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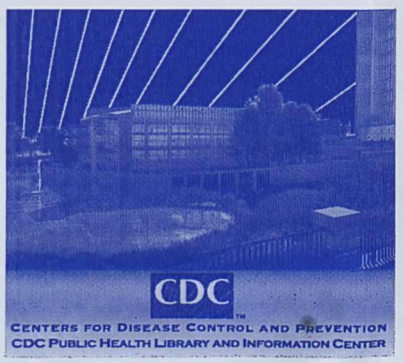

VECTOR TOPICS

NO. 3

Control of Western Equine Encephalitis

OCTOBER 1978

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CENTER FOR DISEASE CONTROL

BUREAU OF LABORATORIES
VECTOR-BORNE DISEASES DIVISION
FORT COLLINS, COLORADO 80522

BUREAU OF TROPICAL DISEASES
VECTOR BIOLOGY AND CONTROL DIVISION
ATLANTA, GEORGIA 30333

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WESTERN EQUINE ENCEPHALITIS

The Disease

Western equine encephalitis (WEE) virus was isolated from sick horses in 1930 and from a fatal human case in 1938. The virus causes an acute febrile illness in equines and humans, characterized in its most severe form by signs and symptoms of inflammation and injury of the meninges, brain, and spinal cord. Large outbreaks occurred in the north central United States in 1941 and in the Central Valley of California in 1952, and both sporadic cases and small epidemics continue to occur in the western states. Western equine encephalitis virus is maintained in a primary enzootic transmission cycle involving wild birds and culicine mosquitoes.

Distribution and Incidence: The virus is widely distributed throughout the United States and southern Canada; however, human and equine disease occurs almost exclusively in the western states and Canadian provinces. Western equine encephalitis virus has also been isolated in Mexico, Argentina, Brazil, Uruguay and Guyana. In South America, both equine epizootics (especially in Argentina) and human disease (Brazil) have occurred.

The virtual absence of disease in the eastern part of the United States probably reflects a paucity of the vector species, *Culex tarsalis*, as well as a lower pathogenicity of local viral strains.

The number of human cases of WEE reported to the Center for Disease Control (CDC) between 1955 and 1976 is shown below. Also indicated is the number of cases and incidence associated with selected epidemic occurrences during that period.

		Selected Epidemic Occurrences		
Year	Total No. Cases	Locality	No. Cases	Attack rates/ 100,000 population
1955	37			
1956	47			
1957	35			
1958	141	Kansas	25	4.8
		Utah	48	-
		California	37	-
1959	14			
1960	21			
1961	27			
1962	17			
1963	56	Hale Co., Texas	47	128.0
1964	64			
1965	172	Colorado	68	4.3
1966	47			
1967	18			
1968	17			
1969	21			
1970	4			
1971	11			
1972	8			
1973	4			
1974	2			

Year	Total No. Cases	Selected Epidemic Occurrences		
		Locality	No. Cases	Attack rates/ 100,000 population
1975	133	N. Dakota-Minn.	39	6.3
1976	1			
Total	897			

The prevalence of WEE viral antibodies in the human population of endemic areas of the western United States, such as Kern County, California, rises from about 5 percent in young children to 20 percent in adults over 20 years of age.[40]

Accurate records of the incidence of equine cases are not available. However, it may be conservatively estimated that for every reported human case there are several hundred horse cases. The occurrence of human disease is always associated with equine encephalitis in the same general area, and an equine epizootic often precedes the appearance of human cases. Where a high proportion of the equine population has been vaccinated against WEE, this early warning of viral transmission may be less obvious.

Western equine encephalitis occurs in early and mid-summer. The case incidence is higher in the rural population of the western United States than in urban residents. About one-third of the cases reported to CDC between 1955-1976 has been in children under 5 years of age; infants under 1 year of age are most susceptible to develop severe encephalitis. Attack rates are higher in adult males than females (probably because of greater exposure to infected mosquitoes during agricultural and recreational pursuits). Infection most often is abortive or mild and undifferentiated. The ratio of inapparent to apparent central nervous system (CNS) infections is approximately 50:1 in children under 5 years of age and over 1000:1 in older individuals. The case-fatality rate is 3-4 percent.

Western equine encephalitis and St. Louis encephalitis (SLE) both occur in the same geographical areas and may share the same vector species (*Culex tarsalis*). Mixed SLE-WEE outbreaks are the rule in the western United States, but generally one or the other infection predominates. The features of the two diseases are so similar as to be clinically indistinguishable, but encephalitis in infants is much more likely to be due to WEE than to SLE virus.

Clinical Features: The incubation period is usually 5 to 10 days. The onset of illness may be sudden, especially in adults, or characterized by a 2-4 day prodrome of lethargy, fever, and headache, especially in children. The acute illness is characterized by a spectrum of symptoms and signs referable to the central nervous system, reflecting infection and inflammation of the meninges and brain parenchyma. Fever, somnolence, headache, anorexia, vomiting, and stiff neck are the most common features of the acute disease. Fever is generally between 102 and 104°F. Confusion, disorientation, stupor, and coma, respectively, reflect increasing involvement of brain parenchyma. Irritability, convulsions and localizing neurological dysfunction, including flaccid and spastic paralyses, cranial nerve palsies, and pathological reflexes are more frequent in infants than in adult patients. Severe hyperthemia may occur. The acute phase generally lasts 3-10 days, after which recovery begins suddenly and proceeds rapidly. Some adult patients have a convalescent syndrome of aesthenia and nonspecific neuropsychiatric complaints, but permanent objective neurological residua are very rare. On the other hand, about half of the affected infants suffer permanent sequelae, including progressive retardation and major motor disorders. Congenital infections have been documented.

Clinical laboratory features include (1) mild-moderate leukocytosis; leukopenia may be found, but only during the very early course of illness; (2) cerebrospinal

fluid (CSF) pleocytosis, only rarely in excess of 500 cell/mm^3, with an early polymorphonuclear and later lymphocytic cell predominance; and (3) elevated CSF protein concentration.

In addition to the patients with full-blown encephalitic infections, cases with milder syndromes, including aseptic meningitis, fever with headache, and other mild undifferentiated febrile illnesses, occur.

Differential Diagnosis: In the western United States, the occurrence of a case of CNS infection during the summer months should raise the suspicion of WEE, especially if equine encephalitis has been reported in the area. Other viral infections (due to mumps, enteroviruses, herpes viruses, etc.), bacterial, fungal, and mycobacterial infections, leptospirosis, cerebrovascular and metabolic diseases enter the differential diagnosis.

Specific Diagnosis and Case Definition: Confirmation of WEE requires viral isolation or demonstration of a rise in specific antibodies in appropriately timed serum samples. Viral isolation from blood or CSF is rarely successful; a postmortem diagnosis may be established by isolation by WEE virus from brain tissue. Hemagglutination-inhibiting and neutralizing antibodies appear within the first week and complement-fixing antibodies in the second week after onset.

