METHODS OF MALARIA VECTOR CONTROL

A STATE OF THE ART LITERATURE REVIEW

Office of International Health
U.S. Department of Health, Education and Welfare

Preface

In May of 1978, AID's Asia Bureau requested that the Office of International Health, DHEW, prepare a Shelf Study of Research on Comprehensive Malaria Control Methods. Given the increasingly adverse impact of malaria around the world, the immediate objective of the study was "to assist AID in making decisions on the choice of methodology for comprehensive malaria control programs."

During the period May - September, 1978, OIH carried out an extensive literature search as well as consultations with numerous experts and institutions involved in malaria research and programs. Several staff members of the World Health Organization, DHEWs Center for Disease Control and AID were especially helpful in this process.

In October 1978 draft copies of the completed study, under the title Malaria Vector Control: A Review, were provided to AID for distribution and comment. The present document represents the final revised version of this study.

TABLE OF CONTENTS

1. INTRODUCTION

1.1. Objectives

The malaria control and eradication programs set up since 1945 have used as their chief attack method the application of measures aimed at the anopheline mosquito which carries the disease. Efforts at vector control have been aimed at the reduction or interruption of transmission by application of residual insecticides, especially DDT, to inside walls and ceiling of houses. The appearance of resistance to DDT and to other, more costly and toxic insecticides has forced a reappraisal of these traditional methods. This has not meant the abandonment of residual spraying as a vector control method, but the recognition that spraying is only one of many measures to be used against malaria vectors. What is needed now is the knowledge to permit national and international authorities to make wise choices of those anti-vector technologies which are appropriate to the objectives of control and eradication projects and consistent with epidemiological, environmental and economic concerns.

This report will attempt to summarize new and old information on all forms of malaria vector control, both those in current use, those under development, and those which were largely displaced with the postwar advent of DDT house spraying. Each technology is reviewed from the standpoint of efficacy, feasibility under field conditions, environmental acceptability, and costs. A list of avenues for possible research is included for the benefit of prospective researchers and sources of research funds.

1.2 Scope

This report will begin with a concise summary of what is known about residual house spraying with the insecticides currently in use or under trial. A detailed description will then be made of the non-spraying measures in past and present use, as well as those currently under development. Possible research topics in vector control methods will be listed, and an effort will be made to outline the requirements for planning, organizing, and evaluating vector control campaigns. Such specialized areas as disinfection of aircraft will not be discussed here; they have formed the subject of reports elsewhere (WHO, 1961b; WHO, 1966; Jensen, 1969). No attention will be given to purely personal protection measures, such as mosquito repellents and bed nets. Measures aimed at the parasite, not the mosquito, will be excluded from this discussion except where they have been used in combination with vector control methods in mass campaigns. Vector resistance and tolerance will be discussed only in the context of their programmatic implications.

This review is restricted to technological control methods. The important area of human ecology, including public acceptance, public education, and movements of human population, lies outside its scope.

2. METHODS IN PAST OR CURRENT USE

2.1 Spraying and other chemical applications against adult mosquitoes

The use of chemicals applied against adult anopheline mosquitoes has been the mainstay of malaria vector control programs since the end of World War II. There are two types of spraying against anopheline adults. Residual spraying is done against house walls and ceilings and kills vectors which rest there before or after taking human blood meals for a period of months; space spraying is done mostly outside houses, kills vectors primarily through physical contact and has no long-term effect. Since most malaria programs rely exclusively on residual spraying, this method will be discussed first.

2.1.1 Residual House Spraying

Spraying of residual insecticides in houses has been the most common method of malaria vector control since shortly after the 1939 discovery that DDT (dichloro-diphenyl-trichloroethane) has a long-term insecticidal effect. Residual house spraying is effective only against those vectors which rest on inside walls or ceilings before or after their blood meals on humans. This tendency to rest inside is called endophily. Exophilic vectors, which rest outside houses, are not affected by house spraying. Three groups of insecticides are in widespread use for house spraying: the chlorinated hydrocarbons, the organophosphates, and the carbamates. These will be discussed in order.

2.1.1.1 Use of Chlorinated Hydrocarbons

There are three chlorinated hydrocarbons which have been commonly used in anti-malaria work: DDT, dieldrin, and HCH. Of these, the one most commonly used is DDT; it has been estimated that DDT accounts for 90 percent of insecticides used against malaria (Brown, 1972).

DDT exerts its lethal effect by acting as a nerve poison on the adult anophelines which rest on sprayed surfaces. The efficacy of DDT has been demonstrated in the large number of countries which have controlled or eradicated malaria using DDT spraying as the chief attack measure. Areas with a population of nearly 800 million people have been cleared of malaria, largely through DDT spraying (Brown, Haworth, and Zahar, 1976).

The feasibility of DDT spraying is well established. DDT house spraying is done with easily transportable water dispersible powder used in aqueous suspensions. The spraying equipment has become standardized through the issuance of CDC and WHO specifications (WHO, 1974c). The methods necessary for safe, effective spraying can be taught to uneducated personnel in a day.

