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**EXPERT COMMITTEE ON  
INSECTICIDES**

**Seventh Report**

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WORLD HEALTH ORGANIZATION

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## EXPERT COMMITTEE ON INSECTICIDES

### Seventh Session

Geneva, 10-17 July 1956

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## **EXPERT COMMITTEE ON INSECTICIDES**

### **Seventh Report \***

The seventh session of the Expert Committee on Insecticides was held in Geneva, Switzerland, from 10 to 17 July 1956. Dr P. Dorolle, Deputy Director-General of the World Health Organization, opened the meeting on behalf of the Director-General.

Dr S. W. Simmons was elected Chairman and Dr C. Mofidi Vice-Chairman; Dr J. A. Reid was appointed Rapporteur. The provisional agenda was adopted.

### **1. RESISTANCE OF INSECTS TO INSECTICIDES**

#### **1.1 International collaborative programme of research on the resistance of insects to insecticides**

Arthropod-borne diseases are very prominent among health problems of concern to Member States of the World Health Organization. Although the advent of DDT and other modern insecticides has resulted in great progress in the control of such diseases as malaria, typhus fever, plague, filariasis, leishmaniasis, onchocerciasis, bartonellosis and shigellosis, the incidence of these infections is still extremely high in many parts of the world.

Initial results from the use of modern insecticides indicated that the majority of vector-borne diseases of man could be controlled effectively, and in some instances eradicated. With the development of resistance by house-flies, certain species of mosquitos, lice and fleas to chlorinated

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\* The Executive Board, at its nineteenth session, adopted the following resolution :  
The Executive Board

1. NOTES the seventh report of the Expert Committee on Insecticides ;
2. THANKS the members of the Committee for their work ;
3. AUTHORIZES publication of the report ;
4. CONSIDERS that this report should be circulated as widely as possible ;
5. CONSIDERS that work in this field should be continued in view of its major importance in the control of insect-borne diseases.

(Resolution EB19.R13, *Off. Rec. Wld Hlth Org.*, 1957, 76, 4)

hydrocarbon insecticides, this optimistic outlook has been somewhat modified. Nevertheless, in spite of the resistance problem, the improved health and subsequent social and economic benefits which have resulted, and are presently being attained, from the use of modern insecticides are outstanding. These gains have stimulated intensive efforts to assure continuation and extension of the control of vector-borne diseases. The principal technical impediment to the success of such efforts is now the problem of insecticide resistance.

As the problem of resistance is growing much more rapidly than are measures to deal with it, the stage has now been reached where a greatly accelerated effort, organized on an international basis, is needed to cope with it. Few practical answers to resistance in the field are available at present, and the purpose of this meeting of the Committee has been principally to study the present status of the resistance problem, to suggest how an international effort could be organized and to indicate the lines of research and measures that are needed.

The Committee noted with satisfaction that staff members and consultants of WHO, during 1955/56, made an extensive, preliminary survey of laboratories throughout the world to ascertain what research was being conducted on the resistance problem and to assess the existing potentialities for research. The results clearly indicated a deficiency in the efforts being expended on this very important problem. Only laboratories that were known or believed to be working on insecticide resistance were visited; even so, about one fifth of them are conducting no work whatsoever relating to the public health aspects of resistance. In most instances the work is confined to field observations to detect resistance or to the random screening of chemicals against resistant species. Research on the problem was largely on a part-time basis, and the number of laboratories with adequate equipment and a staff trained to do much-needed basic research is extremely small. Some workers are not familiar with research in other parts of the world, owing to the lack of adequate exchange of information and the inadequacy of publication channels. Even published material is often not available to workers in remote regions. This lack of information contributes to duplication of effort, increased costs and lowered efficiency.

The Committee, after considering the magnitude and importance of operational programmes for the control of insect vectors of diseases, concluded that the proportion of funds devoted to research on the resistance problem is entirely inadequate and out of line with sound economic practice.

Vector control programmes are being sponsored and financed not only on a national but also on an international basis. Several international agencies, including WHO, are lending support to the eventual global

eradication of malaria and the control of vector-borne diseases. This same international approach must be taken towards the support of research work if the success of the operational programme is to be ensured. The Committee suggested that WHO is an appropriate body to assume leadership in the stimulation and co-ordination of an international research programme on the resistance problem. It considered that a programme of this nature is necessarily an undertaking of several years' duration.

Among the important activities of the co-ordinating agency are the following :

1.1.1 *The collection and dissemination of information on the insecticide resistance problem*

The Committee recommended that WHO act as a depository for information from all sources and undertake its subsequent distribution to research workers. WHO might also stimulate a more extensive direct exchange of reports and visits of personnel between various research establishments. In order to assure the regular receipt of information, a continuing contact with, and occasional reminders to, laboratories might be necessary. The Committee recognized the great deficiency in prompt publication of research information and suggested that WHO might investigate the possibility of improving the situation.

1.1.2 *The promotion of needed research*

After a review of the status of the resistance problem and of information collected on laboratories throughout the world, it was clear that deficiencies existed in all phases of research on the resistance problem. It was the opinion of the Committee, however, that the most serious deficiencies were in basic research, particularly in those fields concerned with the mechanism of resistance—namely, physiology, biochemistry and genetics.

The deficiency of information on biology and ecology was noted by the Committee. A fuller understanding of the biology and ecology of vector species might disclose those behavioural and other characteristics which play a part in giving rise to resistance in the field. It might often be possible with such knowledge to design better and more economical operational patterns and to delay the development of potential resistance.

1.1.3 *The facilitating of procurement of personnel and funds*

The Committee took note of the almost universal scarcity of highly qualified research workers as well as of the shortage of research funds. It was apparent that these two deficiencies did not always occur in the same countries. Some countries had highly qualified people available for

basic research but insufficient funds for their employment in this way, whereas in a few other countries funds were not as serious a limiting factor as the unavailability of such qualified personnel. It was suggested that even relatively small grants for equipment or personnel by WHO to laboratories with adequate leadership and inadequate finances would be a sound investment towards the solution of the resistance problem.

