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Test Methods for Establishing Levels of Susceptibility and Detecting the Development of Resistance in Insects of Public Health Importance

KENNETH D. QUARTERMAN[1]

ABSTRACT

Under the leadership of the World Health Organization (WHO), standard test methods have come into use on a worldwide basis for the detection of resistance in mosquito larvae and adults, and to a slightly lesser extent in body lice (*Pediculus humanus humanus* L.). WHO also has adopted provisional test methods for fleas, bed bugs (*Cimex lectularius* L.) and sandflies (*Phlebotomus* spp.) and tentative test methods for black fly larvae, tsetse flies and adult triatomid bugs. The U.S. Armed Forces Pest Control Board utilizes the same methods as WHO for mosquito larvae and adults, have slightly different methods for bed bugs and body lice, and also have methods for house flies (*Musca domestica* L.) and cockroaches for which there are no WHO methods. Other agencies, laboratories and individuals use these methods or variations of them. Although the different methods vary in some details and in types of equipment used, most of them are based on the establishment and comparisons of dosage-mortality curves. Details of the WHO and Armed Forces Pest Control Board methods may be found in the Tenth Report of the WHO Expert Committee on Insecticides (Technical Report Series No. 191) and the AFPCB Technical Information Memorandum No. 3, respectively.

It is a well-known fact that the discovery of the insecticidal activity of DDT and the subsequent development of many other new, synthetic organic insecticides revolutionized the concept and scope of the control of insects of public health importance. These advancements required the development of new techniques for the evaluation both of the effectiveness of these new insecticides and of the new uses to which they were being put, on a scale much larger than had ever been considered previously. These evaluation techniques varied with the particular insect species involved, the insecticide and formulation employed, the method of application, the ecological conditions under which the control measures were applied, and the ingenuity of the various scientists who developed them. However, most of them had one common factor in that they were based on dosage-mortality comparisons. A few evaluation techniques were based on "knockdown" rather than mortality.

Just as it is necessary to have adequate techniques to evaluate the effectiveness of insecticides under the specific conditions under which they are used, so is it equally necessary to use adequate quantitative tests to detect and evaluate insect resistance to insecticides and to rule out other factors which may be involved in control failures, such as poor quality insecticides or faulty application. Such an adequate quantitative test for the detection of resistance requires preferably simultaneous comparisons between a susceptible population of the same species and the population in which resistance is suspected; but in the absence of a susceptible population, comparisons can be made between the susceptibility level of an insect species obtained at a time when the population was known to be susceptible to the insecticide in question and the level obtained at the time resistance is being evaluated. Laboratory methods involving suitable microapplication techniques are most suitable for the critical evaluation and measurement of the degree of resistance.

With the wide variety of techniques employed in evaluating the effectiveness of insecticides, it was inevitable that there would be also many different techniques developed to detect and evaluate resistance, since most workers tended to adapt their insecticide evaluation techniques to the detection and gross evaluation of resistance. Obviously, some of these were much more suitable for this purpose than others. Since insect vector control problems and operations are global in scope, resistance has frequently occurred in areas where there was a shortage of trained personnel to detect it. Large-scale, nationwide vector control programs were frequently put into operation without obtaining quantitative data on the susceptibility of the vectors to the insecticides employed, and no attention was given to this aspect of insecticidal use until reports of resistance began to appear. Consequently, many of the early reports of resistance were not supported by adequate quantitative test data, making it impossible to assess the accuracy of the reports. Indeed, today the same is still true much too often. As a result, the resistance picture in some areas is still confused, and it is quite likely that control efforts in some situations are not being carried on with maximum effectiveness and efficiency.

CLASSIFICATION OF RESISTANCE TEST METHODS

For the past several years, the World Health Organization (WHO) has been taking the responsibility and initiative to clarify the resistance picture by encouraging the development of standardized detection techniques and stimu-

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lating the use of these standard techniques by supplying test kits and instructions for their use. Cooperating governments, organizations, and institutions have been encouraged to obtain data on the susceptibility of the various vector species before beginning control programs, or as soon thereafter as possible in those cases where such programs were already in operation. The results of their efforts have been most encouraging to date, and should become progressively more productive in the future.

