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WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES
No. 265

INSECTICIDE RESISTANCE
AND VECTOR CONTROL

Thirteenth Report
of the WHO Expert Committee
on Insecticides

WORLD HEALTH ORGANIZATION
GENEVA
1963
WHO EXPERT COMMITTEE ON INSECTICIDES

Geneva, 20-26 November 1962

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INSECTICIDE RESISTANCE AND VECTOR CONTROL

Thirteenth Report
of the WHO Expert Committee on Insecticides

The WHO Expert Committee on Insecticides met in Geneva from 20 to 26 November 1962. Dr M. G. Candau, Director-General, opened the meeting. Dr A. W. A. Brown was elected Chairman and Dr A. A. Shawarby, Vice-Chairman. Dr J. R. Busvine was appointed Rapporteur. The provisional agenda was adopted.

1. PRESENT STATUS OF INSECTICIDE RESISTANCE

In 1956, the WHO Expert Committee on Insecticides reported that there was sure evidence of resistance in 20 insect species of public health importance, and inadequate evidence of resistance in 15 more; at that time it was certain that 5 anophelines had developed resistance. In 1962, the number of species of public health importance for which there is sure evidence of resistance is 81, and there are indications of resistance in about 10 more; 32 anophelines are now classed as resistant to dieldrin or DDT. The extent of the resistance problem is in broad terms some 4 to 5 times as great as it was in 1956.

Of the 81 resistant species, 47 show resistance to DDT and its chemical relatives, and 65 to dieldrin, other cyclodiene derivatives, and BHC. Of the 32 anophelines, 12 show resistance to DDT and 30 show resistance to dieldrin. Resistance to the organophosphorus compounds, which in 1956 had just been discovered in the housefly, is now present in 8 species of insects of public health importance; 3 of these are culicine mosquitoes, but anophelines have not yet been involved. Compared to DDT resistance, dieldrin resistance usually comes faster and is much more decisive; organophosphorus resistance is slower to develop and attains lower intensities than dieldrin resistance.

A total of 35 species show resistance to both groups of chlorinated hydrocarbons. Where this double resistance appears in the same population,
it is necessary to replace the chlorinated hydrocarbons by organophosphorus compounds. These materials are often of limited availability in tropical countries, need to be applied at more frequent intervals than the chlorinated hydrocarbons, and since they are not produced locally are often very expensive. This demand on the foreign exchange supply of a young country often represents the most serious consequence of the resistance phenomenon in insect vectors. The housefly has shown itself capable of developing DDT resistance in any part of the world (Table 1, page 35), instances having recently been reported from the USSR, Japan and China, Eastern Europe and finally India. Resistance to BHC and dieldrin is also becoming ubiquitous. In areas of developing resistance, DDT or BHC can still provide effective control at higher dosages; but with dieldrin the block becomes total, and moreover a plague of flies results. The latter phenomenon, first observed in Georgia (USA) and later found to be of general occurrence, was considered due to the destruction of predacious and competing insects; but observations in Liberia have shown that it also involves an increase in oviposition rate.

Organophosphorus resistance, now present in Western Europe, the USA and Japan, is divided into two types, one to malathion and the other to parathion and diazinon. It may be partly circumvented by new organophosphorus compounds such as Dibrom, fenthion, rotenone, dichlorvos and dimethoate, and partly by the use of baits with organophosphorus compounds such as trichlorfon instead of sprays; but resistance is developing to trichlorfon baits in California, and behaviouristic resistance with avoidance of baits has appeared when malathion was used in Georgia. Carbamate resistance first appeared in Switzerland following the use of Pyrolan; resistance to another carbamate, Sevin, has recently been developed in the laboratory. In general, there is a cross-resistance between most carbamates and most organophosphorus insecticides. A substantial resistance to pyrethrins has developed in multiresistant flies near Stockholm, Sweden, a high pyrethrin tolerance in Central Italy, and an appreciable decline in susceptibility among domestic fly populations in the USA.

A number of midges and gnats have recently developed resistance, such as the midge Culicoides, the Bodega Bay gnat Leptoconops, the Clear Lake gnat Chaoborus (to TDE), the eye gnat Hippelates and the filter fly Psychoda; the Florida midge Glyptotendipes even developed resistance to the organophosphorus compound EPN. Tests performed on Phlebotomus sandflies in the Eastern Mediterranean have shown continuing DDT susceptibility, but there is an unconfirmed report of BHC resistance in P. ferox in Swaziland in 1957.¹

¹ United States Army Environmental Hygiene Agency (1962) Insecticide resistance of medically important arthropods (mimeographed document).
Other synanthropic flies have shown themselves less resistance-prone than the housefly, even *Musca sorbens* still being apparently susceptible. But the latrine fly *Fannia* has become DDT resistant in Northern Spain, and so has the biting housefly *Stomoxys* in Scandinavia. A population of *Stomoxys* in Nebraska, however, proved incapable of developing adult DDT resistance under selection pressure. The *Phaenicia* greenbottles that constitute the sheep blowflies of Australia, South Africa and New Zealand have developed dieldrin resistance. The African coprophagous *Chrysomyia* has shown resistance not only to DDT and to the BHC-dieldrin group, but also to organophosphorous insecticides. Species of *Drosophila*, which carry bacteria from garbage, have developed DDT resistance during public health spraying programmes conducted in Japan. So far *Glossina* tsetse flies have shown no evidence of resistance, despite area-wide spraying of the bush with DDT or dieldrin.

Among body lice (Table 2, page 36), DDT-resistant populations have appeared in all parts of the world as a sequel to mass dusting programmes; however, the resistance of these populations is far from homogeneous. Resistance to BHC has also developed in most regions where it has been extensively used against lice, although it is not as decisive as resistance against dieldrin. The strains resistant to BHC or DDT are very susceptible to malathion, and laboratory selection has not developed malathion resistance. Resistance to pyrethrins, first discovered and later confirmed in France, has been reported from Yugoslavia, Syria, Hong Kong, Nigeria and Mexico, but the test data have not been published. Selection pressure applied to a laboratory strain of body lice developed a tolerance to pyrethrins and a high cross-resistance to DDT.

Chlordane resistance in the German cockroach has spread from the USA to Canada, the Caribbean, Europe and Japan. DDT resistance in this species developed first in Europe and later in the Caribbean area. Organophosphorus resistance in *Blattella* is restricted to diazinon and does not extend to malathion; it appeared in 1960 in Kentucky and is now prevalent in Indiana. A high level of pyrethrin tolerance was found in a chlordane-resistant strain of German roaches from Fort Rucker, Alabama. Chlordane resistance appeared in the oriental roach *Blatta* at Landstuhl, Germany, in 1958.

DDT-resistant populations of the bedbug *Cimex lectularius*, first observed sporadically in the USA, now exist in tropical America, the Congo, Eastern Europe, the Mediterranean region, India and the Western Pacific. Resistance to BHC, chlordane or dieldrin, first observed in Central Italy, now occurs in Western and Eastern Asia and Southern Africa. In the tropical bedbug *C. hemipterus*, DDT resistance has developed in South-East Asia, India, East Africa and West Africa, while resistance to dieldrin or BHC is now of frequent occurrence in India, Malaya, East Africa and West Africa. The bedbug of West African jungle regions, *Leptocimex boueti*,
however, remains susceptible to DDT and dieldrin. Organophosphorus resistance has not yet been reported for field populations of *C. lectularius*, although parathion-methyl, diazinon, and malathion have been used for several years; however, malathion resistance has been developed in a laboratory colony. A 10-fold increase in pyrethrin tolerance was present in a population of *C. hemipterus* resistant to DDT and dieldrin at Mombasa, Kenya, and a 4-fold increase in BHC-resistant population of *C. lectularius* in Israel.

DDT resistance has been reported in the human flea in South America and the Middle East, the cases appearing more than 10 years ago, but direct susceptibility tests were not performed. Dieldrin resistance of a low order developed at Pare, Tanganyika, following antimalaria operations, and a reappearance of fleas after successive dieldrin sprayings has also been observed in West Africa. Resistance in dog and cat fleas to DDT and the BHC-dieldrin group has forced the substitution of organophosphorus compounds in the southern USA while DDT resistance has also been noted in South America and BHC resistance in Eastern Asia. The first case of DDT resistance in the oriental rat flea, *Xenopsylla cheopis*, the primary vector of plague, was proved in a population near Poona, India, in 1959, and in the following years it was found in the state of Mysore; there was an appreciable cross-resistance to BHC, but not to dieldrin. Incipient DDT resistance in *X. astia* has been detected in Mysore and Madras states.

Resistance in ticks is exemplified by two *Boophilus* species, the blue tick in South Africa and the cattle tick in Australia and Brazil; they have become resistant successively to arsenicals, then to BHC and cyclodiene, and then to DDT; in the former species, DDT resistance carried a high cross-tolerance to pyrethrins. Two species of *Rhipicephalus*, the brown dog tick in the USA and the Caribbean area, and the red tick in South Africa, have developed resistance to dieldrin, chlordane, toxaphene and BHC. The wood tick, *Dermacentor variabilis*, a potential vector of Rocky mountain spotted fever, has shown resistance in Massachusetts to both groups of chlorinated hydrocarbon insecticides applied as area sprays.

Resistance in culicine mosquitoes (Table 3, page 37) has now involved 17 species. *Culex fatigans* (*quinquefasciatus*) has developed resistance not only against residual DDT adulticides but also against DDT larvicides in all parts of the world, including finally the USA. Strong resistance to BHC and dieldrin, applied either as adulticides or larvicides, is also of ubiquitous occurrence. Resistance to these two compounds has especially serious consequences in the anti-filaria programme in India. At Douala, Cameroon, a high chlorohydrocarbon resistance was found to be accompanied by a significant malathion resistance, which extended equally to other organophosphorus insecticides. Control failures have appeared with malathion applied against *C. fatigans* in Kern county, California. It
is perhaps significant that the malathion resistance in the Douala strain rapidly reverts to susceptibility when the mosquitoes are reared free of insecticide.

DDT resistance in *C. pipiens* continues to spread in the central and northern parts of the USA. Dieldrin resistance, which followed DDT resistance in Italy, Israel and Japan, is now developing in larvae in the vicinity of Boston and New York. DDT resistance in *C. tarsalis*, vector of western equine encephalomyelitis (WEE), is now present in some Washington and Utah populations; in California there is also resistance to BHC and cyclodiene derivatives, but these are no longer used there. Malathion resistance in *C. tarsalis*, which developed in Fresno county in 1956, has remained localized and has reverted towards susceptibility in California. Malathion resistance has also appeared in Oregon. DDT resistance has developed in two species of *Culex* in Panama and Okinawa, and dieldrin resistance in three species in Dahomey.

In *Aedes aegypti*, which was originally very susceptible in the larval or adult stage, DDT resistance has been discovered in 11 countries of the Caribbean area, and has recently appeared in Southern Vietnam and Southern Florida. DDT resistance has also been reported in Fiji and Japan. A population near San Juan, Puerto Rico, is resistant to dieldrin as well as to DDT; it also shows decreased susceptibility to malathion, diazinon and trichlorfon.

The salt-marsh mosquito *Aedes sollicitans* has shown resistance to DDT and to the BHC-dieldrin group not only in Florida but also in Delaware; and isolated control failures with DDT have occurred in five seaboard states of the USA, ranging from Texas to New Jersey. The smaller and more southern species, *A. taeniorhynchus*, has shown both types of resistance in Florida and Southern Georgia. A decreased susceptibility of the salt-marsh mosquitoes to malathion and other organophosphorus insecticides, first observed in Florida in 1952, has subsequently failed to develop into definite resistance. The irrigation-water mosquitoes *A. nigromaculis* and *A. melaninon* (formerly called *dorsalis*), combated in California with organophosphorus insecticides since the development of both types of chlorohydrocarbon resistance in 1951, have now developed resistance to certain organophosphorus compounds. The parathion resistance of *A. nigromaculis*, which first appeared in Kings county in 1957, has now spread to several other counties of California, although it is not intense. It could be counteracted by substituting parathion-methyl, but this is now failing in Kings county. *A. nigromaculis* is now beginning to show appreciable malathion resistance in Fresno county, while *A. melaninon* is beginning to show parathion resistance in Tulare county. *A. cantator*, which develops in salt-marshes in New Brunswick, has become resistant first to DDT and then to dieldrin. DDT resistance has been reported in *A. cantans* in Germany. The development of dieldrin resistance in the rice-field *Psorophora*
mosquitoes in Mississippi was a consequence of the wide use of cyclodiene insecticides in agriculture.

With the anopheline mosquitoes (Table 4, page 38) there has been a notable increase in the number of resistant species since 1956, most of the records dating from 1958 to 1960 when the WHO test method was widely circulated. The number of DDT-resistant species increased from 3 to 12, and the dieldrin-resistant species from 3 to 29. All the species in which DDT resistance appeared have also developed populations resistant to dieldrin, with the exception of A. numeotovari, in which DDT resistance was only recently recorded. Certain vector species, such as A. pseudopunctipennis in Mexico and A. fluviatilis in the region of the Arabian Sea, have remained susceptible to DDT for nearly 15 years, but became resistant to dieldrin very soon after its introduction. In areas of dieldrin resistance, DDT is used as an effective insecticide; in two areas, Northern Java and Adana, Turkey, where DDT resistance has appeared in A. sundaeicus and A. sacharovi respectively, dieldrin has given excellent control for several years. In areas where both DDT and dieldrin resistance is present in the same species, e.g., Southern Iran (A. stevensi), Central Greece (A. sacharovi), Lower Egypt (A. pharoensis), and Guatemala, El Salvador, Honduras and Western Nicaragua (A. albimanus), it has often been possible to postpone the substitution of the new organophosphorus insecticides as it has been found that DDT at 2 g/m² is still capable of giving satisfactory window-trap mortalities over a period of two to three months. The DDT resistance of A. culicificacies in India appears to be without operational effect, because the species has a low vectorial capacity and is irritated by DDT. Heightened DDT tolerance has been observed in the A. maculipennis group in the North-Eastern Mediterranean region for several years now, without apparent operational significance. Dieldrin resistance was reported in this species in Bulgaria and has also been found in a proportion of the population in Northern Romania, recalling the contamination with dieldrin-resistant genotypes found in some American populations of A. quadrivmaculatus. It is interesting that dieldrin resistance in A. culicificacies of Maharashtra state, Western India, is liable to revert to susceptibility when DDT is substituted; some dieldrin-resistant populations of A. gambiæ in Northern Nigeria have also apparently reverted when dieldrin pressure was released. Reversion of DDT resistance has been observed in A. sacharowi in Greece after aerial spraying was discontinued there; but the change is only slight. However, it is interesting to note that populations consisting mostly of homogyote resistant (i.e., surviving 4 % DDT by the WHO test, according to Davidson's criterion), can still be controlled by DDT at 2 g/m² for two to three months. Organophosphorus resistance has not yet been reported in anophelines, either in the field or in the laboratory.

Behaviouristic resistance is an ill-defined concept and has been used to cover a large number of published instances of great diversity where field
observations have indicated failure of control that could not be attributed to physiological resistance. For example, certain populations of *A. puncti-macula* in Colombia and of *A. cruzii* in Southern Brazil have been described as having developed a behaviour pattern of increased exophily during the course of several years of DDT treatment. The reports of increased exophily in *A. gambiae* in Mauritius and *A. darlingi* in Southern Brazil are not accompanied by proof that it was a developed characteristic; the high exophily of *A. muneztorvani* in Venezuela and of *A. sundaicus* at Tjilat-jap, Java, appears to have been a characteristic already present before the use of DDT. A decrease in anthropophily has recently been reported for *A. sacharovi* in the treated areas of Greece. The classic example of control failure attributed to behaviouristic resistance was in a population of *A. albimanus* in Panama where malaria transmission persisted despite the continued application of DDT. When compared with a normal field population, by step A of the WHO DDT-irritability test (see Annex 16), this population gave slightly lower excitation times, but when tests were performed in window-trap huts, significant differences were found only between laboratory and field material.

Populations of *A. labranchiae* in Morocco were so highly DDT irritable that wall deposits did not kill them, and seven years of DDT spraying evidently did not change that characteristic. On the other hand, a physiologically DDT-resistant population of *A. sacharovi* in the heavily sprayed area of Skala, Greece, was only half as DDT irritable as other populations in Greece and Romania, as judged by the alternative step B of the WHO test. Steps A and B of the test have shown that laboratory strains are significantly less DDT irritable than the F₁ strain of field populations in *A. gambiae* and *A. albimanus*, although there is no difference in the LC₅₀ levels. A change in DDT irritability as a consequence of the selective effect of DDT treatments (which would be classed as behaviouristic resistance in the sense established by usage) has not yet been unequivocally demonstrated, and awaits the use of the WHO test for irritability on populations within a species before and after a spraying regime, or in untreated as against treated areas of a region. It would be expected that if the changed population were also physiologically resistant to DDT the irritability would be reduced, and that only if there were no change in physiological resistance would there be any possibility of an increase in irritability.

The literature on this subject up to 1960 has been reviewed elsewhere.¹ In Table 5 (page 39), references will be found to more recent reports of resistance developing in new species (other than anophelines) and of species developing a new type of resistance.

2. RESEARCH ON INSECTICIDE RESISTANCE

The Committee noted with satisfaction that the recommendations made in its seventh report ¹ had been implemented. The collection and dissemination of information has been proceeding, and the well-known WHO Information Circular on Insecticide Resistance is issued every two months to some 650 collaborators throughout the world. Research on resistance is going on in scores of laboratories throughout the world, supported either by local funds or by WHO itself. Standard test methods for resistance have been developed by the Committee for almost all the main insects of public health importance. Under the WHO insecticide development programme, approximately 600 new insecticides have been examined by various laboratories and the best of them tested in the field or even given operational trials. To ensure efficient liaison, WHO consultants and personnel have made visits to relevant laboratories in different parts of the world, and during the 5 years since publication of the seventh report, a number of seminars and technical conferences have been held and expert groups have been assembled to discuss problems connected with insecticide resistance and vector control.

The Committee considered the lines of future research and recommended that attention now be given to the following topics: (a) discovery of conditions in which DDT can still be used effectively against DDT-resistant populations; (b) determination of the risk of resistance developing to organophosphorus compounds, and of the biochemical mechanisms and cross-resistance spectra of the various instances of organophosphorus resistance. The Committee noted with gratification that as a result of the recommendation made in its eighth report ² regarding the need for information on the ecology of vector species a programme of research on vector ecology and biological control had, in fact, been instituted. A great deal needs to be known about the bionomics of vectors in order that the best use be made of insecticides and the new chemo-sterilants, the ultimate objective being integrated control using both chemical and biological methods. The WHO research project recently initiated in Burma on the control of Culex fatigans and filariasis represents precisely the desired ecological approach to vector control. The Committee recommends that the same approach be followed for other insects of public health importance.

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3. PROCEDURES USED FOR THE DETERMINATION
OF SUSCEPTIBILITY OR PHYSIOLOGICAL RESISTANCE
OF INSECTS TO INSECTICIDES

The Committee noted with satisfaction the wide use of existing standard
methods of detecting and measuring resistance and the great body of
information that had been accumulated. To date, approximately 4000 test
kits had been distributed by WHO.

The existing standard methods were reviewed and were found to need
only very small changes to improve their efficiency. One modification
affecting most of the test methods concerned the concentration chosen
for routine checks after the determination of base-line data. Statistical
considerations suggested that the choice of the lowest concentration giving
100% kill in preliminary tests was not entirely satisfactory, since in some
circumstances the use of this concentration in routine checks might allow
the appearance of survivors due to chance and thus give rise to "false
alarms" of incipient resistance. Certain field evidence suggested that this
did indeed occur. Accordingly, the Committee recommended the use of the
next higher serial concentration to that giving complete kill in preliminary
tests. This level should be used for routine checks.