DDT is the least expensive of the residual insecticides. Its long residual effect, typically six to twelve months, means lower labor and transport costs than for insecticides with shorter residual effect (WHO, 1963). From the standpoint of economy as well as effectiveness, it is clearly the insecticide of choice in those areas where the vector is endophilic and susceptible.

The controversy surrounding DDT has had to do with its environmental acceptability. It is not within the scope of this review to discuss the use of DDT outside the context of malaria vector control, or to examine in detail the very large body of knowledge extant on the toxicity of DDT to humans and to other animals. There is no disputing the toxicity of DDT: the relevant question is whether that toxicity justifies precluding the use of DDT in malaria programs. Findings on the alleged impacts of DDT on humans and animals will be briefly summarized here.

In the case of man, any acute toxic effects of DDT should be evident in the formulators and spray crews who are exposed to it continuously. DDT has been widely used without serious intoxication among spray personnel or the community at large, and there are no reports of acute human intoxication except in circumstances not encountered in malaria campaigns, such as suicidal ingestion (Pampana, 1969; Hayes, 1971).

The absence of reports of acute human intoxication during regular malaria spraying does not mean that DDT is safe to humans over the long run. Longitudinal studies on possible long-term effects on man are rare. Those most relevant to malaria spraying operations are controlled studies of American DDT formulators by Laws and colleagues and of Brazilian malaria spraymen by the Biological Institute of São Páolo (Laws, Curly and Biros, 1967; WHO, 1973d). In the American study, thirty-five DDT formulators with 11 to 19 years' exposure were evaluated on the basis of medical history, physical examinations, and chest X-rays. No ill effects attributable to DDT were found, despite accumulation of DDT in fat and blood (Laws, Curly and Biros, 1967). The Brazilian survey compared sickness among 77 spraymen, 78 men residing in sprayed houses, and a control group of 406 men. Three years of semiannual sickness surveys revealed no differences in sickness levels among the three groups (WHO, 1973d).

Effects of DDT house spraying on nontarget animals are primarily confined to the home, and the quantities of DDT escaping into the open are on the order of one thousandth of the dosages applied in agriculture (NAS, 1976). Leakage of spray to the outside and the floor will vary with house construction and whether eaves are sprayed, but is very small in proportion to the total sprayed. Within the house, nontarget victims of DDT include mice, rats, bedbugs, fleas, and domestic cats, the last because they lick their fur. There is anecdotal evidence that chickens are sometimes affected (Brown, 1972; Newsom, 1967). Toxic effects on pets and domestic animals may be minimized by good spraying practice, such as keeping pets out of doors during spraying and avoiding drips and spills from sprayguns and buckets. Protection of foodstuffs is essential to protection of the human population.

The absence of ecological consequences to humans and animals from supervised DDT spraying has led several national and international bodies to go on record in favor of its continued use.

The WHO Expert Committee on Insecticides has stated:

> In vector control programmes DDT has been in use for over 20 years. Very large quantities continue to be used and no reports of any harmful effects have been recorded among the thousands of people who use it daily in malaria eradication campaigns. Furthermore, DDT remains the insecticide of choice where the vector is susceptible to its action.
>
> The concern that has been expressed in recent years about contamination of the environment by this very stable and persistent insecticide should not, in the opinion of the Committee, be considered sufficient reason for substituting other insecticides for indoor residual spraying against mosquitos. The safety record of DDT remains outstanding. (WHO, 1967)

After reviewing the relevant evidence, the Executive Board of the WHO concluded in 1971 that:

> Indoor spraying of DDT in routine antimalaria operations does not involve a significant risk to man or to wildlife. The withdrawal of DDT from malaria programmes would be fraught with great danger and is unjustifiable in the light of present knowledge. (World Health Organization Executive Board, 1971).

The National Academy of Sciences, the U.S. Environmental Protection Agency, and the Agency for International Development have also taken positions in favor of the supervised use of DDT in public health programs (USAID, 1977).

Biodegradable analogues to DDT have been developed, but their insecticidal effect is not long enough to justify use as residual insecticides (WHO, 1976).

The issue of supposed carcinogenicity has been raised in respect to DDT. In 1972 the Expert Committee on Insecticides reviewed new evidence on association of tumors in mice with prolonged exposure to DDT. It concluded that the new evidence did not provide an adequate basis for recommending the withdrawal of DDT where its continued use for disease control and for protecting food and crops could be life-saving (WHO, 1973d).

The issue of vector non-response to DDT is a concern in some programs. Where DDT spraying has failed to interrupt transmission, the authorities should consider whether the continuation of spraying is suitable in terms of program aims. Especially in Africa, where budgetary constraints are very severe, fiscal considerations may rule out the use of insecticides more expensive than DDT. There, as elsewhere, the retention of DDT in the face of a refractory vector is not inconsistent with the aims of a malaria control program.

