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AIRCRAFT DISINSECTION

**Eleventh Report
of the Expert Committee
on Insecticides**

	Page
1. Introduction	3
2. Insect quarantine procedures	4
2.1 Protection of airports from vectors	4
2.2 Recommendations for aircraft disinsection with aerosols	7
2.3 Specifications for aerosols	10
2.4 Vapour disinsection	11
3. Recommendations for future research and investigation	13
Annex 1. Glossary of terms used in this report	15
Annex 2. Tentative method for the bioassay of candidate aerosols for aircraft disinsection.	16
Annex 3. Test procedures for aerosols and aerosol dispensers	22
Annex 4. Aerosol formulations which have been found effective in practice	25

WORLD HEALTH ORGANIZATION

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EXPERT COMMITTEE ON INSECTICIDES

Geneva, 19-24 September 1960

Members :

Mr R. W. Bonhoff, Facilitation Representative, Deutsche Lufthansa A.G.,
Cologne, Germany

Dr R. A. E. Galley, Research Director and Manager, Woodstock Agricultural
Research Centre, Sittingbourne, England (until 30 June 1960 : Director,
Tropical Products Institute and Officer-in-Charge Research, Colonial
Pesticides Research, London)

Dr Marshall Laird, Associate Professor, Institute of Parasitology, McGill
University, Montreal, Canada (*Chairman*)

Dr J. Lembrez, Directeur du Service de Contrôle sanitaire aux frontières de
la 5^e Circonscription, Marseilles, France (*Vice-Chairman*)

Dr R. Pal, Deputy Director, National Malaria Eradication Programme
(NMEP), Malaria Institute of India, Delhi, India

Mr K. D. Quarterman, Chief, Technical Development Laboratories, Com-
municable Disease Center, US Public Health Service, Savannah, Ga.,
USA (*Rapporteur*)

Representative of ICAO :

Dr F. de Tavel, Medical Adviser, International Civil Aviation Organization,
Montreal, Canada

Secretariat :

Dr P. Bertagna, Research and Technical Intelligence, Division of Malaria
Eradication, WHO

Dr R. W. Fay, Environmental Biology, Division of Environmental Sanitation,
WHO

Dr M. J. Freyche, International Quarantine, Division of Communicable
Diseases, WHO

Mr J. W. Wright, Vector Control and Pesticides, Division of Environmental
Sanitation, WHO (*Secretary*)

AIRCRAFT DISINSECTION

Eleventh Report of the Expert Committee on Insecticides

The Expert Committee on Insecticides met in Geneva, Switzerland, from 19 to 24 September 1960. Dr P. Dorolle, Deputy Director-General, opened the meeting on behalf of the Director-General.

Dr Marshall Laird was elected Chairman and Dr J. Lembrez Vice-Chairman; Mr K. D. Quarterman was appointed Rapporteur. The provisional agenda was adopted.

1. INTRODUCTION¹

The ever-increasing volume of international air traffic, the constantly increasing speed and range of aircraft, the development of insecticide-resistant strains of disease-vector mosquitos and the world-wide activity in the eradication of *Aedes aegypti* and malaria have focused attention on insect quarantine problems in the public health field.

The Committee on International Quarantine in its fifth report² requested the Director-General to ask governments for information on the insecticidal formulations and procedures used or recognized by them for aircraft disinsection and to present the results of this inquiry to the appropriate bodies of the organization. Accordingly, a circular letter was sent in 1958 to Member States requesting them to provide information on their present aircraft disinsection practices.³

As a result of the replies received, the World Health Organization conducted a world survey in 1959 in which insect quarantine was discussed with health authorities, aircraft manufacturers, airline operators and pesticide research organizations. A report of this survey, together with accounts of the introduction of diseases by the accidental importation

¹ A glossary of terms used in this report appears in Annex 1.

² *Off. Rec. Wld Hlth Org.*, 1958, **87**, 399

³ *Off. Rec. Wld Hlth Org.*, 1959, **95**, Annex 1, Appendix 2 (Circular Letter C. L. 15, dated 20 August 1958)

of vectors,^{1, 2, 3} references to the vast literature on the transport of insects by aircraft,^{4, 5, 6, 7} and relevant WHO publications were made available to the Committee and formed the background against which it undertook its work.

Insect quarantine measures take two main forms, the first concerned with airport sanitation and the second with the disinsection of aircraft. It has long been known that too many international airports fall far short of the ideal with respect to freedom from insect vectors of disease. It is also known that at best aircraft disinsection can only be a compromise involving the relative risk of disease introduction, entomological efficiency, passenger comfort, facilitation, and safety of aircraft, since it is not permissible to put the pilot under any additional strain at his periods of peak stress, i.e., during take-off and landing.

These factors have all been taken into account in the following report.

2. INSECT QUARANTINE PROCEDURES

2.1 Protection of airports from vectors

In considering the protection of international airports from mosquito vectors, the Committee bore the following points in mind :

(a) To prevent the accidental extension of the range of yellow fever incidence, Article 20 (1) of the WHO *International Sanitary Regulations*⁸ requires that the area within the perimeter of every airport shall be kept free from *Aedes aegypti* in its larval and adult stages.

(b) Article 20 (3) of the *International Sanitary Regulations*⁸ defines the perimeter of an airport as " a line enclosing the area containing the airport buildings and any land or water used or intended to be used for the parking of aircraft ".

(c) The Eighth World Health Assembly commented upon the area of control as follows :

¹ Hoops, A. L. (1934) *Malay. med. J.*, **9**, 123

² World Health Organization (1955) *Int. Dig. Hlth Legis.*, **6**, 377

³ Soper, F. L. & Wilson, D. B. (1943) *Anopheles gambiae in Brazil, 1930 to 1940*, New York, The Rockefeller Foundation

⁴ Whitfield, F. G. S. (1939) *Bull. ent. Res.*, **30**, 365

⁵ Hughes, J. H. (1949) *Publ. Hlth Rep. (Wash.)*, **210**, 1

⁶ Duguet, J. (1949) *Bull. Wld Hlth Org.*, **2**, 155

⁷ Laird, M. (1951) *J. roy. aero. Soc.*, **55**, 735

⁸ World Health Organization (1957) *International Sanitary Regulations (Annotated ed.)*, Geneva, p. 17

“As regards airports, while it may be unnecessary to clear the full length of runways from *A. aegypti*, a quite extensive area of runways adjacent to the airport buildings should be cleared of this mosquito.

