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VECTOR TOPICS

NO. 2

CONTROL OF DENGUE

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PUBLIC HEALTH SERVICE
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DENGUE

The Disease

Dengue is a viral disease transmitted from person to person by mosquitoes. It may occur in epidemic form in almost any part of the tropical or subtropical world. It is normally an acute, nonfatal disease, characterized by sudden onset, high fever, severe headache, backache and joint pains. Because of these latter symptoms, one of the common synonyms of the disease is "breakbone fever." There may be initial skin manifestations, with early mottling and localized rash; a secondary skin rash occurs somewhat later, usually on the 3rd or 4th day, occurring first on the trunk, but later spreading to arms, legs and face. Although in such uncomplicated cases fatalities rarely if ever occur, the patient usually experiences a prolonged period of convalescence from more severe attacks and may suffer from continued weakness for several weeks.

Epidemiologic data and studies in human volunteers suggest that the dengue viruses often cause an infection which may be very mild or completely without symptoms. On the other hand, in some areas of the world dengue infection commonly results in severe, frequently fatal diseases known as "dengue hemorrhagic fever" (DHF) and "dengue shock syndrome" (DSS). It is likely that these two syndromes are related, with the latter representing a more severe consequence of the hemorrhagic disease. Dengue hemorrhagic fever has been considered to be most frequently a disease of children[1] characterized by high fever and, subsequently, exhibiting a number of hemorrhagic manifestations. A variable proportion of these cases may progress to the characteristic shock syndrome, and the fatality rate is high among such cases. The cause of these more severe manifestations of the dengue infection is still unclear. The viral agents appear to be the same as those responsible for the classical, nonfatal disease. A number of hypotheses have been advanced. One suggests an immunologic phenomenon, where infection with multiple virus types may be involved, either simultaneously or sequentially; however, cases of the hemorrhagic disease have been seen in epidemics involving only one virus type.[2] Other host or virus factors which have been suggested include genetic or racial characteristics of the host, possible viral change through mutation, and dual infection with another viral agent. Further studies will be required before the pathogenesis of this serious disease is resolved. Detailed information on the definitive diagnosis of dengue cases is given in Appendix I.

At present, the hemorrhagic disease appears to be restricted to southeast Asia and Oceania. Dengue in the Western Hemisphere has usually been of the classical, nonfatal type. However, recently cases have occurred in Puerto Rico and Curacao which manifested enough hemorrhagic symptoms to be considered at least borderline cases of dengue hemorrhagic fever.[3,4]

Treatment for uncomplicated disease is totally symptomatic. Severe hemorrhagic cases are normally treated with intravenous fluids or transfusions. Corticosteroids have been used as part of the treatment regimen with some success.

Vaccine development has been under study, but to date there is no commercially available product. The existence of multiple serotypes of dengue virus, as well as the uncertainty of the possible role of immunity in the etiology of dengue hemorrhagic fever or shock syndrome, raises doubts about the development of such vaccines in the near future.

The Viral Agent: As early as 1907, Ashburn and Craig demonstrated the causative agent of dengue in the blood of human patients and also showed it to be "filterable." Studies during World War II summarized by Sabin[5] demonstrated at least two different serotypes (1 and 2) of dengue virus, and Hammon and his associates[6] reported two additional serotypes (3 and 4) in the Philippines a few years later. Presently, there are considered to be 4 distinct types of dengue virus, although there have been suggestions of additional types or sub-types separable by serologic techniques. All four types have been shown to be involved in both uncomplicated dengue and in cases with hemorrhagic complications. The dengue viruses are classified as flaviviruses (Group B arboviruses) and show considerable cross-reactivity in serologic testing with such other flaviviruses as yellow fever, Japanese B encephalitis, and St. Louis encephalitis.

Epidemiology: The dengue viruses are transmitted from person to person by mosquitoes, typically *Aedes aegypti*. Insofar as is known, there is no significant reservoir or amplifying bird or animal host although several species of monkeys are known to be susceptible to dengue, and there are indications that some acquire the infection naturally in endemic areas. When a person is bitten by an infected *Aedes*, a viremia develops, i.e., virus appears in the peripheral blood stream after about 5 or 6 days, at about the same time that the initial symptoms of the disease develop. The viremia may last for 4 or 5 days and during this time mosquitoes taking blood from the person may become infected. To become infective to humans, the virus requires an incubation period in the mosquito, usually of 8 to 10 days duration. The mosquito then may remain infective for the rest of her life, with a potential for transmitting the infection each time she feeds on a new human subject.

Historically, dengue has been seen in sweeping epidemics in many areas of the world. One of the earliest accounts of such an epidemic was that of Dr. Benjamin Rush who described a severe outbreak in Philadelphia during the summer and fall of 1780. Since the 18th century, numerous epidemics have occurred in tropical and subtropical areas throughout the world. Notable among these have been epidemics in the southern United States in 1922, with perhaps as many as 2 million cases, and in Greece in 1927-28, with approximately 1 million cases. Although the last epidemic in the continental United States occurred in Louisiana in 1945, dengue has persisted as an endemic disease in Puerto Rico and other areas in and adjacent to the Caribbean, with occasional epidemics occurring throughout this region. A large epidemic in this region occurred in Colombia in 1972, with an estimated half-million cases. In

Puerto Rico--after outbreaks in 1963, 1969, and 1975[7-9]--a major epidemic caused by dengue virus types 2 and 3 swept the island in 1977 resulting in more than 200,000 cases and estimated economic loss of over 6 million dollars.[11] Hemorrahagic manifestations, which had not been reported from this area prior to the Puerto Rican epidemic in 1975, were also observed in this outbreak; however, clinical illness was mild and no deaths were associated with the 1977 epidemic.

Until 1977 only dengue virus types 2 and 3 had been isolated in Western Hemisphere outbreaks. However, in 1977 dengue type 1 was identified in an abrupt outbreak that resulted in more than 60,000 cases in Jamaica.[10,12] Dengue-1 then spread rapidly to other Caribbean countries and dengue-1 outbreaks were reported from the Bahamas, Cuba, Dominica, Grenada, Guyana, Surinam, and Venezuela. In April 1978, a new epidemic caused by dengue-1 was recognized in Puerto Rico.[11] The epidemic declined by early 1979 having caused an estimated total of more than 400,000 cases. During 1979, epidemic dengue-1 activity was continuing in southern Mexico.

Dengue continues to be endemic and epidemic in wide areas of south and southeast Asia and in the southern and western Pacific. In these regions all four serotypes are found, and hemorrhagic manifestations of the disease are relatively common.