Dieldrin is, like DDT, a chlorinated hydrocarbon with long residual effect. It is as effective as DDT in interrupting transmission by vectors which remain susceptible to it. The gene for dieldrin resistance is dominant or partially dominant, while the gene for DDT resistance is often recessive. This explains why dieldrin resistance has been more rapid in its appearance than DDT resistance. Dieldrin is more expensive than DDT but is usually sprayed at only 0.6 grams per square meter. Unlike DDT, it can be easily absorbed through the skin and has caused death in spraymen. Moreover, dieldrin can cause death of domestic animals and poultry when these are exposed to it through improper procedures, such as poor mixing technique or leaking of dieldrin from defective sprayguns (Sandosham, 1965).

Professional opinion is divided on the suitability of dieldrin for malaria vector control. Its long residual effect makes it suitable for very remote areas where spray crews may find it difficult to get in for the twice-yearly spray rounds required for insecticides with shorter residual effect. But the stringent precautions necessary for its use are hard to maintain in a mass campaign. After reviewing the experiences of national malaria programs with dieldrin, the WHO Expert Committee on Insecticides concluded:

> Because of the hazard to man and domestic animals, the use of this insecticide has been abandoned by most countries. It has been found that in long-term programmes poisoning has occurred among spray operators, even when veils, caps and gloves were worn in addition to hats and overalls. Dieldrin should not be used for indoor spraying without full justification and unless strict precautionary measures and medical supervision are ensured. (WHO, 1970).

HCH (hexachlorocyclohexane) is often grouped together with dieldrin because a vector population resistant to one is very often resistant to the other. HCH spraying is midway in cost between that for DDT and dieldrin. HCH has an airborne (fumigant) as well as a contact effect and is useful on surfaces where sorption (soaking in of the insecticide) takes place (Pampana, 1969).

While HCH is somewhat more toxic than DDT, it has been as incident-free in field use as DDT. Clinical studies on persons with occupational exposure to lindane, the form of HCH most commonly used, have shown generally negative results, except in a few subjects with unusually high blood levels of lindane. The isomer of HCH used in lindane is disposed of rapidly by animals and does not persist long in the environment (WHO, 1974).

HCH fell into disfavor in the early '60s because of the demonstrated cross-resistance between dieldrin and HCH. This, as well as its relatively short residual effect of three months, make it in some respects an unattractive choice. However, HCH has been used successfully in parts of Africa where the gene for dieldrin resistance has not been recorded in the vector. Moreover, it may have potential for savannah areas of West Africa where DDT alone has failed to interrupt transmission. There may be potential for the use of malathion in combination with other insecticides where the danger of dieldrin resistance exists (WHO, 1963).

Of these three chlorinated hydrocarbons -- DDT, dieldrin, and HCH -- DDT is most commonly used, due to its low cost, low acute toxicity, and wide availability. HCH is suitable where a short transmission season justifies the use of an insecticide with short residual effect. Dieldrin is useful, despite its toxicity, in inaccessible areas where operational difficulties make it desirable to spray infrequently with a long-lasting insecticide. Dieldrin does, however, pose the problem of resistance and of cross-resistance with HCH.

Macdonald has made an argument for the choice of DDT over dieldrin which has to do with the development of anopheline resistance to the insecticides:

> Dieldrin resistance is usually incompletely or completely dominant in anophelines. . .DDT resistance, on the other hand. . .does not reach such high levels, and owing to the higher kill with field dosages, the response rate is apparently slow. (PAHO, 1972).

2.1.1.2 Use of Organophosphates

As discussed below, the organophosphates are a more expensive family of insecticides which are used in programs when vector resistance is encountered in the chlorinated hydrocarbons. The only organophosphate approved by the WHO Expert Committee on Insecticides is malathion; however, fenitrothion has shown great promise in advanced field trials and is already being commercially produced.

The efficacy of malathion is firmly established. Residual spraying of malathion at 2 grams per square meter at four-month intervals caused almost total interruption of malaria transmission by *A. gambiae* and *A. funestus* in southern Uganda. No toxic effect on humans was noted. Residual effect was shorter in houses roofed with corrugated iron than in those roofed with thatch. While entomological results were uniformly gratifying, there were some cases at the end of the two year trial, largely imports (Najera, Shidrawi, Gibson, and Stafford, 1967).

Early reports on malathion confirmed its usefulness in malaria control when sprayed on impervious materials like thatch and bamboo, but questioned its usefulness when sprayed on mud walls, which sorb (soak up) the insecticide (WHO, 1962). Subsequent experience from Pakistan suggests the usefulness of malathion on mud walls.

A number of national malaria programs, such as those of Sudan, Pakistan, and Iran have used malathion, and in Pakistan field trials a decrease in recorded incidence of 80 percent has been achieved in one year (De Zulueta, 1977).

Malathion, like all the organophosphates, produces symptoms associated with cholinesterase depression. These are a matter of particular concern in respect to the spraymen, who are exposed to them daily. Their clinical signs and symptoms and suitable treatment have been described (Namba, 1971). Recent tragic experiences from Pakistan demonstrate the need for greater precautions than have previously been in force (Baker *et al.*, 1978). Malathion has been recommended by WHO for use in areas of vector resistance to DDT. The WHO Expert Committee on Vector Biology and Control has pointed to the proliferation of malathion manufacturers in the wake of malathion patent expiration, and has set down standards for quality control, especially in respect to limits on the quantity of isomalathion in water dispersible powders (WHO, 1978).