Although the runways and landing fields of an airport are not necessarily included in the perimeter, they may be so included, wholly or in part, if local conditions call for this.”¹

(d) The Committee on International Quarantine expressed the view in its third report² that “to keep the area within the perimeter of an airport free from *Aedes aegypti* in its larval and adult stages as required by paragraph 1 of Article 20, as amended, it is necessary to maintain active anti-mosquito measures within a protective area extending for a distance of at least 400 metres around that perimeter.”

(d) The fifth report of the Committee on International Quarantine³ stated that “The strongest defence against the carriage of mosquitos by . . . air is the rigid protection of . . . airports by antimosquito measures and the Committee endorses the recommendation of the Study Group on International Protection against Malaria⁴ that health administrations concerned ‘should be asked to take all reasonably possible steps to this end’.”

The efforts at present being made by WHO to assist governments in eradicating malaria and the success of many countries in eradicating *Aedes aegypti*, as well as the emergence of resistance in many mosquito vectors appeared to the Committee good grounds for devoting increased attention to the prevention of accidental transportation of mosquitos from one locality to another. The first step towards preventing such transportation is for all international airports to comply with the requirements and recommendations described above. If this were done throughout the world, the disinsection of aircraft would be relegated, as it should be, to a second line of defence, to guard against the possibility of dangerous insects flying into the control zone, or being carried into it, from the surrounding region.

However, the Committee emphasized that at present too many international airports, while satisfactory from the standpoint of yellow fever hazard cannot be so regarded as far as the other mosquito vectors of diseases of man are concerned. A generally disquieting state of affairs was revealed by the information placed before the Committee on the current status of vector control and the existence of larval habitats for

¹ *Off. Rec. Wld Hlth Org.*, 1956, **72**, 5

² *Off. Rec. Wld Hlth Org.*, 1956, **72**, 36

³ *Off. Rec. Wld Hlth Org.*, 1958, **87**, 413

⁴ Unpublished working document WHO/Mal/183 (8 January 1957), p. 19

mosquitos of public health importance at a number of international airports. Many of these habitats are so located as to present hazards of introduction or exportation of such insects.

The Committee was fully aware of the magnitude of the task of maintaining major airports as mosquito-free zones. Attempts are sometimes made to effect control measures needlessly over wide areas far beyond the physical capabilities and technical facilities of the personnel available for this task, with consequent inability to give the needed attention to the important areas in the immediate vicinity of the airport buildings and aprons. Apart from the chemico-biological aspects of the problem, administrative and practical difficulties may arise associated with the fact that aprons and terminal facilities are frequently located on one side of the airfield adjacent to highways, privately-owned domestic and business premises, or farm or forest land.

National health administrations will readily appreciate that, as long as inadequate mosquito control persists at international airports, the transporting of dangerous mosquitos to receptive areas in other countries or the establishment of dangerous species in their own territories will continue to be a constant and serious danger.

The Committee recommended that :

(1) WHO draw these facts to the attention of Member governments and urge them to initiate measures to correct the unsatisfactory situation prevailing at present ; and

(2) every effort be made by health authorities not only to implement the *International Sanitary Regulations*, which call for freedom from *Aedes aegypti* within the perimeter of the airport, but also to make that zone mosquito-free.

The Committee fully appreciated that it is impossible to achieve this without a high measure of mosquito control in the area beyond the mosquito-free zone, and accordingly further recommended that rigid mosquito control be undertaken in such areas beyond the mosquito-free zone as local conditions may demand.

To achieve these objectives, the Committee recommended :

(a) that mosquito control at international airports be considered as an integral part of the national health programme and that it be devised with the collaboration and advice of the national health authority (it would be of value if the national health authority would check on the effectiveness of the scheme at regular intervals) ;

(b) that on request WHO provide technical advice to governments on the planning and execution of vector control programmes at international airports ;

(c) that as far as possible control measures be of a permanent character, such as land reclamation and drainage, supported by such insecticidal measures as may be considered necessary (techniques involving chemicals have been recommended in the tenth report of the Expert Committee on Insecticides);¹

(d) that routine control be performed by a team or unit specially trained for the purpose, and that advice from WHO on this aspect should be provided, if requested.

The Committee was of the opinion that the establishment of an effective vector control organization at each international airport and the periodical publication by WHO of information on the progress made in making such airports mosquito-free would go a long way towards developing confidence between countries regarding insect quarantine.

2.2 Recommendations for aircraft disinsection with aerosols

The Committee reviewed the evidence that has accumulated since its seventh report in which recommendations were made against in-the-air disinsection of aircraft with aerosols. Notable examples were the report that during the year ending 30 June 1957, out of 1592 mosquitos found aboard aircraft arriving at Miami International Airport following in-the-air treatment with aerosols before arrival, 305 were still alive,² and the more recent advice from the Philippines Government that although its quarantine regulations specify in-the-air disinsection for incoming aircraft, collections made following landing, over a five-year period, showed that 84% of the mosquitos aboard were still alive.³

Replies to Circular Letter C. L. 15 (see page 3) indicated that, despite the ineffectiveness of in-the-air disinsection with aerosols, approximately 30% of the 111 countries replying require or accept this method of aircraft disinsection (6% with some reservations), while about 10% refuse to accept it in all or some instances.

The Committee reaffirmed the previous recommendation that in-the-air disinsection of aircraft with aerosols should not be recognized as complying with the requirements of the *International Sanitary Regulations*.