The resistance detection techniques and test kits have been developed cooperatively by the WHO and those research laboratories and institutions throughout the world which are most qualified to contribute to their development. As a rule, several laboratories collaborate in devising each technique and test kit. In the Tenth Report of the Expert Committee on Insecticides (WHO 1960), the following four categories of test methods were considered:

1. *Promising test methods:* techniques which are still in the earlier stages of development, on which preliminary data are encouraging but for which specific procedures cannot be outlined in detail.

2. *Tentative test methods:* techniques which have been found to be satisfactory by a single investigator, but which require additional evaluation before they can be widely tested in the field. These are referred by the WHO to appropriate cooperating laboratories or investigators with the request that the methods be investigated.

3. *Provisional test methods:* test methods that have been examined and approved by several different investigators, but need wider testing by field workers. At this stage of development, experimental kits with appropriate instructions are prepared and referred to field investigators in suitable geographic locations throughout the world for field evaluation.

4. *Standard test methods:* When "provisional" test methods have been adequately fieldtested, the WHO Expert Committee on Insecticides reviews the results of these tests and the suggestions of the various investigators, and, based on them, recommends the adoption by WHO of the methods as "standard" test methods. Test kits and instructions are then provided by WHO to cooperators for operational use. Replacement items, such as standard insecticidal formulations or insecticide-impregnated papers, are also supplied by WHO. WHO usually requests cooperators to furnish WHO with copies of their test results. Minor modifications in "standard" test methods may be made from time to time as indicated, on review and recommendation by the Expert Committee on Insecticides.

The Armed Forces Pest Control Board (1959), the U. S. Department of Agriculture, the U. S. Public Health Service, the International Cooperation Administration and other large research and operational agencies have developed standardized resistance test methods for use by their respective organizations to meet their own particular problems. For those insect species which are widely distributed and in which there is a mutual interest with WHO, there is an increasing tendency on the part of all agencies concerned to cooperate in establishing a single standard test method for establishing levels of susceptibility and for detecting the development of resistance for each insect species or group.

Uniform standard test methods are now in use for mosquito adults and larvae. The WHO standard test method for body lice (*Pediculus humanus humanus* L.) differs only slightly from that of the Armed Forces Pest Control Board, and investigations are now in progress which may result later in a single uniform method being adopted. The Expert Committee on Insecticides, in September 1959 (WHO 1960), adopted provisional test methods for fleas, bed bugs (*Cimex lectularius* L.), and sandflies, and tentative methods for black fly larvae, tsetse flies, and adult triatomid bugs. Reports received by the Committee indicated that promising methods would soon be available for adult house flies (*Musca domestica* L.) and black flies.

BODY LICE

The first standard test method adopted by WHO was designed to determine the susceptibility of body lice to insecticides and to detect the development of resistance. It was based on recommendations of the U. S. Department of Agriculture laboratory at Orlando, Florida. The test procedure consisted of treating a small square (90 cm^2) of balbriggan type of cloth with dusting powder, placing a certain number of lice on it, and assessing the percentage mortality 24 hours later. Test kits were provided which included four concentrations of DDT, two of lindane, and two of synergized pyrethrins, as well as the cloth squares, a pair of forceps, and a set of instructions for conducting the test. The investigators were asked to supply other necessary items, such as petri dishes, containers, alcohol, etc. Two samples of 10 or 20 lice were tested on three successive days at each concentration. The test was performed in darkness at approximately 25° C. The lice were collected

from the human host shortly before being tested. The cloths were dusted with a specified quantity of dust. After 24 hours of exposure, the lice were divided into three categories: normal, affected (incapable of coordinated movement), and dead (no movement discernible).

Wright and Brown (1957) reported the results of the WHO survey on the susceptibility and resistance of body lice, initiated in 1953 and employing the test kits and standard test method outlined above. The results were based on 177 samples from 27 countries. For the purposes of that survey report, death alone was chosen as the criterion of evaluation to rule out variation in interpretation by the many observers involved in the survey. It was acknowledged that where many tests were run by the same observer, "affected" lice at 24 hours might well be grouped with the dead. The survey results were based on comparisons of the percentages of mortalities obtained in the survey tests with those which might have been expected, based on numerous laboratory tests with lice of normal susceptibility at the USDA laboratory at Orlando, Florida.