Fenitrothion has been shown to be successful for malaria control in East Africa, where *A. gambiae* and *A. funestus* are the main vectors. Several other organophosphate compounds have shown promise in advanced field trials (Pant, Fontaine and Gratz, 1977).

Fenitrothion used against DDT-resistant *A. albimanus* achieved good results in El Salvador when applied at 2 grams per square meter, but the residual effect was only four weeks on wood, thatch, pole, and tile surfaces (Austin, 1972b).

Use of fenitrothion at 2 grams per square meter achieved gratifying results against *A. gambiae* and *A. funestus* when sprayed at three month intervals over two years in Kenya. While transmission was not interrupted, the overall parasite rate declined from 60 to 20 percent in the test area (Payne *et al.*, 1976).

Fenitrothion showed promise in field trials in northern Nigeria, both from the entomological and the epidemiological standpoint. Cholinesterase depression in spraymen was found with this compound, as with other organophosphates. The WHO Expert Committee concluded that fenitrothion is an effective insecticide that can be used safely (WHO, 1967).

Field trials in Kenya using fenitrothion at two grams per square meter showed slight to moderate depression of cholinesterase levels in spraymen, in one case accompanied by symptoms. Poisoning of chickens was also reported, the result of drinking washings from spraycans. Fenitrothion was considered safe by the Expert Committee when used with precautions, including weekly tests of cholinesterase levels in spraymen towards the end of the spraying season (WHO, 1973d).

Excellent control of *A. gambiae* and *A. funestus* was achieved in Kenya with 4 rounds of fenitrothion house spraying at 2 grams per square meter in 1969-1970. Residual effect of the insecticide was observed for as long as 291 days on mud walls. Fumigant effect was observed for as long as 101 days (Anon., 1972).

In southern Iran, where *A. stephensi* is the vector, field trials showed entomological results for fenitrothion which were superior to those for malathion. Fenitrothion had a residual effect of two months. The authors cautioned care in respect to the toxic effect of fenitrothion on work crews (Eshghy *et al.*, 1975). Field trials of fenitrothion have shown good control of *A. aconitus* in Central Java. Residual effect was for about three months (Joshi, Self, Shaw and Supalin, 1977).

Kenyan trials of fenitrothion applied at two grams per square meter over three month intervals showed dramatic reductions in malaria incidence and *A. gambiae* density after eight rounds of spraying. Costs at 1975 levels were US$0.81 *per caput* per cycle. The investigators concluded that under the prevailing epidemiological conditions, eradication would require both residual spraying and such complementary measures as mass drug administration. (Fontaine, Pull, Payne, Pradhan, Joshi, Pearson, Thymakis, and Ramos Camacho, 1978).

Fenitrothion was shown in Indian trials to have a residual effectiveness of five to eight weeks, depending on the dosage sprayed and the sorptive capacity of the sprayable surface. Shelf life did not exceed four months, which could prove to be an operational drawback in programs where insecticide requirements vary unpredictably from year to year (Bhatnagar *et. al.*, 1974).

The degree of acute intoxication by fenitrothion justifies more stringent precautions than are necessary with the chlorinated hydrocarbons and, perhaps, malathion. According to Vandekar:

> Some organophosphorus compounds, like fenitrothion, have a margin of safety which is not very large. When used operationally, weekly cholinesterase monitoring in operators is required whenever a spraying operation lasts for more than one month; in the case of carbamates, however, experience in the field trials has shown that such monitoring is of little value in determining when an operator should be withdrawn from further exposure. (Vandekar, 1975).

In addition to malathion and fenitrothion, which are currently in field use, such other organophosphates as dichlorvos (OMS-14), dicapthon (OMS-214), and chlorphoxim (OMS-1197) have shown promise in advanced field trials. However, recommendations on these last three compounds must await further epidemiological evaluation (Pal, 1978). The only reported slow-release adulticide is the organophosphate dichlorvos, which gave 12 to 13 weeks' protection against anophelines and culicines when dispensed in a wax formulation (Gratz, Bracha and Carmichael, 1963).

2.1.1.3 Use of Carbamates

Where vector resistance has been encountered in both chlorinated hydrocarbons and organophosphates, programs with sufficient resources may wish to consider propoxur, the only carbamate insecticide currently in widespread use as an anopheline adulticide. Propoxur has been found effective against *A. stephensi* in Iran, against *A. gambiae* and *A. funestus* in Nigeria for three to four months, *A. albimanus* in El Salvador for two to four months, and *A. dthali* in Iran for two and a half months. It possesses a fumigant effect and is stable under field storage conditions. Simple safety precautions are sufficient for spraymen and occupants of sprayed houses. Propoxur has an oral LD_{50} of 109 and 82 milligrams per kilograms of body weight for male and female mice, respectively. (The LD_{50} is a measure of the dosage required to kill 50 percent of laboratory animals.) Skin reactions in humans were reported when spilling occurred. Other symptoms included headache, nausea and vomiting; all subsided after brief rests away from the sprayed areas. Propoxur requires stricter safeguards than DDT (Wright *et. al.*, 1969). The airborne (fumigant) effect of propoxur persists for two months in sprayed huts and one month in their immediate vicinity (Pant and Joshi, 1968). In Iranian field trials, propoxur was found effective against *A. stephensi* for three to four months when applied at 2 grams per square meter. A fumigant lethal effect was detected which was important for peridomiciliary transmission (Carmichael *et. al.*, 1968).