#### 1.1.4 *The adoption of standard test methods for use in control programmes*

The Committee was of the opinion that standardized testing techniques for detecting resistance in all arthropods of public health importance are necessary. The use of such techniques would provide an accurate appraisal of the resistance problem throughout the world and early information on potential problem areas. It would also provide a most satisfactory means of verifying reports of suspected resistance. However, if these tests are to be comparable and meaningful, they must be standardized and used universally. The Committee strongly recommended that WHO encourage and support research on the devising of such techniques and sponsor their routine use in vector control programmes.

Another standard field test which would be very valuable in control programmes is a standard bio-assay method for determining the toxicity of residual deposits. The Committee recommended that WHO investigate the possibility of standardizing such a test, and also simplified chemical tests for the field evaluation of the amount of insecticide present on treated surfaces.

All techniques should be thoroughly field-tested by different organizations in various geographical areas before being recommended for general use.

#### 1.1.5 *The procurement and testing of new insecticides*

The world-wide contacts of WHO often enable it to obtain early information on newly developed insecticides. The judicious distribution of these data and, in some instances, of samples to co-operating laboratories for testing would facilitate the early use of worth-while materials.

#### 1.1.6 *Liaison activities*

It was suggested that liaison visits to selected laboratories would do much to stimulate the research needed and to induce the dissemination of the results obtained. Results of the preliminary survey of research laboratories indicate gaps in the research programme and suggest those laboratories that are potentially able to fill them. Visits to these establishments in particular would appear to be especially indicated and would

do much to build a balanced research programme for the elucidation of the resistance problem.

#### 1.1.7 *Meetings and conferences*

The Committee noted with satisfaction that WHO intends sponsoring an early conference of directors of the major laboratories able to participate in the collaborative programme of research. This will ensure better international co-operation in the research effort on the resistance problem. Regional meetings of research workers would further aid in the formulation and execution of the programme.

### 1.2 **Present status of resistance among insect vectors of disease and methods for detecting resistance**

#### 1.2.1 *Definitions*

For the purposes of this report, the term "resistance" is considered to refer solely to developed resistance, be it physiological or behaviouristic. The Committee noted that on many occasions the status of resistance (especially of behaviouristic resistance) of certain insects had been misinterpreted, owing to the lack of distinction between natural and developed characteristics. The Committee was interested to note a helpful statement on the matter made in the sixth report of the Expert Committee on Malaria.<sup>1</sup> In order to clarify the question, the Expert Committee on Insecticides adopted the definition of resistance put forward at the Symposium on the Control of Insect Vectors of Disease held in Rome in 1953,<sup>2</sup> with certain small amendments, so that the full definition now reads as follows:

"Resistance to insecticides is the development of an ability in a strain of insects to tolerate doses of toxicants which would prove *lethal* to the majority of individuals in a normal population of the same species. The term 'behaviouristic resistance' describes *the development of the ability to avoid a dose which would prove lethal*".<sup>3</sup>

#### 1.2.2 *The present status of resistance*

The Committee examined some documents giving lists of insecticide-resistant strains of insects of public health importance. It was agreed

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1957, **123**, section 8.2

<sup>2</sup> *Chron. Wld Hlth Org.*, 1954, **8**, 129

<sup>3</sup> The italics indicate the features of the adopted amendment (e.g., "harmful" changed to "lethal").

that, in many parts of the world, various insects of public health importance have developed high levels of physiological resistance to insecticides. This obviously constitutes a serious threat to the control of insect-borne diseases. These lists provided confirmed evidence of resistance in 10 different disease-vector species. The Committee noted that resistance to chlorinated hydrocarbon insecticides is becoming universal in the house-fly, that it is now of frequent occurrence in the body-louse, that a definite physiological resistance exists locally in at least four species of *Anopheles*, and that DDT-resistance has been found in *Aedes aegypti*, two species of *Culex* and one of *Triatoma*. In addition, there is evidence of resistance in at least 27 other species of insects of actual or potential public health importance. However, in 15 of these instances, the reports have not been sufficiently substantiated.

### 1.2.3 *Methods of detecting and measuring resistance*

The Committee agreed that, as already stated in section 1.1.4 (page 6), in order to evaluate fully the extent and importance of various instances of resistance, it is very desirable to agree upon methods of detecting and measuring physiological resistance which could be universally adopted. The need for such tests is of primary urgency for *adult* and *larval* stages of mosquitos. Subsequently, tests could be developed with advantage for *other insects of public health importance*. The present situation with regard to these tests and the measures recommended by the Committee are as follows :

(1) *Adult mosquitos*. A test method for measuring the resistance of mosquitos was described and advocated by the Expert Committee on Malaria in its fifth report.<sup>1</sup> At the recent sixth meeting of that Committee it was stated that there was "no reason at the present time to modify its opinion. It recognizes, however, that techniques are developing and that others may prove equally valuable or perhaps more applicable to simple laboratory circumstances. It would therefore welcome a careful comparison of the methods of Busvine & Nash and of Fay et al., as well as further development of field testing methods. The further standardization of the Busvine & Nash test by the supply of specially prepared materials from a central source deserves exploration".<sup>2</sup>

The Expert Committee on Insecticides considered the remarks of the Expert Committee on Malaria in this matter and suggested that appropriate members of the Expert Advisory Panel on Insecticides be requested to compare the methods mentioned. This group could possibly derive

<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1954, **80**, 30

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1957, **123**, section 8.4

an improved technique incorporating the best features of both methods, together with some recent suggestions. It was recommended that this work be expedited as far as possible.

(2) *Larval mosquitos*. It may prove somewhat easier to meet the need for a standard method of measuring resistance levels in mosquito larvae, since the common methods already in use are not very dissimilar. Accordingly, the Committee felt able to recommend a tentative method based on a fusion of existing techniques which should be circulated to appropriate panel members for trial and comment, with a view to adopting a form of this test as a standard method.

(3) *Other insects of public health importance*. A test for measuring resistance to insecticides in body-lice had already been developed by WHO in 1953. The test kit has been widely used, and the results obtained to date were examined by the Committee and found to be very valuable in appraising the resistance situation in body-lice.<sup>1</sup> Certain slight modifications suggested as a result of wide field experience were adopted.

