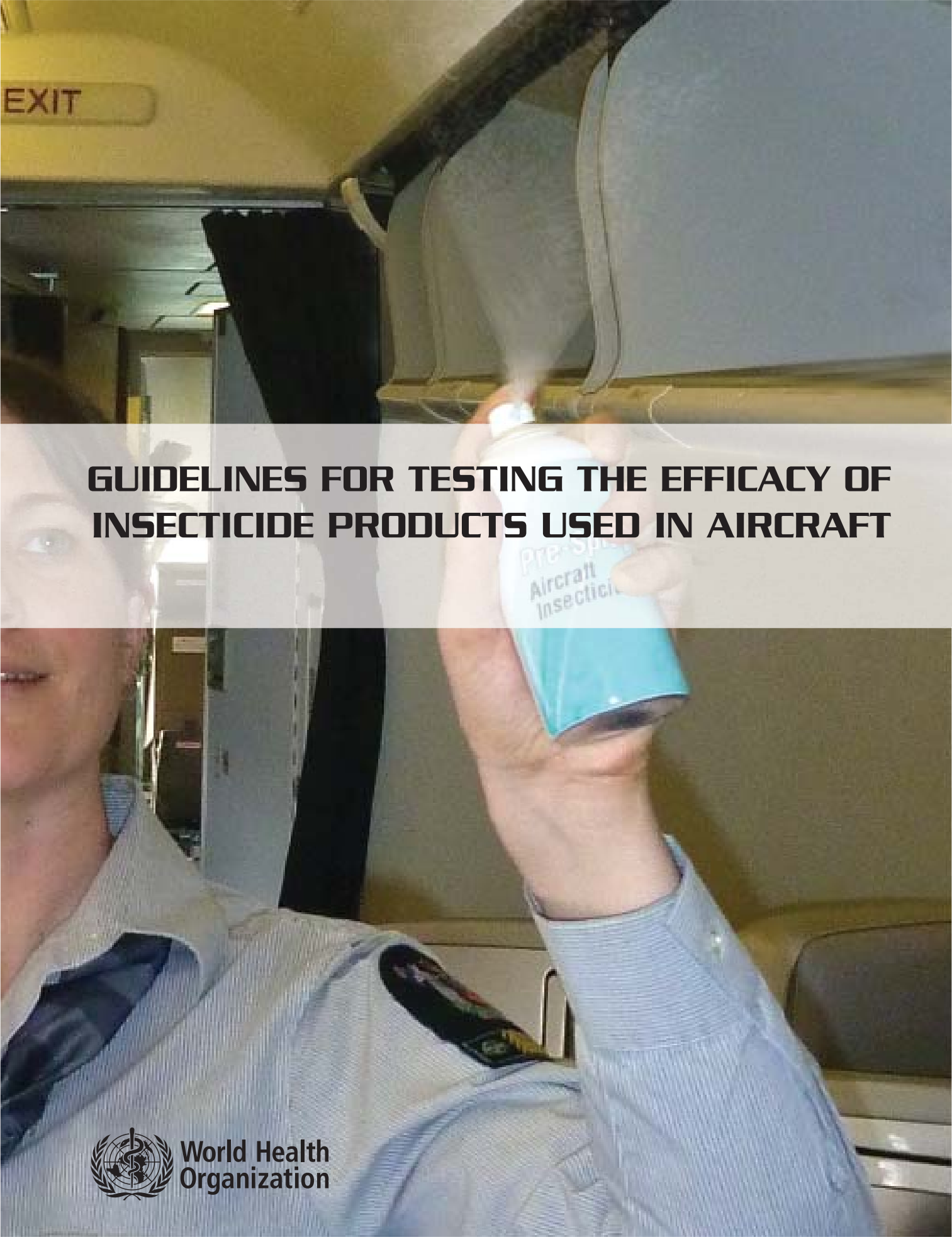

GUIDELINES FOR TESTING THE EFFICACY OF INSECTICIDE PRODUCTS USED IN AIRCRAFT

World Health Organization

Guidelines for testing the efficacy of insecticide products used in aircraft

Control of Neglected Tropical Diseases
WHO Pesticide Evaluation Scheme
and
Department of Global Capacity, Alert and Response
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Contents

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The Department welcomes feedback on the guidelines and suggestions for improvement from national programmes, research institutions and industry.

1. INTRODUCTION

The purpose of these guidelines is to provide specific, standardized procedures and criteria for testing the efficacy of products designed specifically for killing insects (referred to in this document as 'disinsection') in aircraft; and to assist countries in adopting health control measures under the International Health Regulations (2005)[1], hereafter referred to as IHR (2005). The guidelines are intended for use as a companion to other specific WHO technical guidance documents[2] on avoiding the spread of disease vectors through air travel. Their aim is to harmonize the testing procedures used in different laboratories and institutions in order to generate comparable data for registering and labelling such products by national regulatory authorities. Nevertheless, the requirements for registration of pesticides are determined by the national regulatory authorities.

The issue of aircraft disinsection is particularly important for protecting human populations against the spread of disease by vectors that may be inadvertently transported by air and under the scope of the IHR (2005). The range of responsibility for this aspect is wide. It calls for harmonization of practices, shared ownership and collaboration among individual stakeholders and several international bodies that play an important role in adopting scientific-based practices aimed at protecting the health of passengers and crew, including the extermination of insects by airport and aircraft operators. Measures against vector-borne diseases are addressed in several provisions of the IHR (2005), notably Annex 5 (Specific measures for vector-borne diseases), which calls for the use of methods and materials as recommended by WHO to control vectors on board conveyances.

Three methods are currently recommended by WHO for aircraft disinsection: 'blocks away'; preflight and 'top-of-descent' spraying; and residual treatment, as described in Annex 1.

The document provides guidance and stepwise procedures for laboratory studies, aircraft trials and evaluation of products intended for aircraft disinsection against mosquitoes. With some modifications, the guidelines can be used to determine the efficacy of candidate products against other public health and nuisance pests (e.g. cockroaches) that may board aircraft. The guidelines do not cover agricultural pests.

Products submitted for laboratory studies or aircraft trials should be accompanied by a material safety data sheet, the labelling recommendations and the manufacturer's certification that the product is within the company's manufacturing specifications for that product. Independent physical and chemical assessment may be required before the efficacy studies are initiated.

It is presumed that the suitability of a formulation, especially its potential impact on different surfaces of the aircraft, has been assessed before any studies are carried out in aircraft. For

[1] WHO (2005). International Health Regulations (2005). Geneva, World Health Organization (http://www.who.int/csr/ihr/en/).