Another suggestion of fairly general application was that a selection
of typical results already obtained with a particular method should be
included in the test kit. This would be particularly helpful for guidance in
some of the newer and less familiar test methods.

3.1 Development of papers impregnated with organophosphorus insecticides

The widespread resistance to chlorinated hydrocarbon insecticides has
resulted in considerable use of organophosphorus insecticides for vector
control. There was consequently an urgent need for tests for resistance
to these new compounds, particularly as such resistance was beginning
to become an important problem.

Investigations carried out by members of the Expert Advisory Panel
on Insecticides have proved the feasibility of such tests, using papers
impregnated with malathion and fenthion. In both cases, olive oil plus
Ionol CP anti-oxidant has been found the most satisfactory vehicle. These
papers have a maximum life of 4 months in sealed (or re-sealed) boxes.
After removal from the boxes they should not be used for more than 5 days.

In view of the rather limited effective life of organophosphorus impreg-
nated papers, their use in the field should be planned some months ahead
and arrangements made with WHO to supply papers at the appropriate
time.
Most of the trials with these papers have been made with the standard test for adult mosquitoes, but they have also been shown to be suitable for tests with bedbugs, triatomid bugs and fleas.

The obvious need for testing for resistance to organophosphorus insecticides emphasizes the desirability of preparing similar tests in advance for other new insecticides, such as the carbamates. The impregnation of papers with Sevin and with gamma-BHC is now under study. As a general principle, the Committee recommended that when any new compound is found worthy of trial on a field scale, parallel studies should be initiated to develop a resistance test.

3.2 Adult mosquitoes

The Committee gave considerable attention to the test for adult mosquitoes, in view of its great importance in regard to the malaria eradication campaign. In general, the test has been found efficient in detecting and measuring resistance in anopheline mosquitoes, especially towards dieldrin. Some anxiety was expressed on the difficulty of recognizing recessive resistance in its early stages (see Annex 14). This has been encountered in several cases of DDT-resistance in anophelines. As an additional check, the Committee recommended the rearing of survivors from concentration levels expected to give complete kills. The appearance of decreased susceptibility in the progeny would give confirmation of resistance in some cases. (See Annex 1 for revised instructions.)

3.3 Larval mosquitoes

The Committee decided that no change in the present test method, such as lengthening the observation period, was necessary.

However, the extension of the test to the 3 new organophosphorus insecticides presents certain minor technical problems. Thus, the solvent at present used (ethanol) may tend to react with certain organophosphorus esters. It was recommended that appropriate storage tests be made to determine whether this caused serious changes in concentration and, if so, to find a suitable alternative such as isopropyl alcohol. (See Annex 2 for revised instructions.)

3.4 Lice

The Committee reviewed evidence on the comparative values of a dust test, an impregnated paper test, and an impregnated cloth test. It was recognized that the dust test gave less detailed information on the levels of resistance in louse populations; but at the same time it is a useful and practical method of giving warning of resistance.
Accordingly, it was agreed to retain the dust test as the standard method. However, it was recommended that pyrethrum be replaced by malathion at appropriate concentrations. The value of gamma-BHC dust was questioned but it was agreed to retain it for the present.

The Committee agreed that where evidence of resistance was found, the importance of lice as disease vectors was such that more intensive investigation would almost always be required. For this purpose an impregnated-paper test would be most satisfactory. It was noted that the standard test kit for bedbugs could be adapted for this purpose. (See Annex 3 for revised instructions.)

3.5 Bedbugs

The Committee reviewed the data accumulated with the provisional test method and recommended that, with minor modifications, it should be elevated to the status of a standard test method (see Annex 4).

It was agreed that the standard exposure time should be 5 days for DDT, 2 days for dieldrin and 1 day for organophosphorus compounds. It was noted that further data were required to decide the exact concentrations of malathion and fenthion to be supplied with the kit.

The Committee also recognized the importance of the blood meal in affecting the susceptibility (and especially the control mortality) of bedbugs. In this regard it was recommended:

1. where possible to use (exclusively) well-fed bedbugs collected in the field;
2. alternatively, to feed the bedbugs and use them on the following day;
3. if neither alternative is feasible, to record the state of nutrition of the bedbugs.

3.6 Triatomid bugs

The Committee noted with satisfaction that a method almost identical to that for bedbugs had been found satisfactory for triatomid bugs. The only major difference was that DDT papers would not give useful information with triatomid bugs owing to their high natural tolerance; but since DDT was not normally used against these vectors, this fact was not considered important. The only other differences from the bedbug test method were related to the manipulation of the insects, such as the substitution of boiling tubes for test tubes. Accordingly it was agreed to recommend the technique as a standard test method (see Annex 5).
3.7 Fleas

The Committee reviewed the data accumulated with the provisional test method and recommended that, with minor modifications, it should be elevated to the status of a standard test method (see Annex 6).

It was agreed that the standard exposure time should be 1 hour, with mortality counts after 24 hours. However, it was recognized that a 24-hour exposure time might be necessary for resistant strains and the Committee recommended that provision should be made for this in the instructions.

Small amendments to the text were recommended to include advice on the methods of killing rats and collection of fleas. The age and sex of adult fleas need not be determined; but it was suggested that blood-fed fleas should be used, wherever possible. Instructions on methods of feeding fleas would be included in the kit.

3.8 Sandflies

The Committee reviewed the evidence on the use of the provisional test for sandflies and recommended that it should be elevated to the status of a standard test method (see Annex 7).

3.9 Tsetse flies and certain higher Diptera

The Committee reviewed the information on the tentative test method for tsetse flies and agreed that it could be satisfactorily used with Glossina palpalis, G. morsitans and G. swynnertoni, but perhaps not with G. tachinoides, which does not survive well in captivity. It was also noted with satisfaction that the method might be used for other higher Diptera, since it had been found satisfactory with Stomoxys. On the other hand, it was known to be unsuitable for houseflies, the susceptible colonies of which proved too tolerant to give useful mortality levels.

For tsetse flies, it was recommended that the sexes should be tested separately and gravid females excluded. All flies should be fed after capture on an appropriate blood source (which should be recorded) and tested two days later. It was agreed that the exposure should be one hour and the flies be provided with sugar solution during a subsequent 24-hour holding period before making mortality counts (see Annex 8.)

3.10 Houseflies

The Committee considered two alternative methods (see Annex 9): (1) exposure to residues in glass vials and (2) treatment by topical application using a microcapillary apparatus.1

Each method was seen to have advantages and disadvantages and it was agreed to describe both as alternative tentative methods and to await further evidence before accepting either as a provisional or standard test.

It was agreed that the two methods be submitted to appropriate laboratories and field workers for trial; and that tests with the microcapillary apparatus be undertaken in parallel with the more accurate microsyringe.

3.11 Blackflies (Simuliidae)

(1) Larvae

The Committee reviewed the data accumulated during the last 10 years on this subject and considered two methods:

(a) the use of large quantities of water through which air is bubbled to attract the larvae to a glass plate;

(b) the use of shallow trays of water and the manual transfer of larvae.

It was agreed that method (a) should be incorporated in the report as a tentative method and that method (b) should be given as an alternative.

The Committee suggested that both tentative methods should be tried simultaneously in appropriate laboratories to evaluate their comparative efficacy (see Annex 10A).

(2) Adults

The Committee reviewed the very scanty data on an adult test method for blackflies and recommended that this procedure should be further developed (see Annex 10B).

3.12 Ticks

The Committee considered a topical application method using the same microcapillary apparatus as recommended for the tentative method for houseflies. It was agreed to adopt this as a tentative method for ticks and it was suggested that arrangements be made for trials with Ornithodoros by appropriate laboratories (see Annex 11).

3.13 Cockroaches

The Committee agreed that though cockroaches are not major disease vectors, they are definitely of public health importance, and accordingly considered a tentative test method based on that developed by the Entomology Research Division, US Department of Agriculture, Orlando. The test will be based on dieldrin, malathion and diazinon and the time to effect knockdown (see Annex 12).
3.14 Persistent fumigants

The Committee considered a tentative method for detecting and measuring resistance to the vapour of dichlorvos. This depends on producing a standard vapour concentration by enclosing a small quantity of dichlorvos solution in dioctyl phthalate in a glass flask. After a given period, insects (mosquitoes, flies, etc.) are introduced in a small cage and observed for knockdown. Susceptibility or resistance is assessed on the basis of rate of knockdown (see Annex 13). This method might also be used for other persistent fumigants.

It was recommended that further development be done by collaboration between the Insecticide Testing Unit, Nigeria, the WHO Headquarters in Geneva, and the Committee member proposing the method.

4. INTERPRETATION OF RESULTS OBTAINED FROM THE USE OF TEST PROCEDURES FOR PHYSIOLOGICAL RESISTANCE

The various test procedures described in the preceding sections have two purposes:

(a) to determine how susceptible a given species is to various insecticides which either are, or may be, used in practical control work, and

(b) to detect (and measure if possible) any change in susceptibility which may alter the efficacy of control.

Any departure from the base-line characteristic of the species indicates a change in susceptibility. Due regard must be taken of minor variations caused by the non-standard physiological state of the test group or variations in the physical conditions of the test. The most important indication is failure to kill all insects at double the 100% mortality dosage. Any survival at this level in four successive tests is a danger signal indicating the probable existence of resistance in the population and certainly calls for more extensive investigation, using a complete range of concentrations. To evaluate the results of such tests correctly, the explanation in Annex 14 should be consulted.

5. BIO-ASSAY OF INSECTICIDES

5.1 Residues

The instructions given in the Committee's tenth report for the bio-assay of insecticidal deposits on wall surfaces were reviewed in the light of experience with over 600 kits provided to workers in different parts of the world.

A modified procedure based on this is described in Annex 15A.
5.2 Persistent fumigants

The Committee recommended the adoption of a bio-assay method using small cages of the test insect suspended in the treated room or dwelling for a standard period of time (see Annex 15B).

6. THE EFFECT OF INSECTICIDES UPON THE BEHAVIOUR OF MOSQUITOS

6.1 General considerations

In the course of malaria eradication programmes using residual insecticides it has been noted that in some areas of the world the response of malaria vectors to insecticide deposits was unexpected. Some vector species showed a high irritability on contact with the insecticide deposit (mainly DDT) and did not remain sufficiently long on the treated surface, resting for much shorter periods than they did before spraying. Irritability is the main factor in a type of mosquito behaviour operating after the mosquito has had contact with the treated surface. This might prevent the mosquito from absorbing sufficient insecticide to be ultimately fatal; such a condition might be responsible for partial failure of residual spraying but not necessarily so if driving the mosquito out of the human dwelling decreases the amount of man-vector contact. Another result might be increased exposure of the *Anopheles* to natural hazards outside the shelter, thus shortening its mean length of life and reducing transmission of the disease.

Such intrinsic behaviour of the mosquito has been given a number of tentative names, such as "protective behaviour" and "natural protective avoidance".

A different type of behavioural response to contact insecticides was described a few years ago in *A. albimanus* in Panama and generally classed as "behaviouristic resistance". The term describes a character of a population within a normally susceptible species developed by a gradual process of selection.

The tenth report of the Expert Committee on Insecticides stressed that this term should be reserved for populations that have been genetically changed and that it should not be applied to species showing a pronounced irritability as their normal reaction to insecticides; neither should this term be used with regard to species that are normally exophilic or exophagic (*A. darlingi* in Brazil, *A. nuneztovari* in Venezuela, *A. leathosphyrus* in Malaya).

The assessment of the presence and degree of "behaviouristic resistance" and its importance in malaria eradication has high priority, espe-
cially as observations on some vector species indicate that the increasing development of irritability and the selection of highly irritable strains under the continued pressure of insecticides might be a real obstacle to achieving the interruption of transmission. Data on this aspect of mosquito behaviour were originally based on empirical field observations; the Committee recommends the development of a standard irritability test, not only as a necessary objective method for determining changes in the irritability pattern within a given species in areas under continued insecticide pressure, but also for the direct comparison of irritability data on different species.

A provisional irritability test method has been developed, and modified according to varying field conditions. The method has now been tried out extensively in a wide range of laboratory and field conditions, including trials by several entomologists working in malaria eradication programmes.

The results of this collaborative work are now available, although the significance of some data can be only tentatively assessed at the present time. The amount of work done on this difficult subject is truly impressive, and the large body of collected observations will serve as a valuable baseline for many years to come. From this work it is evident that irritability is subject to many variables, such as age, temperature, light, physiological condition, etc., all of which make it difficult on occasions to obtain consistent results or to interpret the data. Perhaps for this reason it is not yet possible to recommend for field use under all conditions one single type of test to determine all the factors bearing on the effects of insecticides on the behaviour of mosquitoes. Different methods have proved to have certain advantages in the hands of different workers, but the ideal of complete uniformity of test method and test procedure appears to be most difficult. The Committee noted that some workers had intimated the possibility of delayed irritability to some insecticides (e.g., dieldrin); it recommends that further study be encouraged on this topic. The results with these tests have revealed distinct differences in irritability between different species and this offers promise that it might be possible to show differences between the strains of the same species. Although it has not yet been possible to relate these differences to field experience in malaria eradication programmes, it seems probable that this could be done.

The Committee considers that, in view of the severely practical needs of the malaria eradication programme, undue emphasis should not be put on the irritability test per se. No matter how desirable it is to adopt strictly standardized test methods, it is becoming evident that, from the practical point of view of vector disease control, empirical field observations may in most cases be a more accurate guide to the significance or otherwise of irritability in nature. There is a need for the development of field tests, such as the use of experimental huts, and of semi-field tests, for example, with so-called “excito-repellency boxes”. Although such methods do not lend themselves to over-all standardization, there is undoubtedly consider-
able room for improvement in the standardization of certain features or certain data.

In connexion with such field observations, it would appear that the irritability tests may still have an important part to play in the measuring and interpreting of certain aspects of behaviour revealed in the field. They have already proved useful in disproving certain rumours of behaviouristic resistance due to hyperirritability where in fact it had not developed. The test may still prove a valuable research tool when used with perception in the interpretation of cruder field observations. In this practical connexion it would appear that there is a need for experiments and assessments to be performed with particular deposits to approximate more closely to the formulations used in the field.

With these and other modifications, there is every likelihood that a standard type of irritability test may still be developed which will be of even greater value in malaria eradication.

6.2 Determination of irritability

Since the publication of the tenth report of the Expert Committee on Insecticides a considerable amount of experimental work has been performed on three alternative laboratory test methods of assessing the irritability of adult mosquitoes to deposits of pure DDT. It has been demonstrated: (1) that species may be subdivided into three classes: hyperirritable, moderately irritable and hypo-irritable; (2) that DDT-resistant strains of culicines (C. fatigans and A. aegypti) are less irritable than susceptible strains; and (3) that laboratory strains of mosquitoes are less irritable than field-collected material. In anophelines, DDT-resistant strains and field populations have not shown a lower irritability than susceptible ones. It is clear that the laboratory test method can illuminate only a small facet of the general problem of irritability to deposits of technical DDT water-dispersible powder in houses. Complete assessment can be made only in typical huts with exit chambers; however, the use of test boxes with exit chambers as used by the Pan American Health Organization, facultative tests such as those described by Mouchet & Cavalli,¹ and laboratory tests with suspensions of pure, technical or formulated DDT, could also provide valuable information on the problem.

The provisional method recommended in the tenth report of the Expert Committee on Insecticides ² involved three alternative determinations in which the light reflected from the test surface of DDT-impregnated paper was 40 foot-candles: the length of time to initial take-off of single mosquitoes (step A), the number of take-offs of single mosquitoes in a given period of

time (step B), and the number of take-offs of 5 mosquitoes in a given period of time (alternative step B). Subsequently, a special apparatus with 8 foot-candles of light transmitted through the test surface was used (Coluzzi method). This had the advantage that the mosquitoes settled more quickly and showed a smaller number of take-offs on control paper; moreover, the apparatus offered much better conditions for observations and the kit provides for a standard pre-exposure to light before the test is performed. Of the three alternative determinations, that provided in alternative step B was found preferable, since the figures obtained showed much the lowest coefficient of variation and thus revealed differences between strains with statistical significance; moreover, there was negligible "proximity irritability" involved in the use of 5 mosquitoes at a time in the same chamber. The method based on time to take-off (step A) proved to be extremely unlikely to show consistent inter-strain differences, although it would appear to have a sound physiological basis. Step B is preferable to alternative step B if the mosquitoes concerned are so highly irritable that the take-offs of 5 cannot be accurately counted. While carrying out step B it is possible to obtain at the same time the figures for step A.

The irritability test is also of value in comparing existing insecticides, and testing new insecticides for their liability to induce irritants in the mosquitoes resting on them. It has so far been found that neither dieldrin nor the two organophosphorus insecticides malathion and fenithion have irritating properties; it is important that new carbamate and organophosphate residual insecticides be tested for irritability during their development to the operational stage, but compounds that are otherwise promising should not be eliminated from further consideration solely because these tests show them to cause irritation. It should be remembered that this test is made with highly purified insecticides and may not guarantee absence of irritating characteristics in formulated preparations which contain various impurities and additional substances.

It is recommended that the distribution of the test kits for irritability be restricted to research laboratories or to specially designated operational staff. Instructions for the use of this kit and the procedure for this method will be found in Annex 16.

7. TRENDS IN VECTOR CONTROL

The Committee reviewed the new trends in research and the efforts to find new control measures rendered necessary by the development of resistance and the possibility of harmful effects from residues of some insecticides.

The new control methods so far investigated fall into 6 general categories: physical methods for the prevention of breeding; the use of new types
of insecticide; more selective or specialized uses of insecticides; the use of attractants and repellents; ecological control; and the induction of sterility.

7.1 Physical methods

There can be no doubt that such long-established methods should be encouraged wherever possible, although, in the past, most of them did not provide the degree of control insisted upon today. Advances in engineering equipment and methods permit sanitation, drainage, and water impoundment operations on a scale that was unattainable in the past, and many populations have acquired the habit, through a decade of excellent control with the chlorinated hydrocarbons, of insisting that pests be reduced not merely to tolerable numbers but almost to the vanishing point.

The foremost example of this trend is to be found in water management for the reduction of mosquito breeding. Methods that were in use for many years and then temporarily neglected, at least in some areas, are being revived and subjected to intensified research to provide the maximum degree of mosquito control, while still maintaining, or even increasing, the wildlife potential. Probably no more has been achieved in any single area than has been accomplished in the past in the reclamation of malarious or pest-mosquito-infested areas, but the measures are now being applied more widely. In many countries the growth of urban and agricultural areas has resulted in the filling-in of breeding areas. Extensive ditching in marshy areas permits the rapid rise and fall of water and affords fish easy access to developing mosquitoes. Impoundments control certain types of mosquito while increasing the wildlife potential, but are not universally adaptable as they may add to the breeding potential of other species, particularly if adequate water for proper management is not available.

The problems of water management in areas where water is scarce are receiving increasing attention. Tremendous water storage and utilization projects are being planned or put into effect, and many of these provide irrigation water to extensive agricultural areas. Through continuous research on mosquito biology, agricultural engineering and soil conservation, methods of conserving and using water without adding to mosquito problems are being continuously improved.