WHO has continued to employ this standard test method and test kit from 1953 to the present time. They have been widely used and have given much useful information. However, the data accumulated do not show a very exact correlation between insecticidal concentration and mortality. At its 10th meeting in Geneva, Switzerland, in September 1959, the WHO Expert Committee on Insecticides (WHO 1960) recommended that dusted filter papers, or insecticide-impregnated papers as used in the standard test method for adult mosquitoes, be studied with a view to later deciding if they might merit formal adoption for use in the standard method. In the meantime, the present method using dusted cloth will continue as the WHO standard test method.

The U. S. Armed Forces Pest Control Board (1959) uses a method for body lice which differs from the current WHO standard test method in the following respects:

1. Formulations of malathion are used rather than synergized pyrethrins.

2. The lice are exposed on filter papers treated with acetone solutions rather than the dusted cloth squares employed by WHO.

3. The use of a different number of insecticidal concentrations is suggested.

4. Each investigator is expected to prepare his own stock solutions, while WHO furnishes the prepared formulations ready for application.

5. Moribund lice are included with dead lice in computing mortalities after 24 hours of exposure.

6. The degree of resistance may be estimated by comparing LC_{50} and LC_{90} values of the resistant strains with similar values of normal lice. Accurate determination of these values cannot be made with the current WHO standard method.

With these exceptions, the WHO standard method and the AFPCB method are substantially the same. It may be reasonably expected that the differences in the two methods will be resolved within the next few years and a single standard method established for use on a global basis.

MOSQUITO ADULTS

A mosquito, *Culex pipiens* issp. *molestus* Forskal, shares with the house fly the dubious honor of being the first species to be reported resistant to DDT in Italy in 1947 (Mosna 1948). Since mosquitoes are such an important group of medically significant insects, since their control is the objective of such large-scale programs throughout the world, and since there are more resistant species of mosquitoes than of any other group of insects, much attention has been given to test methods for detecting mosquito resistance to insecticides. Because mosquitoes are relatively small and fragile, the most convenient methods are based on exposing the adult mosquitoes to surfaces treated with known deposits of insecticides. The earliest use of such methods involved the continuous exposure of the test mosquitoes to the treated surfaces and recording the percentage that was knocked down after specified intervals (Bettini 1948, King and Gahan 1949, and Busvine 1951). However, this procedure assumes that knockdown is synonymous with mortality, and the resistance is expressed in units of time rather than dosage.

In response to a request from WHO in 1953 for a simple, inexpensive field test procedure which could be used in areas where laboratory facilities and supplies were nonexistent, the Public Health Service laboratory at Savannah, Georgia, suggested the use of absorbent paper impregnated with DDT in xylene and the exposure of the mosquitoes to such papers for a specified period of time, followed by a 24-hour recovery period before observations of mortality (Fay *et al.* 1953). This test was designed to detect possible differences between two insect

populations by applying simple statistical methods to the test results.

Busvine and Nash (1953) described a method using a regular progression of concentrations of insecticide dissolved in a nonvolatile mineral oil and impregnated into filter paper. The mosquitoes were exposed for a specific time (e.g., 1 hour) and observed for 24-hour mortalities. Such a procedure permits the calculation of the LC_{50} and allows comparisons between the susceptibilities of two insect populations. It also provides some information on the frequency distribution of the susceptibility of individuals in the population.

After several years of cooperative developmental work by several laboratories at the request of WHO, a standard test method for adult mosquitoes was adopted by WHO in 1957. The Armed Forces Pest Control Board and most organizations and institutions throughout the world have accepted this standard test method for adult mosquitoes. Field kits containing the necessary supplies for making the tests and a set of operating instructions are furnished by WHO. Test papers are impregnated commercially with insecticides in Risella oil at different concentrations. Adult female mosquitoes are collected in the field and held for at least 1 hour to guard against including damaged specimens in the test. The mosquitoes are usually exposed for 1 hour to the impregnated papers, but longer exposure periods may be used, if necessary. The mosquitoes are then transferred to clean holding cages and held for 24-hour mortality observations. Affected specimens which are unable to walk are considered as dead. From such exposures to a range of insecticidal dosages, a dosage-mortality regression line may be constructed, using logarithmic-probability paper, from which the LC_{50} and LC_{90} values may be obtained.