The resistance of *A. albimanus* to chlorinated hydrocarbons and organophosphates has caused several Central American malaria programs to turn to propoxur. But the cost of using propoxur for control of *A. albimanus* is US$11 to $12 per house per year; the comparable cost for the less effective DDT is $1 (NAS, 1976). Moreover, propoxur has produced resistance in some areas.

One highly original effort to overcome the constraints of cost and product volatility has been the partial inside spraying of propoxur at 60 grams per house at 35 day intervals. This effected a 40 percent savings over quarterly spraying in El Salvador, with prevalence in test areas only one-tenth of that in control areas. Sporadic transmission continued at a low level (Lassen, Liu, Lizarzaburu, and Rios, 1972).

Propoxur has produced acute toxic symptoms when taken orally under experimental conditions (WHO, 1974). No effect levels have been established for animals; the acceptable daily intake for man has been placed at 0.02 mg/kg of body weight. Symptoms of cholinesterase depression occurred in Iranian field trials with propoxur, but the compound was judged safe for malaria programs when proper precautions were taken (Vandekar, Hedayat, Pleština and Ahmody, 1968).

Other carbamates have been proposed for use in malaria programs. Carbaryl was tested and found unsatisfactory in reducing the density of DDT-resistant *A. albimanus* in El Salvador when sprayed at two grams per square meter at four-month intervals (Austin, 1972a).

Two carbamates showing promise in field trials are Landrin (OMS-597), (Hobbs and Miller, 1973), and Mobam (OMS-708) (Pal, 1978). Evidence from animal tests suggests that occupational exposure to carbamates will produce incapacitating symptoms at doses well below lethal levels (Vandekar *et al.*, 1965).

2.1.1.4 Use of Sprayed Mixtures

The trial use of mixtures of insecticides has been suggested as a course of action to be taken when resistance to a single insecticide appears. This controversial alternative has not been the object of much field evaluation (Schoof, 1970). Trials of DDT/HCH suspensions against *A. culicifacies* and other mosquitoes in the Indian Punjab showed a definite synergistic effect, both in terms of extended residual effectiveness and reduction of vector density. Dosages were 270 milligrams per square meter of DDT and 54 milligrams per square meter of HCH (Singh, Pal and Sharma, 1951).

Other north Indian trials of DDT/HCH combinations against *A. culicifacies* found the two insecticides to be more effective under some conditions than either one sprayed separately (Pal, Sharma, Krishnamurthy, and Gabba, 1955). Similar trials from Bombay State showed no similar advantage from combined dosages (Viswanathan, Bhatia, and Halgeri, 1955).

In areas of south Gujarat state in India where *A. culicifacies* showed resistance first to DDT, then HCH, subsequent efforts to reduce the vector population by doubling the dosage of DDT and by spraying a combination of DDT and HCH failed to effect any significant change in vector density (Mojamdar, 1975).

African trials of sprayed mixtures have shown similarly mixed results. In comparative trials in Uganda, Supona - DB, a mixed suspension containing 39 percent DDT and 13 percent HCH, achieved greater depression of mosquito density over 29 weeks than 50 percent DDT suspensions and a 30 percent DDT emulsion (van Tiel, 1952). In east African trials, a mixture of HCH and DDT in oil-bound suspension gave the high initial kill characteristic of HCH and the longer residual effect of DDT (Davidson, 1953). However, disappointing results were reported with DDT/HCH mixtures against *A. gambiae* and *A. funestus* in East Africa (Burnett, 1957). Malathion/HCH combinations and propoxur/fenitrothion combinations produced disappointing results against *A. gambiae* in Tanzania and Kenya (Hudson, 1975).

2.1.1.5 Vector Resistance, Spraying Policy, and Changing Control Policies

In 1970, four compounds were recommended by the WHO Expert Committee on Insecticides for interior house spraying in malaria eradication programs: DDT, dieldrin, HCH, and malathion (WHO, 1970). In 1973 the Committee extended its support to propoxur and fenitrothion as alternatives to DDT when necessary (WHO, 1973d).

While fenthion showed promise from the entomological standpoint, it caused acute toxic reactions in work crews, and the precautions necessary to avoid toxic reactions have been deemed too stringent for implementation in a mass campaign (Arnan, 1971).

DDT and dieldrin are thought to have a residual effectiveness of six to twelve months, thus requiring only one or two spraying rounds per year. HCH and malathion are thought to be effective for only three months, requiring four rounds per year in areas with perennial transmission.