7.2 New types of insecticide

As mosquitoes of various species developed resistance to DDT, other chlorinated hydrocarbon insecticides were used for their control. Dieldrin was accepted for use as a residual treatment in buildings, whereas BHC was more widely used as a larvicide and as a spray or fog to control adult mosquitoes in outdoor areas. When some species became resistant to these insecticides too, attention was given to the organophosphorous compounds. On the most effective use of these insecticides, however, opinions have been
divided. In certain areas where larval control was almost a necessity, various organophosphorus compounds, particularly parathion, were applied as larvicides to control the mosquitoes in their breeding places. This was done with the full realization that resistance might develop to these compounds also, but the disease-producing potential of the species concerned made it essential to obtain the maximum degree of control, while past experience supported the hope that other potent larvicides would be available by the time parathion had lost its effectiveness. In other areas, where mosquito control was employed more to abate a nuisance than to control disease, the authorities decided to attempt to use the organophosphorus compounds in a way that might avoid the development of resistance, even though control might be less complete. In these areas the safer compounds, particularly malathion, were used as sprays, fogs or mists, dispersed by aerial or ground equipment, to kill the adult mosquitoes. They were applied only in the populated localities, leaving the mosquitoes untouched in extensive undeveloped breeding areas. In theory this should leave a sizable portion of the mosquito infestation without insecticide pressure, and hence the selection rate should be too low to cause the development of resistance. Neither method of applying the organophosphorus compounds appears so far to have led to a serious resistance problem.

Extensive adulticiding with malathion directed attention towards the possibility of generating aerosols by injecting insecticide solutions into the exhaust manifolds of airplane engines. In previous years this method had been explored with DDT solutions but, although early work was promising, the method was never successfully used on a practical scale with DDT. However, malathion proved well adapted to this method of dispersal, which is the principal one used in a mosquito control district in the USA and is now being initiated in several others.

The replacement of the chlorinated hydrocarbons by organophosphorus compounds for the residual control of mosquitoes in buildings has proved less successful. Particular difficulty was encountered in buildings made of certain absorptive muds. A number of promising organophosphorus compounds still remain to be evaluated for this purpose, however, and some carbamate insecticides also offer promise, particularly as only short exposure periods are required to cause knockdown or kill.

Among the organophosphorus compounds that have been used with success in one or more areas, or that may be considered suitable for recommendation, are the following: (1) larvicides: parathion-methyl, malathion, and fenthion; (2) outdoor treatments for adult mosquitoes: malathion, dichlorvos, fenthion and Dibrom; (3) residual treatments indoors: malathion.

In at least one area there has been a trend back to the use of Paris green as a larvicide, and special granules have been developed for the control of culicine mosquitoes in aircraft.
The organophosphorus compounds have also been used for the control of resistant houseflies, but although a large number of compounds were put into practical use, resistance developed to most of them. None provided the long periods of residual effectiveness that had been obtained with the chlorinated hydrocarbons. The carbamates as a group do not appear as promising for the control of houseflies as for mosquitoes, except when used in baits. Among the more effective organophosphorus compounds are diazinon, malathion, rotenone, Dibrom, trichlorfon and dichlorvos.

Developments in the use of malathion for the control of body lice may be indicative of a future trend. Like so many other vectors, body lice were easily and efficiently controlled with dusting powders containing DDT until resistance to this insecticide developed. Gamma-BHC (lindane) was substituted for DDT with reasonable success, but the duration of effectiveness was so short that weekly treatments were required to provide complete and continuing protection, and some resistance to this insecticide was also encountered. Laboratory, toxicological and field studies in recent years have demonstrated malathion powders to be safe and highly effective for periods of 3 to 4 weeks, and malathion may now be considered as the insecticide of second choice after DDT for use in louse control operations.

Resistance in cockroaches, fleas, bedbugs and ticks has followed the same general pattern, producing the same general trends. Among the organophosphorus compounds that have come into use following the development of resistance to various hydrocarbons are diazinon, malathion and fenithion.

7.3 Selective and special uses of insecticides

Selective and specialized applications of insecticides have been developed to permit the use of compounds that would be too toxic for use by the conventional methods of application, and also to take advantage of special properties of some insecticides, to attempt to avoid the development of resistance, or to avoid hazardous or undesirable residues.

The first organophosphorus compounds were considered to be too toxic for general application for the control of houseflies, but the use of scattered liquid or granular baits permitted their safe use and resulted in control with a minimum of insecticide residue. Treated cords or ribbons served a similar purpose. Dimethane, which has a poor residual action, is effective as a stomach poison in a bait, and fly baits will no doubt continue to be of value for special uses of new insecticides (or perhaps chemosterilants) as they appear.

Reference has already been made to the practice in some regions of restricting the use of malathion to the control of adult mosquitoes in populated
areas, leaving the mosquitoes in the breeding areas untreated in the hope that the development of resistance will thus be avoided. For the control of mosquitoes indoors, there is promise that the use of residual fumigants, such as dichlorvos, will provide a specialized and effective method of control. Dimethrin is moderately effective as a mosquito larvicide; it would not be of outstanding value for general larvicidal operations but, because of its extremely low mammalian toxicity, it may prove to be suitable for the control of mosquito larvae in drinking water, and is now under investigation for this specific use.

7.4 Attractants and repellents

Specific attractants have not been as widely used in the field of vector control as in the field of plant pest control, where sex and food lures play an important part in survey and control operations. A vast amount of research had been devoted to attraction in such vector species as houseflies, mosquitoes, other Diptera, and cockroaches, but even more effort will be required to develop attractants of practical effectiveness. Studies on the factors that attract mosquitoes to hosts are underway in laboratories around the world, and the literature on the subject is voluminous. Chemical factors such as carbon dioxide, water, and components of blood and sweat, as well as physical factors such as heat, movement, colour, and sound, have all been shown to be involved in host-finding behaviour. However, such behaviour is obviously complex, and no attractants of adequate effectiveness for use in control operations have yet been found.

Thousands of compounds have been tested as housefly lures and, again, many substances from the natural foods or oviposition media of houseflies have been demonstrated to possess some attraction. None of these, however, has been shown to be more consistently effective in controlling houseflies than scattered poisoned sugar, which is not an attractant but an arrestance. It should be mentioned that colours and visual patterns show variable attractiveness to houseflies, and with ingenuity may be put to practical use in control and survey methods. Water is attractive to houseflies, and much or all of the action attributed to a “fly-factor” in sugar and other foods on which houseflies have fed has been shown to be caused by a change in the moisture content.

Cockroaches are attracted to certain foods, but no extremely attractive substances have yet been isolated or synthesized. Virgin female cockroaches have been shown to produce a substance attractive to males, but the material has not been identified or put to practical use.

One must bear in mind that useful attractants can only be developed, if the behaviour of the species in question involves response to stimulation of the type under investigation; it would, for example, be unwise to devote
much effort to a search for chemical sex attractants in the housefly unless it can be shown that sexual behaviour in the housefly is stimulated by chemo-reception. However, it seems also self-evident that additional research on the behaviour pattern of vector species in finding food, mates, and oviposition sites can hardly fail to produce leads to chemical or physical attractants which could be turned to destructive ends. Certainly this approach to vector control deserves the most intensive investigation in view of the problem of resistance and toxic residues and the developments in the field of chemosterilants.

The uses of repellents for protection from disease vectors have not changed substantially in recent years, although a few more effective repellents, particularly deet (N,N-diethyl-m-toluamide), have been developed.

None of the repellents now available gives protection for as long as desired—say 24 hours—and all have some undesirable physical characteristics. After examining thousands of compounds in various chemical groups, it is safe to conclude that no compound that must be applied to the skin will remain on the surface in an effective quantity as long as 24 hours. It does, however, seem possible, in the light of the recent development of systemic insecticides for the control of cattle grubs, that systemic insecticides or repellents might be found for use in man. If systemic repellents are discovered, their mode of action will probably bear no relationship to that of conventional repellents, but will involve the counteraction of the chemicals in the host that attract the insects or stimulate them to bite. For this reason, basic studies on the host-seeking and feeding responses of insects should be stressed whenever possible.

7.5 Biological control

This general term embraces both biological control proper and cultural control, the former being defined as the direct or indirect manipulation of the natural enemies of pest species in such a way as to increase mortality among the pest populations, while the latter signifies manipulation of the physical or nutritional environment of a pest species so as to increase the effects of mortality factors other than natural enemies, or to decrease the effects of factors beneficial to the pest. Autocidal control is a form of biological control in which individuals of pest species are manipulated (through sterilization or genetical procedures) to harm their own kind.

There are effective biological and cultural methods for the control of certain agricultural pests. As there are no basic differences between the types of natural enemies of vectors and those of agricultural pests, similar methods and procedures to those used against the latter could be applied effectively against many vectors.
However, before attempting to manipulate living factors in order to control a vector species, it is necessary to know their identity, their relative importance to control, how they impinge upon the vector, and what factors other than the vector itself regulate their occurrence, numbers and attacks. Such information as is available is largely fragmentary and highly incomplete for most vector species in most parts of their range.

To take an instance of biological control, predacious insects and other arthropods, as well as fish and other vertebrates, are important natural enemies of many kinds of biting Diptera in most areas. For example, about 450 species of such predators are known to attack mosquito larvae. Parasitic insects appear to have much less potential against arthropod vectors than they have against agricultural pests, although some may have application against muscoid flies with terrestrial stages. Field trials of sciomyzid flies against certain snail vectors are yielding promising results, whilst entomophilic nematodes and microbial pathogens (e.g., certain bacteria, fungi and protozoa) are considered candidate biological control agents for use against various insects of public health importance. Control by competition may be considered under biological control, and field trials (e.g., against snail vectors of bilharziasis) are yielding hopeful results.

Experience with agricultural pests indicates that to increase supplementary requirements of natural enemies of vectors might often prove valuable at the cost of relatively minor environmental modifications. Consequently, procedures of this sort may prove especially applicable in developing areas.

As regards cultural control, much has been done in the past on the control of tsetse flies and mosquitoes, for example, by means of large-scale alteration of the environment (e.g., bush elimination). Other aspects of cultural control involve the reduction of vectors by reducing or eliminating their hibernation- or shelter-sites and other supplementary requirements.

It is emphasized that the successful use of biotic agents in vector control requires fundamental understanding of the interrelationships between them and their potential hosts or prey. It may also demand basic advances in related fields before the biotic principles can be successfully applied to vector control. Such exact knowledge about the vector and its relation to the environment is generally not required or, at least, has not been considered a vital necessity, in the application of chemical control measures. It is envisaged that for operational use biological control ultimately developed from a research programme along these lines would be integrated with chemical and other procedures. Such integrated vector control procedures based upon sound ecological preparations offer not only a means of minimizing resistance problems, but also the promise of a much more selective form of control that could result in a very significant and economical reduction in vector populations. Furthermore, the long-term
nature of the reduction achieved would help prevent the resurgence of pest populations following the completion of vector control programmes.

7.6 Sterilizing procedures

The use of sterilization techniques for the control or eradication of vector species, although it represents one aspect of autecidal control, embodies the use of chemical as well as biological methods.

Hopes of achieving vector control through sterilization are based partly on the successful eradication of the screw-worm fly (*Cochliomyia hominivorax* (Coquerel)), from the island of Curacao and the south-eastern portion of the insect’s range in the USA, partly on the encouraging results obtained in exploratory studies with chemosterilants, and partly on theoretical considerations of other methods of inducing sterility in natural vector populations.

The eradication of the screw-worm fly from the areas mentioned was accomplished by the release of flies sterilized by gamma radiation. Since the numbers released were several times as large as those of the existing fly populations, the majority of the normal, wild females were rendered sterile by mating with the irradiated males, and the natural infestation was rapidly reduced and soon eliminated. Shortly thereafter, the demonstration that both male and female house flies (*Musca domestica* L.) could be sterilized by radiomimetic chemicals directed attention to the possible advantages of chemosterilants over radiation in applying the sterile-male technique.

Chemosterilants are chemicals capable of causing sexual sterility, that is, failure to reproduce, in insects or other organisms. They might be used in two basic ways — as a substitute for radiation to sterilize insects that have been reared for release in large numbers, or as a means of inducing sterility in a large proportion of the natural population. The second method obviates the necessity of rearing and releasing large numbers of insects, which cannot readily be done in many species.

The principal advantage of a chemosterilant over an insecticide is that it effects a progressively greater reduction in population for the same percentage of exposed individuals. Thus, if a method of application of an insecticide kills 90% of the insects in the population, the 10 out of every 100 females that escape will mate with the 10 out of every 100 males that survive, and there will be 10 fertilized females to produce the next generation. However, if 90% of the population are sterilized by exposure to a chemosterilant, 90 females out of every 100 will be rendered incapable of fertilization. The 10 females that escape sterilization will be subjected to mating competition between 90 sterile males and 10 normal males. From this
ratio it would be expected that only one normal female out of every 10 —i.e., one out of 100 of the total female population—would mate with a normal male and thus be available to produce the next generation.

In many species of insects the females mate only once, but even species in which the females mate repeatedly might be susceptible to control with chemosterilants.

Chemosterilants may be effective when given in the food of insects, when applied topically or used as residues, and when added to the larval medium, although not every compound is effective by all the various methods of administration. The range of insects that have been demonstrated to be susceptible to chemosterilization, includes a number of species of flies, gnats, mosquitoes, moths, beetles, cockroaches, and mites.

Exploratory laboratory studies and small field experiments with house flies encourage the belief that methods can be developed for their control with chemosterilant baits. Both sexes are sterilized by feeding on treated baits at any age, and the sterility is irreversible. The sterile males mate readily with normal females and transfer motile sperm to the spermathecae; females inseminated by these males remain sterile throughout life, even when caged with normal males. The sterile males are fully competitive in mating behaviour with normal males. In three small field tests, the baits caused a high proportion of sterility among the natural population of house flies, and an eventual degree of control exceeding that to be expected with insecticide baits, but eradication was not achieved because of continual reinestation from outside the treated area.

In laboratory experiments in the USA both sexes of *Anopheles quadrimaculatus* Say and *Aedes aegypti* (L.) have been sterilized by chemicals in the food or larval medium or by exposure to treated surfaces. However, the release of males from a laboratory colony of *A. quadrimaculatus* sterilized by chemicals or by radiation has not affected the fertility of the females in the natural population, and it is not yet clear how chemosterilants might be used in the field for mosquito control, unless they prove to be safe for use in larval breeding places, or as residual applications.

More information must be available on the toxicity of the promising compounds before the full range of possible methods of application for the control of various species can be determined. They can certainly be used in the control of some species to sterilize reared insects for release among natural populations. No doubt they will also prove acceptable for use with various baits and attractants, which would bring them into contact only with the species concerned. The demonstration of a somewhat greater degree of safety would permit their use in more general, but still highly selective, methods of application. Their most efficient use will almost certainly require more detailed biological information regarding the species concerned than was required with insecticides.
Other possible methods for inducing sterility in natural populations include the release of strains with lethal or unfavourable genetic factors.

7.7 Organizational trends in vector control

In any discussion of organizational trends in vector control, consideration must be given to the type of programme carried out or envisaged by the country, state or other authority. If the programme is heavily centred on an eradication campaign concerned with a single disease—for example, malaria or yellow fever—there is usually an elaborate independent, or semi-independent organizational division within the health authority dealing with all functions of the programme—personnel, supplies, field operations and evaluation. If, on the other hand, vector control operations are concerned with insect or rodent-borne diseases of low endemicity or with insect pests, the unit dealing with these may often be an offshoot of some larger organizational division, such as a department of epidemiology, a municipal sanitation service, or an environmental health unit. However, whatever the type of programme, there is now a trend towards the development of a separate vector control unit, service or department. In the mass eradication type of programme, which is most often found in developing countries, there are increasing demands on the part of the population, as the country's socio-economic position improves, for additional services to deal with less dramatic if at times more persistent insect- and rodent-borne disease problems. Inasmuch as it is often difficult for the staff of a mass campaign to deal with these problems as well, special units are formed to organize other vector control programmes. In the case of countries or authorities that have established from the beginning a small "offshoot" service, the growing complexity of problems relating to the control of insect- or rodent-borne diseases, usually necessitates the further development of these small units into more substantial independent groups dealing with all aspects of the survey, control and evaluation of the insect or rodent vectors or pests. Under either circumstance it is considered that this evolutionary pressure is desirable and should be encouraged.

The very specialized problems relating to a unit that may have to deal with a multiplicity of disease vectors or pests will not be adequately dealt with if they long remain the responsibility of a unit of secondary importance. However, another trend that may be observed in many countries of Africa and Asia, is the creation of special groups for the study and control of particular insect-borne diseases, such as filariasis, trypanosomiasis or plague, and all problems related to them. In this process, however, the shortage of trained personnel in many of these countries is bringing about the consolidation of several such units into a single authority or department.
8. BHC IN VECTOR CONTROL

In view of the demonstration of cross-resistance between dieldrin and BHC there has been a tendency in recent years to discount the use of BHC in malaria eradication programmes. The disfavour into which BHC has fallen has been due not only to the fact that in some areas (e.g., Nigeria) its use has tended to select out a vector population resistant to dieldrin but also to the fact that, even in the absence of dieldrin resistance, application at three-monthly intervals is necessary if BHC is to be effective.

There is considerable evidence, however, that in future BHC may play a more important role in malaria eradication programmes in the following situations:

1. As the main insecticide against the vector in areas where the gene for dieldrin resistance has not yet been recorded, e.g., in many parts of east, central and southern Africa. It must be stressed that in parts of this area it has already been used with considerable success.

2. In combination with other insecticides—or possibly alone—in areas where the use of dieldrin is contraindicated because of resistance and where DDT alone has failed to interrupt transmission completely (savannah areas of West Africa).

The use of BHC alone has brought about interruption of transmission almost to the point of malaria eradication on tea estates in Assam, and also in Swaziland and the north-eastern part of Southern Rhodesia. Although success in those areas may have been facilitated either by the vulnerability of the vector (A. minimus in Assam), or by the comparatively short transmission season—not more than 6 months—the results are sufficiently striking to merit wider application. The need to re-spray at three-monthly intervals can no longer be regarded as a major handicap in view of the fact that the organophosphorus insecticides, e.g., malathion, may need the same frequency, and that even DDT may require application at four-monthly intervals under African conditions (e.g., at Kigezi in Uganda).

With regard to the use of BHC in combination with another insecticide, two points need elucidation. Firstly, how far does the well-established cross-resistance between dieldrin and BHC really rule out entirely the possibility that BHC alone may still be of operational value against dieldrin-resistant vectors for limited periods of time or to deal with emergencies? The high activity and fumigant effect of BHC, combined with the fact that vector strains resistant to BHC have a level of tolerance only 30 times as great as susceptible strains (as compared with 800 times in the case of dieldrin), suggest that BHC may still possibly have some value in dieldrin-
resistant areas. Perhaps owing to exaggerated fears, this possibility has never really been given a fair, unbiased field trial.

Secondly, there is the possibility of experiments with BHC in combination with an insecticide of a different group, e.g., DDT or malathion, to delay the selection of strains resistant to dieldrin and BHC. The principles involved have recently been investigated in studies on DLI-resistant A. \textit{sundaicus} in Indonesia. For the type of thatch and palm-matting dwelling prevalent in Indonesia, it was found that a mixture of BHC (0.25 g/m²) and malathion (1.0 g/m²) was particularly effective, not only because a 100\% mortality was maintained in hut trials for the first two months, but also because this combination did not favour the selection of dieldrin resistance.

An additional point of some importance is that, while the use of dieldrin in the field calls for protective clothing and a rigid preventive routine because of the toxicity of this compound to spraymen, BHC is free of such drawbacks and has been employed for years with normal, simple precautions.

As the malaria eradication programme—particularly in Africa—has a long way to go, it is by no means too late to explore these possibilities and settle them one way or another on the basis of limited field trials. There is a distinct possibility that such experiments might yet provide a valuable accessory method of insecticide attack in special circumstances.