The lowest concentration of insecticide which consistently gives complete kills in the tests conducted to establish the base line of susceptibility, is chosen for subsequent routine checks. These are made periodically, preferably with five replicates but with no less than two. As long as these routine checks continue to give complete mortalities, no significant change in susceptibility of the mosquito population is indicated. When mortalities begin to fall below 100% in the routine checks, tests should be made with a series of at least four concentrations of insecticide, and a new dosage-mortality regression line constructed. The slope, shape and position of the regression line are important in interpreting whether the change in response of the mosquitoes to the insecticide is due to physiological resistance or to other factors. If there is a relatively uniform decrease in susceptibility of the population as a whole, the slope and shape of the new regression line will be similar to the original, but with a shifting to the right. A plateau response, which is characterized by the failure of the higher concentrations to produce a progressive straight-line increase in mortality, indicates that a proportion of the mosquito population is resistant. The LC_{50} values may be of interest to the worker, but they are unreliable to use as a basis for detecting physiological resistance.

WHO has distributed over 650 test kits to cooperating organizations throughout the world. A great volume of valuable data obtained with these kits has been summarized and released periodically by WHO to cooperators.

Replacement papers for the test kits, impregnated with currently used residual insecticides, are supplied by WHO upon request. As new insecticides come into general operational use, it is expected that WHO also will provide papers impregnated with suitable dosage concentrations of them for use with the test kit.

MOSQUITO LARVAE

Since it is frequently easier to collect mosquito larvae than adults in the numbers needed for adequate testing for resistance or to establish baseline levels of susceptibility, a standard test method for mosquito larvae was developed and adopted simultaneously with the adult mosquito test procedure. The WHO kit supplied for this purpose contains a set of operating instructions, five different concentrations of each of the insecticides provided (currently DDT, lindane, and dieldrin) and other incidental supplies such as pipettes, eye droppers, strainers, etc. Each investigator supplies the test containers. The insecticides are in solution in ethyl alcohol. The tests are made in a specified volume of water in suitable glass containers, at a temperature of approximately 25° C, using 20–25 third- or fourth-stage larvae per container. The larvae are exposed for 24 hours, at the end of which the numbers of dead, living, and moribund larvae are counted. Moribund larvae are combined with the dead in computing the percentage of mortality. At least two replicates are made with each concentration of insecticide. The dosage-mortality regression line may be constructed from the results obtained, using logarithmic-probability paper, and the LC_{50} and LC_{90} read from the graph. The LC_{50} may also be calculated by a method suggested by WHO (Swaroop 1958).

Determination of resistance and susceptibility is made by comparing the LC_{50} values of a given larval population at any time with the baseline LC_{50} values of the same species obtained before the use of insecticides began or with the LC_{50} values of a known susceptible population of the same species (e.g., a susceptible laboratory colony) obtained in concurrent tests with the suspected strain.

This method has been accepted as a standard test method by the Armed Forces Pest Control Board and most other organizations and agencies throughout the world. Field test kits are furnished by WHO, as well as replacement supplies of the insecticidal solutions as needed. Other new insecticides will undoubtedly be added from time to time. WHO has supplied over 450 larval test kits to cooperators throughout the world, and has periodically circulated summaries of test results.

IRRITABILITY OF ADULT MOSQUITOES TO INSECTICIDES

In September 1959, the WHO Expert Committee on Insecticides (WHO 1960) adopted a provisional test method for determining the irritability of adult mosquitoes to insecticides as an aid in detecting and evaluating behavioristic resistance which may be due to increased irritability of a mosquito population to insecticidal residues. The test consists of two procedures: (1) determination of the length of time to the initial takeoff following exposure to insecticide-impregnated paper and (2) counting the takeoffs in a given period of time (15 minutes). A check group of mosquitoes of the same population is exposed simultaneously to untreated paper in the same manner as those exposed to the treated paper. Following exposure, the test mosquitoes are held for 24-hour mortality observations. Results obtained on treated and untreated papers are compared directly. A limited number of test kits containing a set of instructions and testing equipment and materials is being provided by WHO to cooperating investigators for further evaluation of the method.