The Committee noted that, discounting those governments that have no disinsection requirements of any kind, some 60% of the remainder of those replying to the circular letter make provision for post-arrival disinsection. The Committee also considered (1) the frequent opportunities

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1960, 191

² Porter, J. E. (1958) *Florida Ent.*, 41 (1), 41

³ Contained in the reply of the Philippines Government to Circular Letter C. L. 15 (see page 3).

for vectors aboard aircraft to escape after the plane has landed and before disinsection could be accomplished (e.g., through open cockpit windows, from the wheel wells, or through the cabin door as it is opened to admit the Airport Health Officer or his representative), and (2) the undesirable delays in air transportation occasioned by post-arrival disinsection of aircraft, a problem that is being intensified by the rapidly increasing use of jet aircraft with their greater speed and higher operating costs.

The inadequacies of in-the-air disinsection with aerosols and the disadvantages associated with post-arrival treatment, together with the recognized opportunities for vectors to reinfest the aircraft after pre-departure treatment as currently practised, led the Committee to make the following recommendations to replace those made in previous reports of the Expert Committee on Insecticides :

1. Disinsection of the passenger cabin and all other accessible interior spaces of the aircraft, except the flight deck, should be done after the doors have been locked following embarkation and before take-off, this operation to be referred to as "blocks away" disinsection. As visualized by the Committee, single-use hand-operated aerosol dispensers would be used for this purpose. This would avoid the human error inevitably encountered in estimating the aerosol dosage applied with conventional dispensers, and would tend to avoid the multiplicity of dispensers (as well as formulations and dosages) at present in use. Such single-use dispensers would be designed to treat adequately the smaller types of aircraft now in service, and they would be used in multiples for larger aircraft, the number of dispensers to be used aboard each type of aircraft being stated on the label. The dispensers also would be serially numbered. This would permit the entry of serial number(s) by the Airport Health Officer or his representative on the Aircraft General Declaration. The national health authority may wish to delegate authority for the actual discharge of the aerosol to the aircraft operator. In such cases, instructions to this effect would be incorporated in the relevant manuals of the operator. The empty dispenser(s) would be suitably stored in the aircraft, and upon arrival at destination they would serve, together with the entries on the Aircraft General Declaration, as evidence of disinsection. As in current disinsection treatments, all possible mosquito-sheltering places inside the plane should be treated, including cupboards, chests and compartments for clothes, luggage and freight. Foodstuffs and utensils inside the aircraft should be protected from contamination.

2. The flight deck should be treated at a suitable time prior to expected occupancy by the flight crew, the door or curtains of this compartment being then closed and kept closed, except when opened momentarily to permit the passage of the crew members, until the "blocks away" treatment and the take-off of the aircraft are completed.

3. All parts of the aircraft accessible only from the outside and in which insects can find shelter, such as cargo holds and wheel wells, are to be disinfested as nearly as possible to the time the aircraft leaves the apron.

4. For the disinfection of aircraft, the aerosol should be dispersed uniformly throughout the treated spaces at the rate of 35 g of the formulation per 100 m³ (10 g per 1000 cu. ft) of enclosed space. Any aerosol formulation may be used when its biological effectiveness has been shown to be equal to or better than that of the standard reference aerosol (see Annex 2, Appendix 2, page 21) and the other characteristics of the alternative formulation and its dispenser have been shown to conform to the specifications contained in section 2.3 for aerosol formulations and dispensers. Other formulations that have been shown to be satisfactory in service are shown in Annex 4, page 25.

5. The "blocks away" disinfection procedure should be implemented as soon as possible as an interim measure pending the hoped-for early introduction of improved procedures, such as the vaporization method outlined in section 2.4.

6. WHO should encourage the commercial production of single-use aerosol dispensers suitable for aircraft disinfection as outlined above.

2.2.1 *Alternative aerosols*

In view of the ever-increasing prevalence of resistance to insecticides by various vectors of public health importance, the Committee recognized the need for alternative aerosols for aircraft disinfection. Little information is available on aerosol formulations suitable for use in aircraft against resistant vectors, and the Committee recommended that WHO sponsor investigations on the problem.

While recognizing that all governments may not wish to use the standard aerosol formulation recommended by WHO for aircraft disinfection, the Committee believed that it is highly important that no aerosol be used for aircraft disinfection that is not at least equal to the recommended formulation. To this end, the Committee recommended:

(a) that WHO bring to the attention of Member States the Committee's recommended specifications for aerosols, with particular reference to biological performance;

(b) that, to encourage the use of effective aerosols only, WHO distribute to all Member governments Annex 2 of the Committee's report;

(c) that WHO be prepared to make available upon request a standard reference aerosol and a suitable strain of the house-fly for carrying out the bioassay of aerosol formulations.

2.2.2 General

The Committee noted with great satisfaction the potentialities of disinsecting airborne aircraft with insecticidal vapours (see section 2.4, page 11). This would be a vast improvement over existing procedures, and it is hoped that it can replace the use of aerosols for aircraft disinsection in the reasonably near future.

2.3 Specifications for aerosols

The Committee reconsidered the specifications for aerosol formulations recorded in the seventh report of the Expert Committee on Insecticides¹ and reaffirmed:

(a) the principle that permits manufacturers to use their discretion in the choice of suitable solvents, propellents and insecticides, provided the resulting aerosol conforms to the required standards; and

(b) the general requirements of the insecticidal formulation and the design of the dispenser.

The present Committee, however, considered that the insecticidal formulation and its dispenser should be regarded as a single unit required to produce the aerosol.

General. The dispensers may be either a single-shot or a multiple delivery non-refillable type having a capacity not exceeding 490 cm³, with the valve protected against accidental discharge. They must comply with the regulations of governments and of IATA² relating to the carriage of restricted articles by air. The insecticidal formulation must be free from deposit or suspended matter when cooled to -5°C (23°F) or to the lowest temperature encountered in the filling operation, whichever is the lower. The aerosol produced must be non-inflammable, free from human toxicity risks, and non-injurious to materials used in aircraft construction.

Rough usage. The dispenser must not leak when tested as described in Annex 3, section 1 (page 22).