[2] Example include WHO (2009). *Guide to hygiene and sanitation*, 3rd ed. (http://www.who.int/water_sanitation_health/publications/aviation_guide/en/).

assessing the human safety of the product, the WHO *Generic risk assessment model for aircraft disinsection with chemical insecticides*[1] should be used.

As biological tests are subject to variations in living organisms, studies should be conducted under close supervision by qualified staff, with well-characterized insect colonies and experimental procedures. The principles of good laboratory practice or other suitable quality schemes such as those of the International Organization for Standardization should be applied.

2. LABORATORY STUDIES

The objective of laboratory studies is to determine the insecticidal activity of the active ingredient and the formulated product under well-controlled conditions and against well-characterized mosquito colonies for comparative purposes and, as part of research and development, to determine the suitability of the product for aircraft disinsection. The specific objectives of laboratory studies are:

to establish dose–response relations and determine the lethal dosage (LD) of the insecticide active for 50% (LD_{50}) and 90% (LD_{90}) mortality that allow assessment of the intrinsic activity of the insecticide against susceptible adult mosquito species;

to assess the lethal concentration of the insecticide used in making disinsection aerosols, as determined by contact with insecticide spray in wind tunnel tests;

to determine the efficacy and residual action of surface treatment products;

to assess cross-resistance of the insecticide via known resistance mechanisms against commonly used insecticides; and

to determine the biological efficacy of the formulated disinsection insecticide product.

Tests should be conducted with at least one *Aedes* mosquito species *(Ae. aegypti* or *Ae. albopictus*), one anopheline species (e.g. *An. gambiae*) and one *Culex* species (preferably *C. quinquefasciatus*) of known age and susceptibility. The mosquito species and colony (strain) used for the test must be reported.

Standardized mosquito rearing and testing conditions are essential to ensure the reliability and reproducibility of data. The conditions are generally 27 C ± 2 C, 80% ± 10% relative humidity (RH) and photoperiod 12:12 h (light:dark); temperate species may require a different photoperiod. Test mosquitoes are maintained on sugar meals (typically 10% sucrose) and are non-blood fed. Testing should be conducted at a consistent temperature, suitable to most species, i.e. 27 C ± 2 C. The rearing and testing photoperiod for a species should remain consistent.

Inclusion of a positive control (i.e. reference insecticide for which there are data or which is in common use) is highly desirable (e.g. permethrin and D-phenotrin at 0.7 g active ingredient (a.i.)/100 m^3 for aerosol application and 0.2 g permethrin/m^2 for residual treatment).

[1] Currently under development.

Test chambers and equipment must be checked for insecticidal contamination before the start of each test.

2.1 Intrinsic insecticidal activity

The objective of this test is to determine the intrinsic activity of an insecticide on a target species. This is done by topical application of an active ingredient in order to differentiate 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 typically expressed in nanograms of active ingredient per milligram of body weight of live mosquito. Usually, 50 non-blood-fed, susceptible female mosquitoes of the target species are weighed (five batches of 10 previously anaesthetized mosquitoes) to determine the average live weights with their 95% confidence interval.

Fifty susceptible, non-blood-fed, 2–5-day-old female mosquitoes are used at each concentration, with at least five concentrations covering a range of mortality, i.e. two to three doses resulting in mortality <50% and two to three doses resulting in mortality >50% (excluding 0% and 100% mortality). A few mosquitoes at a time are lightly anaesthetized with CO_2 for 15–30 s and then placed on a plate cooled to 4 °C to maintain anaesthesia during manipulations. A constant volume of 0.1 µl should be delivered to the pronotum from an appropriate hand-held or automatic pipetting device (Figure 1). Larger volumes can increase mortality due to solvent toxicity. Two batches of 25 females are used for each concentration of insecticide and for the controls. Control batches are treated with 0.1 µl pure acetone. After dosing, females are transferred to clean holding cups, given 10% sucrose solution on cotton wool and held for 24 h at 27 ± 2 C and 80 ± 10% RH. Mortality is recorded 24 h after the topical applications. Three replicates from separately reared batches (i.e. generation) are tested and the results pooled for statistical analysis. A minimum of 900 mosquitoes are required for this study. Fresh insecticide dilutions should be prepared for each test replicate.

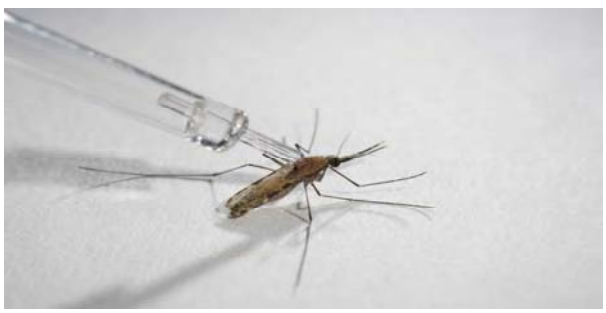


Figure 1. **Topical application of insecticide to the pronotum of an anaesthetized mosquito (courtesy of N. Rahola, Institut de Recherche pour le Développement, Maladies Infectieuses et Vecteurs Ecologie, Génétique et Contrôle, Montpellier, France)**

The relationship between dose and mortality is analysed by log-dose probit regression.[1] Commercial software is available to estimate the LD_{50} and other LD values and their 95% confidence intervals (Annex 2). If mortality in the control group exceeds 20%, the test is rejected. If mortality in the control group is 0–20%, the results with the treated samples are corrected with Abbott's formula:

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

Where *X* is the percentage mortality in the treated sample and *Y* the percentage mortality in the (untreated) control.

A log-probit analysis should be performed for candidate and control insecticides, and their slopes should be compared in a [2] parallelism test. The results of two series of assays are considered not significantly different if the slopes of their log-probit lines are the same (i.e. the null hypothesis of the parallelism test is not rejected) and the confidence limits of their LC_{50} or LD_{50} overlap.

2.2 Insecticidal activity of active ingredients used as aerosols

The objective of the test is to determine the lethal concentration of an insecticide applied as an aerosol.