In setting up malaria control strategies, the pattern has been to start with DDT spraying at two grams per square meter on a twice yearly basis. As early as 1961, the WHO Expert Committee on Malaria recognized that factors influencing dosages and cycles vary markedly from country to country. It was remarkable, therefore, that in the case of DDT 72 out of 82 malaria programs were at that time using the dosage of two grams per square meter (WHO, 1961a). The variety of epidemiological and entomological situations, as well as the difference in surfaces being sprayed justifies departures from standard dosages and cycles when the efficacy of such actions has been demonstrated. Recognition that dosages and frequencies should be tailor-made to suit local conditions has come from the WHO Expert Committee on Vector Biology and Control:

> Fixed schedules and dosages for treatments, although useful operational guides in the past, should now be replaced whenever possible by flexible programmes of chemical treatment aimed at specific targets for disease control and prevention (WHO, 1977).

Since the duration of insecticidal effect varies widely with local humidity and the nature of the sprayable surface, local field tests are required to determine the effective duration of residual effect. Despite their limitations, field bioassays conducted at regular intervals after spraying are considered appropriate for determining effective residual duration where the insecticide is known to be effective (Pampana, 1969).

The dual objectives of insecticide field trials should now be a determination both of the effect of the insecticide on malaria reduction and its effect on resistance development. Optimum dosages and frequencies can be determined by experimentation.

The need for more careful strategic planning, even in areas where the vector is fully susceptible to the insecticide, has come with the emergence of vector resistance. A vector's resistance can be either behavioral (for example, a change in its indoor resting habit after insecticide application) or physiological (a genetic character for survival of lethal dosages).

There are three kinds of mechanisms which influence the development of vector resistance, namely, the genetic, the biological, and the operational. Malaria programs cannot influence the former two. According to Pal, malaria programs can expect to encourage the rapid development of resistance when:

- a residual insecticide is applied which is closely related to an earlier used chemical;

- the compound has prolonged environmental persistence where mosquitos breed or rest;

- applications are made to a low density vector population;

- a high percentage of the vector population is exposed to the insecticidal treatment;

- genetic selection is directed against larvae or against both larvae and adults;

- good coverage is achieved;

- a geographically large area is treated and selection is applied against every generation of the vector population;

- the insecticide deposit is applied at, or deteriorates to, a level at which heterozygotes for the resistance factor will survive.

Conversely, the insecticides will be slow to develop resistance when:

- the vector population is diluted by immigration;

- density of the vector population is drastically suppressed by severe selection; or

- susceptible individuals have a reproductive advantage over their resistant counterparts. (Pal, 1978).

Davidson set out the relevant considerations in planning for avoiding development of resistance in 1958:

> The higher the selection pressure, the quicker any resistance present in the population will appear. Thus, a more efficient insecticide or a combined attack on both larvae and adults could all increase the speed of selection. Conversely, a less efficient insecticide, or a lower dosage,

or a partial attack only on the insect population would delay the appearance of resistance, though, of course, such incomplete measures may not achieve the purpose of disease control (Davidson, 1958).

The practical lessons for malaria programs are clear: the appearance of insecticide resistance will be slowed by a) reduction of agricultural applications of the same family of insecticides being used for house spraying; b) larviciding with substances not used in adulticiding; c) application of adulticides at the lowest dosages and frequencies consistent with program objectives of reducing malaria incidence; and d) avoiding substitution of one kindred adulticide for another, such as HCH for dieldrin. Where the appearance of resistance or of increased tolerance is feared, a reduction in dosage, e.g., from two to one gram per square meter, is said to be advisable to reduce selection pressure (Schoof, 1970).

The development of resistance has been shown to be related to the use of agricultural pesticides. Ways of reducing resistance through coordination between health and agricultural authorities have not yet been elaborated (WHO, 1976).

One practical implication of agricultural applications of insecticides is the need for malaria programs to assess vector susceptibility to alternative insecticides before deploying them into the field (Schoof and Taylor, 1972).

An original approach to agricultural spraying has been suggested for El Salvador:

> . . . during the season of agricultural insecticide spraying there is enough control of vector populations to reduce malaria cases to low levels. Thus, control measures would be needed principally during the wet season. (Mason and Hobbs, 1977).

In practice, resistance is likely to appear after a number of years of house spraying, whatever the precautions taken against its emergence. Malaria control programs, whose task is to reduce malaria to a prevalence where it is no longer a major public health problem, may wish to live with resistance in the case of DDT, to which resistance appears slowly and partially. Like eradication programs, which seek to interrupt malaria transmission, they may wish to change insecticides, adopt supplementary measures, or both. The choice of strategy depends on the objective -- control or eradication -- and the financial resources of the program.

Schoof has pointed out that

> At the present time the principal remedy for resistance is the use of alternate insecticides. Compounds that are effective against dieldrin- and DDT-resistant mosquito populations include malathion, propoxur, and fenitrothion, each of which is far more costly and has a shorter period of residual activity than either DDT or dieldrin. (Schoof, 1970).

The WHO Expert Committee on Malaria has stated that:

> Malathion sprayed at the rate of 2 g/m^2 four times a year can cost about 5 times as much as DDT applied twice a year at the same dosage, while for propoxur (applied at the same rate as malathion) the cost may be 20 times greater than that for DDT. (WHO, 1974b).