Heat stability. No leakage, distortion or other defect shall arise when the dispenser is tested as described in Annex 3, section 2 (page 22).

Discharge rate. The dispenser shall discharge the formulation as an aerosol at the rate of 1.0 g ± 0.2 g per second.

Crazing effect. Crazing of stressed polymethyl methacrylate plastic (Perspex, Plexiglas) must not occur when the aerosol is tested in accordance with the method described in Annex 3, section 3 (page 22).

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1957, 125, 20

² International Air Transport Association (1960) *Restricted Articles Regulations*

Biological performance. The insecticidal action of an aerosol produced from its dispenser shall not be inferior to that of the standard reference aerosol (SRA) produced from its dispenser when tested by the bioassay method described in Annex 2 (page 16).

*Standard reference aerosol*¹

The SRA shall have the following formulation :

	<i>Percentage by weight</i>
Pyrethrum extract (25% pyrethrins)	1.6
DDT technical	3.0
Xylene	7.5
Odourless petroleum distillate	2.9
Dichlorodifluoromethane	42.5
Trichlorofluoromethane	42.5

The net weight of the formulation must be indicated on each container.

SRA dispenser. The dispenser of the SRA shall produce an aerosol complying with the following physical requirements when tested by the method described in Annex 3, section 4 : (a) not more than 20% by weight of the aerosol shall consist of droplets of diameter greater than 30 μ ; (b) not more than 1% by weight of the aerosol shall consist of droplets of diameter greater than 50 μ .

2.4 Vapour disinsection

Although the use of aerosols is the only treatment generally acceptable for aircraft disinsection at this time, the Committee recognized that the method has a number of limitations. Some of the major weaknesses in the aerosol method are : the ineffectiveness of aerosols applied in airborne aircraft ; the difficulty of ensuring that treatment has been carried out ; the delay encountered in airline operations under the application procedures at present practised ; the necessity of treating the baggage holds, the wheel wells and the passenger and crew compartments separately ; and passenger complaints of irritation. The principal reasons why in-the-air aerosol treatments are ineffective are :

- (a) they are rapidly removed from the aircraft through ventilation ;
- (b) they impact on various objects in the aircraft ;
- (c) they fail to penetrate into many parts of the aircraft where insects may be resting.

¹ The SRA may be obtained from the World Health Organization, Geneva.

In contrast to aerosol particles, vapours follow the natural laws for the dispersion of gases. The vapour will penetrate to all required portions of the aircraft and, if an adequate concentration is maintained for a sufficient period of time, will exert its insecticidal effect. The problem of utilizing insecticidal vapours for aircraft disinsection thus resolves itself into two parts :

(a) finding a compound of sufficiently high insecticidal activity to be effective against insects at concentrations that are safe for aircraft passengers and crews, i.e., a compound that vaporizes rapidly at relatively low temperatures, that has no objectionable odour or irritant qualities, that leaves no hazardous or objectionable residues, and that is not deleterious to aircraft construction material ;

(b) developing a simple, effective dispensing system that will produce and maintain the insecticidal vapours at the desired concentrations in the aircraft throughout the required exposure time.

The Committee reviewed recent research reports¹ indicating that O,O-dimethyl O-(2,2-dichlorovinyl) phosphate (DDVP) is a compound which appears to meet all the foregoing requirements and that a satisfactory mechanical system has been developed for its use in vapour disinsection of aircraft in the air. A prototype of one such mechanical dispensing system developed by the United States Public Health Service at their Technical Development Laboratories at Savannah, Georgia, USA, was demonstrated to the members of the Committee, who were impressed by its performance and its potentialities for aircraft disinsection. Not only is vapour disinsection of aircraft more effective than treatment with aerosols against insect vectors of disease, and hence more acceptable to governmental health authorities, but it can also be used for in-the-air disinsection, thereby eliminating the undesirable delays associated with the current use of aerosols and making vapour disinsection more acceptable to the airlines both from the point of view of facilitation and from that of passenger comfort. The Committee recognized, however, that further development work must be accomplished before this highly promising method can be universally adopted. The Committee was of the view that once the remaining development work has been completed, in-the-air vapour disinsection should be adopted as rapidly as possible as the method of choice for disinsecting aircraft.

¹ These reports will be published shortly in the *Bulletin of the World Health Organization*.

3. RECOMMENDATIONS FOR FUTURE RESEARCH AND INVESTIGATION

The Committee's discussion on vapour disinsection (section 2.4, page 11) revealed that more work was necessary on a number of aspects of the use of this technique, including :

- (1) increasing the capacity of the vapour dispenser in order to treat satisfactorily the new larger aircraft coming into commission ;
- (2) developing a device by means of which the port health authority at the airport of arrival can be certain that the dispenser has functioned ;
- (3) investigating the possibility of making even more simple than it is now the introduction of the insecticide-vapour-laden air into the aircraft ;
- (4) seeking new insecticides with the required toxicological, chemical and physical properties as alternatives to DDVP to safeguard against the development of resistance to DDVP by insect vectors of disease likely to be carried by aircraft ;
- (5) obtaining further data, if the Committee of Experts on Toxicology considers this necessary, on the hazards to man associated with the use of DDVP in aircraft ;
- (6) undertaking further trials on the time required for DDVP vapours to effect 100 % knockdown of exposed mosquitos ;
- (7) undertaking further trials of the vapour disinsection technique in airborne aircraft in different parts of the world with different disease vectors.

The Committee recommended that, if necessary, WHO should encourage or sponsor investigations to provide the required information at the earliest possible date.

Until it is possible to equip new aircraft with the vapour dispenser or to fit it into existing aircraft during a major overhaul, it will be necessary to continue to use aerosols for disinsection as described in section 2.2, page 7. The Committee was of the opinion that, in view of the imminent introduction of the vapour technique, so far as the use of aerosols for quarantine purposes in aircraft is concerned only the minimum of additional work is called for. Such work would include as the main items :

- (a) the development of alternative formulations for use in areas where resistance to one or more insecticides has arisen ;
- (b) such studies as may be required to improve, if necessary, the bio-assay technique for comparing candidate aerosol formulations and dispensers with the standard.