In view of the need for tests applicable to other insects of public health importance, the Committee recommended that information be collected on possible test methods. These methods should be put at the disposal of appropriate persons for evaluation and trial, with a view to eventual standardization.

#### 1.2.4 *The uses of a standard test method*

It seems desirable to explain some of the ways in which a standard test method can be used to provide information regarding the emergence and status of resistant strains which might develop in response to insecticidal treatment.

(1) Where an organized insecticidal campaign is being planned, measurements should be made of the initial level of susceptibility of the vector species. This should be done at different places and at different times of the year, so that possible normal variations in susceptibility may be detected. These findings should be taken into consideration in evaluating the results of subsequent tests and deciding whether insecticide resistance has developed.

(2) Where a focus of resistance has already developed, tests should be made in different areas to determine the geographical extent of the strain and to find out whether it is spreading into new areas.

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<sup>1</sup> See: Wright, J. W. & Brown, A. W. A. (1957) *Bull. Wld Hlth Org.*, **16**, 9.

(3) A test that provides a reasonably accurate measurement of the level of resistance should be used as a laboratory method to determine the possibilities of cross-resistance to other insecticides. The information provided will be highly important for selecting possible alternative insecticides which may be employed. Determination of levels of resistance to a range of related insecticides may also reveal a characteristic "resistance spectrum", which might suggest a resistance similar in type to that shown by the house-fly or other cases previously studied.

#### 1.2.5 *Bio-assay and chemical tests for residual deposits*

The Committee felt that the rate of deterioration of deposits of residual insecticides may possibly have some bearing on the development and maintenance of resistance. For example, it has been suggested that deposits giving inadequate kills only<sup>1</sup> may be a contributing cause of resistance.

So far as this is a genetical problem, it would be better investigated in the laboratory (see section 1.3.2, page 14). Field observations may also be important, but they are handicapped by lack of standard methods for measuring the decline of activity of residual insecticides. This has been pointed out by the Expert Committee on Malaria,<sup>2</sup> which hoped that it might be possible for the Expert Committee on Insecticides to bring about some standardization of the methods involved.

The Expert Committee on Insecticides, endorsing the views of the Expert Committee on Malaria, recognized the importance of chemical methods, especially in controlling the application of residual deposits. It considered that chemical determinations of old deposits might well be misleading as measures of biological efficacy, especially where sorption phenomena are important. The Committee agreed further with the Expert Committee on Malaria that bio-assays are also necessary and considered that a simple bio-assay technique for determining the efficacy of residual deposits on walls should be standardized. The results obtained by such a method would, in many instances, be a more useful guide than those obtained chemically.

The Committee recommended that, in order to implement the proposal made in section 1.1.4 (page 6), appropriate members of the Expert Advisory Panel on Insecticides be requested to investigate the possibility of standardizing both the chemical and biological field tests.

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<sup>1</sup> Often referred to incorrectly as "sublethal doses".

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1957, **123**, section 6.1.5

### 1.3 Biological aspects of resistance

#### 1.3.1 *Physiology and biochemistry*

Since resistance is, in most cases, primarily the expression of a shift in the physiological and biochemical capacities of an insect population for dealing with a poison, studies in these fields are essential for detailed understanding of the mode of action of insecticides and of resistance mechanisms. From knowledge thus obtained, one hopes to discover logical means of combating resistance directly, by the use of chemicals designed specifically to nullify the protective changes that have occurred. In addition, such studies are a source of promising leads towards new control agents with modes of action differing from those to which resistance has developed; and, finally, these studies may serve also to reveal other biological weak spots against which alternative control measures could be directed.

The biochemical and physiological line of approach has been rather actively pursued during the past several years, with the production of considerable information concerning the nature of DDT resistance in house-flies. Enzymic degradation of the insecticide appears to be a major factor in most instances, but is evidently seldom the sole cause. Some evidence has been adduced for several other contributory mechanisms, but in general these have been less fully investigated and the data are subject to various interpretations. However, indications are that DDT-resistance is probably not a simple phenomenon, and that a simple physiological solution of the problem is therefore unlikely.

Relatively little is known about the mode of action of other chlorinated insecticides and about pyrethrins<sup>1</sup> and their analogues. Knowledge about the organo-phosphorus compounds is also limited, though most workers believe that their primary toxic action involves inhibition of the enzyme, cholinesterase, in the central nervous system. Resistance has developed to representatives of all these classes of chemicals, yet in no case is the mechanism clearly defined, and currently only moderate effort is being devoted to these problems.

In order to keep physiological understanding in step with resistance developments at the rate at which these are now occurring, it is estimated that at least some four or five times the present effort would be required, but lack of appreciation of the urgency and of the essentially practical nature of fundamental research on these problems, on the part of some administrative and budgetary authorities, is a hindrance to attainment of this objective at the present time.

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<sup>1</sup> For the purposes of this report, this word is used to cover the biologically active complex mixture of substances present in the extract of pyrethrum flowers.

Among specific problems that should receive prompt attention, the Committee wished to draw the notice of WHO to the following :

(1) A broadening of scope is urgently needed : there should be more research with agents other than DDT. Thus, for example, it is most desirable to have full information about the mode of action and mechanism of resistance with organo-phosphorus compounds, both for flies and for mosquitos.

(2) Cross-resistance within this class of compound should also be thoroughly investigated.

(3) Studies of resistance mechanisms should likewise be extended to a wider array of vector species, since the bulk of work to date has been confined to the house-fly.

(4) Attempts should be made at an early stage in a control programme to determine whether a vector is likely to develop resistance. Detoxification and other mechanisms of resistance should be fully explored.

(5) There is also a great need for more extensive study of the fundamental physiology of normal as well as resistant strains and species. A comparative physiological approach, involving a range of species, is regarded as extremely promising, but has hardly been attempted.