Cases of WEE are categorized as clinically suspect until laboratory data (Appendix I) allows a presumptive or confirmed diagnosis.

Transmission Cycle: Western equine encephalitis virus has been isolated from a variety of mosquito species in nature, and occasionally from other arthropods, but the results of these findings when viewed together with the results of experimental transmission studies have led to the incrimination of relatively few species of mosquitoes as major vectors. *Culex tarsalis* is the classic vector in the western United States and Canada. Epidemics and epizootics of WEE in North America have been confined to the range of this species. *Aedes melanimon* has recently been incriminated as a vector of WEE virus in California in a cycle involving jackrabbits.[18] Some of the earlier reports of WEE virus isolations from *Aedes dorsalis* were undoubtedly due, at least in part, to misidentifications prior to the time that this species and *Ae. melanimon* were routinely separated. *Culiseta melanura* serves as the primary enzootic vector in the northeastern United States.[21]

Wild birds serve as the basic viral reservoir hosts during the epidemic season. Both nestling and adult birds of many species serve as effective viremic hosts; some species, such as the house finch and house sparrow appear to play especially important roles. Domestic fowl develop viremias sufficient to infect *C. tarsalis*, but probably contribute relatively little to viral amplification.[29] Some mammals, especially jackrabbits, appear to be involved in transmission cycles in certain areas.

The mechanisms whereby WEE virus reappears each summer in the temperate zone are unknown. No local winter reservoir in hibernating arthropod vectors or vertebrates has been conclusively shown, nor has evidence been found for reintroduction by migrating birds.

Horses and humans are "deadend" hosts for WEE virus; viremia levels are insufficient to serve as a source for vector infection.

Mosquito Vectors of Western Equine Encephalitis Virus

Culex tarsalis occurs from Canada through the United States into Mexico south to the state of Chiapas. In Canada there are records from British Columbia, Alberta, Saskatchewan, Manitoba, and Northwest Territories.[7] In the United States *C. tarsalis* is relatively common west of the Mississippi River. It is usually uncommon or rare in the eastern part of the country, but has been collected in several states and as far east as New Jersey. The distribution of *C. tarsalis* in North America, based upon collection records, shows a clustering effect in the Great Plains, prairie, and other grassland areas. In the western and central United States the distribution and abundance of this mosquito are closely associated with the distribution of irrigated farm and ranch lands.[32] The vertical distribution of *C. tarsalis* extends from below sea level to almost 10,000 feet in California.[5]

Culex tarsalis larvae occur in clear or foul water in practically all freshwater sources except tree holes.[5] Open, sunlit habitats usually are preferred, but shaded pools may also be utilized. Vegetated habitats are most common; however, *C. tarsalis* larvae may thrive in the absence of macroscopic plant growth in habitats such as irrigation standpipes. Irrigation wastewater often is the primary source of water for larval habitats, particularly in pastures and weedy ditches. Habitats include the vegetated margins of lakes, ponds, and marshes as well as ditches, irrigation systems, ground pools, rain-barrels, hoofprints, cesspools, dairy drains, watering troughs, ornamental pools, liquid manure and hot springs. *Culex tarsalis* larvae have been collected in streams receiving sewage effluent, sewage treatment plant percolation ponds, and raw sewage stabilization ponds. Foul water in corrals and around slaughter houses also appears to be a favored habitat in some areas. Rice fields are an important habitat for *C. tarsalis* in areas such as the Sacramento Valley of California.

Inseminated females may seek a blood meal, or in some cases may develop the first egg batch autogenously, i.e., without benefit of a blood meal. Females will take a blood meal as early as the third day after emergence under laboratory conditions (21.1°C and 70% R.H.) and oviposit 4 days later. At 21.1°C, *C. tarsalis* develops from egg to adult in about 2 weeks. The species can complete development during the summer in irrigated pastures in the Central Valley of California within 9 to 10 days following irrigation. Water temperatures between 21.1°C and 29.4°C seem to be optimum for the development of *C. tarsalis* larvae.

Peak flight activity occurs within 2 hours after sunset and most *C. tarsalis* females remain within 50 feet of the ground. Dispersal occurs in all directions at low wind velocities, but mosquitoes orient into the wind as velocities increase. Winds in excess of 6 mph inhibit flight. *Culex tarsalis* females probably can travel 8 to 10 miles in 2 evenings, and may spread as far as 25 miles from breeding sites.

Host-seeking *C. tarsalis* exhibit a strong positive chemotropism to carbon dioxide and, to a lesser degree, to host odor. Precipitin test studies in Washington, California, Kansas, Utah, Colorado, Texas, and Alberta, Canada, have shown that *C. tarsalis* is a general feeder with a preference for avian hosts in some areas during certain seasons of the year.[46] Significant feeding on mammals does occur, and this may become more pronounced as the season progresses. *Culex tarsalis* may feed almost exclusively on birds in the spring in some areas, and then shift to mammalian hosts in mid-summer. Since this shift in the feeding pattern often coincides with the appearance of WEE virus infection in man and other vertebrates, it is perhaps one of the most important factors that makes *C. tarsalis* such an efficient enzootic and epidemic vector. The reasons for the observed seasonal shift in the feeding pattern have not been fully elucidated, but host availability, defensive reactions of the host, mosquito population density, and other seasonal variables may all play a role.

Culex tarsalis feeds readily on man out-of-doors during the summer months. Peak biting activity usually begins about 30 minutes after sunset and lasts for about 1

hour. Human avoidance of exposure to mosquito bites during the first couple of hours after sunset can be a practical prophylactic measure during the WEE transmission season.

Both sexes of *C. tarsalis* seek sheltered resting sites during the daylight hours. Light, temperature, and relative humidity are important variables that determine the suitability of such sites. In an outdoor situation both sexes were found to move out of a box shelter within 30 minutes following sunset, and began returning shortly before sunrise. A variety of natural habitats such as animal burrows and vegetation and artificial shelters such as the underside of bridges and culverts, cellars, chicken houses, and other farm buildings may serve as resting sites.

The seasonal abundance and duration of the period of annual activity of *C. tarsalis* are influenced by latitude and temperature. Throughout much of its range the maximum adult population is generally reached during August or September. Population peaks may, however, occur during June in parts of the Central Valley of California and during July in southern Alberta, Canada, and Washington. Most collection records for *C. tarsalis* east of the Mississippi River are in late autumn. This species occurs in the Tennessee Valley from late August to late November, with a population peak in September. In west Texas *C. tarsalis* is abundant from June through September; however, further south in the Lower Rio Grande Valley, *C. tarsalis* is most abundant during November and occurs throughout the winter in appreciable numbers. Populations then begin to decline and few specimens are collected during April and May, and none from June through September.