Looking further ahead, when high altitude, supersonic flight becomes a reality, aircraft will be subjected to high and low extremes of temperature. Information will be required concerning the possibilities of insect survival under these conditions. Investigations on this subject should be encouraged, and at a later date perhaps sponsored, by WHO, but the Committee was of the opinion that in the immediate future the maximum possible effort should be applied to perfecting the vapour distribution technique.

Annex 1

GLOSSARY OF TERMS USED IN THIS REPORT

Aerosol dispenser. A container holding a pressurized formulation which produces an insecticidal aerosol when the valve is opened. (Note : the use of the earlier term "aerosol bomb" should be discontinued.)

"Blocks away" disinsection. This expression, referring to the removal of the blocks from the wheels immediately before departure, is used to denote the timing of aircraft disinsection which occurs in the interval between the locking of the aircraft doors and actual take-off.

Disinsection ; disinsecting. The words "disinsecting", "disinsectization" and "disinsection" have been used to describe the operation in which measures are taken to kill the insects present in aircraft or ships. It has been decided that, for this report, the operation will be referred to as "disinsection" or "disinsecting" in the English text, and as "desinsectisation" in the French text.

Insect quarantine. This expression is used in this report to denote all the operations associated with preventing the unintentional importation of insects into a territory or their exportation from it.

"In-the-air" treatment. In general aviation usage, the term "in flight" includes the full period from an aircraft getting "off blocks" at departure to "on blocks" on arrival. The expression "in-the-air" treatment is used to specify treatment at that part of a flight between actual take-off and landing.

Annex 2**TENTATIVE METHOD FOR THE BIOASSAY OF CANDIDATE
AEROSOLS¹ FOR AIRCRAFT DISINSECTION**

The test method described herein is recommended as a standardized technique for the appraisal of the relative insecticidal effectiveness of aerosols to be used in aircraft disinsection.

1. Test insects

The test insects must be reared under uniform conditions to minimize variability in vigour, such as is encountered in field-collected specimens or in specimens reared under variable conditions. Factors important in the production of uniform specimens include temperature, humidity, breeding medium, food, and space. A susceptible strain of the house-fly, *Musca domestica*, is required, and suggested methods for rearing this species are outlined in Appendix 1 (page 19). In areas where *M. domestica* does not occur, other suitable susceptible species of *Musca* may be used (e.g., *M. nebulosa*). If needed, a suitable strain of the house-fly to establish a colony may be obtained from WHO.

Flies of the same age (3-6 days) and in a similar state of nutrition must be utilized for all tests.

2. Test chamber

A. *Size.* The volume of the test chamber should be not less than 29 m³ (1000 cu. ft) and not more than 86 m³ (3000 cu. ft). The ceiling height should be not less than 2 m (7 ft) and not more than 2.5 m (8 ft). (These dimensions are approximate, and need not be adhered to strictly.)

B. *Conditions.* It is desirable that the test chamber be conditioned to maintain a temperature variation of approximately 2°C (4°F) and a relative humidity variation of not more than 20%. Temperatures in the range of 24°C-30°C (75°-85°F) and relative humidities of 50%-70% are suitable. Where controlled conditions are not available, the conditions for comparative tests must be similar. Heating elements for chambers should be of a type that does not cause excessive convection currents within the chamber.

¹ The insecticidal formulation and its dispenser shall be considered as a single unit to produce the aerosol (see section 2.3, page 10).

C. *Illumination.* A light intensity of 10-foot candles at 1 m (3 ft) above floor level is suggested. Two 150-watt incandescent bulbs will illuminate properly a chamber of 29 m³ (1000 cu. ft) volume. To facilitate the capture of live flies, it is desirable to incorporate a method for dimming the lights so that the illumination can be reduced by approximately 95%.

D. *Surface covering.* The chamber floor should be covered with uncontaminated paper and the walls and ceiling provided with non-absorbent washable surfaces or a renewable surface.

E. *Ventilation.* The chamber should be so equipped as to provide 1/6 to 1 1/2 air exchanges per minute, the chamber intake and exhaust openings being such that the air velocity in the chamber does not harm the test insects. In a chamber of 29 m³ (1000 cu. ft) volume, a velocity of 180 m (600 ft) per minute has been found satisfactory. The intake openings should be located so that the air is exhausted from all parts of the chamber.

3. Test insecticide

A standard reference aerosol (SRA), according to the specifications given in Appendix 2, page 21, may be obtained from WHO.

4. Test procedure

A. *Chamber preparation.* The test chamber must be free from excessive contamination with insecticidal residues. A test for contamination should follow each day's aerosol tests, using 30-minute exposures of 100 female flies under the usual test conditions, except the application of the aerosol. A chamber is considered excessively contaminated when the 16-hour mortality of the exposed flies exceeds 10%. The walls and ceiling must be thoroughly cleaned whenever excessive contamination is found. To minimize contamination, the chamber floor must be cleaned after each test. Temperature and humidity conditions in the chamber must be checked before each test.

B. *Calibration of dispenser and dosage delivery.* The approximate delivery rates of the dispensers for the standard reference and the candidate aerosols must be determined before use. This is accomplished in the following steps: (1) weigh the container, (2) discharge the aerosol for five seconds (use a stopwatch), (3) reweigh, (4) calculate delivery rate, (5) repeat procedure twice more and average results. The dispenser should be calibrated at the same temperature at which it is to be tested; the desired test dosage is then delivered on a time basis. The actual amount discharged in each test is determined by weighing the dispenser before and after use. A dispenser containing less than 20% of its original content should not

be used, since with repeated discharges the proportion of propellant to non-volatile components changes.