In general, the disease could occur in epidemic form in any area within the range of the appropriate vector mosquitoes. Because of the wide distribution of the principal vector, *Aedes aegypti*, this comprises a large part of the tropical and subtropical world. The potential for the establishment of an epidemic outbreak from an imported case is obvious when one considers the 5- to 6-day incubation period in man after receiving the infective mosquito bite and before the earliest recognizable symptoms occur. With the rapid air transportation available from endemic or epidemic centers to receptive areas, undetected cases could easily be introduced and would be available to vector mosquitoes for initiation of an outbreak, This appears to be a particularly important consideration in the Western Hemisphere, where the disease is endemic and occasionally epidemic in heavily traveled areas in and around the Caribbean, and where, by virtue of the wide occurence of *Aedes aegypti* populations, the adjacent mainland areas of North, South, and Central America would be highly receptive to the establishment and propagation of epidemic foci. Since the last known occurrence of dengue transmission in the continental United States, cases are known to have been imported, but no confirmed secondary cases have been reported. During the admission to the United States of refugees from Vietnam in 1975, at least 6 confirmed cases were imported into Guam from this southeast Asian area; however, because of early detection and extensive efforts toward mosquito control, no spread to the residents of Guam occurred. It seems likely that the occasional dengue epidemics in island situations in the southern Pacific area result from the importation of a case or cases during the incubation period and the subsequent infection of local mosquitoes.

Because of the absence of any form of specific treatment or immunization for dengue, control of the disease relies entirely on the control of the mosquito vector, on the early identification of cases, and, if possible, the isolation of cases from contact with vector mosquitoes.

The Mosquito Vectors of Dengue

Aedes aegypti, Ae. albopictus, and members of the Ae. scutellaris group, including Ae. polynesiensis, are vectors of dengue fever in nature and are the important species in the epidemic transmission of the disease. Aedes aegypti is worldwide in distribution and the only known vector of the disease in the New World, while the other species are considered to be important vectors in Asia and the Pacific islands. Recently, Ae. cooki was suggested as a vector in a dengue epidemic in Niue, southeast of Samoa.[13] Experimental testing of Aedes vexans, Ae. sollicitans, Ae. taeniorhynchus, Ae. cantator, Anopheles punctipennis, A. quadrimaculatus, and Culex pipiens indicate that these species are incapable as vectors of dengue.[5]

Because the virus of dengue fever is present in the patient's bloodstream for only a short time, transmission of endemic dengue takes place only in areas that can supply Aedes vectors throughout the year. Epidemic dengue, on the other hand, can occur throughout the range of the vector species. Since Ae. aegypti is the sole vector in the United States, the following discussion emphasizes this species in the setting of the Western Hemisphere.

Aedes aegypti, as well as the other known vectors, is a semidomesticated mosquito, breeding almost exclusively in artificial containers in and around human habitation. It is a small, dark species that can be recognized by the lyre-shaped, silver-white lines on the thorax and the white bands on the tarsal segments (Fig. 1). Aedes aegypti was originally a tropical species, thought to have been introduced into the Western Hemisphere from Africa.

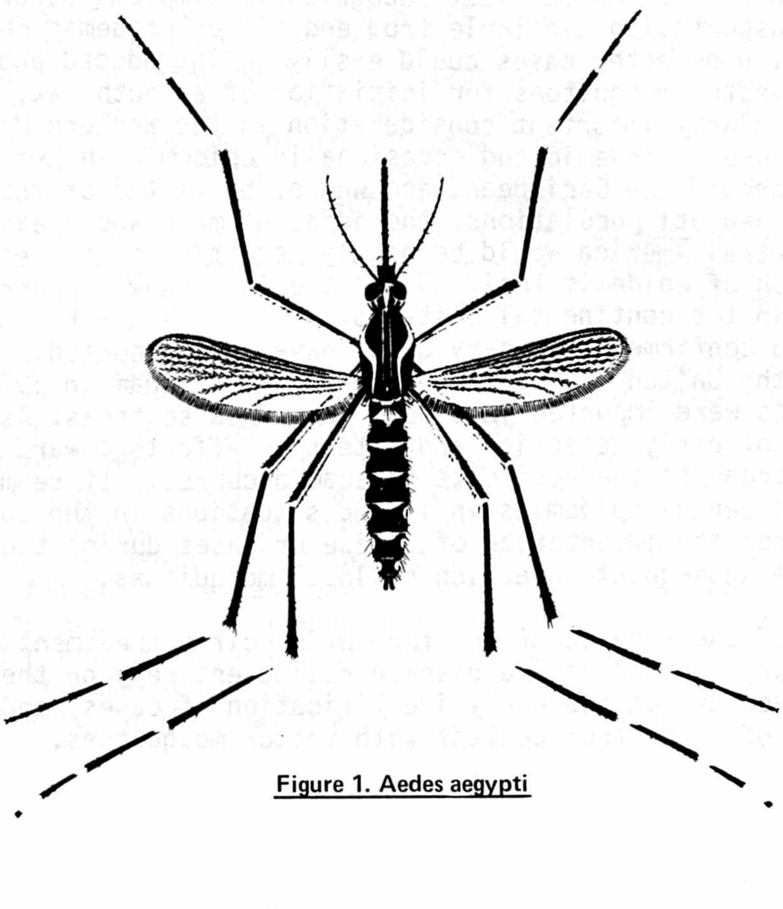

Figure 1. Aedes aegypti

The adult female deposits eggs singly, usually on the side of containers at or above the waterline, or less often on the water surface. The eggs are able to withstand drying for several months and hatch quickly when the container again fills with water. Hatching can take place within two or three days after egg deposition if temperatures are high. Typical habitats for the aquatic stages of this species are flower vases, tin cans, jars, discarded automobile tires, cisterns, rain barrels, sagging roof gutters, and tree holes.

The larvae can complete their development in 6 to 10 days under favorable conditions; in cooler weather a longer period may be required. The pupal period lasts about 2 days. Thus, the life cycle may be completed in as few as 10 days, or it may require as long as 3 weeks or more. Breeding continues throughout the year in the tropics, where generations succeed each other rapidly. In the southern United States, the reproduction rate is slower during the winter. The eggs may remain dormant for several weeks or months, which provides for survival through periods of little or no rainfall. The species is very susceptible to cold and usually does not survive the winter in the northern United States.

The adults of Ae. aegypti enter houses readily and the females apparently prefer to feed on humans rather than on other hosts. This species bites principally during the daylight hours. In large numbers it often becomes a troublesome pest. In laboratory colonies, the adults may live as long as four months or more, but in nature the life span may be considerably shorter. The usual flight range of this species is probably not more than a few hundred feet, although longer distances have been recorded.

Aedes aegypti is widely distributed, generally within the limits of 40 degrees north and south latitude. While it has been known to thrive outside of these limits, it is likely that such populations are introduced during the warm seasons from more temperate areas, multiplying during the summer months, but not surviving the winter. This wide distribution of Ae. aegypti, together with its domesticated habits and adaptability to urban breeding places, make it an important vector of dengue fever. In the Western Hemisphere, Ae. aegypti is known from all countries and territories except Canada. Since the species is a common vector of dengue and yellow fever in the hemisphere, it has been the object of continuing and sometimes intensive control and eradication programs since the turn of the century.