### 1.3.2 Genetics

There is at present no acceptable evidence of resistance arising through processes other than genetic selection. Therefore, studies on the genetics of resistance are essential to provide understanding of how exposure to an insecticide operates to convert a normally susceptible population to a resistant one, and thus to supply information that will permit forecasting (a) the probability of development of resistance in situations where it has not yet occurred ; (b) the rate at which resistance develops under pressure from the insecticide and its rate of spread into a surrounding population from an initial focus ; and (c) the probable extent and rate of regression towards normal susceptibility when insecticidal pressure is relaxed. Genetic knowledge would be of great assistance in planning control operations in such a manner as to avoid the development of resistance in so far as it is possible, or at least to prolong the time required for its development.

Considering the potential significance of genetic information for the understanding of resistance, the work being done is entirely inadequate. Genetic studies to date have been concerned almost exclusively with artificially induced resistance of low degree in *Drosophila* and with DDT-resistance in the house-fly.

The information so far collected on the inheritance of resistance is nevertheless sufficient to allow some generalizations to be made. In all observed cases, the hybrids between susceptible and resistant strains possess a partial or total advantage over the susceptible strains in the presence of the insecticide. This advantage often appears greater if tested at a low dosage level. In all thoroughly studied cases, covering widely different insects resistant to different insecticides as a result of field treatment, it has been ascertained that the resistance involves one major gene, though others may contribute; reliable evidence of multifactorial inheritance of resistance is provided only in laboratory selection experiments. Some suggestions that the genes involved in determining resistance might be homologous in different species of the same genus have been produced. There are no critical data in favour of cytoplasmic inheritance; however, evidence for maternal effects does exist.

The application of genetics to the problem of resistance is possible at present only to a very limited extent. The principal obstacle is the almost complete lack of basic background information on the genetics of species in which resistance is a matter of practical concern. For many of these species, even techniques for the laboratory rearing necessary for any experimental genetic studies are not available. These deficiencies will have to be remedied, wherever possible, if the potential contributions of genetics to the problem of resistance are to be realized.

The lack of background information on the genetics of insects other than members of the genera *Drosophila* and *Habrobracon*, and the absence of suitable rearing techniques, have to a large extent frustrated efforts to interest professional geneticists in working on the resistance problem. Also, the professional geneticist is usually unfamiliar with the techniques of entomology and toxicology; conversely, most entomologists are unfamiliar with the elaborate techniques often required for producing satisfactory genetic information. It is clear, therefore, that this is a field in which collaboration must be achieved, either at the training level or in actual experimental operation, if any worth-while expansion of investigations is to be made.

The Committee invited the attention of WHO to the following list, which outlines a number of typical problems which are of importance in connexion with the resistance phenomenon, and which should be submitted to genetic analysis:

- (1) Is resistance to several different compounds due to multiple effects of a single gene or to distinct genes?
- (2) What are the genetic basis and the biological significance of the delay sometimes involved in development of resistance, and of the subsequent rapid rise in resistance?

(3) Is the localized appearance of resistance in certain widely distributed species due to genetic or to other causes?

(4) Is resistance to a given compound in different populations of the same species due to the same or to different genes?

(5) To what extent do environmental factors affect the resistance of individuals of known genetic constitution?

(6) What is the relative competitive power of (a) homozygous susceptibles, (b) heterozygotes and (c) homozygous resistant, from the same population in the presence or absence of insecticides?

(7) What is the genetic basis of resistance to organo-phosphorus insecticides, to dieldrin and to other insecticides not yet investigated in this respect?

(8) What can be learned about the inheritance of resistance from comparative genetic studies of related strains and species, and is there any correlation between the spontaneous mutation rate of species and their capacity for becoming resistant?

(9) Can any judgement be reached, on genetic grounds, as to the relative efficacy of long-lasting versus short-lasting residual deposits in inducing resistance?

*Cytogenetics.* Cytogenetic studies, such as those already carried out on *Anopheles maculipennis*, should be encouraged, since there are indications that changes in the relative frequency of visibly distinct chromosome types might be associated with the development of resistance, as they are known to be with other competitive advantages.

*Uniform terminology.* In addition to experimental studies on questions such as those mentioned above, there is need for development, in genetic studies on insecticide resistance, of common terminology and symbols, preferably in conformity with accepted practice in genetics.

*List of available strains.* It would also be valuable if WHO would maintain and disseminate to interested laboratories a list of the genetic strains that are available in various research institutions. Additionally, when it is contemplated that a strain will be discarded, participating laboratories should be notified, so that they could take over maintenance of the culture if they should so desire.

### 1.3.3 Ecology and biology

The deficiency in ecological and biological studies has already been noted (section 1.1.2, page 5). There are numerous examples where such information is practically indispensable for the conduct of control

operations, both with resistant and normally susceptible species, and the Committee believes that such data may also be invaluable in gauging the likelihood of development of resistance and in devising measures for dealing with it when it occurs.

*Ecological observations on mosquitos.* The Committee recommended that thorough ecological observations be carried out, if possible, before the initiation of a mosquito control programme. The data will then constitute a basis for the detection of any changes in the pattern of behaviour that may occur with the development of resistance. Such observations should include reliable estimates of population numbers—which still requires development of satisfactory methods—and of the proportion entering sprayed and unsprayed buildings. This proportion can only be determined by quantitative knowledge of the resting and feeding habits, within buildings and outside. These are the principal factors in behaviour that determine what proportion of the population makes contact with the insecticide and the degree of contact realized; thus they have a direct influence on the selection pressure and hence on the rate of development of resistance. The Committee stressed the importance of adequate estimates of these factors.

*Ecological observations on other vectors.* For comparable reasons, similar data should be obtained for all insect vectors of disease.

*Larviciding versus adulticiding.* Another type of problem in which ecological considerations are of importance is the question as to whether larviciding or adulticiding is more likely to cause resistance, and through what mechanism. The Committee considered that this problem should be investigated both in the field and in the laboratory, and that the influence of differences in species and in ecological conditions should be determined.

*Variations in behaviour.* Although, in certain situations, reasonably adequate ecological information is available for a few vector species, there are many others for which this is not so; and there are few recorded data on how and why the activities of a particular species may vary from one locality to another, though the fact of such variations has sometimes been reported. These variations may be concerned in the sporadic occurrence of resistant foci within the distribution of certain species and are no doubt partly responsible also for some of the confusion that at present surrounds the question of behavioural resistance. More thorough ecological observation would help to clarify these important problems. Attention may also be called to the general need for methods for the quantitative measurement of behavioural resistance.