Fifty susceptible, non-blood-fed, 2–5-day-old female mosquitoes are used at each concentration, with at least five doses covering a range of mortality, i.e. two to three doses resulting in mortality <50% and two to three doses resulting in mortality >50% (excluding 0% and 100% mortality). Overall, this will require a minimum of 900 mosquitoes. At each application, duplicate cages of 25 non-blood-fed female mosquitoes are exposed to one of the test concentrations of the atomized insecticide in a wind tunnel (see Annex 3 for equipment specifications, maintenance and procedural details). The apparatus consists of a cylindrical tube (15.2 cm in internal diameter) through which a column of air moves at 1.8 m/s. The mosquitoes are confined in a rimless cylindrical screen cage (mesh openings of 1.22 x 1.60 mm and 0.28 mm diameter wire) made to the exact interior measurements of the wind tunnel (see Annex 3). The cage is inserted into an opening 91.4 cm from the wind tunnel entrance; a flexible, clear plastic sheet is used to close the opening. The technical insecticide in an acetone solution (0.5 ml total volume) is atomized through a nozzle (time, approximately 3 s) to produce droplets with a $Dv_{0.5}$ (the point at which half the volume of droplets is smaller; formerly designated 'volume medial diameter' of 15 ± 2 µm at the position of the cage). Mosquitoes are left in the wind tunnel for a further 5 s. After each test, the mosquitoes are lightly anaesthetized (15–30 s) with CO_2 and transferred immediately into clean holding cups with 10% sugar solution on cotton wool and held for 24 h at 27 °C ± 2 °C and 80% ± 10% RH. Mortality is recorded at 24 h.

[1] Finney DJ. *Probit analysis*. Cambridge, Cambridge, University Press, 1971; Robertson JL et al. *Bioassays with arthropods*. Boca Raton, CRC Press, 2007.

Control tests with acetone alone as the diluent are conducted with each insecticide test, always at the beginning of the test. Tests should begin with the lowest dose and then proceed with increasing concentrations. The wind tunnel is cleaned with a 0.5-ml spray of acetone between each series of concentrations.

Three replicates of separately reared batches of mosquitoes are tested and the results pooled where appropriate (to a total of three duplicate applications per concentration) for statistical analysis. The relation between concentration and mortality is analysed by log-probit regression, as discussed above, and the LC_{50} and LC_{90} values reported.

If mortality in the control group exceeds 20%, the test is rejected. If mortality in the control group is 0–20%, the results with the treated samples are corrected with Abbott's formula (see section 2.1).

2.3 Cross-resistance via known resistance mechanisms

The objective is to determine if there is cross-resistance to an active ingredient via known resistance mechanisms. This test should be performed with well-characterized susceptible and resistant strains. The resistant strains should preferably be homozygous for the selected resistance mechanism (e.g. *kdr* mutation). If homozygosity cannot be achieved, periodic selection is usually necessary to prevent natural selection for susceptible alleles from reducing resistance. Reference strains should be monitored at least twice a year by bioassays or molecular assays so that any reversion in resistance can be detected, assessed and corrected by selection.

Comparison of the values obtained with a susceptible mosquito strain with those obtained with distinct resistant strains (particularly the LD_{50}) gives a good estimation of the existence and level of cross-resistance of the 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.

2.4 Biological efficacy of formulated products

2.4.1 Aerosols for rapid action

The biological activity of an aerosol (a formulation held in a dispenser that is dispersed generally by a propellant as fine droplets or particles upon activation of a valve) intended for rapid action is determined in a Peet-Grady chamber on free-flying mosquitoes (see Annex 4 for specifications). Before the test, the floor of the chamber is covered with absorbent white paper, taped down and divided into quadrants for easy observation and recording of knocked-down mosquitoes. Fifty non-blood-fed, 2–5-day-old females are released into the chamber. Immediately before the test, the aerosol container is shaken, placed in an automatic dispenser and sprayed into a fume hood for 3–5 s to prime the dispenser. Then, 0.65 ± 0.10 g of the formulated product is sprayed, in a single application, towards the centre of the chamber.

The number of mosquitoes knocked down is recorded every minute for 10 min and subsequently at 10-min intervals for a total of 60 min, with a hand counter. Different observers may be assigned to different quadrants for accurate recording of knocked-down

mosquitoes. The chamber is quickly ventilated after a 60-min exposure to the product. The knocked-down and all remaining mosquitoes are carefully collected with an aspirator and transferred into separate clean holding cups. The mosquitoes are given 10% sugar solution on cotton wool and held for 24 h at 27 ºC ± 2 ºC and 80% ± 10% RH. Mortality is recorded 24 h after exposure.

Before each product is tested, free-flying female mosquitoes are released into the chamber for 60 min to serve as a control. After 60 min, mosquitoes are collected with aspirators, transferred to holding cups, given 10% sugar solution on cotton wool and held for 24 h at 27 ºC ± 2 ºC and 80% ± 10% RH to observe mortality.

If mortality in the control group exceeds 20%, the test is rejected. If mortality in the control group is 0–20%, the results with the treated samples are corrected with Abbott's formula (see section 2.1).

2.4.2 Aerosols for combined rapid action and limited residual activity

Aerosols used for preflight application are expected to provide rapid knock-down and mortality with limited residual activity. The biological activity of such products is assessed in two steps. First, the rapid action of the product is measured in a Peet-Grady chamber as described in section 2.4.1. Then, the residual activity is determined by placing a minimum of four samples of absorbent and non-absorbent substrates (e.g. AerFilm®, carpet) on the floor of the Peet-Grady chambers and then spraying the aerosol into the chamber as described above. One hour after spraying, the substrates are removed from the chamber and subjected to WHO cone bioassays.

Ten non-blood-fed, susceptible female mosquitoes aged 2–5 days are introduced into plastic WHO cones[1] secured to four treated substrates for 30 min. The mosquitoes are then transferred into clean cups and given 10% sugar solution on cotton wool. Knock-down is recorded at 60 min, after which the mosquitoes are held for 24 h at 27 °C ± 2 °C and 80% ± 10% RH. Mortality is recorded 24 h after exposure.

Control bioassays are conducted on four untreated substrates in the same manner. If mortality in the control group exceeds 20%, the test is rejected. If mortality in the control group is 0–20%, the results with the treated samples are corrected with Abbott's formula (see section 2.1).

2.4.3 Insecticides for long-term residual activity

Passenger cabins

Residual surface treatments should be evaluated in the laboratory with a minimum of three representative internal aircraft surfaces (e.g. AerFilm®, carpet, curtains, ceiling and wall panels, linoleum) as substrates, with a homogeneous residual deposit of the desired concentration of active ingredient per unit area.

[1] WHO plastic cones are standard, polyvinyl chloride cones of 12 cm in diameter, available at http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.3_eng.pdf.

When available, the target dose claimed by the manufacturer should be tested. If no target dose is available, doses of candidate insecticides can be preselected in bioassays with a range of doses to determine the minimum dose that causes 100% mortality on the most representative substrates of passenger cabins. Then, the substrates are sprayed at two and four times this dose. For each substrate, four samples are tested 24 h after spraying, and then at regular intervals (e.g. weekly) until mosquito mortality and knock-down effect drop below the cut-off point (e.g. 80% mortality after 24 h holding or 95% knock-down after 60 min). The number of weeks during which mortality or the knock-down effect are still above the cut-off point is reported.