FLEAS

The provisional test method for fleas adopted by WHO in September 1959, consists of exposing field-collected adult fleas for 1 hour to insecticide-impregnated papers in glass test tubes under standard test conditions and holding them for 24-hour mortality observations. A test kit will be provided by WHO which will include operating instructions, the glass test tubes, the impregnated papers, and other necessary items. The LC_{50} and LC_{90} may be read from the dosage-mortality regression line or the LC_{50} may be estimated by the graphical method of Litchfield and Wilcoxon (1949). Resistance determinations are made by comparing the LC_{50} values of the flea population under study with those of a population of the same species of known susceptibility.

BED BUGS

The WHO provisional test method for bed bugs, adopted in September 1959, will utilize essentially the same test kit as used for fleas. The exposure period for bed bugs is 5 days as compared to 1 hour for fleas. At the end of the exposure period, mortality counts are made, moribund specimens being counted as dead. Resistance determinations are made in the same manner as for fleas.

The test method for bed bugs in use by the Armed Forces Pest Control Board is based on continuous exposure of the bed bugs to a standard residual deposit on filter paper and periodically recording knockdown. Comparisons are made on the length of time required for knockdown of 50% or 90% (LT_{50} or LT_{90}). These values for the strain under test are compared with values of a strain of known susceptibility obtained in concurrent tests, or with values previously obtained for a known susceptible strain using the same test method. This method is also used by the U. S. Department of Agriculture.

HOUSE FLIES

Resistance to insecticides by this species occurs throughout the world. As a result, the house fly has received attention from more investigators than any other species. Because of the large number of investigators and organizations involved in these studies and the necessity of utilizing laboratory facilities for the wide variety of techniques employed, the development of a standard test method for field usage has lagged behind the progress made with regard to other species.

Many investigators or organizations working on house fly resistance have tended to develop their own individual test method, which may differ slightly in some respect from the methods used by other workers. Exposing the test flies to a single dosage or a series of dosages for a specified period of time and holding them for 24-hour mortality observations has been the procedure followed by many. The kinds of treated surfaces, the type of exposure chamber, the exposure period, the dosages, and the formulations

employed have varied greatly. Concurrent tests with the suspected strain and a normal strain are usually made, and a direct comparison made of the mortality results obtained.

The topical application method of treating individual specimens is also widely used for house fly resistance evaluation, especially by laboratories where basic studies on house fly resistance have been made. Again, a considerable variety of insecticidal formulations, application techniques, and equipment have been employed. In the latter group are included various types of microloop applicators, microcapillary applicators, and microsyringes. Tests are usually made concurrently with the suspected strain and a known susceptible strain. Comparisons are made on the average insecticidal dosage per fly required to produce a 50% or 90% mortality in 24 hours.

The World Health Organization has not adopted a standard test method for house flies but is currently encouraging the development of one. The Armed Forces Pest Control Board has adopted a standard method involving confinement of female adult flies for a specific period of time in 1-pint glass fruit jars treated with various concentrations of insecticides. The exposed flies are held for 24-hour mortality observations. Concurrent tests are made usually with the suspected strain and a known susceptible strain. Resistance determinations are made by comparing the LC_{50} and LC_{90} values of the two strains. If a susceptible strain is not available for the concurrent tests with the suspected strain, the test results with the latter may be compared with those previously obtained with a known susceptible strain in similar tests.

ADULT SANDFLIES

The provisional test method for adult sandflies (*Phlebotomus* spp.) adopted by WHO in September 1959, consists of exposing field-collected specimens for 1 hour to papers impregnated with a series of insecticidal concentrations, and holding them for 24-hour mortality counts. The field test kit used for resistance tests with adult mosquitoes, with minor modifications, will also be used for adult sandflies, and the test procedure is substantially the same. Resistance determinations are made in the same manner as for adult mosquitoes.