*The influence of behaviour on mortality.* Information is needed on the relationship between the susceptibility of various species, as measured by standard tests, and the mortality obtained in practice, as measured for example in window trap-huts, to determine whether discrepancies are due to species differences in behaviour with respect to the insecticide or to some other cause.

*Laboratory culture of vectors.* Mention has been made above, in connexion with genetic studies, of the need for development of methods for the laboratory culture of vector species, including resistant strains. This requirement is just as essential for physiological and biochemical studies, and even for the routine laboratory screening of insecticides, not to mention studies in disease transmission; the successful colonization of such species can only be accomplished as the result of careful ecological observation. Furthermore, it is necessary that such rearing procedures should be carefully standardized, in order to minimize experimental variability and thus produce comparable data. Here again, the optimal procedure can be ascertained only through ecological study.

*Sources of ecological data.* Like the geneticists, ecologists as a group have hitherto taken little interest in the problem of resistance. It seems probable that better publicizing of the urgency of the problem, and of the importance of the type of information sought, might stimulate co-operation from this group. It is believed also that much information of the kind needed exists already, in a scattered and often unpublished form, among experienced field entomologists in various parts of the world. Some means of tapping this buried source of knowledge would be of immediate help in the resistance problem. These entomologists and others in similar situations could probably also be encouraged readily to extend their observations and make them more generally available, if they were made aware of the potential significance of such data.

The Committee emphasized the obvious fact that a great deal of this ecological information can be obtained only in areas where the insect vectors in question are indigenous.

#### **1.4 Possibility of alternative control methods where resistance has developed**

The Committee, in considering alternative methods, was well aware of the need for a comprehensive account of all vector control methods now in common use throughout the world, and recommended that WHO secure information to permit the preparation of such a document at the next meeting of the Expert Committee on Insecticides.

Some of the alternative methods discussed below are recommended for further investigation, and some may be implemented without further research.

#### 1.4.1 *Insecticides*

(1) *Chlorinated hydrocarbons*. Although the successive use of different chlorinated hydrocarbon insecticides has been disappointing in the case of the house-fly after resistance had once developed, nevertheless it is noted that there are instances with other species where another chlorinated hydrocarbon has given extended control after the development of resistance to the one in use. It is suggested that this method might offer some promise of extending the effective control period on malaria control programmes. However, experience in the case of the house-fly has shown that the chances of successfully returning to the original insecticide are not very hopeful.

(2) *Organo-phosphorus compounds*. The early organo-phosphorus compounds were too toxic for general use on public health vector control programmes, but more recently several have appeared that are no more toxic to warm-blooded animals than some other types of insecticides now in common use. These organo-phosphorus compounds, however, characteristically lack the quality of long residual effectiveness. The Committee believed that great possibilities for alternative insecticides may lie in the devising of formulations of these less toxic organo-phosphorus compounds to obtain a satisfactory persistence. This would overcome the present disadvantage of frequent treatment, and render their use economically feasible.

Malathion and diazinon have recently been extensively used in certain countries as surface sprays to control house-flies. Although resistance has developed to these compounds, it is at present of a relatively low order. On the other hand, organo-phosphorus compounds have not been adequately tried against adult mosquitos, and the Committee was of the opinion that experiments should be conducted to determine (a) whether formulations can be devised that will give more durable residues, and (b) whether mosquitos are likely to become resistant to organo-phosphorus compounds. It is suggested that this work should be done on a laboratory and experimental field basis, employing various types of surfaces ordinarily encountered in vector control programmes.

It was also considered that the possibility of using organo-phosphorus compounds to kill mosquito larvae should be further explored, and that the necessary investigations be made concerning health hazards to men and domestic animals and toxicity to fish and birds. It should be noted,

however, that experience with other materials has suggested that there may be greater danger of developing resistance where the larval and adult forms in the same area are treated with insecticides of the same group.

(3) *Insecticide mixtures.* Mixtures of certain organo-phosphorus and chlorinated hydrocarbon compounds have given improved control of certain species resistant to chlorinated hydrocarbons. The Committee believed that this subject should be further investigated.

(4) *Pyrethrins.* While it seems unlikely that it is possible to increase the residual effect of pyrethrins to make them a satisfactory alternative insecticide in residual-spray programmes, the Committee considered that research with this material should be pursued to determine whether its value in public health programmes can be thus increased. Even without further developments, pyrethrin sprays have a definite part to play in individual household use and may be of supplementary value in programmes where resistance has developed.

(5) *Production of new insecticides.* The Committee recommended that every encouragement be given to the production of new insecticides in the hope that, faced as we are with the resistance phenomenon, some material with a new mode of action may be evolved.

(6) *Control of fly larvae.* The Committee noted that certain developments in the control of fly larvae, such as the use of thiourea and rapid composting techniques, offered some promise for improving the control of resistant populations. It recommended that investigations be continued in this matter.

#### 1.4.2 *Synergists*

Under this heading, the Committee discussed materials which when added to an insecticide enhance its effectiveness. There are certain types of synergist that are specifically active solely for resistant pests. The Committee considered that the search for both types of compounds should be continued. Unfortunately, up to the present, those used for chlorinated hydrocarbons have proved disappointing in practice. Moreover, experiments have shown that resistance can develop to the synergized insecticide.

#### 1.4.3 *Repellents*

Repellents are a primary means of personal protection against both vectors and pest insects. There are certain diseases, such as scrub typhus

and tick-borne infections, where repellents are a first line of defence against transmission. In cases of resistance, they may be of supplementary value in vector control programmes.

#### 1.4.4 *Poison baits and attractants*

Recently, some of the organo-phosphorus compounds which possess toxic properties and poor residual qualities, but yet are effective against resistant insects, have been successfully used in baits. Such preparations offer help in the suppression of large populations of resistant house-flies about areas of intensive breeding. The incorporation of such insecticides in baits and other attractants that may make their utilization practical should be explored, particularly with reference to the control of resistant house-flies.