Substrates measuring 15 x 15 cm are sprayed with an appropriate apparatus (e.g. a Potter Spray Tower®). Samples are stored at ambient temperature (preferably 23 °C ± 2 °C and 60% ± 10% RH) under artificial light (out of direct sunlight) between bioassays. Precaution in handling the substrates is highly recommended to avoid substantial removal of insecticide from the treated surfaces. Four untreated samples serve as controls.

The efficacy of the substrates is measured in the WHO cones bioassay, as described in section 2.4.2.

Cargo holds

Surfaces in hold and cargo areas of aircraft consist predominantly of bare metal (e.g. aluminium) and plasticized linings. Products designed for such areas can be evaluated in the same way as described above with a homogeneous residual deposit of the desired concentration of active ingredient per unit area.

3. TRIALS IN AIRCRAFT

Products designed for aircraft disinsection must be tested in the specific environment of aircraft in realistic settings. Such trials are conducted inside aircraft of a size representative of those used on long-haul international flights. The minimum internal cabin dimensions for testing should be 30 m x 5 m. At least two replicates should be conducted with a susceptible mosquito species, and both single- and dual-aisle seating aircraft should be used.

No residual insecticide should have been applied in the aircraft during the previous 3 months. Cleaning and baseline testing with appropriate bioassays (control cages or WHO cones) should be performed in the aircraft to ensure that there is no contamination with pesticide residues. Tests are conducted under ambient climatic conditions. For passenger cabins, the aircraft climate control should be used to maintain a temperature of 23 °C ± 2 °C throughout the test, when possible. Ambient temperature and RH are recorded, as well as the exact dimensions of the cabin or hold used, from actual measurements or technical documentation on individual aircraft.

The requirements and assessment indicators of the WHO-recommended application methods are summarized in Table 1.

Table 1. **Requirements and assessment indicators of WHO-recommended application of disinsection products**

Method of application	Requirements for application	Assessment methods and indicators
Pre-flight spraying	Use of aerosol containing insecticide applied before passengers board, for rapid action and limited residual action (at least 1 h)	Cage bioassays: Assess KD 60 min after application and mortality after 24-h holding Cone bioassays on treated surfaces 1 h after application for 30 min: Assess KD at 60 min after application and mortality after 24-h holding
Blocks-away	Aerosol applied before take-off and after doors are closed, for rapid action	Cage bioassays: Assess KD 60 min after application and mortality after 24-h holding
Top-of-descent	Aerosol applied as aircraft starts its descent, for rapid action	Cage bioassays: Assess KD 60 min after application and mortality after 24-h holding
Residual application	Insecticide applied by compression sprayer for long-term residual activity on aircraft interior surfaces	Cone bioassays on treated surfaces, each for 30 min: Assess KD at 60 min and mortality after 24-h holding One day after application and then at regular intervals (e.g. weekly) until 24-h mortality is < 80%

3.1 Aerosols for rapid action

3.1.1 Study design for passenger cabins

As per the WHO disinsection guidelines, all toilet doors, garment storage lockers and overhead luggage storage compartments should be open, the air-conditioning switched off during spraying and the outer door closed. The flight deck should not be treated, and the access door should remain closed.

The efficacy of the product is assessed with a minimum of two replicates (i.e. two each single- and dual-aisle seating type) tested on separately reared batches of mosquitoes. A minimum of 1 week is required before the aircraft can be used for further testing.

In aircraft with a single aisle, tests are conducted by placing a series of cages of mosquitoes in three areas of the main passenger cabin: in the middle row of seats, in the fifth row of seats from the front of the aircraft and in the fifth row of seats from the rear of the aircraft at least 30 min before the treatment (Figure 2).

In each of these three locations, six nylon or polyester mesh netting (from 1.2 x 1.2 mm to 1.6 x 1.6 mm hole openings), cylindrical steel-frame cages (90 mm diameter x 150 mm height), each containing 25 non-blood-fed 2–5-day-old female mosquitoes, are placed on each side of the cabin, with one in the foot space beneath the window seat, another on the bottom cushion of the aisle seat and a third in the middle of the open overhead luggage

compartment (Figure 2). This placement of six cages (three on the left and three on the right side of the cabin) is repeated in the same manner in the mid, front and rear of the cabin. An additional cage is placed on the lid of a toilet in at least one toilet area and another on a suitable mid-height level surface in at least one galley area.

In aircraft with dual-aisle seating, the placement of cages is the same as for the single-aisle layout but with an additional series of three cages at each of the three locations (mid, front, rear) of the middle seating. An additional cage is placed in a second toilet area and another in a second galley area.

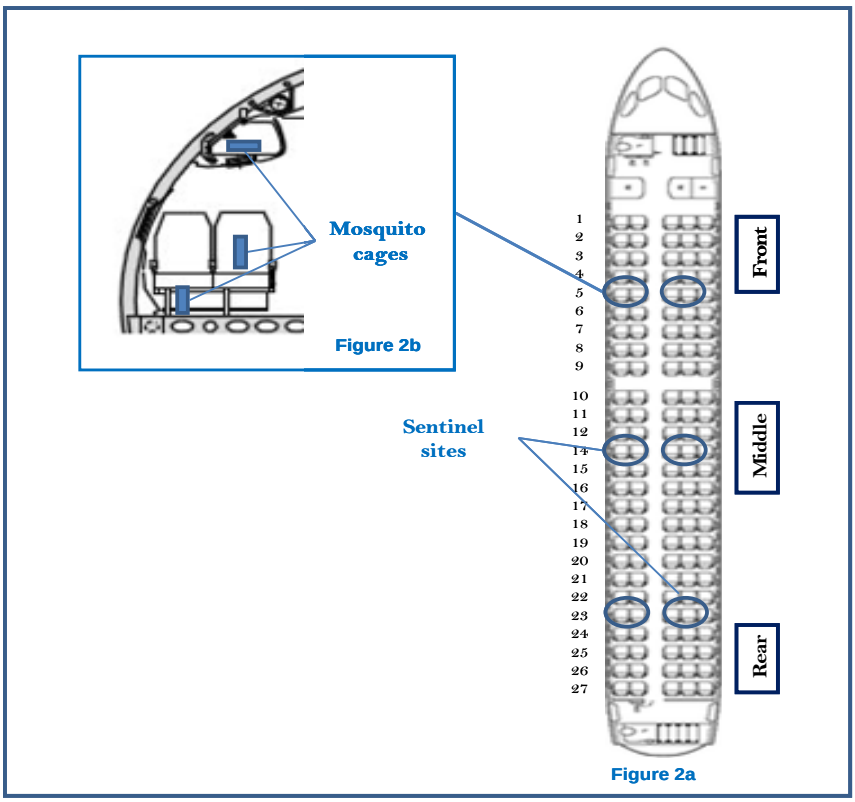


Figure 2. **Layout of mosquito cages in a single-aisle aircraft**

3.1.2 Study design for cargo holds

Aerosols for rapid action can be evaluated in a cargo hold by following procedures similar to those described above. Four nylon or polyester mesh netting (from 1.2 x 1.2 mm to 1.6 x 1.6 mm hole openings), cylindrical steel-frame cages (90 mm diameter x 150 mm height), each containing 25 non-blood-fed 2–5-day-old female mosquitoes, are placed singly on clean paper on the floor 10 cm from each corner of the hold area. A further four cages are hung 10

cm from the ceiling and walls in each corner, and a further three cages are evenly spaced along the central axis of the hold at a height of 1 m.