COCKROACHES

Because of their size and ease of handling, many investigators use topical application or injection techniques for making resistance determinations on cockroaches. This is primarily a laboratory technique, and is not readily applicable for general field use. WHO has not adopted a test method for cockroaches, but the Armed Forces Pest Control Board and the U. S. Department of Agriculture utilize a method involving the continuous exposure of field-collected adult cockroaches, preferably males, to insecticidal residues in glass jars. The percent knockdown is determined periodically, permitting an estimation of the LT_{50}. Concurrent tests are made with cockroaches of a known susceptible strain, if possible, and direct comparisons are made of the test results with the two strains. If a susceptible strain is not available, test results obtained with the suspected strain are compared with results obtained in previous identical tests with a normal strain.

DISCUSSION

Details of the resistance test methods described above may be found in the Tenth Report of the Expert Committee on Insecticides (WHO, 1960) and the Technical Information Memorandum No. 3 of the Armed Forces Pest Control Board (1959). The publication by Brown (1958) on the general subject of resistance also contains some information on test methods. The chapter by Metcalf (1958) in Shepard's publication on methods of testing chemicals on insects is an excellent, comprehensive review of methods of topical application and injection. For those interested in the latter techniques, the following individual references also should be useful: Trevan 1922, Hockenyos and Lilly 1932, McIndoo 1937, McGovran *et al.* 1940, Scholander *et al.* 1943, Richardson 1943, Worthley 1943, Heal and Menusan 1948, March and Metcalf 1949, Yeager and Munson 1949, Woodrow 1949, Roan and Maeda 1953, Kerr 1954, Brezner 1959, and Jones and Perry 1959.

For critical laboratory studies in which it is necessary to measure accurately the amount of insecticide which is brought into actual contact with the test insects, the use of one of the microapplication techniques referred to in the foregoing paragraph is indicated. Detection of resistance can be made by such studies, but they are too exacting and time consuming for routine resistance tests involving many specimens and species. They have the added drawback of not being adaptable for field use or for use against many small and/or fragile species. However, these techniques are preferred for studies designed to obtain critical measurement of the degree of resistance, since the detection techniques discussed previously are not intended for this purpose.

In most of the practical resistance test methods now in use, results are based on mortality of the test insects. A few still employ comparisons of knockdown time. This procedure is based on the assumption that knockdown is equivalent to mortality. For some resistant species this is not the case—specimens which are readily knocked down recover later. Before using a resistance test method which involves comparisons of knockdown time, the investigator should determine that specimens which are knocked-down invariably die within the usual observation period for mortality. Such determinations should be made at frequent intervals, since in a susceptible population knockdown may be synonymous with mortality initially, but not later on when resistance develops.

In many of the resistance test methods, resistance determinations are based on comparisons of KD_{50}, LC_{50}, LD_{50} or LT_{50} values. It has already been demonstrated with adult mosquitoes that comparisons of LC_{50} values are unreliable to use as a basis for detecting physiological resistance in this group of insects. In this group, resistance can be determined most reliably by the appearance in the dosage-mortality regression line of a plateau effect characterized by the failure of the higher concentrations of insecticides used in the test method to produce a progressively straight-line increase in mortality. Investigators should be alert to the possibility that this phenomenon may occur also in other insects for which comparisons of LC_{50} or similar values are currently being used as a basis for detecting resistance.

This discussion has dealt principally with standardized test methods for detecting resistance. While WHO and other organizations have encouraged the development of standard test methods and their common use by scientific workers throughout the world, it should be emphasized that resistance test data obtained with valid techniques of individual investigators are accepted by these organizations even though the techniques employed may differ substantially from the "standard" methods. However, there are many advantages to be derived from the common use of the standard methods by workers in widely separated locations. Much valuable data have been obtained already from the use of available standard methods and the test kits supplied by WHO. The development of test methods and kits for additional vector species will enable the limited number of scientific workers available for such activities to keep more abreast of the rapidly and constantly changing developments in the overall resistance problem.

REFERENCES CITED

Armed Forces Pest Control Board. 1959. Methods for determining the susceptibility or resistance of insects to insecticides. Tech. Information Memorandum No. 3, 34 pp.

Bettini, S. 1948. Contributo allo studio della resistenza alla azione del DDT nelle mosche domestiche. Riv. Parassitol. 9: 137–42; R. C. Ist. sup. Sanitá 11: 1131–6.