In the Imperial Valley of California, where the average July temperature is 34.4°C as compared to 28.9°C in the Lower Rio Grande, *C. tarsalis* adults have been collected each month of the year with population peaks in the spring and fall; however, adults continue to occur in reasonable numbers through the summer as well. The absence of *C. tarsalis* from the Lower Rio Grande during the summer cannot be explained on the basis of temperature alone, since the summer temperatures in that area are not excessive and are much lower than in the Imperial Valley during the same period of the year.

The manner in which *C. tarsalis* passes through the winter in areas where breeding is not continuous throughout the year is of extreme interest since it has been hypothesized that hibernating females might carry arboviruses through the winter. In the northern part of its range, *C. tarsalis* undergoes a facultative diapause in response to decreasing day length and lower temperatures. *Culex tarsalis* females enter shelters in the late fall and winter in those areas where the average January temperature is less than 0°C. Winter dormancy in *C. tarsalis* is more intense and more prolonged in the colder parts of its range, and the inactive winter period may be 6 months or longer in Washington and North Dakota as compared to 2 or 3 months in California.

Inseminated nulliparous females which had emerged in late summer or early fall make up the bulk of the overwintering population. Blood-feeding and ovarian development are greatly reduced during the late summer and fall in populations preparing for overwintering. Nourishment in the form of plant juices rich in sugar is required for development of the fat body which serves as an energy store during winter diapause.

A variety of hibernacula have been described for *C. tarsalis* including: outdoor food storage cellars, abandoned mines, animal burrows, tree holes, buildings, the underside of bridges, loose rock at the base of volcanic outcrops, talus slopes, and culverts.

The emergence of *C. tarsalis* from hibernation in Weld County, Colorado, has been correlated with the time of the spring inversion of soil temperatures.[4] During a 4-year period the first females were observed in the spring within 4 days before or 1 day after the day when the soil temperature at a depth of 2 or 3 feet first exceeded the temperature at 6 feet.

In order for overwintering *C. tarsalis* to carry arboviruses through the winter, it would be essential for some females to take a blood meal from a viremic host prior to entering hibernation, barring the occurrence of transovarial transmission of the viruses. However, the concensus is that *C. tarsalis* does not undergo gonotrophic dissociation, and blood-fed females held under semi-natural conditions are unable to survive the winter in significant numbers. Nonetheless, WEE virus has been isolated from *C. tarsalis* during the winter months,[2,3] and some parous females do enter hibernation and presumably overwinter successfully.

Aedes melanimon has been reported from California, Oregon, Washington, Nevada, Utah, Idaho, Montana, Wyoming, Colorado and New Mexico, and from Alberta, Canada. This mosquito is a typical floodwater species that lays its eggs in moist areas subject to intermittent flooding. The eggs can withstand considerable drying and may survive in the soil for several years; overwintering occurs in the egg stage. Several broods may be produced during the warmer months, their frequency being determined largely by irrigation schedules in agricultural areas and flooding cycles in waterfowl areas. During hot weather, development of the aquatic stages from hatching to adult usually occurs within 5 or 6 days.

Favored habitats for the aquatic stages include irrigated pastures and alfalfa fields, in addition to intermittently flooded waterfowl areas. Irrigation runoff and stream overflow pools, sloughs, roadside ditches, and potholes may also serve as habitats. Sunlit habitats are preferred, but larvae may also occur in shaded and partially shaded situations. Morphologically, *Ae. melanimon* closely resembles *Ae. dorsalis* in all stages;[1] however, *melanimon* tends to occur more frequently in fresher water and *dorsalis* predominates in brackish water. *Aedes melanimon* often is found in association with *Aedes nigromaculis* in irrigated pastures in the Central Valley of California.[4,5] *Aedes melanimon* generally appears first in the spring but is frequently overshadowed by *nigromaculis* during the warmer months, and then becomes predominant again toward the end of the season. *Aedes melanimon* can tolerate higher salinity than *Ae. nigromaculis*.

Peak flight activity occurs during the twilight hours in the spring and summer, but nocturnal flight activity may increase during the fall. *Aedes melanimon* females are strong fliers and may disperse 10 miles or more from breeding sites, particularly when aided by prevailing winds. The females will bite during the day at any time if disturbed, but biting activity is most intense during the evening hours, especially at dusk. *Aedes melanimon* readily bites man, and the species is a major pest in some areas. Other mammals such as cattle, horses, dogs, hares, and rabbits serve as principal hosts. *Aedes melanimon* adults can be collected in large numbers in CDC light traps supplemented with CO_2.

Culiseta melanura occurs in the eastern United States from Canada to the Gulf of Mexico. It has been collected in all of the states east of the Mississippi River except Vermont and West Virginia. However, it is uncommon or rare throughout much of its range due to the lack of suitable breeding habitats. Preferred habitats for the aquatic stages are heavily shaded, permanent, fresh-water swamps and marshes containing cool, acid water. The larvae generally develop in darkness or conditions of low light intensity and in situations where they can have contact with the soil. Such habitats include holes beneath tree roots and stumps, and the underside of root systems of aquatic plants.

Eggs are laid in rafts. In the laboratory they hatch within 2 days at 27°C; pupation follows within 2 or 3 weeks; and adults emerge about 3 days later. In natural swamp habitats, larval development is very slow; 8 to 15 weeks may elapse between oviposition and adult emergence. In nature *Cs. melanura* undergoes an obligatory diapause in the larval stage during the fall and winter months, and in the northern part of its range larvae can be collected in the spring from places that freeze over during the winter.

Larvae are present every month of the year in Maryland but are difficult to find during the winter.[27] Adult emergence begins in late April and oviposition occurs from late May through October. There may be 3 adult emergence peaks between late May and early October. Only 3 complete generations occur during the year. Larvae can be found throughout the year in New Jersey but are most abundant during the summer months.[6] Adults appear in May, and egg laying begins in late June. Adults are most numerous during late summer and early fall and persist until October.

Culiseta melanura feeds primarily on passeriform birds. Other birds and mammals are less frequent hosts. Man is rarely bitten. Reptiles may be an occasional source of blood. Adult females are most active during the evening twilight period, but some activity continues throughout the night. Very little adult activity occurs during the daylight hours.

Adult *Cs. melanura* can be collected in light traps. Adult females are also attracted to bird-baited traps, and can be collected from artificial "resting boxes."

Preventive Measures

Vaccination of Equines and Disease Surveillance: A vaccine for protecting horses against WEE became available in the late 1930's. Subsequently, a bivalent vaccine for both WEE and eastern equine encephalitis (EEE) was developed and has been widely used to protect horses in areas at risk to both of these diseases. More recently, a trivalent vaccine incorporating the antigen of Venezuelan equine encephalitis (VEE) virus has become available. Complete vaccination of the entire horse population against WEE, and periodic booster shots, presumably would solve the veterinary health problem caused by WEE cirus. However, this would not affect the basic WEE virus transmission cycle between mosquitoes and birds, and would do nothing to ameliorate the public health problem.