Preferably, the same number of cages is used for a control group.

3.1.3 Cage bioassay method

Cages containing female mosquitoes are positioned according to the design of the aircraft being tested, as described above, and the insecticide product is used by trained staff as per the manufacturer's instructions or national procedures. Each cage must be properly labeled (e.g. position of exposure, date of test) to ensure traceability and accurate interpretation of the results. Sufficient staff are required for handling the mosquito cages in the aircraft and for timely recording of the results of the bioassays.

The external door is opened 60 min after application, and the area is quickly ventilated before entry. The number of knocked-down mosquitoes is counted, after which the cages are removed from the aircraft. The mosquitoes in each cage are transferred as soon as possible into separately marked, clean holding cups, each with a 10% sugar solution on cotton wool, and held for 24 h at 27 ºC ± 2 ºC and 80% ± 10% RH. The average percentage of knocked-down female mosquitoes after 60 min and average percentage mortality after 24 h, based on pooled data from different test replicates, are reported.

Before the test, an equivalent number of control cages is placed at the same locations, held for 60 min and removed from the aircraft. The mosquitoes are transferred into clean holding cups and held for 24 h at 27 ºC ± 2 ºC and 80% ± 10% RH for observation of mortality. If mortality in the control group exceeds 20%, the test is rejected. If mortality in the control group is 0–20%, the results with the treated samples are corrected with Abbott's formula (see section 2.1).

3.2 Aerosols for combined rapid action and limited residual activity

3.2.1 Study design for passenger cabins

The method of evaluation of products intended for both rapid action and limited residual activity on surfaces in aircraft cabin is to determine the two effects separately. Rapid action activity can be evaluated with the cage bioassay method described above. Residual activity can be evaluated just after completion of a cage bioassay by adding an additional residual surface contact bioassay component.

The product should be applied throughout the cabin by trained staff as per the manufacturer's instructions. As per WHO disinsection guidelines, all toilet doors, garment storage lockers and overhead luggage storage compartments should be open, the air-conditioning switched off and the outer door closed.

Residual activity is evaluated 1 h after application (to allows further tests after the mosquito cage bioassay is completed) in WHO cones secured to sentinel sites in the aircraft. A series of WHO cones is placed on specified surfaces in three different areas of the main passenger cabin: in the middle row of seats, in the fifth row of seats from the front of the aircraft and in the fifth row of seats from the rear of the aircraft. For single-aisle aircraft, at each of these locations (mid, front, rear), the WHO cones are secured with strong, non-marking tape at

various places on the left and the right side of the cabin. As most droplets are expected to sediment onto horizontal surfaces, cones should be placed on the floor of the overhead luggage compartment, on the horizontal surface of the window seat and on the carpet beside the aisle seat. If required, additional cones can be secured at other locations or surfaces (e.g. toilets, galleys).

For dual-aisle aircraft, WHO cones are secured in each of the three locations (mid, front, rear) as above, with additional cones placed on the middle seat and on additional carpet in the central seating area.

3.2.2 WHO cone bioassays

One hour after treatment, 10 non-blood-fed, susceptible female mosquitoes aged 2–5 days are introduced into plastic WHO cones, which are secured in place with adhesive tape at each location in the cabin. Mosquitoes are exposed to the treated surfaces for 30 min and are then rapidly transferred into separately marked, clean holding cups, each with a 10% sugar solution on cotton wool, and held for 24 h at 27 °C ± 2 °C and 80% ± 10% RH. Knock-down is measured 60 min after exposure, and mortality is recorded after a 24-h holding period. A minimum of three replicates from separately reared batches are tested per location and the results pooled for analysis.

Before testing the residual effect of an insecticide product in an aircraft, a series of control WHO cone bioassays are conducted in the same manner and at the same locations. If mortality in the control group exceeds 20%, the results of the entire test should be rejected. If mortality in the control group is 0–20%, the results with the treated samples should be corrected with Abbott's formula (see section 2.1).

3.3 Insecticides for long-term residual activity[1]

Insecticide products intended for long-term residual activity in aircraft cabins and cargo holds are evaluated in the same manner as described in 3.2.2 above. WHO cone bioassays must be performed 24 h after spraying. Residual treatment should be conducted only by professional pest control operators.

Assessment of the long-term residual activity of the insecticide requires minimal interference with the treated surfaces (e.g. cleaning, routine maintenance), as interference can adversely affect the outcome of biological assays. An aircraft that is out of service should be used.

3.3.1 Study design for passenger cabins

Tests are conducted by placing a series of WHO cones on representative surfaces on aircraft in three areas of the main passenger cabin: in the middle row of seats, in the fifth row of seats from the front of the aircraft and in the fifth row of seats from the rear of the aircraft. At each of these three locations (mid, front, rear), WHO cones are well secured with strong,

[1] With some modifications, the guidelines can be used for monitoring efficacy of residual treatments in aircraft in-service.

non-marking tape on both the left and right sides of the cabin, at least on the floor of the overhead luggage compartment, on the vertical surface beside (or just above) the window and on the carpet. If required, additional cones may be secured on other locations or surfaces (e.g. toilets, curtains).

Residual activity can be measured by testing the treated surface weekly until the 24-h mortality is <80%. After residual treatment, controls can be performed at weekly intervals by fixing small pieces of non-permeable plastic sheeting onto the inside wall of the passenger cabin and placing six WHO cones onto the sheeting. Ten non-blood-fed, 2–5-day-old female mosquitoes are introduced per cone for 30 min, then rapidly transferred into separately marked, clean holding cups, each with a 10% sugar solution on cotton wool, and held for 24 h at 27 °C ± 2°C and 80% ± 10% RH.

If mortality in the control group exceeds 20%, the test is rejected. If mortality in the control group is 0–20%, the results with the treated samples are corrected with Abbott's formula (see section 2.1).