C. *Dosage and exposure period.* The dosage employed with the SRA should provide an average 24-hour mortality between 60% and 90%, with a 10-minute exposure period. With house-flies of the CSMA¹ strain, an average dosage of 6 g/29 m³ (1000 cu. ft) has given mortalities within that range. The candidate aerosols shall be applied at the same rate as the SRA. The aerosol should be released by the operator while walking around in the chamber. The dispenser should be held far enough from all surfaces to avoid wetting them with the aerosol.

D. *Insect sample.* Each exposure should be made with approximately 100 adult female flies. If both sexes are employed, approximately 250 specimens should be used per test. If individual cages are used to hold each test sample of flies, each cage should be stocked with sufficient pupae to permit about 10% of the adults to remain in the cage when the insects are liberated. If the test samples of adults are taken from a stock cage, the final 10% of the flies should be discarded. This minimizes the possibility of including non-flying adults in the test.

E. *Exposure of the insects.* The test flies are liberated five minutes before the discharge of the aerosol. The exposure period is started with the discharge of the aerosol. These procedures should be the same in all tests.

F. *Collection of insects.* At the end of the exposure period, ventilation of the chamber is begun, and is continued during the interim between successive tests. Two minutes after ventilation has been started, the operator should dim the lights and begin the collection of the test insects. Any convenient method of picking up the flies without injuring them may be used. A vacuum device with a by-pass to control the degree of suction is recommended. This device should draw the flies into a receptacle large enough to prevent crowding. The flies are placed in holding cages with food and held under uniform conditions for 24 hours, after which mortalities are determined. The same types of collection equipment and holding containers must be used for flies exposed to both the candidate and reference aerosols.

5. Evaluation of results

A test series shall consist of at least eight replicates each of the SRA and the candidate aerosols. An equal number of tests on an alternating sequence should be made each day with the SRA and each of the candidate

¹ Chemical Specialties Manufacturers Association

aerosols. Female flies only should be considered in evaluating test results. All flies unable to walk or fly when observed at the end of the 24-hour period should be recorded as dead.

To assess the efficiency, the percentage mortality from each aerosol is determined by dividing the total number of dead female flies by the total number of female flies in the eight or more replicates. A candidate aerosol is considered equal to the SRA if the percentage mortality is within 5% of the SRA. Comparisons between a candidate aerosol and the SRA can be made only when tested in the same series. It must be emphasized that the basis of these tests is comparative, and the mortality range employed is not to be interpreted as a satisfactory kill.

A specimen evaluation sheet is shown as Appendix 3, page 21.

6. Discussion

Although it would be most desirable that all countries should utilize identical test methods, deviations from the method described herein, with regard to the size of the chamber, test insect, etc., may be made *as long as the conditions employed for testing both the SRA and the candidate aerosol remain the same in each series of tests.*

This recommended test method is for use with flying insects only and does not apply necessarily to other insects, such as roaches, fleas and lice. In general, the use of the house-fly as test insect will give lower mortality values than will be encountered with mosquitos. However, the house-fly is recommended primarily because of the greater ease with which it can be reared and maintained as compared to mosquitos. In addition, aerosols that are effective against flies will be satisfactory against mosquitos, whereas the reverse is not always true.

Appendix 1

SUGGESTED METHOD FOR REARING HOUSE-FLIES

Test house-flies should be from a susceptible strain¹ which has been under laboratory production for at least two generations. This procedure avoids the use of field-caught adults of unknown age and vigour, and the use of eggs from adults of unknown age. The following method has been used successfully for rearing and maintaining house-fly colonies :

Rearing room. The rearing room may be of any convenient size, constructed so as to be free from strong draughts and provided with light for 12 or more hours each day. Temperature and relative humidity should be kept constant, as far as possible, preferably $27^{\circ} \pm 1^{\circ}\text{C}$ ($80^{\circ} \pm 2^{\circ}\text{F}$) and $50\% \pm 5\%$ respectively. Any departure from these conditions must be maintained for all insects used in a given test series.

¹ If needed, a suitable strain of the house-fly to establish a colony may be obtained from WHO.

Colony cages. Colony cages shall have at least three sides of mesh screening (between 250 and 100 mesh per inch, or between 100 and 40 mesh per cm), the size being such as to provide 1-2 cu. in. (approx. 15-30 cm³) of space per fly. The colony cage for each generation shall be stocked with at least 1000 pupae on two consecutive days to provide against inadvertent selection. A new generation shall be established each week.

Adult food. Either a liquid or solid diet containing milk and sugar should be available at all times. A solution consisting of 5% spray-dried non-fat milk solids and 2% sugar (15 ml per 100 flies) has been found adequate. Powdered whole milk and sugar cubes are sufficient, provided that the flies are provided with an independent source of water. Specimens to be used for test purposes only, i.e., not for egg production, may be maintained on sugar and water alone.

Egg collection. Eggs should be taken from a generation between the second and eighth days of egg-laying to avoid (a) the selection of only fast-developing adults and (b) the unfavourable aspects of eggs from aged adults. Each generation is discarded only after the succeeding generation has been laying eggs for two days. Suitable material for oviposition, such as larval-rearing medium or sour milk pads, should be provided. Eggs must be collected at intervals not exceeding 16 hours. Numbers of eggs should be determined volumetrically in water so that, after thorough mixing, appropriate numbers can be placed in each larval-rearing container. If freshly-hatched larvae are used to seed the rearing containers, estimates of numbers can be made gravimetrically.

Larval-rearing medium. Any satisfactory rearing medium may be used, some examples being given in the table below :

Medium A (% w/w)		Medium B		Medium C * (% w/w)	
Wheat bran	24.0	Dried yeast (D.C.L. brand or similar)	100 g	Wheat bran and alfalfa meal	31.0
Grass meal	6.0	Full cream dried milk	100 g	Yeast suspension**	4.5
Bakers' yeast suspension **	0.8	Agar	20 g	Non-diastatic diamalt	1.5
Soya flour	2.0	Water	1 litre	Water	63.0
Malt extract with cod-liver oil B. P.	3.0				
Water	64.2				

* Standard rearing medium recommended by Chemical Specialties Manufacturers Association (CSMA).