As a result of their field exposure, horses are subject to high vector attack rates, and susceptible horse populations may provide a sensitive indicator of WEE virus activity. In areas where WEE virus is endemic and where epizootics among horses occur, clinical illness is usually seen in the younger animals. Subclinical infections and vaccinations against WEE assure that a high proportion of the older population will be immune.

The seasonal occurrence of WEE cases in equines frequently precedes the occurrence of human cases in all phases of an outbreak, i.e., index case, peak in the number of cases, and last case. Therefore, surveillance for equine cases in areas with susceptible horse populations may provide the most practical and sensitive tool for the recognition of a potential public health problem by state and local agencies lacking the resources for monitoring virus activity in birds and mosquitoes. A letter can be sent to all practicing veterinarians in an area advising them of the need for increased vigilance during the WEE virus transmission season. They should be requested to telephone reports of suspected cases of WEE to a central laboratory, and to submit appropriately timed acute and convalescent serum samples from clinical cases to a diagnostic laboratory. Brain tissue from suspect cases which die may also be submitted for virus isolation attempts. Record sheets outlining the type of case history data required by the diagnostic laboratory for all samples submitted can accompany the circular letter. Such data should include an equine encephalitis vaccination history for each suspect case. An individual in the central laboratory should periodically check with key veterinarians in the area to make certain that suspect cases are being reported on a current basis.

Mosquito Surveillance: A sound control program must be based upon a thorough understanding of the biology of the vector species, and adequate survey data concerning the vector mosquito population must be obtained. Breeding sites must be located and the distribution and density of adult mosquito populations determined, particularly in areas in close proximity to human populations. Surveillance should be initiated early enough to permit detection of seasonal changes in the density and distribution of vector mosquito populations. Such information is essential for (1) identifying areas amenable to source reduction campaigns, (2) identifying areas where biological control agents such as larvivorous fish can be used effectively, (3) determining when and where to apply chemical insecticides, and (4) providing baseline data for evaluating the effectiveness of control measures.

Maps of the area encompassed by the control program should be prepared and updated throughout the season to indicate the location of mosquito breeding sites and changes in larval and adult population indices. Aerial maps (vertical aerial photographs) with a scale of 1 in. = 660 ft. or 1/8 mile have proved to be useful for this purpose. A system of mapping and recording based upon this type of map is employed by many mosquito control agencies.

Techniques for collecting larval and adult mosquitoes are presented in Appendices II and III.

Mosquito Control: Success in mosquito control is dependent upon knowledge of the species of mosquitoes and their habits in the control area. Preventive measures include the reduction or elimination of the water in which mosquitoes breed, and other methods which render such water unsuitable for mosquito breeding. If some mosquito breeding continues after this has been accomplished, then this residual production may be controlled with larvicides. Mosquitoes that escape the larvicides may be controlled with space-spray applications of a chemical to which they are susceptible. However, larvicides and space-sprays must always be looked upon as secondary methods of attack with the primary method being elimination of breeding sources through cleanup campaigns, drainage, filling, flooding, controlled reflooding and other water-management practices.

This primary preventive control methodology continues throughout the year, with the use of chemical control only during the breeding season. A consistent long-term effort will produce better results than an intense attack one year and neglect in subsequent years.

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

Virus Surveillance in Birds and Mosquitoes: Since wild birds are the principal vertebrate reservoir hosts of WEE virus during the epidemic season, sampling populations for evidence of infection often provides a sensitive indicator of virus activity. In one study conducted by CDC in West Texas, virus recovery rates from viremic nestling house sparrows varied directly with human attack rates of WEE.[25] This was the most reliable of several indices tested for estimating the likelihood of clinical WEE occurring in humans.

The selection of particular bird species for inclusion in a surveillance operation will depend upon (1) the density and distribution of bird populations in the area, (2) their susceptibility to WEE virus infection, and (3) their potential for infecting arthropod vectors. As indicated above, house sparrows and house finches play a major role in the dissemination of WEE virus in some areas. Other passerine birds may also be involved, and may be of major importance in some areas.

Sentinel bird flocks, usually domestic chickens, can be used for two purposes in a WEE virus surveillance system. The level of WEE virus activity can be monitored by bleeding the flocks periodically and testing the blood samples for WEE antibody; furthermore, the flocks can be used to attract mosquitoes which can be collected and tested for virus. A standard sentinel chicken-shed, fitted with two baffle-type mosquito traps, has been designed for such use. This is described in the CDC publication "Collection and Processing of Vertebrate Specimens for Arbovirus Studies."[16]

Arrangements should be made with a laboratory for testing blood samples from wild or domestic birds prior to their collection. Personnel responsible for obtaining and shipping the specimens may require additional training. A discussion of useful techniques can be found in the CDC publication referred to above. Both federal and state scientific collecting permits are required to capture wild birds and these should be obtained beforehand.

For practical purposes WEE virus surveillance in mosquitoes can be limited to the collecting and testing of *C. tarsalis*. Occasional WEE virus isolates may be obtained from other mosquito species collected concurrently, or sometimes earlier in the season, but the significance of such findings and their relationship to the degree of WEE virus activity that can be expected are unknown.

Culex tarsalis females can be collected by a variety of methods. The CDC miniature light trap used in combination with dry ice is effective, and lard-can bait traps using only dry ice also attract large numbers of specimens. Mosquitoes must be handled and processed carefully to optimize the chances for virus recovery. Techniques for sampling mosquito populations for arboviral surveillance have been described in the CDC publication "Collection and Processing of Medically Important Arthropods for Arbovirus Isolation."[9] Again, arrangements for testing the specimens should be made prior to their collection, as well as for training personnel in the use of acceptable techniques.

Emergency Measures

A multidiscipline approach to secure and evaluate virologic, entomologic and morbidity data is essential for planning and managing emergency control measures in a WEE epidemic. The techniques employed for virus identification and definition of the vector situation require time and effort. It is urgent, therefore, that a well organized assessment of the situation be quickly undertaken to expedite decisions on emergency measures. With optimal environmental and climatic factors, proliferation of vectors and amplification of the virus reservoir accelerates. Impact of control measures on the course of the epidemic will be greatly diminished by even small delays in proceeding.

Important assessments crucial to the emergency control plan include the following:

(1) infection rates in mosquitoes and birds,
(2) size of the adult mosquito population,
(3) extent of human and equine disease,
(4) extent of mosquito breeding,
(5) anticipated changes in mosquito activity due to seasonal effects, and
(6) climatic factors which may affect mosquito production and behavior.