Dengue appears to have been common as late as the 1940's along the United States Gulf Coast and was the stimulus for a number of Ae. aegypti control campaigns.[14] These campaigns were limited, however, and were not aimed at the elimination of the vector species. From 1964 to 1968, the United States did engage in an eradication campaign. This resulted, however, in only limited and temporary success. There remain significant infestations in urban centers of States bordering the Southern Atlantic and Gulf Coasts of the United States, and relatively little is being done toward the control of this species in these areas. The introduction of dengue virus into these receptive areas in persons traveling from endemic or epidemic areas has been reported on several occasions, and the establishment of local transmission remains a continuing threat.

Because of the probable universal susceptibility to dengue in the population of the southern United States and the opportunity for repeated introduction into this area of the virus from endemic and epidemic areas in tropical America, it seems important to be prepared for control of the vector species on a contingency basis. To bring up to date the information on the distribution of Ae. aegypti in the United States, CDC's Bureau of Tropical Diseases conducted a survey in 1976 which showed the presence of the species in 29 of 30 cities checked in 10 southern States. Laboratory tests to determine insecticide susceptibilities did show, however, that the species was sensitive to malathion, the insecticide most likely to be used if adulticiding programs are needed in epidemic situations.

In the early 1920's some antimosquito programs in South America succeeded in eliminating Ae. aegypti from defined areas. Such successes were often cited in support of a growing concept of species eradication for the control of yellow fever. In 1947, the member nations of the Pan American Health Organization (PAHO) resolved to eradicate the species from the Western Hemisphere. Nearly all of the involved countries and smaller political entities subscribed to this concept, and during the last three decades, at one time or another, have planned or conducted a program with Ae. aegypti eradication as the objective. Based on standard criteria developed by PAHO, 17 countries had eradication confirmed by 1965. In 1979 only seven countries and the Panama Canal Zone were shown by PAHO to have completed eradication and were under a surveillance program.[15] Many countries have now experienced the discouragement of administrative, technical, or financial problems, and regardless of the level of control effort, few areas, if any, have remained permanently free of resurgent Ae. aegypti populations.

Preventive Measures

The prevention and limitation of epidemic dengue relies entirely on the control of the vector mosquito. Knowledge of when, where, and how to control this vector is in turn dependent on adequate and efficient disease and vector surveillance measures, and on a knowledge of the natural history of this vector in relation to disease transmission.

Disease Surveillance: The timely initiation of vector control procedures relies entirely on early information on the presence or importation of dengue infections in the community. Particularly in areas not known to be endemic for dengue, efforts should be made in any clinically suspect case to confirm the diagnosis by serologic methods and by isolation and identification of the viral agent. Although specific serologic or virologic diagnosis may be of little value to the patient, it is highly important in confirming the presence of endemic or epidemic dengue foci.

Dengue is a little-known disease in the continental United States, not having been seen in this area since 1945, and would be unlikely to be considered in routine diagnosis. Dengue is not a notifiable disease, nor is it subject to quarantine regulations. Areas receptive to epidemic dengue, i.e., those where *Ae. aegypti* is prevalent, should maintain an alert for dengue or dengue-like illnesses. It is essential that cases be discovered and identified as early as possible so that steps can be taken to avoid the initiation of an epidemic, or in any event to prevent its continuation or extension. It is the responsibility of public health authorities in receptive areas to note the presence of dengue epidemics in areas of commercial or tourist interest to their constituency and to alert their diagnostic resources to the possibility of imported or introduced cases.

Detailed information on clinical and serologic diagnosis of dengue and techniques for virus isolation are given in Appendix I.

Mosquito Surveillance: *Aedes aegypti* is a highly adaptable urban mosquito whose pre-adult stages (eggs, larvae, and pupae) are found in a wide variety of natural and artificial containers in or near human dwellings. Its close association with man and its utilization of nearly any type of neglected, water-holding receptacle as a breeding site are factors which contribute significantly to its role as a vector of dengue fever. Knowledge of the presence, relative abundance, and distribution of *Ae. aegypti* populations is necessary if the potential for dengue transmission in a given urban area is to be realistically estimated. Surveillance begun early and continued systematically throughout the mosquito breeding season will provide a record of seasonal changes in distribution and population levels. When tabulated and mapped, surveillance data constitute baseline information about *Ae. aegypti* occurrence and densities which is of great importance in determining when, where, and how to employ control measures, and in evaluating the effectiveness of those measures.

Methods of sampling the adult *Ae. aegypti* population are not well developed and the results of adult sampling are less reproducible than the results of egg, larval and pupal sampling. Sampling of the adults can be important, however. The occurrence of large numbers, and especially of males, in a particular place, is a reliable indicator of a nearby source of production.

Techniques for conducting egg, larval, and adult surveys are detailed in Appendices II, III, and IV.

Mosquito Control: Because of the "domestic" habits of the *Ae. aegypti* mosquito, much can be done toward reducing or eliminating populations of this vector through elimination of breeding sources in peridomiciliary situations. Periodic cleanup campaigns can eliminate cans, jars, discarded tires, and other common collectors of rainwater which serve as breeding sources in urban or suburban neighborhoods. Rain barrels, cisterns, and other collections of water for domestic use can be screened; ornamental ponds can be stocked with larvivorous fish.

Where breeding cannot be controlled by such source elimination, chemical larvicides or adulticides may have a useful role in reducing *Ae. aegypti* populations. However, the use of larvicidal and adulticidal chemicals must be considered a secondary method, with the primary measure being the elimination of breeding sources. Where emergency control measures are required due to transmission of dengue, priority must be given to adulticiding operations to kill infected adult mosquitoes.

Methods used to control mosquitoes are outlined in Appendices V and VI.

Emergency Measures

A recognized or suspected dengue outbreak requires immediate measures to halt transmission. It is urgent that a well organized assessment of the situation be quickly undertaken to expedite decisions on emergency control measures. Important considerations in the design of the emergency control plan include assessment of the following factors:

1) Extent and distribution of human disease;

2) size and distribution of the adult mosquito population;

3) extent of mosquito breeding;

4) anticipated changes in mosquito activity due to seasonal effects; and

5) climatic factors which may affect mosquito production and behavior.

An emergency vector control plan based on measures which will eliminate or reduce adult mosquito populations will include four essential activities: 1) human case surveillance, 2) intensified mosquito surveillance, 3) area-wide mosquito control, and 4) public information.

Human Case Surveillance: The occurrence of an unusual number of dengue-like illnesses in an area where dengue is endemic, or even a single case in a non-endemic area known to be receptive to dengue transmission, requires a careful assessment of the origin of the case or cases and the institution of intensified case surveillance to detect possible extension of activity to epidemic proportions. A simple and practical system of human case surveillance facilitates recognition of the disease in the community, decisions to institute emergency control measures, and assessment of the effectiveness of control measures. When there is a high level of suspicion on clinical grounds that dengue cases have occurred, an active case search should be instituted by the responsible health department officials, with daily communication with hospitals, clinics, and other available medical care facilities in the community. Such frequent communication enhances the early reporting of suspect cases and also alerts medical care personnel of the need to consider dengue in the differential diagnosis of patients presenting with an acute febrile illness of unknown etiology.