A variety of considerations may require implementation of strategies other than insecticide substitution. Where resistance has been encountered, Zahar and Davidson advocate raising the dosage and frequency of spraying where indicated (1973). When DDT proved ineffective against *A. pulcherrimus* and *A. hyrcanus* breeding in rice fields in Afghanistan, spraying was discontinued in favor of drainage, filling, water management, use of larvivorous fish, and larviciding of rice nurseries (Dukhanina and Quadeer, 1974).

The appearance of resistance to the insecticide in use requires a needs assessment which takes into account the objectives of the vector control program, the alternatives open to the program, of which insecticide substitution is only one, and the choice of the measure or measures which are most suited to the local entomological, epidemiological, ecological and financial situation.

2.1.2 Space Spraying

Space spraying is the application of contact insecticides to vectors in space. It is effective in bringing about a short-term reduction in vector density but lacks the long-term residual effect of residual house spraying.

If space spraying is to be done on a large scale, in conjunction with house spraying, the space and residual insecticides should be from different groups, so as not to speed the development of resistance.

2.1.2.1 Pyrethrum House Spraying

The earliest form of space spraying was pyrethrum spraying. Pyrethrum is the common name for an extract of the dried flowers of *Chrysanthemum cinerariaefolium*; the active ingredients of pyrethrum are called pyrethrins. Native to Persia and Dalmatia, pyrethrum is now grown chiefly in Kenya, with some production in other parts of Africa, in Asia, and in South America. Pyrethrum was in use as a space insecticide before World War II, but was displaced when the residual effect of DDT was discovered. It is still used in malaria work for quick knockdown of mosquitoes in entomology work, and is an ingredient in "mosquito coils." It is noted for its lack of toxicity to men and animals (Nelson, 1975).

Where pyrethrin injury has occurred in man, it has come most frequently in the form of allergy, rather than as other forms of toxicity (WHO, 1973a).

New synthetic pyrethroids are superior in stability to the natural pyrethrins (Nishizawa, 1971). They have been found to be highly toxic to *A. stephensi* and to have some residual effect when the sprayed surface is kept in darkness. Exposure to normal sunlight causes these substances to lose their insecticidal effect (Hadaway *et. al.*, Turner and Flower, 1970).

Reviewing the evidence from a number of field trials, Busvine concluded that reduction of malaria transmission with pyrethrins could be effected only with frequent application, preferably twice a week (Busvine, 1960). Pampana has expressed the view that interruption of transmission with pyrethrum might require not only weekly house spraying, but also vector endophily, at least during spraying. He called for *ad hoc* experiments with this insecticide in 1969. Concerns about environmental safety and vector resistance weigh more heavily in choice of insecticide now than then, and pyrethrum is ecologically safe and not known to produce malaria vector resistance (Pampana, 1969). The WHO Expert Committee on Insecticides concluded in 1960 that, while pyrethrum's limited residual effect posed operational problems in vector control programs, it possessed value in situations where immediate but transitory control are important, such as larviciding in certain areas (WHO, 1960). Meeting in 1968, before the development of widespread propoxur resistance, the Committee found that interior space spraying with pyrethrum and other substances "is useful as a temporary means of personal protection but has no practical value in a vector control programme (WHO, 1970)." Very recent developments of synthetic pyrethroids stable to light and air hold out promise for their practical use in space applications. Considerations of cost make it likely that these stable formulations would be most suitable at low dosages (WHO, 1978). The future of pyrethorids has been recently reviewed by Elliott and colleagues (1978).

A form of space spraying which has already come into widespread use in American vector control is ultra-low volume application of natural and synthetic insecticides.

2.1.2.2 Ultra-low Volume and Other Exterior Space Applications With Non-Botanical Substances

ULV spraying is the dispersal of fine liquid particles of pesticide concentrates containing 90 percent or more of active ingredients at application rates of 1 liter or less per hectare (WHO, 1973d). ULV application can be done from aircraft, vehicle-mounted ground units, or portable units carried by spraymen.

The advantages of ULV application include savings in formulation costs, reduction in operating time for equipment, and reduced probability of community intoxication. The disadvantage of ULV application is its lack of residual effect.

To infiltrate the outside resting places of the adult mosquito, the ULV droplets must be very small, probably on the order of 5-10 microns MMD (mass median diameter) for ground applications and 10-25 microns MMD for aerial applications (Lofgren, 1972).

The efficacy of ULV spraying against adults and larvae of other mosquitoes is not in dispute. For example, ULV was used successfully to eliminate almost completely the culicine population in a 1966 encephalitis outbreak in the U.S. (WHO, 1970). ULV malathion applications of 0.62 pounds per acre have shown good results against anophelines in Panama (Lofgren, 1970).

Published reports have shown ULV applications to be successful against a variety of vectors in a number of environments, but little consensus has emerged about insecticides, equipment or dosages of choice. In what follows, an effort will be made to discuss these subjects.

There are three ways to make ULV applications: from aircraft, from vehicle-mounted units, and from portable units carried on foot.