An effective emergency vector control plan based on the use of adulticides consists of five essential activities: (1) intensified mosquito surveillance, (2) human case surveillance, (3) equine case surveillance, (4) area-wide adult mosquito control, and (5) public information.

Intensifying Mosquito Surveillance: Information concerning the relative abundance and distribution of vector mosquitoes must be obtained quickly in an emergency situation. It is often necessary to increase the number of sampling sites and the frequency of sampling in order to obtain this information. It is particularly important to identify residential areas with high vector mosquito population levels.

When vector mosquitoes are abundant and large numbers of live adult mosquitoes are obtained with normal sampling of the established sites, these same mosquitoes may also be used for surveillance of WEE virus activity. However, it is frequently necessary to make special collections in order to have sufficient numbers of mosquitoes in suitable condition for virus isolation tests.

Human Case Surveillance: In the event of an equine outbreak or detection of an index case in humans, active surveillance of human CNS infections should be instituted and an attempt made to secure specimens for laboratory diagnosis. Surveillance depends upon the coordinated participation of public health and medical personnel. Information about suspect cases is sought, usually by telephone and on a routine schedule, from a specifically designated representative at each hospital serving the affected area. Follow-up of suspect cases by public health and medical personnel will usually be required in order to obtain diagnostic specimens. Data on the geographic origin, date of onset, age, sex, clinical features, and laboratory test results are tabulated and used to prepare current summaries which describe the course of the outbreak and serve as a guide for other investigations, control efforts, and press releases.

Equine Case Surveillance: The methods used for monitoring WEE in equines during an emergency are essentially the same as those outlined in the section dealing with preventive measures.

Area-wide Adult Mosquito Control: When the presence of WEE virus in mosquitoes, horses or birds is determined and human cases have occurred, the existing infective adult mosquitoes must be killed as rapidly as possible to prevent more human cases. Once adult mosquitoes become infective with WEE virus they remain so for life with the possibility of transmitting infection each time they bite. Adult mosquitoes may live a month or longer.

Ultra-low volume (ULV) application of insecticides, with ground-based or aerial equipment has been used successfully for adult mosquito control in emergency situations.

Specific instructions on the methods of area-wide adult mosquito control and on the insecticides approved for use by these methods are presented in Appendix V. Generally, ground-based space-spray operations (ULV, fogging, and 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 particles closer to the ground, enhancing effectiveness. If the winds are excessively strong or the ground temperature is too high, the treatment is likely to be ineffective, since these conditions may cause rapid dispersion of the insecticide particles. Aerial ULV is usually applied during the early morning hours.

Where large areas are involved, aerial ULV application is most appropriate; adequate coverage of a large area by this method is effective in killing a high percentage of the adult mosquitoes and in preventing rapid reinfestation from surrounding areas. While a single efficient application may halt transmission of the virus, retreatment may be necessitated by a variety of factors, such as the size of the area covered, the percentage kill resulting from the prior treatment, and the seasonal timing of the application, i.e., repeat applications might be required when the initial spraying is done early in the season, while in late season a single application might suffice.

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 V). 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 VII.

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

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 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 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 VI). This can be augmented by community participation and reduction of peridomestic breeding by eliminating water-holding containers and standing

water which act as breeding sources.

One individual should coordinate the dissemination of information. Efforts should be made to reach the 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 emergency situations. 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 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: The use of pesticides for mosquito control requires a high level of care in their application to assure safety of the operator and the public and to avoid adverse environmental effects. Only pesticides approved by the Environmental Protection Agency for the intended use should be considered. When used according to label directions and local, state and federal regulations, these compounds are not hazardous to people. Experience to date indicates no adverse human health effects following ultra-low volume aerial applications in large area emergency mosquito 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.[15]

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. It is important to take precautions by notifying beekeepers of a planned application; they may protect their hives by moving them, closing them, or by turning on water 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.

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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.

APPENDICES

I - VII

APPENDICES

APPENDIX I: Criteria for Classification of Human Western Equine Encephalitis Infections

Clinical Categories

I. Encephalitis -- including meningoencephalitis, encephalomyelitis (both signs under A & B)

A. Acute febrile illness (temperature $\geq 100^{o}F$).

B. One or more signs under (1) or (2) or both.

1. Profound alteration in state or level of consciousness (confusion, disorientation, delirium, lethargy, stupor, coma, etc.).

2. Objective sign of CNS dysfunction (dysarthria, pathological reflexes, hyperreflexia, rigidity, cranial nerve palsy, convulsion, paralysis, tremor).

II. Aseptic Meningitis (all signs under A, B, and C)

A. Acute febrile illness (temperature $\geq 100^{o}F$).

B. Occurrence of either (1) or (2) or both.

1. One or more signs of meningeal irritation (stiff neck, positive Kernig or Brudzinski signs).

2. Pleocytosis (5 or more WBC/cc).

C. Absence of encephalitis and meningitis of bacterial or other nonviral etiology.

III. Other Illness

Febrile headache or other syndromes, but not encephalitis or aseptic meningitis.

IV. No Clinical Disease

No symptoms

V. No Clinical Data

Unable to obtain any clinical information

VI. Case Under Investigation

Aware of existence of case or possible case, but clinical data pending.

Laboratory Categories

I. Confirmed WEE

A. 4-fold or greater rise or fall in antibody titer by CF, HI, or serum-dilution neutralization test (1.3 logs by virus-dilution neutralization).

B. Isolation of virus from patient.

II. Presumptive WEE

A. Single serum titers - HI $\geq$ 1:80
CF $\geq$ 1:16
N $\geq$ 1:160

B. Stable high titers in paired sera.

C. Cases that are fatal 5 days or more after onset, with presence of detectable antibody and postmortem findings consistent with WEE infection.

III. Inconclusive

A. Highest titer HI $<$1:80 or CF $<$1:16 and not satisfying criteria for confirmed or negative case, or

B. Unsatisfactory serologic data.

IV. Negative

No titer or stable low titers (e.g., HI in range of 1:10-1:20) in appropriately paired sera and no virus isolation.

V. No Laboratory Data

APPENDIX II: Techniques and Equipment for Larval Mosquito Surveys

The larval survey is a rapid, reliable, and inexpensive method of estimating mosquito populations. The survey delineates breeding areas and establishes the relative abundance of vector species important in the planning, execution, and evaluation of control programs.

In areas of WEE activity, *Culex tarsalis* is the most important vector species sought during larval surveys, with *Aedes nigromaculis*, *Ae. dorsalis*, *Ae. melanimon*, and *Ae. vexans* of ancilliary importance.

