feed inh. (%)	-	3.33	19.23	9.54	27.20	NS
	divided by	-	1.03	1.24	1.11	1.37	1.01
	blood fed dead	15	108	182	101	149	12
	ratio / blood fed (%)	1.49	9.82	36.84	14.64	35.73	1.14
	blood fed dead (%)	1.44	9.20	28.84	12.83	25.21	1.09
	P	-	0.00	0.00	0.00	0.00	NS
Mortality	**overall mortality**	**18**	**140**	**281**	**156**	**274**	**16**
	mean number / night	0.25	1.97	3.96	2.20	3.86	0.23
	overall mortality (%)	1.73	11.93	44.53	19.82	46.36	1.46
	95% Conf.limits	0,94-2,52	10,07-13,78	40,65-48,41	17,04-22,61	42,34-50,38	0,75-2,17
	P	-	0.00	0.00	0.00	0.00	0.62
	corrected (%)	-	10.38	43.56	18.41	45.42	-0.28
	95% Conf. limits	-	8,63-12,12	39,69-47,43	15,70-21,12	41,40-49,43	-
	mortality unfed	3	32	99	55	125	4
	ratio / total mortality (%)	16.67	22.86	35.23	35.26	45.62	25.00
	mort. unfed (%)	0.29	2.73	15.69	6.99	21.15	0.36
	P	-	0.00	0.00	0.00	0.00	NS
	corrected (%)	-	2.44	15.45	6.72	20.92	0.08
	immediate mortality	6	110	259	136	253	10
	ratio / total mortality (%)	33.33	78.57	92.17	87.18	92.34	62.50
	immediate mort. (%)	0.58	9.37	41.05	17.28	42.81	0.91
	P	-	0.00	0.00	0.00	0.00	NS
	corrected (%)	-	8.84	40.70	16.80	42.48	0.34

World Health Organization

Control of Neglected Tropical Diseases
WHO Pesticide Evaluation Scheme
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