** Yeast suspension should be prepared by dispersing 230 g of yeast in 1 litre of water. The mixture can be stored at 0°C (32°F) and used as required.

The rearing medium may be prepared 1 to 16 hours before seeding. It should be placed in containers that retain fermentation heat, but temperatures in excess of 57°C (135°F) should be avoided. Rearing containers should be stored at least 1 inch apart on shelves to avoid overheating of the medium. Any medium is suitable if the time for larval development is 4-6 days. The rearing technique may be taken to be satisfactory if the average weight of pupae is constant. The same rearing medium and containers should be used consistently throughout the production of flies for a given series of tests.

Pupae. Mature larvae migrate to the top portion of the medium or to a layer of vermiculite, dry sand, or similar dry agent if it is added on top of the medium four to five days after seeding. Normally, pupation is complete by the seventh day after seeding eggs. The top layer of the medium, or the layer of vermiculite or sand, containing the pupae, should be removed and the pupae separated mechanically by winnowing or sifting. All pupae collected on a given day should be combined into one lot, mixed, and measured into units by predetermined weight or volume. Units of pupae are placed in

a cage that provides at least 1 cu. in. (approx. 15 cm³) of space per adult fly. Pupae for stocking new colony cages are handled in a similar manner. Under normal rearing conditions at least 80 adult flies should be obtained from each 100 eggs seeded.

Appendix 2

STANDARD REFERENCE AEROSOL

The standard reference aerosol shall have the following formulation :

	<i>Percentage by weight</i>
Pyrethrum extract (25% pyrethrins)	1.6
DDT technical	3.0
Xylene	7.5
Odourless petroleum distillate ¹	2.9
Dichlorodifluoromethane	42.5
Trichlorofluoromethane	42.5

The net weight of the formulation must be indicated on each container.

Appendix 3

AEROSOL EVALUATION SHEET (WHO)

Candidate Aerosol No. _____ Date _____
 SRA No. _____ Laboratory _____
 Test insect _____ Investigator _____

Test	SRA				CA			
	g/29 m ³	Total	Dead	Mortality %	g/29 m ³	Total	Dead	Mortality %
Average								

Conclusion : CA is inferior — equal — or superior to SRA

Remarks : _____

¹ Now more commonly called odourless kerosene

Annex 3

**TEST PROCEDURES FOR AEROSOLS AND
AEROSOL DISPENSERS****1. Rough-usage test**

Drop the full dispenser under its own weight through a height of 75 cm (30 inches) on to a hardwood surface, so that it receives the impact (a) end down, (b) top down, and (c) sideways.

2. Heat-stability test

Immerse the full dispenser in a water-bath until the contents of the container reach 54°C (130°F). This may be accomplished by immersing the full container for three minutes in a water-bath held at 60°C (140°F). Leaks may be detected by bubbles issuing from the surface of the container.¹

3. Crazing test

(To be carried out at a room temperature of 20°C ± 5°C (68°F ± 9°F).)

A strip of heat-treated polymethyl methacrylate plastic (Perspex, Plexiglas) of good quality (as used for aircraft) and about 18 cm × 2.5 cm × 0.6 cm (7 inches × 1 inch × 1/4 inch) in size must be clamped in a horizontal position as a lever with a fulcrum 5 cm (2 inches) from the clamp. At the free end remote from the clamp, at a distance of 10 cm (4 inches) from the fulcrum, a load of 1.2 kg (2.6 pounds) shall be applied. A spray of the material under test shall be directed from a nozzle held at 2.5 cm (1 inch) above the fulcrum so that the surface of the plastic is thoroughly wetted. After 24 hours, during which the ambient air temperature shall be 20°C ± 5°C (68°F ± 9°F), the plastic shall be wiped clean and examined for crazing at varying angles of incident light.

4. Determination of droplet size of aerosols

A method that is satisfactory for determining the droplet size of insecticidal aerosols is to deposit a sample on a glass slide and to measure the droplets under a high-power microscope. In this manner, droplets of relatively non-volatile materials can be measured before they evaporate.

¹ Purchasers wishing to receive dispensers with temperature stability exceeding 54°C (130°F) should state their requirements when placing the order. The test at the higher temperature is performed under conditions similar to those described here.

To prevent excessive spreading, filming, or coalescence, the slide may be coated with an oleophobic substance that will cause the individual droplets to maintain their convexity to some degree. Two satisfactory materials for this purpose are a 1% alcoholic solution of mannitan monolaurate,¹ and a 2% solution of a silicone in carbon tetrachloride.² The slides are first immersed in a cleaning solution, dried, then immersed in the oleophobic coating solution, and redried. The dry slides should be lightly polished with a soft cloth and stored in ordinary slide boxes for several days before they are used.

4.1 *Deposition of droplets on slides*

The aerosol sample may be deposited by impingement or by settling, but as the second method is generally limited to droplets below 20 μ in diameter details are provided for the more generally applicable impingement method only.

4.1.1 *Impingement*

With the aerosol projected horizontally, the slide, held at arm's length, should be inserted in the spray in a plane perpendicular to the axis of the spray and at a distance of about 1.5 m (5 feet) from the dispenser. The slide should then be moved as rapidly as possible towards the dispenser in an arc and be brought out of the spray again at arm's length. To avoid excessive deposits an exhaust fan—capacity 1133 litres per second (2400 cubic feet per minute)—may be used to draw the aerosol past the point at which the slide is waved. The velocity at which the slide is waved is not critical. A good slide should have only 1/1500 of its surface covered.

The waving procedure may be modified by using an impactor consisting of a small variable-speed motor equipped with a counter-balanced slide-holder. The slide rotates on a 10-cm (4 inch) radius in a direction perpendicular to the shaft of the motor. The motor unit is mounted at the centre of a tube 53 cm (21 inches) in diameter and 91 cm (36 inches) long, and air containing the aerosol is drawn through with a ventilating fan. A fan with a capacity of 1133 litres per second (2400 cubic feet per minute) has been found satisfactory for this purpose. The maximum speed at which the slide can be rotated to obtain a good sample is approximately 800 revolutions per minute, which is equivalent to about 30 km (19 miles)

¹ Marketed by the Atlas Powder Company, Wilmington, Del., United States of America, and by Honeywill-Atlas Ltd, Great Britain, as G-772.