Confirmation of the disease as dengue is highly important and should be accomplished as rapidly as possible with the collection of serum samples from the earliest suspect cases (see Appendix I). The results of serologic diagnosis {hemagglutination-inhibition (HI), complement-fixation (CF), or neutralization (NT)} are most significant when acute and convalescent sera are collected, a 4-fold rise in titer representing a significant positive result. The tests should include all 4 types of dengue virus. Although virus isolation is a procedure which is not commonly available, it would be highly desirable in the definitive identification of virus type.

Although an elevated serologic titer in a single sample does not guarantee a definitive diagnosis because of the possibility of prior experience with other flaviviruses, in association with a highly suggestive clinical syndrome, it may be sufficient evidence to justify the institution of control measures. In view of the relatively long period of time required for completion of the incubation period and for the completion of even acute serology, it may not be practical to delay the initiation of emergency control measures until such time as the diagnosis is confirmed.

Intensified Mosquito Surveillance: Ideally, information on the abundance and distribution of the vector, *Aedes aegypti*, should be available through an ongoing vector surveillance program in any dengue-receptive area. If such is the case, the need for additional information would require only an increase in the number of larval or adult sampling sites, particularly in relation to geographic location of human cases, and an increase in the frequency of sampling at key sites. In localities or areas where routine mosquito surveillance has not been established prior to the threat of a disease outbreak, it is essential to initiate without delay at least an efficient adult mosquito sampling program. The several methods of sampling for *Ae. aegypti* adults are described in Appendix IV.

Where area-wide control measures are contemplated, it is important to establish mosquito population baselines which can be used to determine the effectiveness of the control measures subsequently employed.

Area-Wide Adult Mosquito Control: The presence of confirmed cases of dengue, or even illnesses with a high probability of being dengue, in a community where *Ae. aegypti* populations are known to exist should signal the initiation of area-wide mosquito control efforts. If these cases can be classified as having been locally acquired, full-scale control efforts toward the immediate reduction of adult mosquito populations are essential. Under these conditions it must be assumed that there are infected mosquitoes present in the area, and efforts toward their immediate elimination to prevent additional human cases are highly important. Under ideal laboratory conditions the adult *Ae. aegypti* may survive as long as 4 months or more, but in nature a one-month survival would be more likely. Once an adult *Ae. aegypti* female has become infective for dengue, she probably remains so for life, with the possibility of transmitting the infection each time she feeds on a human host.

Ultra-low volume (ULV) application of insecticides, using ground-based or aerial equipment has been used successfully for adult mosquito control in emergency situations. While used mainly for the emergency control of vectors of the viral encephalitides, these measures have been tested against *Ae. aegypti* in several trials and are adaptable for use in emergency situations. Adult *Ae. aegypti* populations are difficult to reach with effective dosages of insecticide dispersed as ULV sprays because of their habit of resting indoors and in well-protected areas.

Specific instructions on the methods of area-wide adult mosquito control and on the insecticides approved for use by these methods are in Appendix VI. Generally, ground-based space spray operations (ULV, fogging, misting, and dusting) are conducted during late afternoon, at night, or early in the morning. During these hours there is usually an inversion of air temperature and a lower wind velocity, conditions which hold the insecticide droplets close to the ground, enhancing effectiveness. If the winds are stronger than 6 mph or the ground temperature is high, the treatment is likely to be ineffective, since these conditions disperse and dilute the treatment. Aerial ULV is usually applied only during the early morning hours.

The effects of mosquito control measures must be determined periodically to ensure continuing effectiveness. Results of ground or aerial insecticide applications should be monitored to ensure that proper droplet size and distribution as well as reduction of vector species are achieved (Appendix VI). Poor results and/or resistance to an insecticide can occur and alternative methods or a different insecticide must then be employed. Methods of evaluating chemical control are outlined in Appendix VIII.

Area-wide mosquito control programs may be augmented by other methods in localized situations, for example, residual treatment of areas where adult mosquitoes rest in large numbers (Appendix VI).

It would be desirable to conduct an intensive area-wide campaign of source reduction, i.e., a "cleanup" campaign, along with emergency measures for adult *Ae. aegypti* control; however, few areas have sufficient resources available for such a campaign on an emergency basis. Such a measure, if well organized and efficiently managed, could prevent new populations of adult *Ae. aegypti* and the extension of a dengue epidemic.

Public Information: Release of accurate and well-timed information to the public is extremely important because an informed public is more likely to cooperate with and support mosquito control efforts; further, they may even be encouraged to protect themselves personally and to reduce mosquito breeding on their own property.

The public should be made aware of the real threat of disease and to understand the role mosquitoes play in its transmission. It is important for the public to know the character and extent of mosquito control operations, the schedule and locations of spraying, and how the mosquito control operations may affect them. Announcements should be made immediately preceding application so that the public is not surprised by either the smell of insecticide or the noise associated with its application. In addition, information on simple measures for personal protection against mosquito bites should be disseminated (Appendix VII). This can be augmented by community participation and reduction of peridomestic mosquito breeding by eliminating water-holding containers which act as breeding sources.

Information dissemination should be the responsibility of one individual. Effort should be made to reach the largest population quickly and in the most efficient manner. Radio and TV-spot announcements, along with newspaper coverage, will generally reach most of the population of the area. Well prepared presentations to key civic groups or at public gatherings can be useful in certain emergency situations during any mosquito-borne disease outbreak. There will be numerous telephone inquiries. One particularly important aspect of good public relations is providing well prepared responses to these callers. To do so requires the personnel of the health department and vector control personnel to be current on all aspects of the situation.

In an epidemic, information should be released as early as possible and continued on a daily basis for as long as necessary.

Human Safety and Environmental Considerations: Current standards of practice of mosquito control through use of pesticides require a high level of care in their application to assure safety of the operator and the public, and to avoid adverse environmental effects. Only those pesticides approved by the Environmental Protection Agency for the specified use should be considered. When used according to label directions and local, State and Federal regulations, these compounds are not considered hazardous to people. Experience to date indicates no adverse human health effects following ultra-low-volume aerial applications in *Aedes aegypti* control. In one study of people working in an urban area during a large-scale emergency control application, risks to human health were determined to be negligible.[16]

Adult mosquito control operations, especially aerial applications, can present a hazard to certain nontarget species. Honeybees are particularly susceptible to such treatments, although most public health aerial ultra-low-volume applications have not resulted in serious harm to bees because of the low dosages used. It is important to take precautions by notifying beekeepers of a planned application; they may protect their hives by closing them or turning on sprinklers over the hives before daylight (when early morning applications are used) to keep the bees inside during the spray application. The beekeepers association and/or State experiment station should be contacted for advice regarding methods of protecting bees. During the last 10 years a few instances of fish kill have occurred following aerial ULV applications. These have occurred in shallow, warm water where there appeared to have been other environmental stresses on the fish prior to the insecticide application.