\section{9. RECOMMENDED METHODS FOR VECTOR CONTROL}

The Committee noted that the annex “Recommended methods for vector control”, originally issued in the eighth report of the Expert Committee on Insecticides\textsuperscript{1} and since revised in the tenth report,\textsuperscript{2} has received wide acceptance. By revising this document at intervals, WHO is providing the field worker with up-to-date information on the latest advances in arthropod and rodent control by pesticides. The newly-revised recommendations are given in Annex 17.

In the tenth report it was recommended that priority be given to information on the control of blackfly vectors of onchocerciasis. Accordingly a special section of the “Recommended methods” has been devoted to this.

It was also observed in the tenth report that while certain insect groups (cockroaches and bedbugs) that have been included in the previous and present “Recommended methods” are not vectors in the strict sense, they are included, along with certain others, e.g., blowflies and pest mos-

\begin{enumerate}
\end{enumerate}
quitoes, in the vector control programmes of many health agencies; their control is often essential to ensure public acceptance of a campaign directed against vector species. Therefore data on their control have been retained in the annex.

The Committee reviewed the pesticides for which chemical and physical specifications were needed and recommended that such specifications be established for dichlorvos, fenthion, parathion-methyl, diethyltoluamide and diphenacine.

In addition to these compounds, four more were considered, namely rotenone, Dibrom, dimethoate, and Sevin, for which the present demand did not appear to be great enough to warrant the drafting of specifications but for which standards may be required in the near future.

It was recommended that the decision to draft specifications for a given compound be based on the degree to which it is used in international health programmes.

ACKNOWLEDGMENTS

The Expert Committee on Insecticides appreciates the contribution and assistance given it in the consideration of the agenda items by:

Dr L. J. Bruce-Chwatt, Chief, Research and Technical Intelligence, Division of Malaria Eradication, WHO
Mr B. Grab, Health Statistical Methodology, Division of Health Statistics, WHO
Mr N. Gratz, Vector Control, Division of Environmental Health, WHO
Dr M. Laird, Chief, Environmental Biology, Division of Environmental Health, WHO
Dr J. W. Miles, Vector Control, Division of Environmental Health, WHO
Dr R. C. Muirhead-Thomson, Division of Malaria Eradication, WHO
Dr R. Pal, Vector Control, Division of Environmental Health, WHO
Mr K. Uemura, Health Statistical Methodology, Division of Health Statistics, WHO
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<thead>
<tr>
<th>Species</th>
<th>DDT group</th>
<th>BHC-dieldrin group</th>
<th>Organophosphorus group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Area</td>
<td>Year</td>
</tr>
<tr>
<td>Musca domestica</td>
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<td>1949</td>
</tr>
<tr>
<td></td>
<td>1947</td>
<td>USA, Mediterranean</td>
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<tr>
<td></td>
<td>1948</td>
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<td>1951</td>
</tr>
<tr>
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<td>Western Europe, Canada</td>
<td>1952</td>
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<tr>
<td></td>
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<td>1958</td>
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</tr>
<tr>
<td></td>
<td>1960</td>
<td>India</td>
<td>1962</td>
</tr>
<tr>
<td>Gypseolripes paripes</td>
<td>1961</td>
<td>California</td>
<td>1953</td>
</tr>
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<td>Chaoebora xistaloporus</td>
<td>1949</td>
<td>Illinois</td>
<td>1953</td>
</tr>
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<td>Psychoda alternata</td>
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<td>California</td>
<td>1953</td>
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<tr>
<td>Leptocorys kelebazi</td>
<td>1961</td>
<td>California</td>
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</tr>
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<td>Calicoides furans</td>
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</tr>
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<td>Hippelotes collasor</td>
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<td>1957</td>
</tr>
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<td>Leucocera birtula</td>
<td>1955</td>
<td>Malaya</td>
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</tr>
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<td>Drosophila virilis a</td>
<td>1952</td>
<td>Japan</td>
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<tr>
<td>Fannia canicularis</td>
<td>1953</td>
<td>Spain</td>
<td>1958</td>
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<td>Stomoxys calcitrans</td>
<td>1948</td>
<td>Sweden</td>
<td>1958</td>
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<tr>
<td>Phaenicia cuprina</td>
<td>1957</td>
<td></td>
<td>1959</td>
</tr>
<tr>
<td>Phaenicia sericata</td>
<td>1959</td>
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</tr>
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<td>Chrysomya putoria</td>
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</tr>
<tr>
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<td>1960</td>
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</table>

* This table covers only the first reported instance of resistance in any area.

a Also D. melanogaster both-field strains.
### TABLE 2. RESISTANCE OF CERTAIN ARTHROPODS OF PUBLIC HEALTH AND VETERINARY IMPORTANCE *

<table>
<thead>
<tr>
<th>Species</th>
<th>DDT group</th>
<th>BHC-dieldrin group</th>
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</thead>
<tbody>
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<td>Area</td>
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<td></td>
<td>1952</td>
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<tr>
<td></td>
<td>(UNRWA camps)</td>
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<tr>
<td></td>
<td>1955</td>
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<tr>
<td></td>
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<td>West Africa, Peru, Chile</td>
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<tr>
<td></td>
<td>1955</td>
<td>France</td>
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<td></td>
<td>1958</td>
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<td></td>
<td></td>
<td>Afghanistan, India</td>
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<tr>
<td></td>
<td>1959</td>
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<td></td>
<td>1959</td>
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<td>1961</td>
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<td></td>
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<td>1957</td>
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<td>1960</td>
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<td></td>
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<td>1954</td>
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<td></td>
<td>1955</td>
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<td>1957</td>
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<td>1950</td>
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</tr>
<tr>
<td></td>
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<td>X. astia</td>
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<td>Boophilus decoloratus</td>
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<td>1954</td>
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<td>Boophilus microplus</td>
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* This table covers only the first reported instance of resistance in any area.
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<th>BHC-dieldrin group</th>
<th>Organophosphorus group a</th>
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<td>Year</td>
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<td>1957</td>
<td>Puerto Rico</td>
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<td>C. coronator</td>
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<td>C. triaeniorhynchus</td>
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<td>1958</td>
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<td>1959</td>
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<td>A. melanism b</td>
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<td>A. canadensis</td>
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<td>A. cantans</td>
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<td>Psorophora confinna</td>
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<tr>
<td>P. discolor</td>
<td>1954</td>
<td>Mississippi</td>
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* This table covers only the first reported instance of resistance in any area.

a M = malathion resistance; P = parathion resistance; O = general organophosphorus resistance.

b Formerly called A. dorsalis.
### TABLE 4. RESISTANCE OF ANOPHELINE MOSQUITOS TO DDT AND DIELDRIN *

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Mosquito</th>
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<th>Area</th>
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<tbody>
<tr>
<td>DDT</td>
<td>A. sacharovi</td>
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<td>Greece, Lebanon, Iran, Turkey</td>
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<td>A. sundalicus</td>
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<td>Java, Burma</td>
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<tr>
<td></td>
<td>A. stephensi</td>
<td>1955</td>
<td>Arabia, Iraq, Iran, S. India</td>
</tr>
<tr>
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<td>A. subpictus</td>
<td>1955</td>
<td>N. India, W. Pakistan, Nepal</td>
</tr>
<tr>
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<td>A. albimanus</td>
<td>1958</td>
<td>Salvador, Nicaragua, Guatemala, Honduras</td>
</tr>
<tr>
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<td>A. pharoensis</td>
<td>1939</td>
<td>Egypt, Sudan</td>
</tr>
<tr>
<td></td>
<td>A. quadrimaculatus</td>
<td>1939</td>
<td>Georgia, Maryland, Mexico</td>
</tr>
<tr>
<td></td>
<td>A. annularis</td>
<td>1939</td>
<td>W. India</td>
</tr>
<tr>
<td></td>
<td>A. culicifacies</td>
<td>1960</td>
<td>W. India, S. India</td>
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<td>A. albitalris</td>
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<td>Colombia</td>
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<tr>
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<td>A. nuneztovari</td>
<td>1961</td>
<td>Venezuela</td>
</tr>
<tr>
<td></td>
<td>A. aconitus</td>
<td>1962</td>
<td>Java</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>A. sacharovi</td>
<td>1952</td>
<td>Greece</td>
</tr>
<tr>
<td></td>
<td>A. quadrimaculatus</td>
<td>1953</td>
<td>Mississippi, Georgia, Mexico</td>
</tr>
<tr>
<td></td>
<td>A. gambiae</td>
<td>1955</td>
<td>Nigeria, Liberia, Ivory Coast, Dahomey, Upper Volta, Cameroun, Sierra Leonne, Togo, Ghana, Mali, Congo (Brazzaville)</td>
</tr>
<tr>
<td></td>
<td>A. subpictus</td>
<td>1957</td>
<td>Java, Ceylon, N. India</td>
</tr>
<tr>
<td></td>
<td>A. constanti and</td>
<td>1957</td>
<td>Arabia</td>
</tr>
<tr>
<td></td>
<td>A. punctulinus</td>
<td>1958</td>
<td>Salvador, Guatemala, Nicaragua, Honduras, Jamaica, Ecuador, Mexico, British Honduras, Cuba, Dominican Republic, Haiti</td>
</tr>
<tr>
<td></td>
<td>A. albimanus</td>
<td>1958</td>
<td>Mexico, Nicaragua, Peru</td>
</tr>
<tr>
<td></td>
<td>A. pseudopunctipennis</td>
<td>1958</td>
<td>Trinidad, Venezuela, Brazil</td>
</tr>
<tr>
<td></td>
<td>A. aequus</td>
<td>1958</td>
<td>W. India, Nepal</td>
</tr>
<tr>
<td></td>
<td>A. barbirostris and A. annularis</td>
<td>1958</td>
<td>Java</td>
</tr>
<tr>
<td></td>
<td>A. sergenti</td>
<td>1958</td>
<td>Jordan</td>
</tr>
<tr>
<td></td>
<td>A. flaviallis</td>
<td>1958</td>
<td>Arabia</td>
</tr>
<tr>
<td></td>
<td>A. splendens</td>
<td>1958</td>
<td>N. India</td>
</tr>
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<td>A. stephensi</td>
<td>1959</td>
<td>Iran, Iraq</td>
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<td>A. minimus flavirostris</td>
<td>1959</td>
<td>Philippines</td>
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<td>Egypt, Sudan, Israel</td>
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<td>A. albitalris</td>
<td>1959</td>
<td>Colombia, Venezuela</td>
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<td></td>
<td>A. labrocinus</td>
<td>1959</td>
<td>Morocco, Algeria</td>
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<tr>
<td></td>
<td>A. strobil</td>
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<td>Venezuela</td>
</tr>
<tr>
<td></td>
<td>A. triannulatus</td>
<td>1959</td>
<td>Venezuela, Colombia</td>
</tr>
<tr>
<td></td>
<td>A. sundaculus</td>
<td>1960</td>
<td>Java</td>
</tr>
<tr>
<td></td>
<td>A. aconitus</td>
<td>1960</td>
<td>Java</td>
</tr>
<tr>
<td></td>
<td>A. neomaculipalpus</td>
<td>1960</td>
<td>Trinidad, Colombia</td>
</tr>
<tr>
<td></td>
<td>A. crucians</td>
<td>1960</td>
<td>S. Carolina, Dominican Republic</td>
</tr>
<tr>
<td></td>
<td>A. filipinae</td>
<td>1960</td>
<td>Philippines</td>
</tr>
<tr>
<td></td>
<td>A. maculipennis</td>
<td>1961</td>
<td>Bulgaria</td>
</tr>
<tr>
<td></td>
<td>A. rangeli</td>
<td>1961</td>
<td>Venezuela</td>
</tr>
</tbody>
</table>

* This table covers only the first reported instance of resistance in any area.
<table>
<thead>
<tr>
<th>Species</th>
<th>Insecticide</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedes cantans</td>
<td>DDT</td>
<td>Bunn, R. W., cited in: United States Army Environmental Hygiene Agency (1962) Insecticide resistance of medically important arthropods (mimeographed document)</td>
</tr>
</tbody>
</table>

* Not included in published review up to 1962.
* These authors also report "resistance of medium degree" to gamma-BHC.
* This author also reports increased tolerance to gamma-BHC and dieldrin.
Annex 1

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOS TO INSECTICIDES

1. Introduction

(a) This method measures the susceptibility levels of a population of adult mosquitoes to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. In many cases, the first indication of incipient resistance may be an increase in the number of mosquitoes in the sprayed houses. Nevertheless, it is desirable to detect resistance as early as possible, before it is widely established in the insect population. For this purpose, it is necessary (i) to establish the susceptibility levels of normal populations of mosquitoes of the species concerned, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. The test is specifically devised to detect physiological resistance. It is stressed that this test is not designed to evaluate the effectiveness of insecticides in the field; for this purpose other entomological techniques must be used.

(b) Establishing the base-line. Batches of mosquitoes are exposed to different concentrations of insecticide and the kills obtained at each level are determined. It is suggested that a preliminary test be made at each concentration in the complete range provided, using the standard exposure of one hour. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of four concentrations should be chosen, some of which will give partial mortalities (i.e., at least one of them should give a complete kill, and one should give less than 50% mortality). Tests at these concentrations should be repeated four times with samples from the same population of mosquitoes. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable. Even if a true base-line (i.e., for an untreated population) cannot be established owing to previous applications of insecticides for any purpose, the susceptibility level of the vector species should be determined immediately.

(c) Subsequent routine checks. A concentration double the lowest concentration that has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with five replicates; when mosquitoes are scarce, a minimum of two replicates is permissible. The occasional appearance of survivors in such checks
may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include four tests at each of the concentrations used in establishing the original baseline. To validate the results, the investigator must make every effort to collect sufficient specimens for providing at least 15 mosquitoes per tube. It should be emphasized that the confirmation of resistance in its early stages is difficult when the genes responsible are recessive (as has been noted in certain cases of DDT resistance). In such cases it may be helpful to keep survivors from the exposure at the check concentration and rear their progeny. If the progeny give a distinctly lower level of susceptibility than the parents, this is a strong indication of resistance (however, this does not always occur with a recessive type of resistance).

(d) Condition of mosquitoes. Females should be used exclusively. It is recommended that the mosquitoes selected for test be females which have recently fed and show the presence of a blood meal. If mosquitoes are scarce, it is permissible to use a mixture of fed and unfed females, provided the proportion of each is recorded. Mosquitoes may be collected from sprayed and unsprayed premises in the zone, but their source should be reported on the form provided. In instances where it is not possible to collect a sufficient number of adult mosquitoes for testing, these specimens may sometimes be provided by collecting the immature stages and rearing the adults therefrom. In some circumstances females without a blood meal may be used exclusively, e.g., those recently emerged from a collection of larvae.

(e) Conditions of test. The experiments should be done indoors, if possible, in buildings free from insecticidal contamination and extremes of temperature, humidity, illumination, and wind. Where possible, subsequent comparison tests should be made under similar conditions of temperature and humidity. Transportation of insects to a base laboratory often results in mortality from causes other than the insecticide; this will be evident as high mortality in the controls.

2. Composition of test kit

(a) Twenty plastic tubes, 125 mm long by 44 mm in diameter: 8 of these (with red dot) are used for exposing the mosquitoes to the insecticide; 2 (with green dot) are used for the control exposure without insecticide; 10 (with green dot) are used as holding-tubes for pre-test sorting and post-
exposure observation. Each tube is fitted at one end with a 16-mesh screen. In order to identify the concentrations used with them, the red exposure tubes should be numbered 1 to 8, the green control exposure tubes 9 and 10, and the holding-tubes 1a to 10a.

(b) Ten slide-units, each with a screw-cap on either side and provided with a 20-mm filling hole.

(c) Five packages of papers impregnated with DDT (p,p' isomer) in mineral oil (Risella 17, Shell) at concentrations of 0.25%, 0.5%, 1.0%, 2.0% and 4.0%, respectively; and one package treated with oil only. Seven packages of papers impregnated with dieldrin (purified HEOD) in mineral oil at concentrations of 0.05%, 0.1%, 0.2%, 0.4%, 0.8%, 1.6% and 4.0%,1 and one package treated with oil only.2

(d) Sheets of clean paper (12 × 15 cm) for lining the holding-tubes.

(e) Twenty spring-wire clips to hold the papers in position against the walls of the tubes. The 12 silver clips should be used only for the holding-tubes and the control exposure tubes; the 8 copper clips should be used only for the insecticide exposure tubes.

(f) Two glass aspirator tubes, 12 mm in internal diameter, together with 60 cm of tubing.

(g) One roll of self-adhesive plastic tape.

(h) Instruction sheets and a set of report forms; three sheets of log-probability paper for plotting regression lines.

3. Procedure

(a) First a preliminary test is performed with the complete range of concentrations. Into each of the holding-tubes (6 for DDT; 7 for dieldrin), insert a piece of clean white paper rolled into a cylinder to line the wall and fasten it in position with a spring-wire clip (silver). Attach the slides to the tubes.

(b) Collect approximately 200 female mosquitoes with the aspirator provided (Fig. 1, A). Damage resulting from careless handling of mosquitoes during collecting may produce misleadingly high mortalities. Mosquitoes

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1 The following impregnated papers are also available on request:

(i) DDT and dieldrin at lower concentrations.

(ii) DDT at 3% for special investigations.

(iii) Malathion at 0.4%, 0.8%, 1.6%, 3.2%, 6.4% and 12.8%.

(iv) Fenthion at 0.1%, 0.2%, 0.4%, 0.8%, 1.6% and 3.2%.

(Malathion and fenthion papers lose their efficacy on long storage; their use should therefore be planned well in advance.)

2 Each package contains eight papers.
should be collected in lots of not more than 10 (Fig. 1, B) and gently transferred to the holding-tubes through the filling-hole in each slide (Fig. 1, C) to give 15 to 25 per tube. Any departure from these figures may impair the reliability of the results.

(c) A pre-test holding period may be necessary to guard against including damaged specimens in the test. For this purpose, the holding-tubes are set upright, screen end up, for one hour or more. At the end of this time, the damaged insects are removed.

(d) Into each of the exposure tubes (6 for DDT, 7 for dieldrin) introduce a sheet of impregnated paper, rolled into a cylinder to line the wall, and fastened into position with an appropriate spring-wire clip. One paper impregnated with each of the concentrations of insecticide provided should be used, and one control paper impregnated with oil alone.

(e) Introduce the mosquitoes into the exposure tube by attaching it to the vacant screw-top in the slide (Fig. 1, D). The slide should be pulled out to a point beyond the filling hole so that no part of it occludes the tube openings; the mosquitoes are then blown gently down into the exposure tube. (If necessary, the small safety knob in the slide may be filed down to facilitate this operation.) Close the slide. Detach the holding-tube and set it aside.

(f) Leave the exposure tubes standing upright with screen end up for one hour (Fig. 1, E) under conditions of moderate, diffuse illumination.

(g) At the end of the exposure period, transfer the mosquitoes to the holding-tubes by reversing the process described under (e). When some mosquitoes have been knocked down in the course of an exposure, the exposure tubes should be held horizontally and tapped to dislodge the insects from the slide before withdrawing it. Attach the holding-tube, open the slide, and gently blow the mosquitoes into the holding-tube; close the slide and remove the exposure tube. Then set the holding-tube so that it stands on the slide and place a pad of wet cotton-wool on the screen (Fig. 1, F). Cardboard cartons or cups or other suitable containers may be used instead of the holding-tubes, provided that they are used consistently.