C. tarsalis larval production sites can be rapidly determined by field reconnaissance. Knowing the affinity of the species for waste irrigation water, it is possible to readily locate suspected problem areas. Each site surveyed is assigned a number which is then entered on a map or an aerial photograph. An inspection of a specific site is made, and when production is found, that site is designated as a collecting station, and noted for subsequent inspections.

C. tarsalis larvae are commonly found in the marginal areas of habitats with a water depth of 4-6 inches. They are usually associated with grasses, other types of vegetation, and debris which serve as protection. Larvae tend to "bunch up" in groups within a given habitat rather than being uniformly distributed over the entire habitat. This necessitates sampling several parts of a likely-appearing site.

From the foregoing, it is apparent that the knowledge and judgement of the inspector are important for a complete and representative survey. It is also true that collecting technique influences the accuracy of the collected data. The inspector must be aware of the importance of not casting his shadow over a larval habitat, or some of the larvae will dive down and remain for a few moments, and thus be missed in the sample. The inspector also must not disturb the water before sampling as the larvae may react as they do to shadows.

Inspections should be made at frequent intervals during the breeding season because areas which are entirely free of larvae on one occasion may have many larvae at other times.

Equipment

A pint-sized white dipper, with the cup portion approximately 4 inches in diameter, is frequently used for making *C. tarsalis* larval collections. Such dippers, found in hardware and general merchandise stores, may be enamel or plastic. The handle portion can be extended by adding a length of wood dowel or cane to suit the convenience of the inspector.

When making extensive field collections, the larvae must be handled properly in order that the specimens remain in good condition for identification. The larvae may be stored temporarily in polystyrene coffee cups containing a few ounces of water from the collection habitat. The 4th instar larvae can be removed from the dipper by use of a wide-mouthed pipette ("eye dropper"). The larvae are later transferred to properly labeled vials containing either 70% alcohol or cellosolve as a preservative until they can be identified and counted.

Field notes pertaining to the collection are necessary for analyzing the results of the larval survey. A record sheet should include the following data: station number, date, number of dips made, the number of positive dips, the number of larvae collected, the source of the water, approximate size of the breeding area, and a brief description of the site. Later, the number of larvae per dip and a breeding index can be calculated in order to assess the relative importance of breeding sites.

APPENDIX III: Techniques and Equipment for Adult Mosquito Surveys

Adult mosquito surveys provide information on the relative abundance of species involved in WEE virus transmission. Light traps are particularly valuable as a surveillance tool because the main vector, *C. tarsalis* can be collected in large numbers by this method. Specimens from these collections can be tested for WEE virus. Adult surveys can also provide data on seasonal and spatial distribution of the vector (s).

Light Traps

Light traps are probably the best means of surveying WEE vectors. *Culex tarsalis* is readily attracted to light traps, enabling sampling from dusk to dawn. The light trap is suspended from a tree or post so that the light is approximately 6 feet above the ground. It should be located 30 feet or more from buildings, in open areas near trees and shrubs. It should not be placed near other lights, in areas subject to strong winds, or near industrial plants which emit smoke or fumes. Traps should be operated on a regular schedule from one to seven nights per week, from just before dark until just after daylight.

The New Jersey-type light trap is widely used in adult surveys because of its attraction to mosquitoes and its durability. This is a standard device used by mosquito control agencies in the United States and one which can be operated manually or used with an automatic timer or photo-electric cell to start and stop the motor and light. The collection may be funneled into a killing jar, thus making the collection acceptable for density studies but unacceptable for arboviral studies which require live specimens. A fine-mesh collecting bag can be substituted for the killing jar when living specimens are required. The collection should be gathered each morning and placed into a properly-labeled container until the mosquitoes can be sorted, identified, and counted, or the live catch processed immediately. The New Jersey-type trap also is dependent upon a 110-volt source of electric power which somewhat restricts its use.

The CDC miniature light trap was developed for greater portability and can be taken to remote areas which could not otherwise be sampled by a trap dependent upon electricity. It is commonly operated with four 1-1/2-volt "D" cell flashlight batteries, or one 6-volt motor cycle battery as the power source, either of which provides sufficient power for one night's trapping.[44] It weighs only 1-3/4 pounds and is easily disassembled for transport. The CDC trap is fitted with a large, collapsible, nylon collecting bag instead of a killing jar and, in this way, the catch is captured alive and kept in this manner until the specimens can be frozen. The trap has a large metal or plastic canopy which shields the operating mechanism from rain. The CDC light trap does not compete well with other light sources and smaller catches may result during a full moon.

Solidified carbon dioxide (dry ice) is frequently used as an attractant in conjunction with light trap operation.[35] It has been demonstrated that dry ice greatly improves *Culex tarsalis* trapping results. A small block of dry ice is placed in a padded shipping envelope or wrapped tightly in newspaper, and then suspended a few inches above the light trap.

Bait Traps

Dry ice has also been used as the bait in other types of traps. An economical, portable mosquito trap, made from a 12-inch lard can, has been developed, and is effective in capturing large numbers of *Culex tarsalis*.[3] Equipped with inwardly directed screen-wire funnels on each end, this trap utilizes about 3 pounds of dry ice (wrapped in newspaper) which is placed inside the can. This trap can also be baited with a live chicken or other animal.

Daytime Resting Places

Adult *C. tarsalis* are inactive during the day, resting quietly in dark, cool, humid places. An index of the population density can be obtained by carefully counting the number of adults found in a resting station. These sampling sites are also a source of specimens for arboviral tests. Mosquito resting stations are divided into two general types, natural and artificial.

Natural resting stations are usually present in houses, barns, stables, chicken houses, privies, culverts, and bridges. With experience one becomes capable of evaluating the suitability of shelters as adult mosquito resting stations. It is essential that collections be made in the same manner and at the same time of day for accurate comparison of results.

Artificial resting stations may be constructed when suitable natural resting stations are unavailable, and many types have been devised. They may be placed near suspected breeding places. Chicken-baited sentinel traps have also been used for collecting *C. tarsalis*.[36]

APPENDIX IV: Control of Mosquito Larvae

There are three basic approaches to the control of mosquito larvae:

(1) Source reduction -- the elimination of mosquito-producing sites by environmental modification;
(2) Biological control -- the introduction of predators or pathogens of mosquito larvae into the aquatic environment; and
(3) Chemical control -- the use of petroleum products, organic insecticides or the newer insect growth regulators as larvicides.

The first two approaches are highly desirable from the environmental point of view, but are often difficult to achieve over large areas or are exceedingly expensive. Thus, chemical control may still be the primary method available for the control of larval populations in areas where WEE may occur.

The major sources of *C. tarsalis* are irrigation waste water collections, playa lakes of western Texas, reservoirs, rice fields, and sewage stabilization ponds. Control of mosquito larvae in irrigation wastewater can be solved by eliminating the habitats. This can be achieved through better design of the delivery and drainage systems. Many main supply canals are earthen, without impermeable linings, and water percolates through the canal banks and collects in adjacent low areas, thus creating ideal breeding sites. This source can be eliminated by providing canal linings of concrete, polyethylene, or other impervious materials.