In planning control measures in areas where delicate ecosystems could be disrupted by mosquito control practices, assistance and cooperation should be sought from competent conservationists, fish and game specialists, and biologists.

A P P E N D I C E S

I - VIII

APPENDIX I: Definitive Diagnosis of Dengue Cases

The definitive diagnosis of dengue cases requires not only clinical and serologic diagnoses, but also virus isolation. As with a number of exotic diseases, the question, "where have you been?" may elicit from a patient information which is highly important in the inclusion of dengue in the differential diagnosis. Once the identity of an epidemic is established, clinical diagnosis may become more reliable, but it usually must be considered a presumptive rather than a confirmed diagnosis in the absence of virologic or serologic confirmation.

The differential clinical diagnosis of dengue may be difficult in initial cases in areas when dengue is not endemic and where epidemic occurrences have been rare. The occurrence of such symptoms as headache, backache, fatigue, stiffness, anorexia, chilliness, malaise, and rash, either preceding or accompanying a temperature rise, should suggest a dengue infection in persons living in or having recently arrived from areas where dengue is currently endemic or epidemic. The typical dengue rash usually appears 3 to 4 days after onset of fever and may be maculopapular or occasionally scarlatiniform. It is usually seen first on the trunk but eventually spreads to the face and limbs. During the last day of fever or soon thereafter, petechiae may appear on the feet and legs, wrists and hands, in the axillae, and on the palate. Leucopenia and lymphadenopathy may occur. While classical dengue is characterized by self-limited clinical disease and rarely terminates fatally, the more serious complications of dengue infection present a different picture. Dengue hemorrhagic fever (DHF) is a severe illness characterized by abnormal vascular permeability, resulting in blood plasma loss, and abnormal blood clotting mechanisms. Early symptoms include sudden onset of high fever, headache, abdominal pain, vomiting, and anorexia. Hemorrhagic manifestations are seen frequently and include a positive tourniquet test, bleeding of gums or at venipuncture sites, epistaxis, or a fine petechial rash. Thrombrocytopenia is characteristic, and in more severe cases there may be an elevated hematocrit, low serum albumin, elevated transaminases and a prolonged prothrombin time.

In some patients, usually after a few days of fever, dengue shock syndrome (DSS) may ensue, with development of circulatory failure, manifested by tachycardia, hypotension, cold clammy skin, and restlessness. Unless detected and treated, this condition can worsen to profound shock, with imperceptible pulse and blood pressure.

The serologic methods available include hemagglutination-inhibition (HI), complement-fixation (CF) and neutralization (NT). A four-fold rise in titer in any of these tests in a series of two or more paired sera would be significant in diagnosis. The tests should be conducted for all 4 types of dengue virus as well as locally occurring flaviviruses and yellow fever. Because of the occurrence of cross-reactivity among the flaviviruses, test results in persons who had previously experienced infections with one or more of these agents (including 17D yellow fever vaccination) are more difficult to interpret.

Dengue virus isolation has been difficult using animal or cell culture systems. Infections can be established by intracerebral inoculation of acute serum into suckling mice, but often several blind passages are required for adaptation before a reproducible pattern of illness and death in mice is established. It has been further shown that some strains of dengue virus do not cause illness in newborn mice despite many blind passages.[17] The dengue viruses can be propagated in cell cultures of several types, but in none of the cell culture systems used to date is the cytopathic effect consistent for all dengue virus isolates.

At present, the best available method for detection and isolation of dengue viruses in cell cultures is by plaque assay in a continuous monkey-kidney cell line (LLC-MK_2 cells).[18] Dengue viruses can be recovered by this method from mosquitoes and from human sera collected in the course of epidemiologic studies. Identification of virus type can be accomplished using the same cell culture systems by a plaque reduction neutralization method.[19] Because of the one- to two-week period required for isolation and identification of dengue viruses by this method, it cannot be useful for the immediate diagnosis of individual cases, but, nevertheless, can provide essential information in the identification of endemic foci or epidemic outbreaks.

A new technique[17] has been developed for isolation and amplification of dengue virus by parenteral inoculation of Aedes mosquitoes with sera or, presumably, other virus-containing fluids. This method is apparently more sensitive in isolating the virus than animal or cell culture systems, and can provide quantities of virus sufficient for further study and identification by fluorescent antibody, complement-fixation or cell culture methods.[20,21]

APPENDIX II: Techniques and Equipment for Surveys of *Aedes aegypti* Using Oviposition Traps

In recent years a technique of demonstrating the presence of *Aedes aegypti* by collecting its eggs in an "oviposition trap" or "ovitrap" has proven highly effective. The ovitrap consists of a pint-sized black jar with a narrow paddle (3/4" x 5") clipped vertically to the inside. The paddle is made from dark-colored hardboard with its rough side facing the center of the jar and its lower end standing in an inch or more of clean water which is added to the jar. As it absorbs water, the hardboard paddle becomes an attractive surface on which the mosquitoes deposit their eggs. The trap works by taking advantage of certain natural responses of the gravid mosquito which include attraction to dark objects and to water vapor, and a preference for a rough moist substrate for egg laying.

Proper placement of the ovitrap in the field is crucial to its success and requires that certain other aspects of the mosquito's oviposition behavior be kept in mind. Adherence to the guidelines listed below will help realize the ovitrap's full potential as a sampling tool.

1. The female normally flies near the ground, so the trap must be placed at or near ground level.

2. The mosquito's responses are in part visual, so the trap must be visible to a female flying over it.

3. The trap should not receive excess water from such sources as garden sprinklers or runoff from eaves or broadleaf plants.

4. Adult mosquito resting places such as shrubbery and junk piles are good trap locations.

5. Ovitraps should be placed in partial or total shade. Avoid direct afternoon sunlight and fully exposed paved areas.

6. The rear of the property is usually a better location for traps than the front because generally there are more mosquito breeding places and shelter for the trap in the backyard than near the street.

7. The ovitrap does not compete well with tires and should not be located in tire yards or near piles of tires. Tires are themselves excellent ovitraps since they are large, black, water-retaining receptacles.

Ovitrap surveys are conducted by pre-selecting trap sites with the aid of zone maps and a grid. Commonly a 500-foot grid is used and actual trap placement is within 100 feet of the spot defined by the crossed grid lines.

After placement, ovitraps are serviced once a week. At each trap site the paddle, identified by location and week, is placed in a plastic bag for later examination under the low power of a dissecting microscope. The jar is scrubbed clean (without soap or detergents), re-supplied with water and a new paddle and returned to its position.

The occurrence and distribution of *Ae. aegypti* in an area are revealed by the presence of its eggs on ovitrap paddles, and weekly counts of eggs and positive paddles serve as an index of changes in its population density.