(h) Keep the holding-tubes for 24 hours in a secluded, shaded place, where the temperature does not exceed 30°C. Wherever feasible, the maximum and minimum temperature of the room during the 24 hours should be recorded. If necessary, the tubes should be protected from ants by placing them on a platform standing in a pan of water. If conditions are very hot and dry, a moist chamber may be prepared by suspending damp towelling in a container.

(i) Mortality counts are made after 24 hours. Remove the dead mosquitoes by gently detaching the slide and cautiously moving the tube aside. Affected specimens that are unable to walk are to be counted as dead.
FIG. 1. METHOD FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOS TO INSECTICIDES

A

HOLDING-TUBE

B

HOLDING-TUBE

C

EXPOSURE TUBE

D

EXPOSURE TUBE

E

HOLDING-TUBE

F
As an aid to counting the living specimens, they may be stunned by a sharp jerk of the tube or stupefied by chloroform. The results should be recorded on the forms provided. Copies of completed forms should be distributed in accordance with the instructions on page 47.

(j) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of four concentrations. Four replicates, including the preliminary test where appropriate, should be performed at each of these chosen concentrations. The 20 tubes provided in the kit are sufficient for one series of two replicates at each of the four chosen concentrations, together with two controls.

(k) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(l) When the test has been repeated four times with the same population of mosquitoes, adequate data should be available for constructing a base-line of susceptibility as described below (section 5).

4. General remarks

(a) Each impregnated paper may be used up to 20 times, and up to three weeks after removal from the package, provided all possible precautions are taken against evaporation of the oil. To this end, the papers should be left in the tubes, with the open end well wrapped, and placed in the kit box, which in turn should be kept in a cool place. No paper should be used more than three weeks after removal from the package.

(b) After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The packages should be kept in a cool place, but not in a refrigerator, as too low a temperature may cause crystallization in the higher insecticidal concentrations. Prolonged storage at high temperatures should be avoided. Papers should not be used after the expiry date shown on the box. The period of three years (for chlorinated hydrocarbon papers) from the date of impregnation presupposes that the packages are kept sealed at all times.

(c) If the species of mosquito concerned is exceptionally insensitive, the exposure period may be increased to two, four, or eight hours. In all cases, the one-hour exposure period should be used first and the results recorded.

5. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-
probability paper provided. The regression line may be fitted by eye, and the LC$_{50}$ or LC$_{90}$\(^1\) read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC$_{50}$, the worker is referred to the methods described by Swaroop\(^2\) and Litchfield & Wilcoxon.\(^3\)

\((b)\) In cases where the control mortality is between 5\% and 20\%, the percentage mortalities should be corrected by Abbott's formula:

\[
\frac{\text{% test mortality} - \text{% control mortality}}{100 - \text{% control mortality}} \times 100
\]

\((c)\) The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per tube (25) decreases the effect of individual differences in response. Regression lines based on similar numbers of specimens are more reliable.

6. Interpretation of results

See Annex 14: Criteria and meaning of tests for determining the susceptibility of insects to insecticides.

7. Distribution of reports

It is of considerable importance to WHO to receive copies of results obtained from the use of this test kit. It is therefore requested that copies of all reports be sent to the following addresses: \(^4\)

\(^1\) The LC$_{50}$ and LC$_{90}$ represent respectively the insecticide concentrations at which 50\% and 90\% of the specimens are killed. LC$_{50}$ values for various anopheles will be found in mimeographed documents WHO/Mal/224; WHO/Insecticides/95-WHO/Mal/222; and WHO/Insecticides/100.


\(^3\) Litchfield, J. T., Jr & Wilcoxon, F. (1949) J. Pharmacol. exp. Ther., 96, 99

\(^4\) Addresses of WHO offices are as follows:

Headquarters:
World Health Organization, Geneva, Switzerland

Regional Offices:
- World Health Organization, Regional Office for Africa, P.O. Box No. 6, Brazzaville, Republic of the Congo
- World Health Organization, Regional Office for the Eastern Mediterranean, P.O. Box 1517, Alexandria, Province of Egypt, United Arab Republic
- World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India
- World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 1501 New Hampshire Avenue, N.W., Washington 6, D.C., USA
- World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark
- World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, Manila, Philippines
For anopheline species:

1. World Health Organization, Division of Malaria Eradication, Geneva, Switzerland;
2. WHO Regional Office;
3. Project Headquarters;
4. Investigator.

For non-anopheline species:

1. World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland;
2. WHO Regional Office;
3. and 4. Investigator.
### Specimen Report Form

**WHO TEST FOR INSECTICIDE-RESISTANCE IN ADULT MOSQUITOS**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Preliminary</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Totals (for comparable tests only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature during exposure period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humidity during exposure period (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature during 24-hr holding period (°C)</td>
<td>Max.</td>
<td>Min.</td>
<td>Max.</td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Insecticide concentration (%)</td>
<td>Dead</td>
<td>Total</td>
<td>Mort. (%) cor.</td>
<td>Dead</td>
<td>Total</td>
</tr>
<tr>
<td>Control (oil alone)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Cross out what does not apply
2. Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions)
3. Whose collected: shelters sprayed/unsprayed/outdoors/biting/bred out
4. Type of test on population: first time/routine check/complete retest

Remarks:

Interpretation of results (optional):

Signature of investigator:

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland. A second copy to be sent on completion to the appropriate WHO Regional Office.
Annex 2

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF MOSQUITO LARVAE TO INSECTICIDES

1. Introduction

(a) In order to detect the emergence of an insecticide-resistant strain of a mosquito, it is necessary to establish a base-line for the species, either before the wide use of insecticides or with specimens from an untreated area. Where regular larvicide operations are undertaken to control mosquitoes, the normal susceptibility levels of the larvae should be determined as early as possible. To this end, several tests (a minimum of eight) should be performed at various localities and seasons, to assess normal biological variation. Tests should then be continued at regular intervals to determine any significant reduction in susceptibility.

(b) The previous history of the use of insecticides in the area, both in mosquito control and major agricultural uses, should be noted. It should be stressed that this test is not designed to indicate the relative effectiveness of the insecticides in the field.

2. Composition of test kit

(a) Five different concentrations of each of three insecticides are provided, namely DDT (p,p' isomer), gamma-BHC or lindane (pure gamma isomer) and dieldrin (HEOD). They are in the form of 50-ml standard solutions in ethanol; the final concentrations indicated on the labels are those obtained when 1 ml is added to 249 ml of water, namely 0.004, 0.02, 0.10, 0.50 and 2.50 p.p.m. A standard for 0.0008 p.p.m. dieldrin is also provided for very sensitive species, such as certain anophelines. Fifty ml of ethanol for the control are also provided.1

(b) Four 1-ml pipettes are provided, one for each insecticide and one for the ethanol. Each pipette is equipped with a small rubber suction bulb for drawing up test solutions. Two eye-droppers and one strainer are also provided for transfer of the larvae. The user is expected to provide his own collecting and test vessels.

(c) Instruction sheets and a set of report forms; three sheets of log-probability paper for plotting regression lines.

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1 In addition to the chlorinated hydrocarbon solutions, standard solutions of malathion, diazinon and fenthion are available on request at the following concentrations: 0.0008, 0.004, 0.02, 0.1, 0.5 and 2.5 p.p.m.
3. Procedure

(a) For a complete test with one insecticide, sufficient larvae should be collected from the field in order that about 300 individuals of the same species may be selected; they should be in their third or early fourth instar and should be retained in the water in which they were collected until selection for testing. Any larvae showing abnormalities, for example a fuzzy appearance due to the presence of parasites on the body surface, should be discarded. Lots of 20-25 larvae are distributed in each of 12 small beakers, each containing 25 ml of water. Their transfer is effected either by means of the strainer provided, or by means of an eye-dropper and a filter-paper cone; during the process they should be rinsed lightly in clean water.

(b) Into each of 12 glass vessels approximately 7.5-10 cm in diameter (jars, bowls or 500-ml beakers) place 225 ml of water. The vessels should be such that the depth of water is between 2.5 and 7.5 cm. Distilled water, rain-water or tap-water may be used, or even water obtained from a well or stream, but it should be as free as possible from chlorine or organic contaminants. It should be noted that distilled water obtained commercially may contain traces of poisonous heavy metals. Certain species, such as salt-marsh or tree-hole mosquitoes, may suffer on transfer to relatively pure water, an effect that will be reflected in high control mortalities; in this case, water from the breeding site should be used, provided that it is free of insecticides and care is exercised to exclude detritus. The average temperature of the water should be recorded and should be approximately 25°C; it must not be below 20°C nor above 30°C.

(c) Prepare the test concentrations by pipetting 1 ml of the appropriate standard insecticide solution under the surface of the water in each of the glass vessels and stirring vigorously for 30 seconds with the pipette. In preparing a series of concentrations, the most dilute should be prepared first. There should be two replicates at each concentration, and two control replicates. The two controls should be prepared by the addition of 1 ml of ethanol to the water in each container.

(d) Within 15-30 minutes of the preparation of the test concentrations, add the mosquito larvae to them by tipping the contents of the small beakers into the vessels.

(e) After a period of 24 hours, make mortality counts. In recording the percentage mortalities for each concentration, the moribund and dead larvae in both replicates should be combined. Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface (within a reasonable period of time) or of showing the characteristic diving reaction when the water is disturbed; they may also show discoloration, unnatural positions, tremors, incoordination, or rigor.
(f) Discard the larvae that have pupated during the test. If more than 10% of the control larvae pupate in the course of the experiment, the test should be discarded. Tests with a control mortality of 20% or more are unsatisfactory and should be repeated.

(g) It is of importance to obtain not less than three mortality counts between 10% and 90%. With some species it has not been possible to do this using only the standard concentrations in the test kit, and it is then necessary to prepare additional concentrations both above and below 0.004 p.p.m. Additional intermediate concentrations may be prepared by diluting a portion of a standard solution with pure ethanol (e.g., a concentration of 0.01 p.p.m. may be obtained by diluting the 0.02 p.p.m. standard with an equal quantity of ethanol before taking the 1 ml for addition to the water in the vessel). If higher concentrations are required, they may be obtained from WHO.

(h) When four replicates have been performed with the same population of mosquito larvae, adequate data should be available for constructing a base-line of susceptibility. The results should be recorded on the forms provided. Completed forms should be distributed in accordance with the instructions on page 54.

4. General remarks

(a) The accuracy of the concentrations provided will be affected if the alcohol is allowed to evaporate from the standard solutions. The bottles should therefore be tightly stoppered after use. The contents should no longer be used when they have decreased below 5 ml; fresh standard solutions should then be obtained from WHO.

(b) Test vessels should be carefully cleaned after use to remove traces of insecticide. They should be thoroughly rinsed, scrubbed with detergent and water, or cleaned with potassium dichromate solution, and rinsed again. Pipettes should be thoroughly cleaned with acetone or alcohol.

5. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC$_{50}$ or LC$_{90}$ read from the graph. The

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1 When physiological resistance is demonstrated in a population, the effect on the transmission of the disease and on the advisability of continuing to use the same insecticide in the area can be evaluated only by epidemiological appraisal and by entomological assessment by other means.

2 The LC$_{50}$ and LC$_{90}$ represent respectively the insecticide concentrations at which 50% and 90% of the specimens are killed.
regression line should not be extended (extrapolated) beyond the highest mortality obtained.

(b) In cases where the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

\[
\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100
\]

(c) The accuracy of the interpretation of results depends on the reliability of the data obtained. Utilizing the maximum number of specimens per test (25) decreases the effect of individual differences in response. Regression lines based on similar numbers of specimens offer greater reliability.

(d) It appears that resistance may be suspected in mosquito larvae if there is an increase of 10-15 times over the original LC50, or when a proportion of the population can no longer be killed by the highest concentration in the kit. From the comparatively few data available at present, the indications are that when an LC50 for DDT in excess of 0.1 p.p.m. is found for Aedes or Anopheles spp., or an LC50 above 1 p.p.m. for Culex spp., resistance should be suspected.

6. Interpretation of results

See Annex 14: Criteria and meaning of tests for determining the susceptibility of insects to insecticides.

7. Distribution of reports

It is of considerable importance to WHO to receive copies of results obtained from the use of this test kit. It is therefore requested that copies of all reports be sent to the following addresses:

1 Addresses of WHO offices are as follows:

*Headquarters:*
World Health Organization, Geneva, Switzerland

*Regional Offices:*
World Health Organization, Regional Office for Africa, P. O. Box No. 6, Brazzaville, Republic of the Congo
World Health Organization, Regional Office for the Eastern Mediterranean, P. O. Box 1517, Alexandria, Province of Egypt, United Arab Republic
World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road New Delhi, India
World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 1501 New Hampshire Avenue, N. W., Washington 6, D. C., USA
World Health Organization, Regional Office for Europe, 8 Scherigovej, Copenhagen 0, Denmark
World Health Organization, Regional Office for the Western Pacific, P. O. Box 2932, Manila, Philippines
For anopheline species:

1. World Health Organization, Division of Malaria Eradication, Geneva, Switzerland;
2. WHO Regional Office;
3. Project Headquarters;
4. Investigator.

For non-anopheline species:

1. World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland;
2. WHO Regional Office;
3. and 4. Investigator.
### Specimen Report Form

**WHO TEST FOR INSECTICIDE-RESISTANCE IN MOSQUITO LARVAE**

**Date:** __________________________

*Insecticide*: DDT/dieldrin/BHC/other [1]

**Species:**

1. **Investigator:** __________________________
2. **Country:** __________________________
3. **Province:** __________________________
4. **Locality:** __________________________
5. **History of insecticide treatment (including agriculture):** __________________________

6. **Condition of larvae**: Instar __________________________

7. **Results of test** (abbreviations: "M"—moribund; "D"—dead)

<table>
<thead>
<tr>
<th>Tests</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Replicate 4</th>
<th>Totals (for comparable tests only)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Date of test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temperature during test</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

[1] Cross out what does not apply

[1] Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions)

**Remarks:** __________________________

**Signature of investigator:** __________________________

---

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.

A second copy to be sent on completion to the appropriate WHO Regional Office.
Annex 3

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF LICE TO INSECTICIDES

1. Introduction

(a) This method measures the susceptibility levels of a population of lice to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose it is necessary (i) to establish the susceptibility levels of normal lice, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance.

(b) Establishing the base-line. Batches of adult lice are exposed to different concentrations of insecticide and the kills obtained at each level are determined. It is suggested that a preliminary test be made at each concentration in the complete range provided. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of concentrations should be chosen, some of which give partial mortalities (i.e., at least one of them should give a complete kill and one less than 50% mortality). Tests at these concentrations should be repeated four times with samples from the same population of lice. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable.

(c) Subsequent routine checks. The lowest concentration that has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with two to five replicates. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include four tests at each of the concentrations used in establishing the original base-line. In this way, it is possible to detect the presence of physiological resistance and to determine the approximate proportion of resistant lice in the population.

(b) Condition of lice. Adult lice of either sex can be used provided they are not obviously starved.

(c) Conditions of test. Tests should be done in a room free from insecticidal contamination. The lice are exposed in darkness and held at a temperature between 20° and 30°C and at a relative humidity above 25%.
2. Composition of test kit

(a) Three packages, each containing 0.5 g of insecticide powder, of each of the following concentrations:

- DDT, technical: 0.04%; 0.2%; 1.0%; 5.0%
- gamma-BHC: 0.02%; 0.1%; 0.5%; 2.5%
- malathion: 0.0025%; 0.01%; 0.04%; 0.16%; 0.64%.

(b) 50 pieces of cloth, 12.5 cm square.

(c) 1 pair of forceps.

(d) Instruction sheets and a set of report forms; three sets of log-probability paper for plotting regression lines.

Other items not included in the kit but which the investigator needs to conduct the test are as follows:

- 14 halves of 9-cm Petri dishes or metal rings from preserving jars (to confine lice on treated cloth);
- 14 clean containers (beakers, salve tins, or other smooth-sided containers);
- 1 spatula or table knife (for spreading powders on cloth).

A small amount of acetone or alcohol should be available for cleansing the spatula and forceps after each use.

3. Method of test

(a) Each package contains 0.5 g of powder, or enough to treat one square of cloth. The cloth should be placed on a glass surface (or a piece of white paper), the contents of a package sifted over it, and the powder spread uniformly over one side of the cloth with a spatula or table knife and worked gently into the nap of the fabric. The treated cloth should be carefully placed on a rigid surface (wood or stiff cardboard) and the corners fastened thereto by thumb tacks or narrow strips of tape. Three cloths should be treated as replicates with each concentration of powder, making a total of 39 treated cloths. Three untreated cloths should be similarly mounted for use as controls.

(b) Adult lice should be collected in a single container from one individual or, if this is not possible, from a small group of individuals. To assess the resistance level in a locality, additional samples should be taken from a sufficient number of infested individuals. The lice should be divided by random selection into lots of twenty in small containers (beakers, salve tins or other clean containers). Although it is desirable to use 20 lice in each replicate, if this is not possible the number may be reduced down to an absolute minimum of 10 per replicate.
(c) One lot of adult lice should be placed on each treated cloth and confined under half a Petri dish or inside a metal ring. Rubber bands or weights should be employed to hold the dish or metal ring so that the lice cannot escape.

(d) Observations of mortality should be made after 24 hours. Only lice capable of co-ordinated movement should be counted as "alive".

(e) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of four concentrations giving partial and complete mortality. Three replicates should be performed at each of the chosen concentrations.

(f) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(g) When the test has been repeated four times with the same population of lice, adequate data should be available for constructing a base-line of susceptibility as described in section 4. The results should be recorded on the forms provided.

4. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC$_{50}$ or LC$_{90}$

\[^{1}\]

read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC$_{50}$ the worker is referred to the methods described by Swaroop \(^{2}\) and Litchfield & Wilcoxon.\(^{3}\)

(b) In tests where the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

\[
\frac{\% \text{ test mortality } - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100
\]

5. Interpretation of results

See Annex 14: Criteria and meaning of tests for determining the susceptibility of insects to insecticides.

\[^{1}\] The LC$_{50}$ and LC$_{90}$ represent respectively the insecticide concentrations at which 50% and 90% of the specimens are killed.


6. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland, and one copy to the appropriate WHO Regional Office.¹

¹ Addresses of WHO offices are as follows:

Headquarters:
World Health Organization, Geneva, Switzerland

Regional Offices:
World Health Organization, Regional Office for Africa, P. O. Box No. 6, Brazzaville, Republic of the Congo
World Health Organization, Regional Office for the Eastern Mediterranean, P. O. Box 1517, Alexandria, Province of Egypt, United Arab Republic
World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India
World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 1501 New Hampshire Avenue, N. W., Washington 6, D. C., USA
World Health Organization, Regional Office for Europe, 8 Schonfigsvej, Copenhagen Ø, Denmark
World Health Organization, Regional Office for the Western Pacific, P. O. Box 2932, Manila, Philippines
Specimen Report Form

WHO TEST TO DETERMINE THE EFFECTIVENESS OF INSECTICIDE
DUSTING POWDERS
AGAINST UNFAMILIAR STRAINS OF BODY LICE

1. Investigator: ___________________________ Date: ___________________________

5. Background information (in narrative form, using additional sheets if needed):
   (a) How prevalent are body lice among the population?
   (b) What insecticides or other control measures are in general use?
   (c) For how many years have these insecticides, particularly DDT, been used for control of lice?
   (d) How effective are the various insecticidal treatments against lice at present?
   (e) Is there any evidence that the insecticides in common use are less effective now than in
       previous years?