3.3.2 Study design for cargo holds

As the interior surfaces of cargo holds consist predominantly of a single material, fewer WHO cones are required for the test than recommended for passenger cabins. Tests are conducted by placing a minimum of six cones in different locations of the hold, with one cone fixed on each wall and two on the ceiling. Immediately before treatment, control bioassays are conducted in exactly the same locations and with the same number of cones to ensure that no residues remain on the surfaces. Residual activity can be measured by testing the treated surface weekly until 24-h mortality is <80%.

After residual treatment, controls can be performed at weekly intervals by fixing small pieces of non-permeable plastic sheeting onto a wall (at predefined locations) and by placing six WHO cones onto the sheeting. Ten non-blood-fed, 2–5-day-old female mosquitoes are introduced per cone for 30 min and then rapidly transferred into separately marked, clean holding cups, each with a 10% sugar solution on cotton wool, and held for 24 h at 27 °C ± 2 °C and 80% ± 10% RH. Mortality is recorded 24 h after exposure. If mortality in the control group exceeds 20%, the test is rejected. If mortality in the control group is 0–20%, the results with the treated samples are corrected with Abbott's formula (see section 2.1).

ANNEX 1. METHODS CURRENTLY RECOMMENDED BY WHO FOR AIRCRAFT DISINSECTION

Three methods are currently recommended by WHO for aircraft disinsection: 'blocks away'; preflight and 'top-of-descent' spraying; and residual treatment, This involves, in practice, four techniques.

Pre-flight: A pre-flight aerosol containing an insecticide with rapid action and limited residual action is applied by ground staff to the flight deck, passenger cabin including toilet areas, open overhead and side-wall lockers, coat lockers and crew rest areas. The spray is applied before the passengers board the aircraft but not more than 1 h before the doors are closed. A 2% permethrin cis:trans (25:75) formulation is currently recommended for this application, at a target dose of 0.7 g a.i./100 m^3. This requires application at 35 g of formulation per 100 m^3 to various types of aircraft, with a droplet size of 10–15 µm. Preflight spraying is followed by a further in-flight spray, i.e. top-of-descent as the aircraft starts its descent to the arrival airport.

Blocks away: Spraying is carried out by crew members when the passengers are on board, after closure of the cabin door and before the flight takes off. An aerosol containing an insecticide for rapid action is used. The air-conditioning system should be switched off during cabin spraying. The flight deck is sprayed before the pilot boards (when no passengers are on board). The doors of overhead luggage racks should be closed only after spraying has been completed. An aerosol containing 2% D-phenothrin is currently recommended by WHO and should be applied at a rate of 35 g of formulation per 100 m^3 (i.e. 0.7 g a.i./100 m^3). Cargo holds should also be disinsected.

Top-of-descent: Top-of-descent spraying is carried out as the aircraft starts its descent to the arrival airport. An aerosol containing 2% D-phenothrin is currently recommended by WHO for this purpose and is applied with the air recirculation system set at from high to normal flow. The amounts applied are based on a standard spray rate of 1 g/s and 35 g of the formulation per 100 m^3 (i.e. 0.7 g a.i./100 m^3).

Residual: The internal surfaces of the passenger cabin and cargo hold, excluding food preparation areas, are sprayed with a compression sprayer that has a constant flow valve and flat fan nozzle according to WHO specifications.[1] Permethrin 25:75 (cis:trans) emulsifiable concentrate is currently recommended by WHO at a target dose of 0.2 g/m^2 applied at intervals not exceeding 2 months. The emulsion is applied at 10 ml/m^2 to avoid run off. Residual sprays are applied by professional pest control operators and are intended for long-term residual activity on aircraft interior surfaces. In electrically sensitive areas, it may be necessary to use an aerosol instead of a compression sprayer. After treatment is completed, air-conditioning packs should be run for at least 1 h before the crew and

[1] *Equipment for vector control specification guidelines*. Geneva, World Health Organization, 2010 (also available at http://whqlibdoc.who.int/publications/2010/9789241500791_eng.pdf).

passengers embark to clear the air of the volatile components of the spray. Areas that undergo substantial cleaning between treatments require supplementary ‘touch-up’ spraying.

The pesticide formulations, including spray cans, should comply with national regulations and international standards as well as with WHO specifications for pesticides. Spray operations should follow international regulations and WHO recommended procedures and comply with quarantine requirements in the country of arrival.

ANNEX 2. EXAMPLE PRINT-OUT OF A 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 3. WIND TUNNEL SPECIFICATIONS

The wind tunnel (Figure A3.1) is constructed of galvanized duct pipe with an internal diameter of 15.2 cm. The entrance to the tunnel is covered with an end cap. A series of 30 1-cm holes, 1.27–2.54 cm apart and arranged in three concentric circles, are drilled in the end cap to reduce the volume of air pulled through the tunnel and to even flow across the tunnel. An eight-bladed fan and a 120-V AC variable speed motor are installed 366 cm from the tunnel entrance. The fan motor is connected to a powerstat, which is used to regulate the fan motor speed and the resulting air velocity in the tunnel.

The insects to be tested are retained in a screen cage made to fit the exact interior measurements of the wind tunnel, i.e. 15.2 cm diameter x 2.5 cm depth (Figure A3.2). The sides of the cylindrical disc cages are made of brass, with a 1.9-cm hole for introducing the insects. After introduction, the hole is sealed with a strip of masking tape, and plain paper is attached at the position of the hole to protect the mosquitoes from sticking to the tape. The tape is also used as a label.

A 1.22 x 1.6 mm mesh opening brass screen, thread diameter 0.28 mm, covers the ends. The screen is soldered only to the solid edge of the cages, so that there is no lip to block the free flow of the aerosol or to give protection to the insects. The cage is inserted in an opening 10 cm long and half the diameter of the wind tunnel. This opening is 91.5 cm from the wind tunnel entrance. A flexible clear plastic sheet is used to close the opening (Figures A3.3 and A3.4).

An atomizer delivering droplets of 15 ± 2 µm $Dv_{0.5}$ is mounted so that the nozzle is centred on a 2.5-cm hole in the end cap ($Dv_{0.5}$ represents the point at which half the volume of droplets is smaller). The nozzle is inserted about 2.5 cm into the wind tunnel. Because it is inert, nitrogen is used as the propellant. The pressure in the cylinder is reduced to 103.35 kPa by means of a valve and gauge mounted on the cylinder. Another valve and gauge are mounted near the wind tunnel to ensure that the pressure is maintained at this level. The air velocity of the wind tunnel is adjusted to 1.8 m/s by means of the powerstat.