APPENDIX III: Techniques and Equipment for Surveys of *Aedes aegypti* Larvae

The systematic collection of larvae serves the same purpose as the ovitrap survey, namely, to determine the presence of *Aedes aegypti*, its distribution, and the relative abundance of infestations. This is done by inspecting premises to look for *Aedes* larvae (and pupae) in whatever kinds and numbers of water-holding containers may be present. When a positive container is found, a sample of the larvae is withdrawn and placed in a vial in clean water or alcohol for species determination with a microscope in the laboratory.

Since larvae may occur in a wide variety of receptacles ranging in size from wading pools and boats to tin cans and fence pipes, a variety of collecting devices is necessary for taking samples. A dipper, preferably the long-handled, white enamel type, is most frequently used in sampling from large containers. Since larvae are most easily seen against a white background, it is often worthwhile to pour the contents of small containers such as cans and jars into the dipper for examination. Even such a simple task as dipping requires care because *Ae. aegypti* larvae are easily alarmed by any disturbance. Moving or jarring the container or even the passage of a shadow will send them quickly to the bottom where they can remain undetected for several minutes.

An ordinary squeeze-bulb syringe of the type used for servicing auto batteries or for basting food is well suited for removal of water from narrow-mouth receptacles or those too small for a dipper. It is particularly useful for taking samples from tree holes. For especially deep holes a length of rubber or plastic tubing may be added to the syringe. Other items of equipment which will prove valuable in the course of a larval survey include a flashlight, a tea strainer, which is used for transferring specimens from debris-laden or dark-colored water to clean water, a white plastic or enamel tray for examining material from the dipper, syringe or tea strainer and a medicine dropper for moving individual larvae into collection vials.

Three types of larval surveys have been employed. For a very rapid appraisal of an *Ae. aegypti* infestation a 12.5 percent premise sample is made by inspecting half the premises in one of every four blocks. Another rapid procedure entails the inspection of all premises in all blocks until an infestation of *Ae. aegypti* is found. The remainder of this positive block and the blocks immediately adjacent to it are then omitted from inspection. A comprehensive survey in which no premises in the survey area are omitted requires more time and manpower than other kinds of survey, but gives the most reliable and detailed results.

APPENDIX IV: Techniques and Equipment for Surveys of *Aedes aegypti* Adults

In circumstances such as a sudden dengue fever outbreak where it is necessary to assess quickly the effect of emergency adulticiding operations, adult sampling, before and following treatment, must be done. Collection of blood-seeking females attracted to a human host may prove feasible in productive *Aedes aegypti* breeding places such as tire yards, cemeteries, and junk yards. The mosquitoes are captured individually with mouth- or battery-powered aspirators as they come to feed. A half-hour collecting period is appropriate. It has been shown than human attractiveness varies for *Ae. aegypti* by as much as 5 times from person to person. In choosing individuals to serve as attractants, therefore, it is advisable to make preliminary trials and select those who prove most attractive. All such collections must be standardized, i.e., done by the same persons, in the same manner, at the same place, for the same period, and at equal time intervals. Non-standardized collections are not comparable and can lead to incorrect conclusions regarding population changes.

Another method of collecting is to search for adults in houses, garages, outbuildings, sheds and similar adult resting places. Since *Ae. aegypti* is in general active throughout the day, resting specimens found during the day will usually be those which are in the process of digesting a recent blood meal and developing their eggs. Resting individuals will be found most often in dark corners, under tables and desks and in similar places where light intensities are low, so a flashlight, in addition to an aspirator, will be required for their capture.

APPENDIX V: Control of Mosquito Larvae

Measures for the control or elimination of Aedes aegypti populations are primarily directed at the larval stages, either through the elimination of the larval habitats ("source reduction") or through destruction of the larvae in these habitats with insecticides or other means.

Source reduction: Because Ae. aegypti utilizes artificial water containers for the vast majority of its breeding sites, and because these containers represent man's discarded wastes, man can control the availability of most of these breeding sites, either by preventing their accumulation or by eliminating those which already exist. Organized efforts toward the proper disposal of cans, bottles, tires, appliances, plumbing fixtures, or any other discarded materials which might hold water can be useful in reducing Ae. aegypti populations.

Source reduction through community cleanup campaigns requires careful organization and the full cooperation of private citizens and businesses with the public health authorities. Such campaigns can be conducted periodically, e.g., annually, or may be stimulated as an emergency measure on the threat of epidemic disease. Skillful use of the news media and other communication channels, such as schools, civic clubs, churches, unions, etc., is highly important in the dissemination of information and assuring the successful organization and operation of such campaigns. Community-wide cleanup has certain public health side benefits, including the reduction of rat and fly populations and the elimination of accident hazards. The aesthetic values are also considerable.

The conduct of such campaigns in depressed and economically less favored urban areas may meet with numerous problems and require somewhat different approaches. Similarly, certain non-residential situations may be responsible for large accumulations of water-containing materials and may require special treatment; these include cemeteries, roadside dumps, used tire and automobile junk yards, vacant lots, partially demolished or unused buildings, etc. Ornamental pools, cisterns, rain barrels, and other water collections which are intentionally maintained, and inadvertent collections in poorly maintained roof gutters, boats, or other non-disposable containers, require additional measures to eliminate mosquito breeding.

Larviciding: The use of certain chemical larvicides is appropriate in situations where source reduction is not feasible or is incomplete. The insecticides that are currently registered for use as mosquito larvicides are listed in Table 1.

Larviciding for Ae. aegypti control can be accomplished with hand sprayers for most premises, or with power spraying equipment for larger breeding sites, using one of several appropriate liquid formulations of insecticides. Certain dusts, pellets, or granules may also be useful. The method for larviciding usually involves the selective treatment of all actual or potential water-holding containers in the premises being treated.

The amount of insecticide sprayed on each such container is a matter of judgment on the part of the spray operator. The treatment should exceed the minimum level for larval kill. Although one would expect little hazard to non-target organisms in such breeding sites, excessive treatment should be avoided. Great care should be used in applying insecticides in and around human dwellings because of the presence of children, pets, and possible valuable ornamental plants.

Other methods of larval control include the stocking of ornamental ponds with mosquito-eating fish, such as *Gambusia* or guppies; cisterns, rain barrels or other water storage containers can be covered or screened to preclude mosquito breeding; animal watering pans or tanks can be emptied and cleaned periodically to prevent the maturation of larval *Ae. aegypti* and the emergence of adults.

Environmental Aspects: Control measures aimed at mosquito larvae can have adverse effects on other species, requiring care in choosing a method of control and in performing the control operation. Fortunately the breeding of *Ae. aegypti* usually results from man-made sources which can be eliminated or treated without significant impact on other species. In mosquito control aimed at disease prevention, only those pesticides, formulations and dosages approved by the Environmental Protection Agency for the planned use should be considered.

Table 1. Insecticides for Use as Mosquito Larvicides

These recommendations are guidelines only. User must ensure that insecticides are applied in strict compliance with the label, as well as local, State, and Federal regulations.

Only those pesticides approved by the U.S. Environmental Protection Agency can be considered for use in the United States. When applied according to label directions, these compounds are not considered hazardous to human health.