6. Source of lice: ___________________________

7. Temperature at which test was performed: ___________________________

<table>
<thead>
<tr>
<th>Concentration of toxicant in powder</th>
<th>Condition of lice after 24 hours' exposure to powder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First replicate</td>
</tr>
<tr>
<td></td>
<td>No. tested</td>
</tr>
<tr>
<td>0.04% DDT</td>
<td></td>
</tr>
<tr>
<td>0.2% DDT</td>
<td></td>
</tr>
<tr>
<td>1.0% DDT</td>
<td></td>
</tr>
<tr>
<td>5.0% DDT</td>
<td></td>
</tr>
<tr>
<td>0.02% gamma-BHC</td>
<td></td>
</tr>
<tr>
<td>0.1% gamma-BHC</td>
<td></td>
</tr>
<tr>
<td>0.5% gamma-BHC</td>
<td></td>
</tr>
<tr>
<td>2.5% gamma-BHC</td>
<td></td>
</tr>
<tr>
<td>0.0025% malathion</td>
<td></td>
</tr>
<tr>
<td>0.01% malathion</td>
<td></td>
</tr>
<tr>
<td>0.04% malathion</td>
<td></td>
</tr>
<tr>
<td>0.16% malathion</td>
<td></td>
</tr>
<tr>
<td>0.64% malathion</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>

Remarks: ___________________________ Signature of Investigator: ___________________________

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit,
Division of Environmental Health, Geneva.
A second copy to be sent on completion to the appropriate WHO Regional Office.
Annex 4

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT BEDBUGS TO INSECTICIDES

1. Introduction

(a) This method measures the susceptibility levels of a population of adult bedbugs to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose, it is necessary (i) to establish the susceptibility levels of normal bedbugs of the species concerned, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance. It is stressed that this test is not designed to evaluate the effectiveness of deposits of insecticides in the field; this must be ascertained by the use of other entomological techniques.

(b) Establishing the base-line. Batches of adult bedbugs are exposed to different concentrations of insecticide and the kills obtained at each level are determined. It is suggested that a preliminary test be made at each concentration in the complete range provided, at the standard exposure of one day for organophosphorus compounds, two days for dieldrin, and five days for DDT. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of four concentrations should be chosen, some of which give partial mortalities (i.e., at least one of them should give a complete kill and one less than 50% mortality). Tests at these concentrations should be repeated four times with samples from the same population of bedbugs. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable.

(c) Subsequent routine checks. A concentration double the lowest concentration that has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with two to five replicates. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include four tests at each of the concentrations used in establishing the original base-line. In this way, it is possible to detect the presence of physiological resistance and to determine the approximate proportion of resistant bedbugs in the population.
(d) *Condition of bedbugs.* Adult bedbugs of either sex can be used and their nutritional status should be recorded. Wherever possible fully fed bedbugs collected in the field should be used. If fully fed bedbugs are not available (starved specimens are very thin and transparent) the specimens collected may be fed on a suitable host and used on the following day.

(e) *Conditions of test.* Tests should be done in a room free from insecticidal contamination. The bedbugs are exposed and held at a temperature between 20°C and 30°C and at a relative humidity above 25%. Some uniformity in these conditions can be secured by the use of the kit box.

2. *Composition of test kit*

   (a) Five packages each containing 40 papers (5 cm long by 1.5 cm wide, tapered at one end) impregnated with DDT (p,p' isomer) in mineral oil (Risella 17, Shell) at concentrations of 0.25%, 0.5%, 1.0%, 2.0% and 4.0% respectively; and one package treated with oil only. Six packages of papers impregnated with dieldrin (purified HEOD) in mineral oil at concentrations of 0.05%, 0.1%, 0.2%, 0.4%, 0.8% and 1.6%, and one package treated with oil only.¹

   (b) 24 glass test-tubes, 15 cm long by 15 mm diameter.

   (c) Test-tube rack, plastic covered, holding 24 tubes, together with a black foam plastic mat.

   (d) 30 pieces of fine-mesh gauze to fit over the tubes.

   (e) 50 rubber bands.

   (f) One nylon brush, one pair of forceps, two fine paint brushes.

   (g) A holding container.

   (h) Pill boxes (for feeding bedbugs) with fine-mesh gauze and rubber bands.

   (i) Roll of self-adhesive plastic tape.

   (j) Instruction sheets and a set of report forms; three sheets of log-probability paper for plotting regression lines.

   (k) 40 sheets of clean typing paper.

3. *Method of test*

   (a) Having located infested houses, collect as many adult bedbugs as possible (approximately 100). The area from which collections are made

¹ The following impregnated papers are also available on request:

   (i) Dieldrin at 0.025% and 4.0%.

   (ii) Malathion at 0.05%, 0.1%, 0.2%, 0.4%, 0.8% and 1.6%.

   (Malathion papers lose their efficacy on long storage; their use should therefore be planned well in advance)
should be restricted as far as possible for tests in a given series. The bedbugs may be gathered by brushing them from infested sites (bed frames etc.) into the collecting vessel supplied, taking care not to damage them. The specimens are then brought to the locality of the test. If the bedbugs collected are unfed they are introduced into the pill boxes provided, which are then closed with the fine-mesh gauze secured with rubber bands and applied to suitable surfaces of a convenient host.

(b) Set up sufficient test-tubes to include two replicates at the range of concentrations required. Into each tube place one of the treated papers. Two papers impregnated with each of the concentrations of insecticide provided should be used and one control paper impregnated with oil alone.

(c) Transfer 10 adult bedbugs to each tube and close with the fine-mesh gauze. The tubes are then set upright in the holding rack in the box. If necessary, damp cloths are placed in the box to obviate very low humidity. Close the box for the appropriate exposure period (5 days for DDT, 2 days for dieldrin, or 1 day for organophosphorus compounds).

(d) At the end of the exposure time, the bedbugs are examined and the mortality recorded. Bedbugs incapable of any movement, or unable to cling to the test paper, are taken as dead. At the conclusion of the test, the test papers are discarded, the bedbugs destroyed and the tubes thoroughly cleansed.

(e) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of four concentrations giving partial and complete mortality. Three replicates should be performed at each of the chosen concentrations.

(f) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(g) When the test has been repeated four times with the same population of bedbugs, adequate data should be available for constructing a base-line of susceptibility as described in section 5. The results should be recorded on the forms provided.

4. General remarks

After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The packages should be kept in a cool place, but not in a refrigerator, as storage at too low a temperature may cause crystallization in the higher concentrations. Prolonged storage at high temperatures should be avoided. Papers should not be used after the expiry date shown on the box. The period of three years (for chlorinated hydrocarbon papers) from the date of impregnation presupposes that the packages are kept sealed at all times. Additional papers are available on request.
5. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC50 or LC95,1 read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC50, the worker is referred to the methods described by Swaroop 2 and Litchfield & Wilcoxon 3.

(b) In tests where the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

\[
\frac{\text{% test mortality} - \text{% control mortality}}{100 - \text{% control mortality}} \times 100
\]

6. Interpretation of results

See Annex 14: Criteria and meaning of tests for determining the susceptibility of insects to insecticides.

7. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland, and one copy to the appropriate WHO Regional Office.4

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1 The LC50 and LC95 represent respectively the insecticide concentrations at which 50% and 90% of the specimens are killed.
4 Addresses of WHO Regional Offices are as follows:

**Headquarters**:
World Health Organization, Geneva, Switzerland

**Regional Offices**:
- World Health Organization, Regional Office for Africa, P. O. Box No. 6, Brazzaville, Republic of the Congo
- World Health Organization, Regional Office for the Eastern Mediterranean, P. O. Box 1517, Alexandria, Province of Egypt, United Arab Republic
- World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India
- World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 1501 New Hampshire Avenue, N. W., Washington 6, D. C., USA
- World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen 0, Denmark
- World Health Organization, Regional Office for the Western Pacific, P. O. Box 2932, Manila, Philippines
Specimen Report Form
WHO TEST FOR INSECTICIDE RESISTANCE IN ADULT BEDBUGS

Date: .................................................................

1. Investigator: .................................................................
2. Species: .................................................................
3. Country: .................................................................
4. Province: .................................................................
5. Locality: .................................................................
6. History of insecticide treatment (including agriculture): .................................................................
7. Condition of bedbugs: blood-fed/unfed  
8. Where collected: human habitation/animal shelter/other...  
9. Type of test on population: first time/routine check/complete retest  
10. Exposure period: .................................................................

<table>
<thead>
<tr>
<th>Tests</th>
<th>Preliminary</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Totals (for comparable tests only)</th>
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<td>Date of test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature range</td>
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<td>Max.</td>
<td>Max.</td>
<td>Max.</td>
<td></td>
</tr>
<tr>
<td>during exposure</td>
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<td>Min.</td>
<td>Min.</td>
<td>Min.</td>
<td></td>
</tr>
<tr>
<td>Humidity range</td>
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<td>Max.</td>
<td>Max.</td>
<td>Max.</td>
<td></td>
</tr>
<tr>
<td>during exposure</td>
<td>Min.</td>
<td>Min.</td>
<td>Min.</td>
<td>Min.</td>
<td></td>
</tr>
<tr>
<td>Insecticide conc. (%)</td>
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<td></td>
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<tr>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>Dead</td>
<td>Total(1)</td>
<td>Dead</td>
<td>Total(1)</td>
</tr>
<tr>
<td>Corr.(2)</td>
<td></td>
<td>Corr.(2)</td>
<td></td>
<td>Corr.(2)</td>
<td></td>
</tr>
</tbody>
</table>

Control (oil alone) ........................................................................................................

\(1\) Cross out what does not apply.
\(2\) Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks: ................................................................................................................
Signature of Investigator: .................................................................

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.
A second copy to be sent on completion to the appropriate WHO Regional Office.
Annex 5

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF TRIATOMID BUGS TO INSECTICIDES

1. Introduction

(a) This method measures the susceptibility levels of a population of triatomid bugs to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose, it is necessary (i) to establish the susceptibility levels of normal triatomid bugs of the species concerned, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance. It is stressed that this test is not designed to evaluate the effectiveness of deposits of insecticides in the field; this must be ascertained by the use of other entomological techniques.

(b) Establishing the base-line. Batches of triatomid bugs are exposed to different concentrations of insecticide and the kills obtained at each level are determined. It is suggested that a preliminary test be made at each concentration in the complete range provided, at the standard exposure of one day for organophosphorus compounds and two days for dieldrin. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of four concentrations should be chosen, some of which give partial mortalities (i.e., at least one of them should give a complete kill and one less than 50% mortality). Tests at these concentrations should be repeated four times with samples from the same population of triatomid bugs. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable.

(c) Subsequent routine checks. A concentration double the lowest concentration that has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with 2-5 replicates. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include four tests at each of the concentrations used in establishing the original baseline. In this way, it is possible to detect the presence of physiological resistance and to determine the approximate proportion of resistant triatomid bugs in the population.

— 69 —
(d) Condition of triatomid bugs. Last-stage nymphs should preferably be used—if adults are used, the results should be kept separate.

(e) Conditions of test. Tests should be done in a room free from insecticidal contamination. The triatomid bugs are exposed and held at a temperature between 20° and 30°C and at a relative humidity above 25%. Some uniformity in these conditions can be secured by the use of the kit box.

2. Composition of test kit

(a) Five packages each containing 40 papers (2.5 cm × 15 cm) impregnated with dieldrin (purified HEOD) in mineral oil at concentrations of 0.2%, 0.4%, 0.8%, 1.6% and 4.0% and one package treated with oil only.¹

(b) 24 glass test-tubes, 25 cm long by 3 cm diameter.

(c) Test-tube rack, plastic covered, holding 24 tubes, together with a black foam plastic mat.

(d) 30 pieces of fine-mesh gauze to fit over the tubes.

(e) 50 rubber bands.

(f) Two pairs of collecting forceps.

(g) A holding container.

(h) Roll of self-adhesive plastic tape.

(i) Instruction sheets and a set of report forms; three sheets of log-probability paper for plotting regression lines.

(j) 40 sheets of clean typing paper.

3. Method of test

(a) Having located infested houses, collect as many last-stage nymphs as possible (approximately 100). The area from which collections are made should be restricted as far as possible for tests in a given series. The triatomid bugs collected from infested sites are placed in the holding tubes taking care not to damage them. The specimens are then brought to the locality of the test.

(b) Set up sufficient test-tubes to include two replicates at the range of concentrations required. Into each tube place one of the treated papers, folded once lengthwise into the shape of a letter “V”. In doing so care should be taken to avoid contamination. Two papers impregnated with

¹ The following organophosphorus impregnated papers are available on request: Malathion and fenithion at 0.8%, 1.6%, 3.2% and 6.4%. (These papers lose their efficacy on long storage; their use should therefore be planned well in advance.)
each of the concentrations of insecticide provided should be used and one control paper impregnated with oil alone.

(c) Transfer 10 adult bugs to each tube and close with the fine-mesh gauze. The tubes are then set upright in the holding rack in the box. If necessary, damp cloths are placed in the box to obviate very low humidity. Close the box for the two-day exposure period for dieldrin and one day for the organophosphorus compounds.

(d) At the end of the appropriate exposure time, the triatomid bugs are examined and the mortality recorded. Bugs incapable of any movement, or unable to cling to the test paper, are taken as dead. At the conclusion of the test, the test papers are discarded, the bugs destroyed, and the tubes thoroughly cleansed.

(e) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of four concentrations giving partial and complete mortality. Three replicates should be performed at each of the chosen concentrations.

(f) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(g) When the test has been repeated four times with the same population of bugs, adequate data should be available for constructing a base-line of susceptibility as described in section 5. The results should be recorded on the forms provided.

4. General remarks

After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The packages should be kept in a cool place, but not in a refrigerator, as storage at too low a temperature may cause crystallization in the higher concentrations. Prolonged storage at high temperatures should be avoided. Papers should not be used after the expiry date shown on the box. The period of three years (for dieldrin papers) from the date of impregnation presupposes that the packages are kept sealed at all times. Additional papers are available on request.

5. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted
by eye, and the LC₅₀ or LC₉₀ read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC₅₀, the worker is referred to the methods described by Swaroop and Litchfield & Wilcoxon.

(b) In tests where the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott’s formula:

\[
\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100
\]

6. Interpretation of results

See Annex 14: *Criteria and meaning of tests for determining the susceptibility of insects to insecticides.*

7. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland, and one copy to the appropriate WHO Regional Office.

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1 The LC₂₀ and LC₉₀ represent respectively the insecticide concentrations at which 50% and 90% of the specimens are killed.
4 Addresses of WHO offices are as follows:

**Headquarters:**
World Health Organization, Geneva, Switzerland

**Regional Offices:**
- World Health Organization, Regional Office for Africa, P. O. Box No. 6, Brazzaville, Republic of the Congo
- World Health Organization, Regional Office for the Eastern Mediterranean, P. O. Box 1517, Alexandria, Province of Egypt, United Arab Republic
- World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India
- World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 1501 New Hampshire Avenue, N. W., Washington 6, D. C., USA
- World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen 5, Denmark
- World Health Organization, Regional Office for the Western Pacific, P. O. Box 2932, Manila, Philippines.
Specimen Report Form

WHO TEST FOR INSECTICIDE RESISTANCE IN TRIATOMID BUGS

Date: 

1. Investigator: 

2. Species: 

3. Country: 

4. Province: 

5. Locality: 

6. History of Insecticide treatment (including agriculture): 

7. Condition of triatomid bugs: blood-fed/unfed 

8. Stage used: nymphs/adults: 

9. Where collected: human habitation/animal shelter/other: 

10. Type of test on population: first time/routine check/complete retest: 

11. Exposure period: 

<table>
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<th>Tests</th>
<th>Preliminary</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Totals (for comparable tests only)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of test</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Insecticide conc. (%)</td>
<td>DDT/dieldrin/other</td>
<td>Dead</td>
<td>Total</td>
<td>Mort. (%) corr.</td>
<td>Dead</td>
</tr>
<tr>
<td>Control (oil alone)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

1 Cross out what does not apply. 
2 Correct by applying Abbott’s formula if control mortality is between 5% and 20% (see instructions).

Remarks: 
Signature of investigator: 

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland. 
A second copy to be sent on completion to the appropriate WHO Regional Office.
INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF FLEAS TO INSECTICIDES

1. Introduction

(a) This method measures the susceptibility levels of a population of adult fleas to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose, it is necessary (i) to establish the susceptibility levels of normal fleas of the species concerned, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance. It is stressed that this test is not designed to evaluate the effectiveness of deposits of insecticides in the field; this must be ascertained by the use of other entomological techniques.

(b) Establishing the base-line. Batches of adult fleas are exposed to different concentrations of insecticide to determine the kills obtained at each level. Fed fleas must be used wherever possible. It is suggested that a preliminary test be made at each concentration in the complete range provided at the standard exposure of 60 minutes followed by a 24-hour recovery period. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of four concentrations should be chosen, some of which will give partial mortalities (i.e., at least one of them should give a complete kill and one less than 50% mortality); tests at these concentrations should be repeated four times with samples from the same population of fleas. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable. If 60 minutes’ exposure regularly gives low mortalities at the highest available concentrations, the base-line shall be established by exposing continuously for 24 hours without recovery period.

(c) Subsequent routine checks. The concentration double the lowest concentration that has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with two to five replicates. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert
signal calling for further investigations. Such investigations will include tests at each of the concentrations used in establishing the original base-line with 1-hour exposure as well as tests with 24 hours’ exposure. In this way, it is possible to detect the presence of physiological resistance and to determine the approximate proportion of resistant fleas in the population.

(d) Sex of fleas. Adult fleas of either sex may be used.

(e) Conditions of the test. Tests should be done in a room free from insecticidal contamination. The fleas are exposed and held at a temperature between 20° and 30°C and at a relative humidity above 25%, in the dark. Some uniformity of these conditions can be secured by the use of the kit box.

2. Composition of test kit

(a) Five packages each containing 40 papers (5 cm long by 1.5 cm wide, tapered at one end), impregnated with DDT (p,p’ isomer) in mineral oil (Risella 17, Shell) at concentrations of 0.25%, 0.5%, 1.0%, 2.0% and 4.0% respectively; six packages of papers impregnated with dieldrin (purified HEOD) in mineral oil at concentrations of 0.05%, 0.1%, 0.2%, 0.4%, 0.8% and 1.6% respectively; and one package of papers impregnated with mineral oil only. The papers are impregnated at the rate of 3.6 mg of oil solution per cm² of paper. Papers impregnated with organophosphorus insecticides will be supplied on request.¹

(b) A supply of non-impregnated papers (5 cm by 1.5 cm, tapered at one end) for use in the holding tubes.

(c) Twenty-four glass test tubes, 15 cm long by 15 mm inside diameter.

(d) Test-tube rack, plastic-covered, holding 24 tubes, together with a small black foam-plastic mat.

(e) Thirty pieces of fine-mesh gauze to fit over the tubes.

(f) Fifty rubber bands.

(g) Collecting apparatus—one pair of ophthalmic forceps and two nylon brushes.

(h) Holding container for fleas.

(i) One pair of tongs, preferably with locking device.

¹ The following organophosphorus-impregnated papers are available on request:

(i) Malathion at 0.2%, 0.4%, 0.8%, 1.6%, 3.2% and 6.4%.

(ii) Fenthion at 0.05%, 0.1%, 0.2%, 0.4%, 0.8% and 1.6%.

(These papers lose their efficacy on long storage; their use should therefore be planned well in advance.)
(j) Two aspirators fitted with rubber suction bulbs.
(k) Two pairs of rubber (latex) gloves.
(l) Two rolls of self-adhesive plastic tape.
(m) Instruction sheets and a set of report forms; three sheets of log-probability paper for plotting regression lines.
(n) Forty sheets of clean typing paper.