The material to be sprayed is placed in a volume of 0.5 ml in the cup of the atomizer. Then the valve is turned on, allowing the insecticide to be sprayed into the airflow, so that the droplets are directed towards the cage. The valve is turned off 5 s after all the material has been sprayed (within about 3 s) or until the sound of atomization changes significantly.

Before testing each dose, a ‘blank’ or test without a cage of insects is run to expel from the system any material remaining from the previous dose. The wind tunnel is cleaned after each dose series by running three or four blanks of 0.5 ml of diluent through the system.

The most important parameters to be standardized are wind speed, tunnel diameter, cage size and droplet size distribution. Small deviations in the other parameters are generally acceptable.

The care and equipment decontamination procedures are as follows:

1. Wash in hot soapy water to remove as much oil and residue as possible. Rinse well and dry.
2. Rinse in solution (about 1–5%) of acetic acid and water.

3. Rinse in two consecutive technical acetone baths and dry.
4. Bake in an oven for 24 h at about 150 ^{o}C.
5. Bake glassware for a shorter time at a higher temperature.
6. Dispose of used wash solutions properly. The acetic acid solution should be changed every other day if the equipment is washed every day. If washing is sporadic, it should be mixed freshly each time. Acetone can be kept longer and changed as needed.
7. Decontaminate the wind tunnel between each treatment dose by applying 0.5 ml acetone or other suitable solvent through the nozzle. Repeat this procedure four times at the end of each experiment.

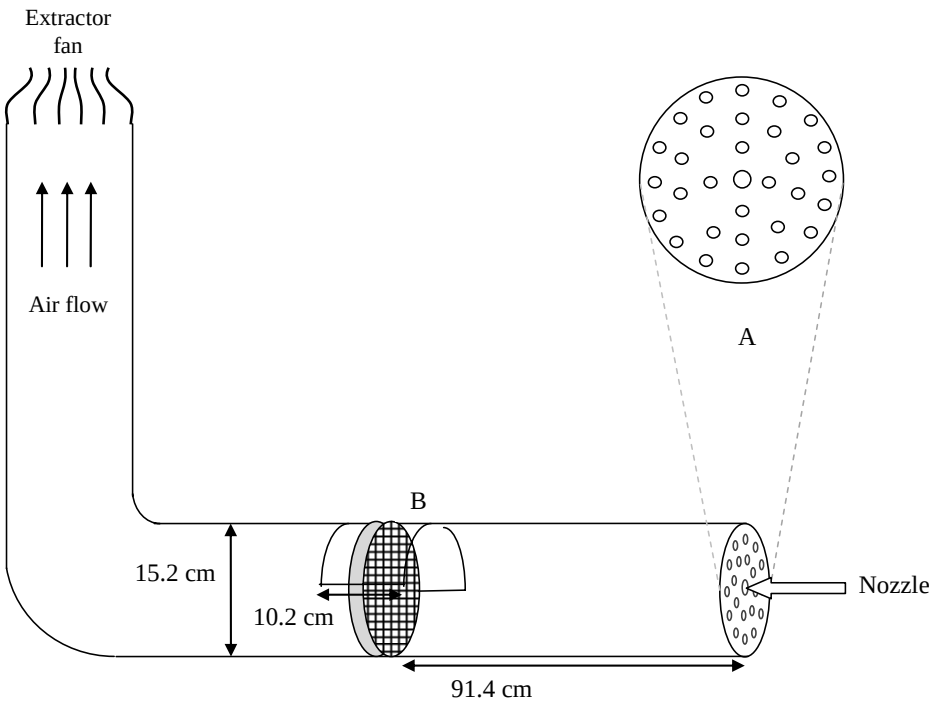


Figure A3.1. **Wind tunnel design**

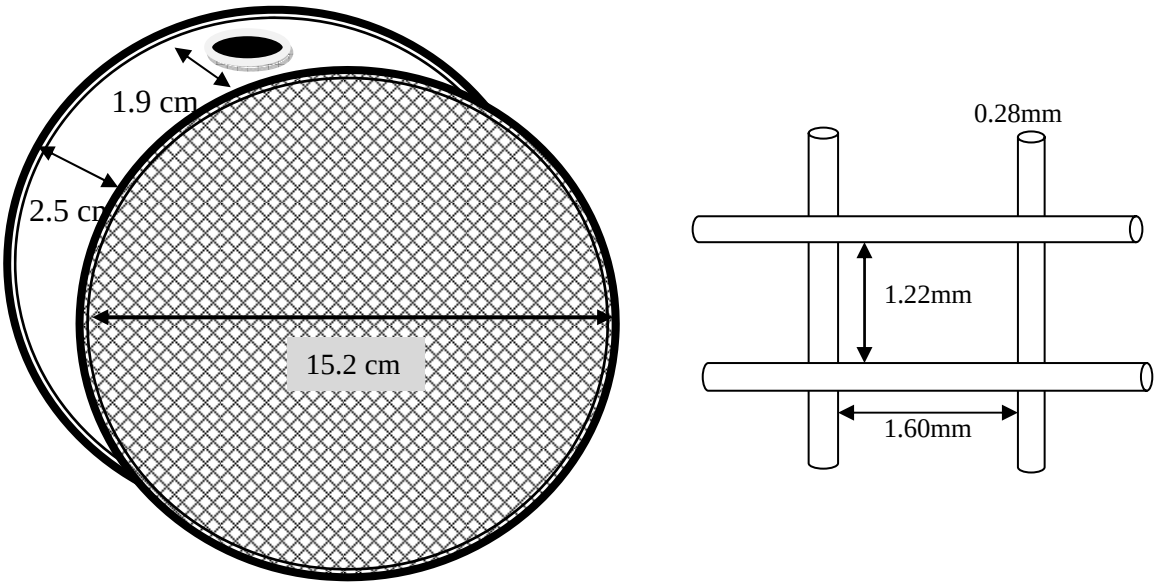


Figure A3.2. **Wind tunnel cage**

Figure A3.3. **Wind tunnel (courtesy of Dr Jane Bonds, Florida A&M University, Panama City, Florida, USA)**

Figure A3.4. **Sealed cage being positioned in wind tunnel (courtesy of Dr Jane Bonds, Florida A&M University, Panama City, Florida, USA)**

ANNEX 4. PEET-GRADY CHAMBER SPECIFICATIONS

The Peet-Grady chamber was designed to test the efficacy of household insecticide products.[1] A suitable testing room of any convenient size should be selected that is capable of holding the chamber, with adequate additional space to permit efficient performance of the tests.

The chamber has internal dimensions of 180 cm x 180 cm x 180 cm (Figure A4.1). It should be constructed from smooth internal wall panels made of either stainless steel, aluminium, glass or another suitable material to allow easy cleaning of insecticide or solvent residues.