Insecticide	Rate of application (AI/A*)	Formulation
Organophosphates		
chlorpyrifos (Dursban®)	0.0125-0.05 lb	2E (2 lb/gal emulsifiable conc.)
fenthion (Baytex®)	0.05 lb	4EC (4 lb/gal emulsifiable conc.)
malathion	0.4-0.5 lb	57E (57%; 5 lb/gal emulsifiable conc.)
temephos (Abate®)	0.05-0.1 lb	sand, celatom, 4E (4 lb/gal emulsifiable conc.)
Organochlorines		
methoxychlor	1 lb	50% WP (wettable powder)
Insect Growth Regulators		
methoprene (Altosid®)	0.025-0.05 lb	CR and others (controlled release, microencapsulated flowable, sand granular)
Oils		
diesel fuel oil No. 2	1-5 gal	with spreading agent only
proprietary mosquito control oils (Flit MLO, ARCO larvicide, GB-1313)	1-5 gal	with spreading agent only

*Active ingredient/acre

APPENDIX VI: Control of Adult Mosquitoes

Area-wide spraying of insecticides, i.e., "space spraying," provides an important means for reducing or eliminating adult mosquito populations on an emergency basis. Such space spraying has been applied using dusts, thermal fogs, or ultra-low volume cold fogs, either with ground-based or aerial equipment. Control of adult mosquitoes by space spraying of any kind is only temporary, since mosquitoes from nonsprayed areas can move rapidly into the sprayed area following spray applications, and there is usually little or no effect on the aquatic stages and emergence of adults will continue.

Space spraying operations are most effective when conducted during the late afternoon and early evening, at night, or in the early morning when the air is cool and wind velocity is not excessive. If air movement is excessive, the small droplets used in space spraying are dispersed so swiftly than effectiveness is reduced or prevented. Similarly, during the middle of a hot day the droplets are dispersed by rising currents of warm air known as thermals. At night there may be an inversion of air temperature, holding small droplets close to the ground and usually producing excellent control of mosquitoes.

Outdoor space treatments with ground or aerial applications have been used effectively against a number of mosquito vectors, including *Aedes aegypti*. The insecticides considered useful for such applications are listed in Tables 2 and 3.

Ultra-Low Volume Application with Ground Equipment

Ultra-low volume (ULV) treatment is defined as the application of less than 2 quarts of insecticide per acre, usually with ground ULV less than 1 fluid ounce per acre. Since 1970, great advances have been made in the development of ground-based ULV equipment, and a number of different machine types are now commercially available. The ULV method has a number of advantages: ULV equipment utilizes insecticide concentrate with little or no diluent or carrier, resulting in significant savings in fuel costs and loading time; further, the "cold" ULV aerosols do not produce dense fogs, as do "thermal" aerosols, which constitute a traffic hazard by reducing visibility when used along roads. The ground ULV machine usually has an insecticide tank of 5- to 10-gallon capacity, and is small enough to be mounted on a small vehicle, such as a ½-ton pickup truck.

Performance requirements for ground ULV equipment have been published as follows:

1. The ULV cold aerosol nozzle for dispersal of malathion to control adult mosquitoes must have the minimum capability of producing droplets in the 5- to 27-micron range. Large droplets may permanently damage automobile paint. The average diameter should not exceed 17 microns. Determination of droplet size should be made after collecting a sample

of the aerosol on a teflon- or silicone-coated glass slide and measuring the droplets under a microscope with an ocular micrometer.

2. Tank pressure should be not less than 2 pounds nor greater than 6 pounds per square inch (psi).

3. Flow rate must be regulated by an accurate flow meter. Flow meter data (gal/hr) should be recorded at the end of each day's operation.

4. The nozzle should be in the rear of the truck and pointed upward at an angle of 45^{o} or more.

5. Vehicle speed should not be greater than 10 miles per hour. The ULV machine should be shut off when the vehicle is stopped.

Insecticides having EPA label approval for application as ULV aerosols by ground equipment are listed in Table 2.

Ultra-Low Volume Application with Aerial Equipment

The aerial ULV technique uses the application of 0.5 to 3 ounces of highly concentrated insecticide per acre for the control of adult mosquitoes. Two insecticides are currently approved for adult mosquito control by ULV application from aircraft: Malathion at 3 fluid ounces per acre, and naled at 0.5-1 fluid ounce per acre.

Special airplane equipment for ULV application includes special insecticide tanks, electrically driven pumps, spray booms, and small orifice nozzles.

In general, aerial ULV applications should be made only:

1. When temperatures are below $80^{o}F$ (usually early morning).

2. With droplet size of not more than 50 microns MMD (Mass Median Diameter) and no more than 10% of the droplets should exceed 100 microns. In some areas damage to car paint has occurred when larger droplets were dispersed or more than 10% of the droplets exceeded 100 microns. Effectiveness against adult mosquitoes requires 10 or more drops per square inch. Determination of droplet size should be made by collecting a sample of the aerosol on a teflon- or silicone-coated glass slide and measuring the droplets under a microscope with an ocular micrometer.

3. By multi-engine aircraft flying at a height of 100-150 feet, at speeds of about 150 miles per hour or more, with swath widths of 300-1000 feet, with pump pressures and

Table 2. Adulticides Applied Aerially or by Ground Equipment at Ultra-Low Volume (ULV)

Insecticide	Rate of application (AI/A*)	Formulation
	Aerial ULV	
malathion (Malathion ULV Concentrate®, Cythion®)	3 fl oz	ULV concentrate
naled (Dibrom®)	0.5-1.0 fl oz	ULV concentrate
	Ground-applied ULV	
Organophosphates		
chlorpyrifos (Dursban®)	0.005-0.01 lb	ULV concentrate
fenthion (Baytex®)	0.005 lb	ULV concentrate
malathion	0.08-0.1 lb/acre (3-4.6 oz/min at 5 miles per hour)	ULV concentrate
naled (Dibrom®)	0.01-0.02 lb	10% in heavy aromatic naptha
Pyrethroids		
pyrethrum (Pyrocide®)	0.002-0.0025 lb	5% with 25% piperonyl butoxide
resmethrin (SBP-1382®)	0.007 lb	10% solution 40% oil-based ULV concentrate

*Active ingredient/acre

> nozzle sizes and positions adjusted to provide the proper droplet size. Single-engine fixed wing and rotary wing aircraft are undesirable for this technique because of their slower air speed and resulting problems with droplet break-up. There are additional factors related to safety over urban areas with single-engine aircraft and with their limited "pay load" which need to be considered.

Aerial ULV has been used on several occasions for adult *Ae. aegypti* control during outbreaks of dengue, but, because of the emergency nature of the operations and the use of multiple control measures, there has been little opportunity to fully evaluate its effectiveness in disease control. However, experimental trials in Florida[22] demonstrated significant control of *Ae. aegypti* populations, using malathion at 3 fluid ounces per acre on a weekly or twice-weekly schedule. Similar trials in Thailand demonstrated moderate to good control with malathion at 3 to 6 ounces per acre applied by aircraft.[23-25]

On occasion, car spotting, bee kills, and fish kills have occurred as a result of ULV aerial applications. It is essential to follow closely the label directions and to assure the proper size and distribution of droplets and to take additional steps necessary to avoid undesirable side effects.