Collections of water on unleveled irrigated fields and pastures is a source of *C. tarsalis* production. Important variables are: condition of the field prior to irrigation, the rate and amount of water applied to the field, and proper drainage. Grading and leveling fields may eliminate much of this type of larval habitat, and also produce more arable land.

In the West, the indiscriminate flooding of great expanses of unleveled and unmodified pastures may allow water to collect over large areas and produce large numbers of mosquitoes. In such areas modifications of the terrain and proper water management can reduce larval habitats. Similarly, the excessive applications of irrigation water to crops which will tolerate such excesses often results in persistent water collection and mosquito production.

Poor drainage of irrigation wastewater is probably the most important factor affecting *C. tarsalis* production in the irrigated areas of the western United States. Irrigation runoff frequently does not drain efficiently, and excess water impounds in roadside ditches and borrow pits, which then become excellent larval habitats. Routine maintenance of drainage systems is often neglected and the drains themselves may become larval habitats. Improved drainage is needed on the majority of irrigation developments in order to lessen *C. tarsalis* production.

Extensive studies have shown the relationships of mosquito breeding to natural lake conditions and have demonstrated the restriction of breeding through shoreline modification and deepening of these lakes. Without such modification the applications of chemical larvicides may be required to maintain mosquito populations at low levels.

Impounded reservoirs may produce *C. tarsalis* when the water level is static during the summer months. Production may be found in shallow, vegetated ("feather edge") areas of the upper reaches of reservoirs. Production could be greatly reduced by deepening the water-land interface areas and by fluctuating the water level during the mosquito season by drawing it down by as little as 0.1 foot per week.[13]

Rice fields are flooded during the mosquito season and thus present unique problems in *C. tarsalis* larval control. *Gambusia* may be effective in such situations, but each field must be restocked with fish annually. Appropriate chemical control frequently is required to keep mosquito populations manageable in areas of rice cultivation.

Sewage stabilization ponds can produce *C. tarsalis*. Much of the production in such impoundments is found where vegetation becomes established and when the ponds are allowed to fill slowly. The process of slow-filling creates habitats a few inches deep in some areas of the ponds thus favoring breeding conditions.[2] Vegetation control and rapid filling tend to reduce larval production significantly.

Floodwaters which remain for extended periods in potholes and depressions may produce large populations of mosquitoes, including *C. tarsalis*. In 1975 such residual water collections following extensive flooding in Montana, North Dakota and Minnesota were responsible for excessive *C. tarsalis* breeding. In Montana, extensive and timely larviciding was employed to arrest a potential threat of WEE transmission. In North Dakota and Minnesota the floodwaters contributed to huge *C. tarsalis* populations and outbreaks of WEE in humans and equines necessitated the emergency use of aerial ULV spraying for vector control in a large area.

An integrated control strategy which includes all methods of reducing mosquito populations and exerts minimal harmful effects on the environment is the preferred approach to larval control. This includes environmental management and judicious application of insecticides, including growth regulators, plus the use of predator fish. This approach is especially necessary in areas where there is widespread resistance to insecticides, and it may retard development of resistance in areas where little or none exists.

If areas cannot be managed by source reduction at an acceptable cost, or if biological control is not feasible, larviciding is a reasonable alternative. A variety of dry compounds, emulsions, and solutions may be used. Insecticides that are currently registered for use as larvicides are listed in Table 1.

Table 1. Insecticides for use as mosquito larvicides

Insecticide	Rate of application (AI/A)	Remarks
Organophosphates		
chlorpyrifos (Dursban®)	0.0125-0.05 lb/acre	Mix 0.8-1.6 oz chlorpyrifos 2E with water, kerosene, or fuel oil to make 1 gal. Apply at 1 gal/acre. In heavy vegetation apply at 1.6-3.2 oz chlorpyrifos 2E per gal. Apply at 1 gal/acre.
	1.5 ppm	Apply Dursban 10CR pellets uniformly over the flooded area according to label instructions. Use of this slow release formulation at 1.5 ppm requires an estimate of the volume of water to be treated or, in the case of prehatch treatment, an estimate of anticipated water volume.
fenthion (Baytex®)	0.05 lb/acre	Apply in sufficient water, kerosene, or diesel oil to obtain uniform coverage. Allow at least 3 weeks between applications.
malathion	0.28 lb/acre	Mix 2.5 oz of malathion 57E with water to make 1 gal. Apply up to 5 gal/acre depending on flotage and vegetation.
temephos (Abate®)	0.05-0.1 lb/acre	Apply 5-10 lb of 1% Abate sand and celatom granular/acre. Apply 2.5-5 lb of 2% Abate sand and celatom granular/acre. Apply 1-2 lb of 5% Abate sand and celatom granular/acre.
temephos (Abate®)	0.1-0.5 lb/acre	In water with high organic or pollution content, apply up to 25 lb of 2% Abate sand and celatom granular, or up to 10 lb 5% Abate sand and celatom granular.

Continued--

Table 1. (Continued)

Insecticide	Rate of application (AI/A)	Remarks
Organophosphates (cont'd)		
temephos (Abate®)	0.016-0.048 lb/acre	Mix 0.5-1.5 oz of Abate 4E per gal of water. Apply at 1 gal/acre.
Chlorinated hydrocarbons		
methoxychlor	1 lb/acre	Apply up to 2 lb of 50% methoxychlor WP to dried-up breeding places, as a prehatch treatment.
Petroleum oils		
Diesel fuel oil No. 2 with spreading agent	1-5 gal/acre	Dosage depends on amount of flotage and vegetation in water. In catch basins cover water surface.
Proprietary mosquito control oils (as Flit MLO, ARCO larvicide, and GB-1313)	1-5 gal/acre	As above.
Insect Growth Regulators		
Altosid®	0.025-0.5 lb/acre	Mix 3-5 oz of 10% Altosid in 0.5 to 5 gal of water and apply to 1 acre. Apply to water with 2nd, 3rd and 4th instar larvae.

Em or EC = Emulsifiable concentrate
WP or WW = Water wettable powder
AI/A = Active insecticide per acre

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

APPENDIX V: Control of Adult Mosquitoes

Area-wide spraying of insecticides, i.e., "space spraying," provides an important means for reducing or eliminating adult mosquito populations during an emergency. Space sprays may be applied as thermal fogs, or ultra-low volume cold fogs, either with ground-based or aerial equipment. Control of adult mosquitoes by space spraying is only temporary, since mosquitoes from adjacent nonsprayed areas can move rapidly into the sprayed area following spray applications; 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 that effectiveness is reduced or eliminated. 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 many mosquito vectors, including *C. tarsalis*. The insecticides considered useful for such applications are listed in Tables 2 and 3.