3. Collection of fleas

(a) Fleas for susceptibility testing may be obtained from different sources. They may be obtained direct from rats that have been trapped, or as adults—or pupae—from rat burrows or from litter in houses. In places where there is a risk of contracting plague or other rat-borne zoonoses, handling of rats by means of tongs and rubber gloves will help to minimize direct contact.

With regard to the collecting of fleas from rats, three methods of killing or immobilizing rats are suggested:

(i) Killing by strangulation, preferably by means of tongs or forceps with a locking device.

(ii) Putting the rat into a bag, and killing it by means of an injection of formalin.

(iii) Using carbon dioxide to asphyxiate the rat (the fleas themselves quickly recover from this treatment).

For the purpose of removing fleas from the dead rat, the following methods may be used:

(i) Brushing the fleas into a deep tray or pan from the rats by means of the nylon brush supplied.

(ii) Putting the dead rat into a polythene bag or a deep enamelled container, and allowing the fleas to leave the dead host naturally overnight. The fleas are then collected with an aspirator. With either of these methods, fleas may be further encouraged to leave the body of the host by blowing on the animal fur.

In some areas it may prove more convenient to recover fleas from litter or burrows, rather than from the rat itself. The material is placed in a deep container (at least 20 cm deep). Fleas emerging from the pupae or the litter will require feeding before testing; this can be done conveniently by placing a white mouse or rat in the container.

(b) As the fleas are collected they are transferred to the holding container. A single piece of circular paper, smaller than the diameter of the
holding container, should be dropped into the container to furnish a suitable resting surface for the fleas and to facilitate aspiration of individual specimens. Filter paper or clean typing paper is suitable for this purpose.

4. Method of test

(a) Sufficient test tubes are taken for the complete range of concentrations. A test paper of each concentration of insecticide and one control paper impregnated with oil alone is inserted into each of the test tubes.

(b) Into each tube 10 fleas, fed if possible, are transferred by means of the aspirator unit. This is accomplished by fitting the aspirator unit to each test tube in turn and aspirating the fleas from the holding container. Each tube is closed by the fine-mesh gauze and the exposure period begins. The tubes are placed vertically in the rack which in turn is placed under one of the halves of the kit box so that the fleas are in darkness during the exposure period.

(c) At the conclusion of a 60-minute exposure period, the tubes should be removed in the order in which they were set up and all the fleas transferred (by means of the aspirator) to clean tubes containing a clean non-impregnated paper. The holding tubes are then closed with gauze, returned to the rack, and placed in darkness. To minimize contamination of test insects with high concentrations of insecticides during the transfer from treated to clean papers, aspiration should be done with the control fleas first, then with the lowest concentration of DDT or dieldrin, working up to the highest concentration. The aspirator tube should be thoroughly rinsed with acetone or alcohol and dried before transferring the fleas from papers treated with different insecticides. If the exposure period is extended to 24 hours the mortality is recorded at the end of the exposure period.

(d) After 24 hours, the fleas are examined for mortality. Fleas unable to stand should be counted as dead. At this time, the fleas should be carefully examined to make sure that only specimens of the desired species are being recorded. The results should be recorded on the forms provided (see section 8 for distribution of report forms). At the conclusion of the test, the exposure papers are discarded, the fleas destroyed, and the tubes thoroughly cleansed.

(e) After the preliminary test has been performed with the complete range of concentrations, the test should be continued with the chosen series of four concentrations. This test involves three replicates at each of the chosen concentrations giving partial and complete mortality.

(f) Tests with control mortality in excess of 20% are unsatisfactory and should be repeated.
(g) When the test has been repeated four times with the same population of fleas, adequate data should be available for constructing a base-line of susceptibility as described in section 6.

5. General remarks

After an impregnated paper has been removed, the package should be carefully resealed with the plastic tape provided. The packages should be kept in a cool place, but not in a refrigerator, as storage at too low a temperature may cause crystallization in the higher concentrations. Prolonged storage at high temperatures should be avoided. Papers should not be used after the expiry date shown on the box. The period of three years (for chlorinated hydrocarbon papers) from the date of impregnation presupposes that the packages are kept sealed at all times. Additional papers are available on request.

6. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC$_{50}$ or LC$_{90}$ read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC$_{50}$, the worker is referred to the methods described by Swaroop$^2$ and Litchfield & Wilcoxon.$^3$

(b) In tests where the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

\[
\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100
\]

7. Interpretation of results

See Annex 14: Criteria and meaning of tests for determining the susceptibility of insects to insecticides.

---

$^1$ The LC$_{50}$ and LC$_{90}$ represent respectively the insecticide concentrations at which 50% and 90% of the specimens are killed.


8. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland, and one copy to the appropriate WHO Regional Office.¹

¹ Addresses of WHO offices are as follows:

_Headquarters_

World Health Organization, Geneva, Switzerland

_Regional Offices_

- World Health Organization, Regional Office for Africa, P. O. Box No. 6, Brazzaville, Republic of the Congo
- World Health Organization, Regional Office for the Eastern Mediterranean, P. O. Box 1517, Alexandria, Province of Egypt, United Arab Republic
- World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Ring Road, New Delhi, India
- World Health Organization, Regional Office for the Americas (Pan American Sanitary Bureau), 1501 New Hampshire Avenue, N. W., Washington 6, D. C., USA
- World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark
- World Health Organization, Regional Office for the Western Pacific, P. O. Box 2932, Manila, Philippines
# Specimen Report Form

**WHO TEST FOR INSECTICIDE RESISTANCE IN ADULT FLEAS**

1. Investigator: __________________________  2. Species: __________________________


6. History of insecticide treatment (including agriculture): __________________________

7. Condition of fleas: blood-fed/unfed

8. Where collected: host animal/animal burrow/other

9. Type of test on population: first time/routine check/complete retest

10. Exposure period (if not one hour): __________________________

<table>
<thead>
<tr>
<th>Tests</th>
<th>Preliminary</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
</tr>
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<tr>
<td>Date of test</td>
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<td>Temperature during exposure period</td>
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<tr>
<td>Humidity during exposure period</td>
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</tr>
<tr>
<td>Insecticide conc. (%) DDT/dieldrin/other</td>
<td>Dead</td>
<td>Total</td>
<td>Mort. (%) corr.</td>
<td>Dead</td>
</tr>
</tbody>
</table>

Totals (for comparable tests only)

---

1 Cross out what does not apply.
2 Correct by applying Abbott's formula if control mortality is between 5% and 20% (see instructions).

Remarks: __________________________

Signature of investigator: __________________________

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.

A second copy to be sent on completion to the appropriate WHO Regional Office.
Annex 7

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT SANDFLIES TO INSECTICIDES

1. Introduction

(a) This method measures the susceptibility levels of a population of adult sandflies to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose, it is necessary (i) to establish the susceptibility levels of normal sandflies of the species concerned and (ii) to make subsequent routine checks of susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance in sandflies. It is stressed that this test is not designed to evaluate the effectiveness of deposits of insecticides in the field; for this purpose other entomological techniques must be used.

(b) Establishing the base-line. Batches of sandflies are exposed to different concentrations of insecticide, and the kills obtained at each level are determined. It is suggested that a preliminary test be made at each concentration in the complete range provided, using the standard exposure of one hour. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of four concentrations should be chosen, some of which will give partial mortalities (i.e., at least one of them should give a complete kill and one less than 50% mortality). Tests at these concentrations should be repeated four times with samples from the same population of sandflies. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable.

(c) Subsequent routine checks. The lowest concentration which has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with five replicates; when sandflies are scarce a minimum of two replicates is permissible. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include four tests at each of the concentrations used in establishing the original base-line. To validate the results, the investigator must make every effort to collect sufficient specimens for providing at least 15 sandflies per tube.
(d) Condition of sandflies. Sandflies may be collected from sprayed and unsprayed premises indoors and in the open at night, either when resting on outside walls or in the act of biting man or animals or both. Their source should be reported on the form provided.

(e) Conditions of test. The experiments should be done indoors, in a selected building free from insecticidal contamination and extremes of temperature, humidity, illumination, and wind. In practice, it is usually possible to find a suitable room near the centre of the locality where the sandflies were collected. Transportation of insects to a distant base laboratory often results in mortality from causes other than the insecticide; this will be evident as high mortality in the controls. Where possible the comparable tests should be made under similar conditions of temperature and humidity.

2. Composition of test kit

(a) Twenty-two plastic tubes, 125 mm long by 44 mm in diameter; 8 of these (with red dot) are used for exposing the sandflies to the insecticide; 2 (with green dot) are used for the control exposure without insecticide; 10 (with green dot) are used as holding-tubes for pre-test sorting and post-exposure observation, and 2 (with green dot) are extra tubes to which those sandflies alive at the end of the 24-hour holding period may be transferred for chloroformizing and placing in separate pillboxes prior to identification. Each tube is fitted at one end with a double thickness of fine-mesh nylon netting. In order to identify the concentrations used with them, the red exposure tubes should be numbered 1 to 8, the green control exposure tubes 9 and 10, and the holding-tubes la to 10 a.

(b) Ten slide-units, each with a screw-cap on either side and provided with a 20-mm filling hole.

(c) Five packages of papers impregnated with DDT (p,p' isomer) in mineral oil (Risella 17, Shell) at concentrations of 0.25%, 0.5%, 1%, 2% and 4%, respectively; and a package treated with oil only. Six packages of papers impregnated with dieldrin (purified HEOD) in mineral oil at concentrations 1 of 0.05%, 0.1%, 0.2%, 0.4%, 0.8% and 1.6%, and one package treated with oil only.²

(d) Sheets of clean paper (12×15 cm) for lining the holding-tubes.

(e) Twenty-two wire clips to hold the papers in position against the walls of the tubes. The 14 silver clips should be used only for the holding-tubes and control exposure tubes; the 8 copper clips should be used only for the insecticide exposure tubes.

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1 Papers impregnated with 0.025% and with 4.0% dieldrin are available on request.
² Each package contains eight papers.
(f) Two glass aspirator tubes, 12 mm in internal diameter, together
with 60 cm of tubing. Ordinary test tubes may be used, if provided by the
investigator.

(g) One roll of self-adhesive plastic tape.

(h) Instruction sheets and a set of report forms; three sheets of log-
probability paper for plotting regression lines.

3. Procedure

(a) First a preliminary test is performed with the complete range of
concentrations. Into each of the holding-tubes (6 for DDT; 7 for dieldrin)
insert a piece of clean white paper rolled into a cylinder to line the wall and
fasten it in position with a spring wire clip (silver). Attach the slides to the
tubes.

(b) Collect the sandflies with the aspirator provided (Fig. 1, A). Sand-
flies should be collected in lots of not more than 5 (Fig. 1, B) and gently
transferred to the holding-tubes through the filling-holes in each slide
(Fig. 1, C) to give 25 per tube.

(c) Into each of the exposure tubes (6 for DDT or 7 for dieldrin) intro-
duce a sheet of impregnated paper, rolled into a cylinder to line the wall,
and fastened into position with an appropriate spring-wire clip. One paper
impregnated with each of the concentrations of insecticide provided should
be used and one control paper impregnated with oil alone.

(d) Introduce the sandflies into the exposure tube by attaching it
to the vacant screw-top in the slide (Fig. 1, D). The slide should be pulled
out to a point beyond the filling hole so that no part of it occludes the
tube openings; the sandflies are then blown gently into the exposure
tube. (If necessary, the small safety knob on the slide may be filed down
to facilitate this operation.) Close the slide. Detach the holding-tube
and set it aside.

(e) Leave the exposure tubes standing upright with the screen end up
for one hour (Fig. 1, E) under conditions of moderate, diffuse illumination.

(f) At the end of the exposure period, transfer the sandflies to the
holding-tubes by reversing the process described under (d). The exposure
pipes should be held almost vertically with the screen end down and the
slide tapped to dislodge any insects resting on it before it is withdrawn.
Then attach the holding-tube, withdraw the slide but do not detach it,
and blow the sandflies gently into the holding-tube; close the slide and
remove the exposure tube. Then set the holding-tube so that it stands on
the slide and place a pad of wet cotton wool on the screen (Fig. 1, F).
Cardboard cartons or cups or other suitable containers may be used
instead of the holding-tubes, provided that they are used consistently.
FIG. 1. METHOD FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT SANDFLIES TO INSECTICIDES
(g) Keep the holding-tubes for 24 hours in a secluded part of the room where the temperature does not exceed 30°C. Wherever feasible, the maximum and minimum temperature of the room during the 24 hours should be recorded. If necessary, the tubes should be protected from ants by placing them on a platform standing in a pan of water. If conditions are very hot and dry, a moist chamber may be prepared by suspending damp towelling in a container.

(h) Mortality counts are made after 24 hours. The dead sandflies can be clearly seen through the nylon netting and accurately counted. The number of living insects is determined by chloroforming them and counting the total number present. In instances where a mixture of species is tested, those flies alive after 24 hours should be transferred to the extra tubes provided in the same manner already described in section 3 (f). The living sandflies are chloroformed and counted and a count also made of the dead ones remaining in the holding-tubes. Each group exposed to each concentration should be placed in a separate pillbox and labelled for later identification.

(i) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of four concentrations. Four replicates, including the preliminary test where appropriate, should be performed at each of these chosen concentrations. The 20 tubes provided in the kit are sufficient for one series of two replicates at each of the four chosen concentrations, together with two controls.

(j) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(k) When the test has been repeated four times with the same population of sandflies, adequate data should be available for constructing a base line of susceptibility as described in section 5. The results should be recorded in duplicate on the forms provided.

4. General remarks

(a) Each impregnated paper may be used up to 20 times, and up to three weeks after removal from the package, provided all possible precautions are taken against evaporation of the oil. To this end, the papers should be left in the tubes, with the open end well wrapped, and placed in the kit box, which in turn should be kept in a cool place. No paper should be used more than three weeks after removal from the package.

(b) After an impregnated paper has been removed, the package should be resealed carefully with the plastic tape provided. The packages should be kept in a cool place, but not in a refrigerator, as too low a temperature may cause crystallization in the higher insecticidal concentrations. Prolonged storage at high temperatures should be avoided. Papers should
not be used after the expiry date shown on the box. The period of three years from the date of impregnation presupposes that the packages are kept sealed at all times.

(c) If the species of sandfly concerned is exceptionally sensitive (so that it shows high mortality at the lowest concentration provided), the exposure period may be reduced to half or even a quarter of an hour. If the species is exceptionally insensitive, the exposure period may be increased to two, four or eight hours. In all cases, the one-hour exposure period should be used first and the results recorded.

5. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the LC$_{50}$ or LC$_{90}$ read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC$_{50}$, the worker is referred to the methods described by Swaroop $^4$ and Lichtfield & Wilcoxon.$^5$

(b) In cases where the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

(c) On establishment of a change in the population presumed to be due to resistance, there still remain several questions to answer. For example, it is of interest to know whether there has been a change in the entire population or an appearance of complete resistance in a section of the population only. Such questions can only be answered by tests over a range of concentrations, including those employed in establishing the original base-line. The user may therefore wish to perform this test at multiple concentrations and derive the new dosage-mortality relationship.

6. Interpretation of results

See Annex 14 : Criteria and meaning of tests for determining the susceptibility of insects to insecticides.

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7. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland, and one copy to the appropriate WHO Regional Office.¹

¹ Addresses of WHO offices are as follows:
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World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark
World Health Organization, Regional Office for the Western Pacific, P. O. Box 2932, Manila, Philippines
**Specimen Report Form**

**WHO TEST FOR INSECTICIDE RESISTANCE IN ADULT SANDFLIES**

1. Investigator: ......................................................... 2. Species: ............................................................
5. Locality: .............................................................
6. History of insecticide treatment (including agriculture): .................................................................
7. Condition of sandflies: blood-fed/unfed
8. Where collected: human habitation/animal shelter/host/other
9. Type of test on population: first time/routine check/complete retest
10. Exposure period (if not 1 hour):

<table>
<thead>
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<th>Tests</th>
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<tr>
<td>Temperature during exposure period</td>
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<td>Humidity during exposure period</td>
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<tr>
<td>Insecticide conc. (%) DDT/dieldrin/other</td>
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<td>Dead Total</td>
<td>Dead Total</td>
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<tr>
<td></td>
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<td>Mort. (%)</td>
<td>Mort. (%)</td>
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<td>corr.</td>
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</tr>
</tbody>
</table>

Control (oil alone)

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1 Cross out what does not apply.  
2 Correct by applying Abbott’s formula if mortality is between 5% and 20% (see instructions).

Remarks: ..........................................................  
Signature of investigator: ....................................

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.  
A second copy to be sent on completion to the appropriate WHO Regional Office.
Annex 8

PROVISIONAL INSTRUCTIONS FOR DETERMINING
THE SUSCEPTIBILITY OR RESISTANCE OF TSETSE FLIES
AND CERTAIN HIGHER DIPTERA TO INSECTICIDES

1. Introduction

(a) This method measures the susceptibility levels of a population of adult tsetse flies and of Stomoxys to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose, it is necessary (i) to establish the susceptibility levels of normal tsetse flies of the species concerned and (ii) to make subsequent routine checks of susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance. It is stressed that this test is not designed to evaluate the effectiveness of deposits of insecticides in the field; for this purpose other entomological techniques must be used.

(b) Establishing the base-line. Batches of tsetse flies are exposed to different concentrations of insecticide, and the kills obtained at each level are determined. It is suggested that a preliminary test be made at each concentration in the complete range provided, using the standard exposure of one hour. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of four concentrations should be chosen, some of which will give partial mortalities (i.e., at least one of them should give a complete kill and one less than 50% mortality). Tests at these concentrations should be repeated four times with samples from the same population of tsetse flies. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable.

(c) Subsequent routine checks. A concentration double the lowest concentration that has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with five replicates; when tsetse flies are scarce a minimum of two replicates is permissible. The occasional appearance of survivors in such checks

---

1 This test has been proved satisfactory for use with Glossina palpalis, G. swynnertoni and G. morsitans and also for Stomoxys. It has not been proved satisfactory for house-flies and would probably be unsuitable for G. tachinoides, which does not survive well in captivity.
may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include four tests at each of the concentrations used in establishing the original base-line. To validate the results, the investigator must make every effort to collect sufficient specimens for providing ten to twelve tsetse flies per tube.

(d) *Capture, transport and storage of tsetse flies.* Wild tsetse flies may be captured with a butterfly net and transferred to test tubes. For transport to the laboratory, they are preferably transferred to small cages, separating males from females and discarding the gravid females (which, in any case, are comparatively rare). About 25 specimens should be put into each cage and the cages immediately transferred to a wooden box containing a damp cloth to maintain adequate humidity during transport.

In the laboratory, the specimens are fed as soon as possible (e.g., on a guinea pig for 30-40 minutes). The unfed ones are removed and given a second opportunity of taking a blood meal a few hours later; finally those that do not feed are discarded. The fed specimens are kept for 48 hours before the test.

(e) *Conditions of test.* The experiments should be done indoors, in a selected building free from insecticidal contamination and extremes of temperature, humidity and illumination, and away from draughts. In practice, it is usually possible to find a suitable room near the centre of the locality where the tsetse flies were collected. Transportation of insects to a distant base laboratory often results in mortality from causes other than the insecticide; this will be evident as high mortality in the controls. Where possible the comparable tests should be made under similar conditions of temperature and humidity.