Thermal Fog and Dust Applications

Tests have shown ULV cold aerosols and thermal fogs to be similar in effectiveness. The disadvantages of the thermal fogs include the hazard of reduced visibility due to the dense smoke-like fog produced and the additional expense of carrying and using the fuel oil additive. Thermal fogs are still widely used and several types of equipment for their dispersal are commercially available. Insecticide dusts have also been used successfully for area-wide adult mosquito control. The insecticides currently used for ground-based adult mosquito control are listed in Table 3.

Other Methods of Adult Mosquito Control

Residual treatment outdoors for mosquito control does not always provide good control. However, some benefit may be derived by applying residual insecticide outdoors on vegetation, in storage sheds and other buildings in close proximity to where cases of dengue have occurred and infected mosquitoes may still be present. Water suspensions or emulsions with a low percent of insecticide (rather than oil solutions) are used in order not to "burn" vegetation. These applications can be made with power sprayers or with hand sprayers using nozzles which provide a broad fan or cone and a coarse spray, such as the Tee-Jet® 8004. The insecticides used for such outdoor applications include methoxychlor (50% wettable powder, 2 lb per 100 gal water) and fenthion (emulsifiable concentrate, 2 to 4 oz per gal water).

Table 3. Adulticides Used in Foggers, Misters and Dusters

Insecticide	AI/A**	Examples of concentrations of finished spray or dust*		
		Thermal fogs	Dusts	Emulsions***
carbaryl (Sevin®)	0.2-1.0 lb	-	2.5-5 lb/acre	-
chlorpyrifos (Dursban®)	0.025-0.05 lb	2 gal fogging conc/ 98 gal oil	-	3.2 fl oz/acre of 2E
fenthion (Baytex®)	0.01-0.1 lb	2 gal spray conc/ 100 gal oil	-	-
malathion	0.075-0.2 lb	-	-	2% spray (4.5-11.2 oz of 5E/gal water)
naled (Dibrom 14®)	0.02-0.1 lb	100 fl oz 14 conc/ 99 gal oil	-	100-230 fl oz 14 conc. in 100 gal oil
propoxur (Baygon®)	0.05-0.07 lb	-	-	4.25-6 fl oz 5E in 2-4 qt water for aerial use
pyrethrins (synergized)	0.002-0.0025 lb	-	-	5% oil-based ULV conc. with 25% piperonyl butoxide
resmethrin (SPB-1382®)	0.007 lb	-	-	10% solution

*Examples do not represent all acceptable pesticide formulations
**Active ingredient/acre
***2E = 2 lb/gal emulsifiable concentrate; 5E = 5 lb/gal emulsifiable concentrate

Apply the methoxychlor spray to vegetation, trunks of trees, outside walls of buildings, walls and fences in a drenching spray to the point of runoff. The fenthion spray should be applied at a rate of 2 gallons per 1000 square feet.

Environmental Aspects

Assistance should be sought from competent conservationists, fish and game specialists, and others in planning control measures in areas where delicate ecosystems could be disrupted by mosquito control practices. Only those pesticides, formulations and dosages approved by the Environmental Protection Agency for the planned use should be considered.

APPENDIX VII: Personal Protection from Mosquitoes

Aedes aegypti bite primarily during daylight hours but will also attack at night. They readily enter dwellings and often breed in flower vases and other water-holding containers indoors. People can protect themselves from mosquitoes by eliminating indoor and outdoor breeding places, using proper window screens, protective clothing, or repellents. Consequently, in an epidemic situation people should be encouraged to avoid mosquito contact as much as possible. The ordinary window screen with 16-x-16 or 14-x-18 meshes to the inch will keep out most but not all *Aedes aegypti*. Screen doors should open outward and have automatic closing devices. Residual insecticide applications on and around screen doors give added protection. The use of mosquito netting to protect infants in their cribs may also be indicated in high risk circumstances.

Relief from mosquito attack may usually be obtained by applying insect repellents to the skin and clothing. Repellents are available as liquids in bottles, pressurized spray cans, and in stick form.

When applied to exposed areas of skin, liquid repellents will prevent mosquito bites for 2 hours or more, depending on the person, species of mosquito attacking, and abundance of mosquitoes. *Aedes aegypti* is especially prone to bite on the ankles and lower legs so care should be taken to apply repellents to these areas. Repellents can also be sprayed on clothes to make them repellent. Many repellents are solvents of paints, varnishes, and plastics such as watch crystals, rayon fabrics, and fountain pens, and caution should be used to avoid contact with such materials. Care should be taken not to apply repellents to the eyes, to the lips, or to mucous membranes.

Pressurized aerosol insecticide dispensers can be used in the home to kill adult mosquitoes. Most of these contain pyrethrum or allethrin because these insecticides have low human toxicity and cause a quick knockdown of mosquitoes. These aerosol dispensers may also contain a synergist such as piperonyl butoxide and another insecticide such as diazinon to kill the insects. Release of the aerosol for a few seconds usually kills most insects in an ordinary-sized room, tent, or trailer. These aerosols are not hazardous if used as directed on the container, except in rare cases where persons are allergic to pyrethrum or the synergist.

APPENDIX VIII: Methods for Assessing Chemical Control of Mosquitoes

Evaluating the results of the treatments applied as larvicides and adulticides is important to any control effort. Resistance to the insecticide being used may become a problem, or improper application techniques may reduce the effectiveness of the method, or possibly increase the risk of killing nontarget species. Standard resistance/susceptibility test kits are available from the World Health Organization, Geneva, Switzerland, and periodic tests may indicate a change in the susceptibility of a mosquito species from an established baseline.

The basic approach used in evaluating larviciding or adulticiding applications is comparison of the number of specimens per collection made before and after the application. For this purpose collections should be made on each of several days before and after the application and as many sampling sites as possible should be included.

Another useful method is that of bioassay tests with caged specimens. A bioassay test for space sprays may be done by using the following technique:

> Treatments may be applied by fog, dust, mist, or ULV machines using the recommended label dosage. Field-collected, caged specimens (100-150/cage) are hung 3-6 ft above the ground at stations 150-300 ft from the point of discharge of the machine along each of three streets (270-300 ft apart). Ten to 15 minutes after exposure the cages are removed and the insects are transferred to holding cages, given food and held for a 24-hr mortality count. Seventy percent or better kill is expected.
>
> If the kill at either the 150' or 300' station is less than 70%, then the equipment and timing of application of insecticide should first be examined and adjusted. If, after these adjustments have been made, the kills are still unsatisfactory, then a change of insecticide should be recommended. Aerial applications may also be evaluated using the bioassay method.

Bioassay tests for larvicides are of less value than sampling of natural larval habitats for larvae before and after an application is made.

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