Certain work has been performed on attractants to be used to localize insect populations at specified points for subsequent destruction. The Committee advised that this subject be kept in mind and that encouragement be given to the development of the idea.

#### 1.4.5 *Control by environmental and biological methods*

The Committee, recognizing the increasing resistance problem, re-emphasized the necessity of source reduction of vector species. This would involve all types of applicable environmental control work, such as drainage, filling, dyking, flooding and herbiciding for mosquito control, and proper waste disposal to prevent the breeding of flies and other filth-inhabiting insects. Environmental techniques should, so far as possible, be adapted to multi-vector control problems.

Evidence was considered concerning the possible use of parasites, predators and other biological control agents in mosquito control, and the Committee recognized the role of such agents in localized situations. Owing to the diversity of species and environments concerned, it appears most unlikely that such methods would find universal application, but they should be investigated for use in localities where applicable.

The Committee also studied the report of the eradication of *Callitroga hominivorax* on the island of Curaçao by the liberation of large numbers of males previously rendered sterile by irradiation. This technique is an entirely new one in the field of insect control and it is recommended that WHO maintain close liaison with workers on this subject to determine if subsequent extension to public health problems is possible.

## 2. DISINSECTIZATION OF AIRCRAFT

The attention of the Committee was drawn to section 5, XV, of the first report of the Committee on International Quarantine,<sup>1</sup> in which the Expert Committee on Insecticides was "requested to keep the Quarantine Committee constantly informed on the progress and development of insecticides applicable to aircraft" for quarantine purposes. The Expert Committee on Insecticides considered the various aspects of this problem and wishes to draw the attention of the Committee on International Quarantine to the present position as described below.

The Committee reconsidered the specification for aerosol formulations recorded in the fourth report of the Expert Committee on Insecticides<sup>2</sup> and the recommendations concerning the use of aerosols<sup>3</sup> for the disinsectization of aircraft for quarantine purposes, contained in the second report of the Expert Committee on Insecticides.<sup>4</sup>

### 2.1 Aerosol formulations

The Committee noted the composition of two new (medium pressure) aerosol formulations which had been approved by the Foreign Quarantine Division of the US Public Health Service for use on aircraft entering the USA. These, and other formulations which have been shown to be satisfactory in service, are recorded in Annex 1 (page 25).

Attention was given to the question of specifying particular solvents for use in aerosol formulations. The Committee, however, reaffirmed the principles on which the earlier specification was based—which permitted manufacturers to use their discretion in the choice of suitable solvents, propellents and insecticides—provided that the finished product complied with all the following requirements:<sup>5</sup>

#### 1. Insecticide solution

##### 1.1 General

The insecticide solution must be suitable for use inside aircraft. When dispersed as an aerosol at the prescribed rate, it must be free

<sup>1</sup> *Off. Rec. Wld Hlth Org.*, 1954, **56**, 66

<sup>2</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1952, **54**, 30 (section 2.4)

<sup>3</sup> For the purposes of this report, an insecticidal aerosol is defined as a dispersion in air of the insecticide solution in which the majority of droplets, on a weight basis, are of a diameter of 5-25  $\mu$ .

<sup>4</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1951, **34**, 4 (section 1.1)

<sup>5</sup> These supersede all those contained in the second and fourth reports of the Expert Committee on Insecticides.

from fire hazard, human toxicity risks and injurious effects on fabrics, metals, woodwork, rubber, and surface furnishings used in aircraft. It must be free from visible impurities and foreign matter and must remain free from deposit or suspended matter when cooled to  $-5^{\circ}\text{C}$  ( $23^{\circ}\text{F}$ ) or to the lowest temperature encountered in the filling operation—which-ever is the lower.

### 1.2 Composition

	<i>Percentage by weight</i>
DDT, technical. . . . .	3.0
Pyrethrum, refined extract, total pyrethrins equivalent to . . . . .	0.4*
Non-volatile oil, suitable solvents and propellents <sup>1</sup> . . . . .	96.6

\* 2% of a 20% pyrethrum extract

### 1.3 Ingredients

The DDT and pyrethrum extract must comply with the appropriate, current specifications recommended by the Expert Committee on Insecticides. The other ingredients must be of good commercial quality.

### 1.4 Performance

The insecticidal action of the aerosol produced from its dispenser must not be inferior to that of the following standard formulation :

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

### 1.5 Crazing effect

Crazing of stressed Perspex (Plexiglas) must not occur when the solution is tested in accordance with the method described in Annex 2, section 1 (page 26).

<sup>1</sup> Aerosol formulations containing 15%-20% non-volatile oil and solvents and 80%-85% propellents have been found to be effective.

## 2. Dispenser

### 2.1 Design

The design of the container shall be such that it will withstand the tests described in Annex 2, section 2 (page 26). The valve must be protected from accidental discharge.

### 2.2 Performance

The physical performance of the dispenser shall be such that, when operated in accordance with the manufacturer's instructions, 1.0 g  $\pm$  0.20 g of the formulation is dispersed per second as an aerosol. The aerosol, when tested by the method described in Annex 2, section 3 (page 28), shall comply with the following requirements :

(1) Size of droplets: (a) Not more than 20% by weight of the aerosol shall consist of droplets of diameter greater than 30  $\mu$ . (b) Not more than 1% by weight of the aerosol shall consist of droplets of diameter greater than 50  $\mu$ .

(2) The valve shall not dribble before, during, or after the release of the aerosol.

With reference to section 1.1 of the above specification, however, the Committee considered that it would be desirable for WHO to enquire if user countries were satisfied that  $-5^{\circ}\text{C}$  ( $23^{\circ}\text{F}$ ) was a sufficiently low temperature at which freedom from separated DDT and/or other solid matter should be determined. The Committee would be prepared to make this requirement more stringent if it were considered that difficulty on this account had been experienced or that the margin of safety was insufficient.

## 2.2 Disinsectization procedures

The Committee endorsed the majority of the recommendations made at its second session regarding the establishment of *cordons sanitaires* round airfields and the optimum time and method of treating aircraft as recorded below. The dosage which is recommended for use in the absence of passengers is slightly irritating and may result in objections if used in their presence.