Ultra-low Volume Application with Ground Equipment[30]

Ultra-low volume (ULV) treatment is defined as the application of less than 2 quarts of insecticide per acre; usually with ground ULV this is less than one 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 1/2-ton pickup truck.

Six insecticides have EPA label approval for application as ULV aerosols by ground-based equipment; these insecticides are listed in Table 2.

Performance requirements for the correct application of ULV insecticides using ground-based equipment include the following:

1. For most formulations, tank pressure should be not less than 2 pounds nor greater than 6 pounds per square inch (psi). For naled, a maximum of 1.5 psi is recommended.

2. Flow rate must be regulated by an accurate flow meter, and flow rates should be recorded for each day's operation to ensure continued satisfactory performance of equipment.

3. The aerosol nozzle for ULV dispersal of insecticides must have the minimum capability of producing droplets in the 5- to 27-micron range, with the average diameter not exceeding 17 microns. Droplet size should be determined by collection of an aerosol sample on a teflon- or silicone-coated glass slide and the measurement of the droplets under a microscope equipped with an ocular micrometer. Sprays containing droplets which are too large may permanently damage automobile paint or produce other undesirable side effects.

4. The ULV equipment should be mounted in the carrying vehicle so that the nozzle is pointed to the rear and upward at an angle of 45° or more.

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

For calibration and operation of the ULV equipment, directions of the manufacturers of the equipment and of the producers of the insecticide being used should be followed closely to assure proper application.

Ultra-low Volume Application with Aerial Equipment[15,28,33,34]

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°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 large 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 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 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 breakup. 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.

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 and in storage sheds or other buildings in close proximity to cases which have occurred and where 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 (Baytex 4 Emulsifiable Concentrate, 2 to 4 oz per gal water).

The methoxychlor spray is applied 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.

Table 2. Insecticides currently used for control of adult mosquitoes with ultra-low volume ground equipment

Insecticide	Formulation	Remarks
chlorpyrifos (Dursban®)	Dursban Dow Mosquito Fogging Concentrate	At vehicle speed of 10 mph, 0.67 to 1.33 fl oz/min.
fenthion (Baytex®)	Baytex Liquid Concentrate	At vehicle speed of 10 mph, 1 fl oz/min.
malathion	Cythion ULV Concentrate	At vehicle speed of 5 mph, 1-2 fl oz/min. At vehicle speed of 10 mph, 2-4.3 fl oz/min.
naled * (Dibrom®)	10% Dibrom 14 in HAN	At vehicle speed of 10 mph, 6-12 fl oz/min. At this rate persons may have serious irritation of eyes and respiratory tract.
pyrethrum	5% pyrethrins - 25% piperonyl butoxide	At vehicle speed of 5 mph, 2-2.25 fl oz/min. At vehicle speed of 10 mph, 4-4.5 fl oz/min.
resmethrin	SBP-1382® - 40 MF 12.5 fl oz with 1 gal light mineral oil	At vehicle speed of 5 mph, 3.0 fl oz/min.

Note: mph = miles per hour; fl oz = fluid ounce; max = maximum; HAN - heavy aromatic naphtha.

*With naled, tank pressure should not be greater than 1.5 lb psi because of overatomization and poor mosquito control.

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

Table 3. Insecticides currently used for adult mosquito control with ground foggers, misters, and dusters.

Insecticide	Rate of Application lb/acre (AI/A)*	Remarks
carbaryl (Sevin®)	0.2-1.0	Dosage based on swath width of 300 ft. Apply during period from dusk to dawn. Mists are usually dispersed at rates of 7 to 25 gallons per mile at a vehicle speed of 5 mph. Fogs are applied at a rate of 40 gal/hr dispersed from a vehicle moving at 5 mph; occasionally, 80 gal/hr and 10 mph. Finished formulations for thermal foggers contain from 0.5 to 8 oz/gal actual insecticide in oil. For nonthermal foggers or misters, water emulsions can be used. Dusts can also be applied with ground equipment.
chlorpyrifos (Dursban®)	0.005-0.01	
fenthion (Baytex®)	0.01-0.1	
malathion	0.075-0.2	
naled (Dibrom®)	0.02-0.1	
propoxur (Baygon®)	0.05-0.07	
pyrethrins (synergized)	0.002-0.0025	
resmethrin (SBP - 1382®)	0.007	

* AI/A = Active insecticide per acre.

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

APPENDIX VI: Personal Protection from Mosquitoes

People can protect themselves from mosquitoes by using proper window screens, protective clothing, or repellents. The principal vector of WEE, *C. tarsalis*, is active from dusk through the evening hours. Consequently, in an actual or potential epidemic situation people should be encouraged to avoid mosquito contact at that time of day. The ordinary window screen with 16x16 or 14x18 meshes to the inch will keep out most mosquitoes including vectors of WEE. Frequently, mosquitoes follow people into buildings or enter on the human host. For this reason, screen doors should open outward and have automatic closing devices. Residual insecticide applications on and around screen doors give added protection.

Long-sleeved clothing of tightly woven material offers considerable protection against mosquito bites. Sleeves and collars can be kept buttoned and trousers tucked in socks when mosquitoes are biting. This type of protection may be necessary for people who must work in areas where infected vector mosquitoes are particularly abundant. 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. A number of these have given adquate protection against mosquitoes. Effective protection may be obtained through the use of diethyl toluamide or deet, dimethyl phthalate, Rutgers 612, and 6-2-2. Repellents are available as liquids in bottles, pressurized spray cans, and in stick form. When applied to the neck, face, hands, and arms, liquid repellents will prevent mosquito bites for 2 hours or more, depending on the person, species of mosquito attacking, and abundance of mosquitoes. These repellents can also be sprayed on clothes to make them repellent. Many repellents are solvents of paints and varnishes, and will damage plastics and other synthetic materials (e.g. watch crystals, fountain pens, rayon fabrics, etc.). Care should be taken not to apply repellents to the eyes, lips, or 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 VII: 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,[16,17] 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 spray may be done by using the following technique:

Treatments may be applied by fogger, duster, mister, or ULV machine mounted on a vehicle and moving at 5 mph or 10 mph and using the recommended label dosage. Field-collected, caged specimens (100-150/cage) are hung 6 feet above the ground at stations 150-300 feet from the point of discharge of the machine along each of three streets (270-300 feet 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-hour female 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.

Bioassay tests for larvicides are of less value than sampling of natural larval habitats for larvae before and after an application is made. A useful technique to improve reproducibility of larval sampling is that of placing numbered stakes at various sites and then taking a prescribed number of dips at the site each time it is sampled. A 70% or greater reduction in the number of larvae per dip is expected.

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