A tight-fitting entrance door (approximately 165 x 90 cm) is fixed on one of the side walls. The chamber has a fluorescent light, as well as an exhaust fan in the ceiling to remove insecticide vapour after each test. Four hooks are fitted in the corners of the ceiling about 20 cm from the side walls to suspend test cages. For air circulation in the chamber, a 30-cm diameter fan with a flat dish of 30 cm diameter attached on top of the fan rail guard is placed on the floor of the chamber, facing upwards. Two glass observation windows and four mosquito introduction or utility windows are set in each of the side walls (see Figure A4.1) for easy introduction of insects and counting of those knocked-down during the test.

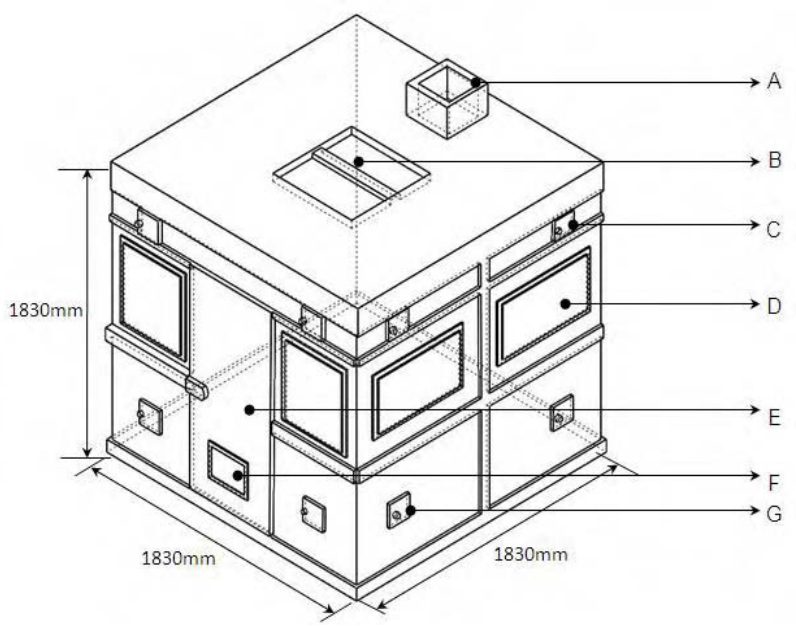
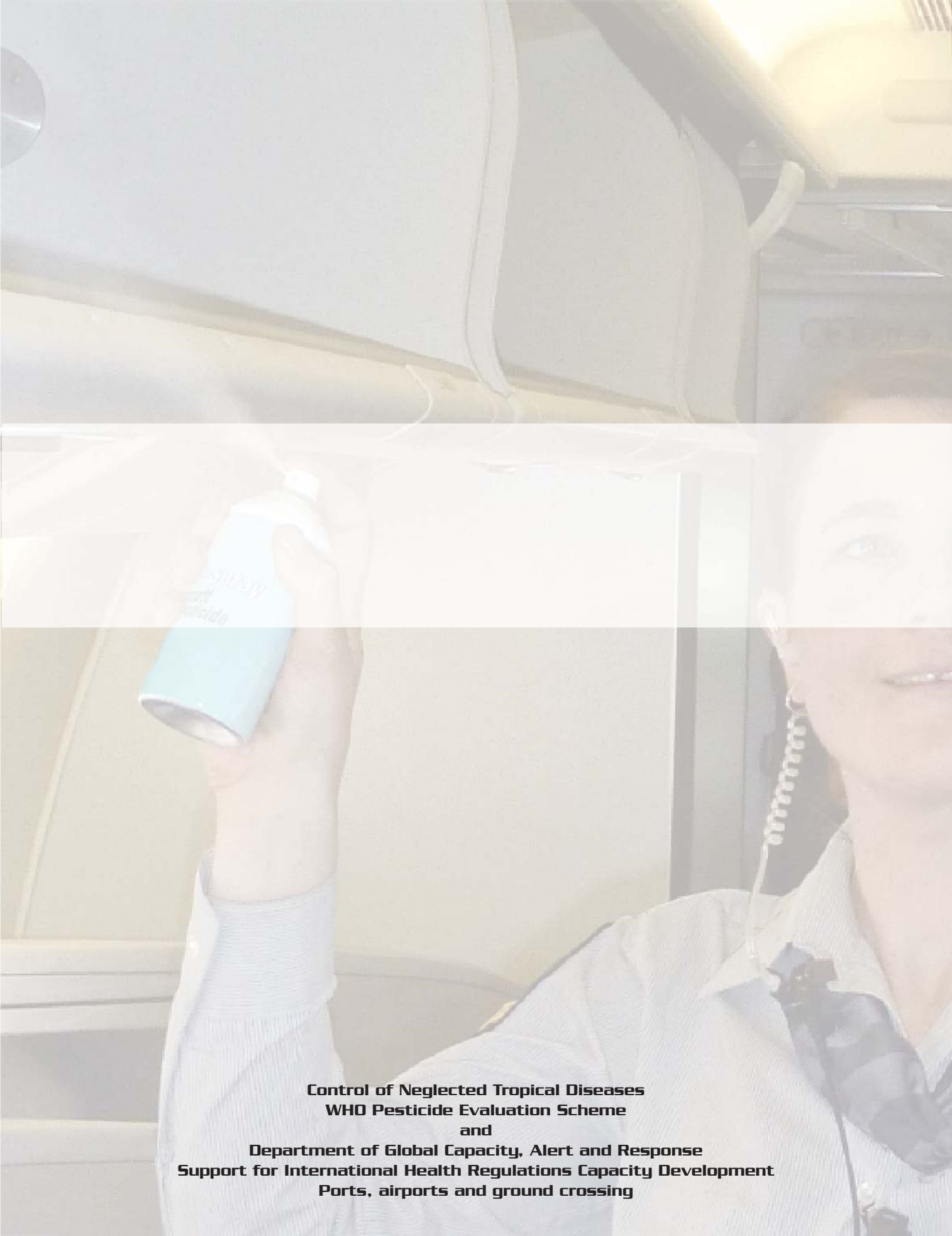


Figure A4.1. Peet-Grady chamber (A. Exhaust fan; B. Fluorescent light; C. Top introduction or utility window; D. Glass observation window; E. Entrance door; F. Insect introduction window; and G. Bottom introduction or utility window)

[1] Busvine JR. *A critical review of the techniques for testing insecticides*. Dorchester: Henry Ling Ltd, The Dorset Press, 1971:345.



Control of Neglected Tropical Diseases
WHO Pesticide Evaluation Scheme
and
Department of Global Capacity, Alert and Response
Support for International Health Regulations Capacity Development
Ports, airports and ground crossing