The Committee recommended that :

(1) airports open to international traffic should be kept as free as possible from mosquitos and other disease-bearing insects within a protective area extending for a distance of 400 metres around their perimeter ;

(2) for disease-bearing insects other than mosquitos, appropriate measures should be taken in the area of the airport and, in so far as possible, in crew and passenger quarters when located outside the airport area ;

(3) (a) disinsectization should take place before take-off, with all luggage and/or freight loaded, but without passengers. All possible mosquito-sheltering places inside the plane should be sprayed, including cupboards, chests, clothes, luggage and freight compartments. Particular attention should be given to spaces under seats and behind crates and luggage, where diffusion of the insecticide would otherwise be slow. Foodstuffs and utensils which may be inside the aircraft should be protected from gross contamination with the insecticidal spray ;

(b) the passenger, crew and freight compartments, the ventilators and all external apertures of the aircraft must be kept tightly closed during the spraying and for a period of not less than five minutes following the operation, and every care must be taken to prevent the entry of mosquitos into the aircraft after disinsectization and before take-off ;

(c) if, for any reason, passengers and/or crew have to disembark and re-enter the plane and there is danger of reinfestation of any part of the plane, the whole or any such part of the spraying operation may, at the discretion of the health authority, be carried out again ;

(d) the recesses provided for the landing-gear, and all parts of the aircraft accessible only from the outside and in which insects can find shelter, are to be disinsectized as nearly as possible to five minutes before starting the engines ;

(4) disinsectization during flight by the crew of an aircraft should not be recognized as complying with the requirements of the International Sanitary Regulations ;

(5) if, for any reason, the presence on board of live insect-vectors is suspected, additional disinsectization should be carried out at the discretion of the health authorities after landing ;

(6) for the disinsectization of the interior of the aircraft and any exterior parts which might constitute shelter for insects, an aerosol of pyrethrins and DDT as specified above should be dispensed uniformly through these spaces at the rate of 35 g of the formulation per 100 m<sup>3</sup> (10 g per 1000 cubic feet) of enclosed space.

In order to eliminate the human factor in giving the correct dosage of insecticide, the Committee at its sixth session had considered the design

of small, single-dose, disposable dispensers which, once opened, completely emptied their contents.<sup>1</sup> The simultaneous discharge of an appropriate number of these dispensers would ensure that the correct dose had been applied. The Committee was shown prototypes of different models of these dispensers and considered that their use in aircraft in the way proposed would ensure liberation of the recommended dosage and make for greater efficiency and uniformity in the application of the aerosol than is achieved at the present time. The Committee recommended that WHO sponsor trials with these dispensers on commercial airlines under normal operating conditions.

The Committee recognized that in some instances additional disinsectization is carried out in flight. It is believed that the procedure, as ordinarily performed, is ineffective. The use of the disposable dispenser would improve the situation by ensuring liberation of the recommended dosage. As stated above, the Committee does not wish to imply that in-flight disinsectization, even with the recommended dosage, constitutes satisfactory disinsectization.

### 2.3 Bio-assay test for aerosols

Attention was given to the development of a standard bio-assay test for the evaluation of aerosols for aircraft disinsectization. Two methods of test had been submitted for consideration.

The Committee noted that there was agreement between the two methods on a number of important principles, including the rearing conditions of the test insects and the desirability of using insects of defined size and age, although on points of detail there were minor discrepancies.

On one important aspect, the size of the test chamber, there was disagreement. As there had been insufficient time for experimental work to be undertaken on these tests by workers other than the authors, the Committee recommended that these methods should be passed to appropriate members of the Expert Advisory Panel on Insecticides for practical critical assessment.

The Committee hoped that early agreement on a bio-assay test method for aerosols would be reached, so that new formulations submitted may be tested by an approved technique.

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<sup>1</sup> *Wld Hlth Org. techn. Rep. Ser.*, 1956, **110**, 28

## Annex 1

AEROSOL FORMULATIONS WHICH HAVE BEEN FOUND  
EFFECTIVE IN PRACTICE

<i>Formulation</i>	<i>Percentage by weight</i>
<i>Formulation G-382</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 AR60 or Socony Vacuum 544G) . . . . .	8.0
Dichlorodifluoromethane (Freon-12 or Genetron-12) . . . . .	84.0
<i>Formulation G-1029</i>	
Pyrethrum extract (20% pyrethrins) . . . . .	6.0
DDT . . . . .	2.0
Aromatic petroleum derivative solvents :	
Velsicol AR60 or Socony Vacuum 544G . . . . .	6.0
Velsicol AR50 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 US Public Health Service at the rate of 18 g per 100 m<sup>3</sup> (5 g per 1000 cubic feet) (spraying time, 7-10 seconds with average dispenser).

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

## Annex 2

### TEST PROCEDURES FOR AEROSOLS AND AEROSOL DISPENSERS

#### 1. Crazing Test

(To be carried out at a room temperature of  $20^{\circ}\text{C} \pm 5^{\circ}\text{C}$  ( $68^{\circ}\text{F} \pm 9^{\circ}\text{F}$ ))

A strip of heat-treated Perspex (Plexiglas) of good quality, as used for aircraft, about  $18\text{ cm} \times 2.5\text{ cm} \times 0.6\text{ cm}$  (7 inches  $\times$  1 inch  $\times$   $\frac{1}{4}$  inch) in dimensions, 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 Perspex (Plexiglas) is thoroughly wetted. After 24 hours, during which the ambient air temperature shall be  $20^{\circ}\text{C} \pm 5^{\circ}\text{C}$  ( $68^{\circ}\text{F} \pm 9^{\circ}\text{F}$ ), the Perspex (Plexiglas) shall be wiped clean and examined for crazing at varying angles of incident light.

#### 2. Container Tests

Non-refillable aerosol containers of a capacity not exceeding  $490\text{ cm}^3$  (30 cubic inches or 16.6 American fluid ounces) and to contain pressures not exceeding 3.7 atm. (55 pounds per square inch (p.s.i.)) at  $21^{\circ}\text{C}$  ( $70^{\circ}\text{F}$ ) are exempt from the provisions contained in sections 2.1 and 2.2 below, but are subject to those of sections 2.3 and 2.4.