2. Composition of test kit

(a) Twenty plastic tubes, 125 mm long by 44 mm in diameter; 8 of these (with red dot) are used for exposing the tsetse flies to the insecticide; 2 (with green dot) are used for the control exposure without insecticide; 10 (with green dot) are used as holding-tubes. Each tube is fitted at one end with a double thickness of fine-mesh nylon netting. In order to identify the concentrations used with them, the red exposure tubes should be numbered 1 to 8, the green control exposure tubes 9 and 10, and the holding-tubes 1a to 10a.

(b) Ten slide-units, each with a screw-cap on either side and provided with a 20-mm filling hole.

(c) Five packages of papers impregnated with DDT (*p,p'* isomer) in mineral oil (Risella 17, Shell) at concentrations of 0.25%, 0.5%, 1%, 2%
and 4%, respectively; and 1 package treated with oil only. Six packages of papers impregnated with dieldrin (purified HEOD) in mineral oil at concentrations\(^1\) of 0.05%, 0.1%, 0.2%, 0.4%, 0.8% and 1.6%, and one package treated with oil only.\(^2\)

\((d)\) Sheets of clean paper (12 × 15 cm) for lining the holding-tubes.

\((e)\) Twenty wire clips to hold the papers in position against the walls of the tubes. The 12 silver clips should be used only for the holding-tubes and control exposure tubes; the 8 copper clips should be used only for the insecticide exposure tubes.

\((f)\) Two glass aspirator tubes, 12 mm in internal diameter, together with 60 cm of tubing.

\((g)\) One roll of self-adhesive plastic tape.

\((h)\) Instruction sheets and a set of report forms; three sheets of log-probability paper for plotting regression lines.

3. Procedure

\((a)\) First a preliminary test is performed with the complete range of concentrations. Into each of the holding-tubes (6 for DDT; 7 for dieldrin) insert a piece of clean white paper rolled into a cylinder to line the wall and fasten it in position with a spring wire clip (silver). Attach the slides to the tubes.

\((b)\) Collect the tsetse individuals with the aspirator provided (Fig. 1, A). Gently transfer them to the holding-tubes through the filling-holes in each slide to give 10 to 12 per tube (Fig. 1, B & C).

\((c)\) Into each of the exposure tubes (6 for DDT or 7 for dieldrin) introduce a sheet of impregnated paper, rolled into a cylinder to line the wall, and fasten it in position with an appropriate spring-wire clip. One paper impregnated with each of the concentrations of insecticide provided should be used and one control paper impregnated with oil alone.

\((d)\) Introduce the tsetse flies into the exposure tube by attaching it to the vacant plastic cap in the slide (Fig. 1, D). The slide should be pulled out to a point beyond the filling-hole so that no part of it occludes the tube openings; the tsetse flies are then blown gently into the exposure tube, which should be pointed towards the light. (If necessary, the small safety knob on the slide may be filed down to facilitate this operation.) Close the slide. Detach the holding-tube and set it aside.

\(^1\) Papers impregnated with 0.025% and with 4.0% dieldrin are available on request.

\(^2\) Each package contains eight papers.
FIG. 1. METHOD FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF TSETSE FLIES TO INSECTICIDES

A

B

C

D

E

F
(e) Leave the exposure tubes standing upright with the screen end up for one hour (Fig. 1, E) under conditions of moderate, diffuse illumination.

(f) At the end of the exposure period, transfer the tsetse flies to the holding-tubes by reversing the process described under (d). The exposure tubes should be held almost vertically with the screen end down and the slide tapped to dislodge any insects resting on it before it is withdrawn. Then attach the holding-tube, withdraw the slide but do not detach it, and blow the tsetse flies gently into the holding-tube; close the slide and remove the exposure tube. Then set the holding-tube so that it stands on the slide and place a pad of cotton wool on the screen (Fig. 1, F). Cardboard cartons or cups or other suitable containers may be used instead of the holding-tubes, provided they are used consistently.

It may be found more satisfactory to transfer the tsetse flies to clean paper cups covered with nylon netting secured by an elastic band. The insects can be introduced through a hole cut in the middle of the netting which is subsequently plugged with a pad of cotton wool soaked in sugar solution. This procedure may perhaps give lower control mortality than the use of holding-tubes.

(g) Keep the holding-tubes (or paper cups) for 24 hours in a secluded part of the room where the temperature does not exceed 30°C. Wherever feasible, the maximum and minimum temperature of the room during the 24 hours should be recorded. If necessary, the tubes should be protected from ants by placing them on a platform standing in a pan of water. If conditions are very hot and dry, a moist chamber may be prepared by suspending damp towelling in a container.

(h) Mortality counts are made after 24 hours. The dead tsetse flies can be clearly seen through the nylon netting and accurately counted. The number of living insects is determined by chloroforming them and counting the total number present.

(i) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of four concentrations. Four replicates, including the preliminary test where appropriate, should be performed at each of these chosen concentrations. The 20 tubes provided in the kit are sufficient for one series of two replicates at each of the four chosen concentrations, together with two controls.

(j) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(k) When the test has been repeated four times with the same population of tsetse flies, adequate data should be available for constructing a base-line of susceptibility as described in section 5. The results should be recorded in duplicate on the forms provided.
4. General remarks

(a) Each impregnated paper may be used up to 20 times, and up to
three weeks after removal from the package, provided all possible pre-
cautions are taken against evaporation of the oil. To this end, the papers
should be left in the tubes, with the open end well wrapped, and placed
in the kit box, which in turn should be kept in a cool place. No paper
should be used more than three weeks after removal from the package.

(b) After an impregnated paper has been removed, the package should
be resealed carefully with the plastic tape provided. The packages should
be kept in a cool place, but not in a refrigerator, as too low a temperature
may cause crystallization in the higher insecticidal concentrations. Prolonged
storage at high temperatures should be avoided. Papers should not be
used after the expiry date shown on the box. The period of three years from
the date of impregnation presupposes that the packages are kept sealed
at all times.

(c) If the species of tsetse fly concerned is exceptionally sensitive (so
that it shows high mortality at the lowest concentration provided), the
exposure period may be reduced to half or even a quarter of an hour. If the
species is exceptionally insensitive, the exposure period may be increased to
2, 4 or 8 hours. In all cases, the one-hour exposure period should be used
first and the results recorded.

5. Results

(a) The user may desire to construct the dosage-mortality regression
line from the results obtained in the quadruplicate tests at the chosen
concentrations and, in many cases, from those of the single preliminary
test as well. For this purpose, the results should be plotted on the loga-
ithmic-probability paper provided. The regression line may be fitted
by eye, and the LC₅₀ or LC₉₀¹ read from the graph. The regression line
should not be extended (extrapolated) beyond the highest mortality obtained.
For more accurate methods of computing the LC₉₀, the worker is referred
to the methods described by Swaroop² and Lichtfield & Wilcoxon.³

(b) In cases where the control mortality is between 5% and 20%, the
percentage mortalities should be corrected by Abbott’s formula:

\[
\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100
\]

¹ The LC₅₀ and LC₉₀ represent respectively the insecticide concentrations at which
50% and 90% of the specimens are killed.

² Swaroop, S. (1958) Statistical methodology in malaria work, unpublished mimeo-
graphed document WHO/Mal/240

6. Interpretation of results

See Annex 14: Criteria and meaning of tests for determining the susceptibility of insects to insecticides.

7. Distribution of reports

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland.
### Specimen Report Form

**WHO TEST FOR INSECTICIDE RESISTANCE IN TSETSE FLIES**

1. Investigator: 
2. Species: 
3. Country: 
4. Province: 
5. Locality: 
6. History of insecticide treatment (including agriculture): 
7. Where collected: 
8. Type of test on population: first time/routine check/complete retest ¹
9. Exposure period (if not 1 hour):

<table>
<thead>
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<th>Preliminary</th>
<th>Replicate 1</th>
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<td>Humidity during exposure period</td>
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</table>

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<tr>
<th>Insecticide conc. (%) DDT/ diethrin/other ²</th>
<th>Dead</th>
<th>Total</th>
<th>Mort. (%) corr. ²</th>
<th>Dead</th>
<th>Total</th>
<th>Mort. (%) corr. ²</th>
<th>Dead</th>
<th>Total</th>
<th>Mort. (%) corr. ²</th>
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<tr>
<td>Females</td>
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<tr>
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</table>

¹ Cross out what does not apply.  
² Correct by applying Abbott's formula if mortality is between 5% and 20% (see instructions). 

Remarks: 
Signature of Investigator:

*One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.*
Annex 9

TENTATIVE INSTRUCTIONS FOR DETERMINING
THE SUSCEPTIBILITY OR RESISTANCE
OF HOUSEFLIES TO INSECTICIDES

A. Treated Vial Exposure Method

1. Introduction

(a) This method measures the susceptibility levels of a population of houseflies to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose it is necessary (i) to establish the susceptibility levels of normal houseflies of the population concerned, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. This test is devised specifically to detect physiological resistance. It is stressed that this test is not designed to evaluate the effectiveness of deposits of insecticides in the field; this must be ascertained by use of other entomological techniques.

(b) Establishing the base-line. Batches of flies are exposed to different concentrations of insecticide and the kills obtained at each level are determined. It is suggested that a preliminary test be made at each concentration in the complete range provided. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of four concentrations should be chosen, some of which give partial mortalities (i.e., at least one of them should give a complete kill and one less than 50% mortality). Tests at these concentrations should be repeated four times with samples from the same population of flies. To reveal the full range of natural variation, this set of tests should be made at several locations and at different seasons, so far as this is practicable.

(c) Subsequent routine checks. A concentration double that which has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with 2-5 replicates. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include four tests at each of the concentrations used in establishing the original base-line. In this way, it is possible to detect the presence of physiological resistance and to determine the approximate proportion of resistant houseflies in the population.
(d) **Sex of test insects.** Female flies only should be used, since control mortalities in males may be high.

(e) **Conditions of test.** Tests should be done in a room free from insecticidal contamination. The flies are exposed and held at a temperature between 20° and 30°C and at a relative humidity above 25%.

2. **Composition of test kit**

(a) 50 ml of malathion concentrate (0.075% in acetone).
(b) 50 ml of diazinon concentrate (0.02% in acetone).
(c) 4 assorted pipettes.
(d) 4 formulating bottles with 6-ml calibration.
(e) 24 vials, 75 mm × 25 mm, with lip.
(f) 4 50-ml bottles of acetone.
(g) 100 No. 8 elastic bands.
(h) 24 nylon-net holding-cage covers.
(i) Nylon mesh to cover vials.
(j) One collecting net.
(k) Instruction sheet and a set of report forms; three sheets of log-probability paper for plotting regression lines.

3. **Method of test**

(a) To prepare the serial dilutions, 3 ml of the test concentrate are added to a formulating bottle, the formulation being made up to 6 ml by the addition of acetone. 3 ml of this formulation are then added to a second vial and the latter made up to 6 ml with acetone, and so on. The final series of dilutions will consist of four concentrations which will be added to the vials by means of the pipette.

(b) Vials are treated with the serial dilutions by adding to each vial 0.5 ml of the appropriate solution. The acetone is evaporated by rotating the inclined vial by hand, or, for larger numbers, by using a mechanical rotator, set up over a source of gentle heat, e.g., a light bulb.

(c) The flies are collected in a net and transferred to small cages before use. It is advisable to keep wild flies overnight before exposure. Female flies, lightly anaesthetized with carbon dioxide or by chilling, are placed in untreated vials and allowed to recover completely. They are then transferred rapidly to the treated vials, 25 flies to each vial, and the vials kept in a horizontal position for one hour. The vials are then closed with gauze and rotated a quarter of a turn every 15 minutes. At the end of the exposure period, the batches of flies are transferred to holding-cages (e.g., paper cups covered by nylon netting), given sugar and water, and mortality-counts made at the end of 24 hours.
(d) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of four concentrations giving partial and complete mortality. Three replicates should be performed at each of the chosen concentrations.

(e) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(f) When the test has been repeated four times with the same population of flies, adequate data should be available for constructing a base-line of susceptibility as described in section 4. The results should be recorded on the forms provided.

(g) Vials should be decontaminated in the laboratory before use in the field.

4. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the log-logistic-probability paper provided. The regression line may be fitted by eye, and the LC$_{50}$ or LC$_{90}$ read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the LC$_{50}$ the worker is referred to the methods described by Swaroop and Litchfield & Wilcoxon.

(b) In tests where the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

\[
\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100
\]

5. Interpretation of results

See Annex 14: Criteria and meaning of tests for determining the susceptibility of insects to insecticides.

6. Distribution of report forms

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland.

---

1 The LC$_{50}$ and LC$_{90}$ represent respectively the insecticide concentrations at which 50% and 90% of the specimens are killed.
**Specimen Report Form**

**WHO TEST FOR INSECTICIDE-RESISTANCE IN HOUSEFLIES**

1. Investigator: ____________________________________________________________
2. Species: ______________________________________________________________
3. Country: ______________________________________________________________
4. Province: ______________________________________________________________
5. Locality: ______________________________________________________________
6. History of insecticide treatment (including agriculture): ____________________
7. Type of test on population: first time/routine check/complete retest

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<th>Replicate 2</th>
<th>Replicate 3</th>
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<td>Temperature during exposure period</td>
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<td>Humidity during exposure period</td>
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<table>
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<th>Total</th>
<th>Mort. (%) corr.¹</th>
<th>Dead</th>
<th>Total</th>
<th>Mort. (%) corr.¹</th>
<th>Dead</th>
<th>Total</th>
<th>Mort. (%) corr.¹</th>
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¹ Cross out what does not apply.
² Correct by applying Abbott’s formula if control mortality is between 5% and 90% (see instructions).

Remarks: ________________________________________________________________
Signature of investigator: ____________________________________________________________________________

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.
B. Topical Application Method

1. Introduction

(a) This method may be used to measure susceptibility levels of a population of houseflies to a given insecticide. The technique is designed to detect the emergence of an insecticide-resistant strain if and when it appears. For this purpose, it is necessary (i) to establish the susceptibility levels of normal houseflies of the populations concerned, and (ii) to make subsequent routine checks of the susceptibility levels at periodic intervals. The test is devised specifically to detect physiological resistance.

(b) Establishing the base-line. Batches of adult houseflies are treated topically with different concentrations of insecticide to determine the kills obtained at each level. It is suggested that a preliminary test be made at each concentration in the complete range provided for each species at the standard dosage suggested. This will indicate the general level of susceptibility; further tests should then be made on this basis. A series of four concentrations should be chosen, some of which will give partial mortalities (i.e., at least one of them should give a complete kill and one less than 50% mortality); tests at these concentrations should be repeated four times with samples from the same population. To reveal the full range of natural variation, this test should be made at several locations and at different seasons, so far as this is practicable.

(c) Subsequent routine checks. A concentration double the lowest concentration that has consistently given complete kills in the four successive tests is chosen for the routine checks. These should be made periodically with 2-5 replicates. The occasional appearance of survivors in such checks may be due to normal variation. However, the regular occurrence of survivors (e.g., on three successive occasions) constitutes an alert signal calling for further investigations. Such investigations will include four tests at each of the concentrations used in establishing the original baseline. In this way, it is possible to detect the presence of physiological resistance and to determine the approximate proportion of resistant individuals in a population.

(d) Sex of test insects. Female flies only should be used, since control mortalities in males may be high.

(e) Conditions of the test. Test should be done in a room free from insecticidal contamination. The insects are treated and held at a temperature between 20° and 30°C and at a relative humidity above 25%.

2. Composition of the test kit

(a) Five glass microcapillary tubes for delivery of 0.38-microlitre doses.
(b) Five glass holding-tubes, 14 cm in length and 7 mm in external diameter; the microcapillary is fixed at one end by means of a plug 3 mm long, with 0.5-1.0 mm of the capillary tube projecting from one end of the plug; the other end is connected by rubber tubing to a glass mouthpiece or a rubber bulb.

(c) Five reservoir tubes.

(d) Five dip tubes with a rubber ring.

(e) Five metal clips for holding the reservoir tube.

(f) Five 50-ml bottles containing the following concentrations of insecticides in methyl ethyl ketone:

- DDT (p,p'-isomer) 10%
- Dieldrin (purified HEOD) 5%
- Malathion 10%
- Fenthion 5%
- Control methyl ethyl ketone.

(g) One collecting net.

(h) Twenty-five holding-containers for insects (plastic tubes with coarse mesh screen).

(i) Instruction sheets and set of report forms, plus three sheets of log-probability paper for plotting regression lines.

3. Method of test

(a) The houseflies are collected in a net and transferred to small cages pending use. Small batches are taken out for treatment in test-tubes. The insects are anaesthetized (if necessary) either by carbon dioxide or by chilling. The required concentration of insecticide is then applied topically on the dorsal side. The houseflies are held in the holding-containers and the percentage mortality recorded after 24 hours. Insects unable to move are counted as dead.

(b) After the preliminary test has been performed with the complete range of concentrations, tests should be carried out with the chosen series of four concentrations. Three replicates should be performed at each of the chosen concentrations giving partial and complete mortality.

(c) Tests with a control mortality in excess of 20% are unsatisfactory and should be repeated.

(d) When the tests have been repeated four times with the same populations of insects, adequate data should be available for constructing a base-line of susceptibility (see section 5).

(e) After the required quantity of insecticide solution has been removed, the stock bottles should be carefully closed, and stored in a cool place.
5. Results

(a) The user may desire to construct the dosage-mortality regression line from the results obtained in the quadruplicate tests at the chosen concentrations and, in many cases, from those of the single preliminary test as well. For this purpose, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye and the \( LC_{50} \) or \( LC_{90} \), read from the graph. The regression line should not be extended (extrapolated) beyond the highest mortality obtained. For more accurate methods of computing the \( LC_{50} \), the worker is referred to the methods described by Swaroop \(^2\) and Litchfield & Wilcoxon.\(^3\)

(b) In tests where the control mortality is between 5% and 20%, the percentage mortality should be corrected by Abbott’s formula:

\[
\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100
\]

6. Interpretation of results

See Annex 14: Criteria and meaning of tests for determining the susceptibility of insects to insecticides.

7. Distribution of report forms

One copy of each report form as completed should be sent to the World Health Organization, Vector Control Unit, Geneva, Switzerland.

---

\(^1\) The \( LC_{50} \) and \( LC_{90} \) represent respectively the insecticide concentrations at which 50% and 90% of the specimens are killed.


\(^3\) Litchfield, J. T., Jr. & Wilcoxon, F. (1949) \textit{J. Pharmacol. exp. Ther.}, 96, 99
Specimen Report Form

WHO TEST FOR INSECTICIDE-RESISTANCE IN HOUSEFLIES

1. Investigator: .................................................. 2. Species: ..................................................


6. History of insecticide treatment (including agriculture): ...........................................

7. Type of test on population: first time/routine check/complete retests 1

<table>
<thead>
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<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Totals (for comparable tests only)</th>
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<tr>
<td>Temperature during exposure period</td>
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<tr>
<td>Humidity during exposure period</td>
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<tr>
<td>Insecticide conc. (%)</td>
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<tr>
<td>DDT/allethrin/ malathion/diazinon 1</td>
<td>Dead</td>
<td>Total</td>
<td>Dead</td>
<td>Total</td>
<td>Dead</td>
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<td>Total</td>
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<td>Mort. (%) cor. 1</td>
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<td>Mort. (%) cor. 2</td>
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<td>Mort. (%) cor. 2</td>
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<td>Mort. (%) cor. 3</td>
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<td>Mort. (%) cor. 3</td>
</tr>
</tbody>
</table>

Control

1 Cross out what does not apply.

Correct by applying Abbott's formula if mortality is between 5% and 95% (see instructions).

Remarks: .................................................. Signature of investigator: ..................................................

One copy of this form to be sent on completion to: World Health Organization, Vector Control Unit, Division of Environmental Health, Geneva, Switzerland.