² Solution marketed by Midland Silicones Ltd, London, England, as MS 1208; silicone alone marketed by General Electric Company, Schenectady, New York, United States of America, as Dri-film 9987; both silicone and solution marketed by Hopkins and Williams Ltd, Great Britain.

per hour. At greater speeds, the large aerosol droplets are found to move across the slide becoming distorted so that their true size cannot be determined. For all types of commercial aerosols, a speed equivalent to 16 km (10 miles) per hour is recommended. The aerosol should be released at a distance of 120 cm (4 feet) from the impactor, using about 0.3 g of total aerosol. Air should be drawn past the impactor at approximately 20 km (12 miles) per hour.

4.2 *Measurement of droplets*

Using a microscope with eyepiece micrometer and mechanical stage, measure at least 200 droplets from the lenses they produce on the slide. The slide should be moved by the stage and measurements made at various points, from one edge of the slide to the other, of all droplet-lenses that pass through the micrometer scale, avoiding the smaller sized groups congregated along the margin of the slide. Express the number of droplets of each size (correct the diameter as described in section 4.3) as a percentage of the total and plot the cumulative percentage less than a given size against size on an arithmetic probability scale. This gives the droplet-size distribution in terms of the number of droplets. To convert the result to a mass basis, read from this graph the mean diameter in each 5% interval in the cumulative percentage. Take the *cube* of this diameter to represent the mass in the size-range bounded by the 5% interval. Express the mass in each size-range as a percentage of the total and plot the cumulative percentage mass against the size as before. Read off the diameters corresponding to 80% and 99% cumulative mass.

This method of deriving the mass-distribution curve is approximate only. Where the difference between the two bounding sizes of the 5% intervals in the first curve are large, greater accuracy in the final result can be obtained by taking 2.5% or even 1% intervals among the larger sizes. In calculating the contribution of each interval to the total mass, allowance must then be made for the varying widths of the intervals.

4.3 *Procedure for correcting the measured diameter of droplets*¹

Use a compound high-power microscope with a flat mirror; remove the condenser; use an external source of light; focus on the droplets; measure and record the exact diameter, setting the reading on the fine-focus adjustment at zero.

Manipulate the coarse-focus adjustment and mirror until some distant object (e.g., window frame) is in as sharp a focus as possible, using the droplet as a lens; then focus downwards with the fine-focus adjustment

¹ See: May, K. R. (1945) *J. sci. Instrum.*, **22**, 187.

until the droplet is in clear focus. The difference between the zero fine-focus adjustment and the final fine-focus adjustment is the focal-length change.

Correction factors *

$\frac{f'}{2A}$	Correction factor
1.48	0.60
1.55	0.55
1.80	0.50
2.3	0.45
3.3	0.40
4.8	0.35
7.0	0.30

* Interpolation is permissible, if necessary.

Calculate the ratio $\frac{f'}{2A}$

where f' = focal-length change, as determined above,

and $2A$ = measured diameter of the lens produced by the droplet.

Look up the corresponding correction factor in the adjoining table and multiply the lens diameter by this factor to obtain the true diameter of the droplet.

Example : The diameter of a droplet-lens covering 4 divisions in an eyepiece micrometer (one division = 15.4 μ) is $4 \times 15.4 \mu$, or 61.6 μ .

With a focal-length change of 206 μ , the ratio $\frac{f'}{2A}$ for this droplet is $206/61.6$, or 3.3. With this ratio of 3.3, the correction factor for the droplet-lens is 0.40 (see table) and the true diameter of the droplet is $61.6 \mu \times 0.40 = 24.6 \mu$.

Annex 4

AEROSOL FORMULATIONS WHICH HAVE BEEN FOUND EFFECTIVE IN PRACTICE

<i>Formulation G-382</i>	<i>Percentage by weight</i>
Pyrethrum extract (20% pyrethrins)	5.0
DDT	3.0
Cyclohexanone (water-free)	5.0
Lubricating oil (SAE 30)	2.0
Dichlorodifluoromethane (Freon-12 or Genetron-12)	85.0
 <i>Formulation G-651</i>	
Pyrethrum extract (20% pyrethrins)	6.0
DDT	2.0
Aromatic petroleum derivative solvent (Velsicol AR 60 or Socony Vacuum 544G)	8.0
Dichlorodifluoromethane (Freon-12 or Genetron-12)	84.0

<i>Formulation G-1029</i>	<i>Percentage by weight</i>
Pyrethrum extract (20% pyrethrins)	6.0
DDT	2.0
Aromatic petroleum derivative solvents :	
Velsicol AR 60 or Socony Vacuum 544G	6.0
Velsicol AR 50 or Socony Vacuum 544C	2.0
Trichlorofluoromethane (Freon-11 or Genetron-11)	25.2
Dichlorodifluoromethane (Freon-12 or Genetron-12)	58.8

<i>Formulation G-1152</i>	
Pyrethrum extract (20% pyrethrins)	5.0
DDT	3.0
Cyclohexanone (water-free)	5.0
Lubricating oil (SAE 30)	2.0
Trichlorofluoromethane (Freon-11 or Genetron-11)	25.5
Dichlorodifluoromethane (Freon-12 or Genetron-12)	59.5

NOTE : The above formulations are generally used by the United States Public Health Service at the rate of 18 g per 100 m³ (5 g per 1000 cubic feet), delivered in 7-10 seconds by the average dispenser.

<i>Formulation CMR/IDC/1</i>	
Pyrethrum extract (25% pyrethrins)	1.6
DDT	3.0
Xylene	7.5
Odourless petroleum distillate	2.9
Dichlorodifluoromethane	42.5
Trichlorofluoromethane	42.5