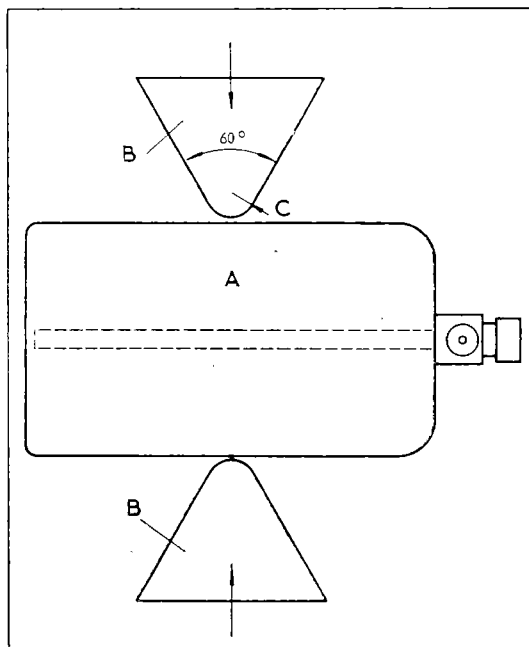
## 2.1 Pressure tests

(1) Each container shall be tested at an internal pressure of at least 27.2 atm. (400 p.s.i.) for refillable containers—or 13.6 atm. (200 p.s.i.) for non-refillable containers—and not exceeding 40.8 atm. (600 p.s.i.), held for at least 30 seconds, and shall show no leak or other defect when inspected by suitable means.

*Warning :* Where air or gas pressure is used for testing, means designed to protect personnel are recommended.

(2) One out of each 1000 refillable containers or 3000 non-refillable containers (or less), successively produced, shall be hydrostatically tested to destruction and must not burst below 81.6 atm. (1200 p.s.i.). Each such 1000 or 3000 containers (or less) successively produced shall constitute a lot; if the test container should fail, then the entire lot must be

### FLATTENING TEST FOR AEROSOL CONTAINERS



- A = aerosol dispenser
- B = wedge
- C = angle rounded to 13-mm ( $\frac{1}{2}$ -inch) radius

rejected. All containers constituting a lot shall be of identical size, design, construction, heat treatment, finish and quality.

## 2.2 Flattening test

Between knife-edges, wedge-shaped at a 60° angle and rounded to 13-mm (½-inch) radius, test one container taken at random after the pressure test out of each lot of 1000 refillable or 3000 non-refillable containers (or less). The test container shall not have cracked when the outer surfaces of the walls are apart not more than a distance of six times their thickness (see the figure).

## 2.3 Heat-stability test

Each dispenser is filled to the extent that the liquid does not completely fill the container when it is heated to the test temperature. It is then immersed in a water-bath held at 54°C (130°F) until the contents of the container reach the same temperature. To comply with the test, the container must give no evidence of leakage, distortion, or other defect; leaks may be detected by bubbles issuing from the surface of the container.<sup>1</sup>

## 2.4 Rough-usage test

The dispenser is allowed to drop under its own weight through a height of 75 cm (30 inches) on to a hardwood surface. The dispenser is arranged so as to receive the impact (a) end down, (b) top down, and (c) sideways. The dispenser must not leak after being subjected to this test.

## 3. 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. 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 mono-

<sup>1</sup> 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 temperatures is performed under conditions similar to those described here.

laurate,<sup>1</sup> and a 2% solution of a silicone in carbon tetrachloride.<sup>2</sup> 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.

### 3.1 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  $\mu$  in diameter details are provided for the more generally applicable impingement method only.

#### 3.1.1 *Impingement*

Wave the slide through the aerosol or draw the aerosol past the fixed slide, increasing the velocity as the droplet size decreases, since the rate of deposition is proportional to the square of the diameter. The slide should be inserted in the aerosol in a plane perpendicular to the axis of spray, at a distance of about 1.5 m (5 feet) from the dispenser, and moved rapidly towards the dispenser and out of the spray. 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 revolves at a 10-cm (4-inch) radius around the centre of the motor in a direction perpendicular to its movement. 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 revolved to obtain a good sample, or approximately 800 revolutions per minute, is equivalent to about 30 km (19 miles) per hour. With greater speeds the large aerosol droplets were found to move across the slide and distort their true size. For

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<sup>1</sup> Marketed by Atlas Powder Company, Wilmington, Del., USA, and by Honeywell-Atlas Ltd, Great Britain, as G-772.

<sup>2</sup> Solution marketed by Midland Silicones Ltd, London, England, as MS 1208; silicone alone marketed by General Electric Company, Schenectady, N.Y., USA, as Dri-film 9987; both silicone and solution marketed by Hopkins & Williams Ltd, Great Britain.

all types of commercial aerosols, a speed equivalent to 16 km (10 miles) per hour is recommended. The aerosol should be released 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.

### 3.1.2 *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. At various points along the slide, measure all droplet-lenses from one edge of the slide to the other that pass through the micrometer scale as the slide is moved by the stage, avoiding the smaller sized groups congregated along the margin of the slide. Express the number of droplets of each size—the size corrected as described in section 3.2—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 data 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 differences 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.

### 3.2 Procedure for correcting the measured diameter of droplets<sup>1</sup>

Use a compound high-power microscope with a flat mirror; remove the condenser; use outside 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 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.

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

**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, if necessary, is permissible.

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 four divisions in an eyepiece micrometer (one division =  $15.4 \mu$ ) is  $4 \times 15.4 \mu$ , or  $61.6 \mu$ .

With a focal-length change of  $206 \mu$ , 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  $61.6 \mu \times 0.40 = 24.6 \mu$ .

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No. 127 <b>Expert Committee on Biological Standardization</b> Tenth report . . . . .	1/9	0.30	1.—
No. 128 <b>The Public Health Laboratory Service</b> First report of the Expert Committee on Health Laboratory Methods . . . . .	<i>In preparation</i>		
No. 129 <b>General Principles Governing the Use of Food Additives</b> First report of the Joint FAO/WHO Expert Committee on Food Additives . . . . .	1/9	0.30	1.—