Brezner, Jerome. 1959. An electrically operated micro dispenser. Jour. Econ. Ent. 52(5): 1019–20.

Brown, A. W. A. 1958 Insecticide resistance in arthropods. W.H.O. Monogr. Ser. No. 38, 240 pp.

Busvine, J. R. 1951. Mechanism of resistance to insecticides in houseflies. Nature (London) 168: 193–5.

Busvine, J. R., and **R. Nash.** 1953. The potency and persistence of some new synthetic insecticides. Bull. Ent. Res. 44: 371–6.

Fay, R. W., J. W. Kilpatrick, R. L. Crowell, and **K. D. Quarterman.** 1953. A method for field detection of adult-mosquito resistance to DDT residues. Bull. World Health Organ. 9(3): 345–51.

Heal, Ralph E., and **Henry Menusan, Jr.** 1948. A technique for bloodstream injection of insects and its application in tests of certain insecticides. Jour. Econ. Ent. 41(4): 535–43.

Hockenyos, George, and **John Lilly.** 1932. Toxicity studies by hypodermic injection of *Celerio lineata* larvae. Jour. Econ. Ent. 25(2): 253–60.

Jones, J. C., and **A. S. Perry.** 1959. A nib-point microloop and its calibration. Mosquito News 19(1): 26–7.

Kerr, R. W. 1954. A method for the topical application of small measured doses of insecticide solution to individual insects. Bull. Ent. Res. 45: 317–21.

King, W. V., and **J. B. Gahan.** 1949. Failure of DDT to control house flies. Jour. Econ. Ent. 42(3): 405–9.

Litchfield, J. T., Jr., and **F. Wilcoxon.** 1949. A simplified method of evaluating dose-effect experiments. Jour. Pharmacol. and Exptl. Therap. 96: 99–113.

March, R. B., and **R. L. Metcalf.** 1949. Laboratory and field studies of DDT-resistant house flies in southern California. California Dept. Agric. Bull. 38: 93–101.

McGovran, E. R., G. L. Phillips, and **E. L. Mayer.** 1940. A measured drop method of applying liquid insecticides. U.S. Bur. Ent. and Plant Quarantine Circ. ET-165. 3 pp.

McIndoo, N. E. 1937. Quantitative injection and effects of nicotine in insects. Jour. Agric. Res. 55: 909–22.

Metcalf, R. L. 1958. Methods of topical application and injection. Chapter VIII *in* "Methods of testing chemicals on insects," H. H. Shepard, Editor, Vol. I, Burgess Publ. Co. 356 pp.

Mosna, E. 1948. *Culex pipiens* autogenicus DDT-resistenti e loro controllo con Octa-Klor e esachlorocicloesano. Riv. Parassitol. 9: 19–25; R. C. Ist. sup. Sanitá 11: 425–32.

Richardson, C. H. 1943. Testing contact insecticides. Amer Assoc. Adv. Sci. Publ. 20: 126–35.

Roan, C. C., and **S. Maeda.** 1953. A microdevice for rapid application of toxicants to individual insects. U. S. Bureau Ent. and Plant Quarantine Circ. ET-306, 2 pp.

Scholander, P. F. G. A. Edwards, and **L. Irving.** 1943. Improved micrometer burette. Jour. Biol. Chem. 148: 495–500.

Swaroop, S. 1958. Statistical methodology in malaria work. World Health Organ./Mal/174 Rev. 1; World Health Organ./HS/58 Rev. 1.

Trevan, J. W. 1922. An apparatus for the measurement of small quantities of fluid. Lancet 202: 786.

Woodrow, A. W. 1949. A convenient microsyringe. Science 110: 142–3.

World Health Organization, Expert Committee on Insecticides. 1960. Tenth Report. Tech. Rept. Ser. No. 191.

Worthley, H. N. 1943. A drop test. Amer. Assoc. Adv. Sci. Publ. 20: 140–3.

Wright, J. W., and **A. W. A. Brown.** 1957. Survey of possible insecticide resistance in body lice. Bull. World Health Organ. 16: 9–31.

Yeager, J. Franklin, and **Sam C. Munson.** 1949. Relationship between knockdown and survival time for DDT-poisoned flies and roaches. Jour. Econ. Ent. 42(6): 874–7.