The advantage of aircraft ULV dispersion is that it is indispensable in those situations where a large, dispersed area, logistically difficult to cover from the ground, requires fast and thorough treatment. ULV spray has been shown to penetrate jungle canopy, with 50 percent loss, in the Panama Canal Zone (Mount et al., 1970). Aerial application of malathion at six US fluid ounces per acre for control of urban Aedes aegypti produced very high mortalities among larvae and adults in north central Thailand. When the dosage was halved in urban areas near Bangkok, less satisfactory results were achieved (Lofgren, Ford, Tonn and Jatanasen, 1970; Lofgren, Ford, Tonn, Banq and Siribodhi, 1970).

In non-ULV aircraft operations against the vector of onchocerciasis, it was noted that

> The most striking advantage of aerial application of larvicides as compared with ground treatment is the rapidity with which breeding sites can be treated. For example, all of the 30-40 dry-season breeding sites of the White Volta drainage system, encompassing an area of more than 10 000 mi^2 (26 000 km^2), could be treated in about 5 1/2 hours using the relatively slow Beaver aircraft that has a cruising speed of 95 mi/h (152 km/h). A faster twin-engined aircraft could treat these same sites in about 2 1/2 hours. In contrast, weekly treatment of these sites using hand application methods would require 5 teams

of 6 men each with Land Rovers.

A further advantage of using aircraft is that breeding sites can be detected and mapped from the air. (While it requires about 1 year for an entomologist to survey and map the breeding sites in 100 miles (160 km) of river from the ground, using aircraft the same breeding sites can be detected and mapped in a few hours per month). These sites are often difficult and sometimes impossible to reach from the ground and may change from one year to the next or during the course of the dry season. (Jamnback, Duflo, and Marr, 1970).

The limitation on aircraft application of ULV is the cost. Aircraft rental of $100 to $200 per hour is a severe constraint. "The initial cost of aircraft and equipment may be high, but a well-organized vector control operation also has very high productivity. The final cost per unit of area treated from the air is often less than the cost per unit treated from the ground." (WHO, 1971). Aerial ULV application should not be ruled out on financial grounds unless comparisons with labor costs of ground application show it to be more expensive. Ground ULV applications generally require dosages which are several times lower than those needed for air ULV (Lofgren, 1970). Ground ULV applications can be done from vehicle-mounted units when all sprayable areas lie within the swath of the aerosol stream. In areas where human settlements or breeding places are not accessible, portable applicators are the equipment of choice. Backpack sprayers can be converted for ULV ground application (Lofgren, 1970).

In the area of ULV insecticides, there seems as yet to be no single insecticide of choice.

In ground ULV applications, such compounds as malathion, fenitrothion, methyl Dursban[R] and chlorpyrifos have shown good results (Schoof and Taylor, 1972).

East African applications of malathion succeeded in reducing larval and vector populations of Aedes simpsoni by 93 to 100 percent at dosages of 20.2 US fluid ounces per acre. ULV dosage of six ounces per acre produced less satisfactory results. The trials were made in areas with some canopy of false banana (Musa ensetta) ranging in height from one to six meters (Brooks, Neri, Gratz, and Weathers, 1970).

Ground ULV trial applications of chorpyrifos, fenthion, Actellic[R], Dowco 214, malathion, and malathion-naled have been shown to give good control of Aedes taeniorhynchus (Rathburn and Boike, 1975). On Grand Cayman Island, trial ULV application of Abate[R] against Aedes sollicitans by airplane at four fluid ounces per acre effected 95 to 99 percent mortality after 24 hours (Armstrong, 1970).

Among the carbamates, field trials of propoxur (BAYGON[R] MOS) showed it to be two to three times as effective as malathion against caged A. quadrimaculatus adults when used in ULV form (Mount, Pierce, and Baldwin, 1975b).

Synthetic pyrethroids show promise in ULV operations, as evidenced by kills of A. albimanus showing toxicity 24 to 80 times that of malathion (Lofgren, 1974). Wind tunnel tests with pyrethroids and organophosphates against A. quadrimaculatus and A. albimanus showed that the pyrethroids were more highly toxic (Mount and Pierce, 1975). In Maryland, ULV field trials of synergized pyrethrins against caged Culex pipiens showed rapid knockdown and kill at distances up to 200 feet. However, Minnesota ULV trials against free flying Aedes vexans showed only modest success as measured by vector density after 24 hours (Preiss, 1973). Successful use of synergized pyrethroids has been reported when used as inside space sprays in the Panama Canal Zone against anopheline and other mosquitoes (Sullivan et. al., 1976). Weekly ground ULV application of synergized pyrethrins during the four-month malaria transmission season in El Savador brought about very great reductions in vector density and in incidence of falciparum malaria. While the cost of the pyrethrins was high, manpower costs were low (Hobbs, 1976).

Information on environmental impact of ULV applications is very fragmentary. There are legitimate concerns about ULV intoxication in spray personnel due to the high concentration of the active ingredient. Spills and leaks from equipment are, therefore, of more concern in ULV than in conventional spraying. Intoxication of householders is less of a problem with ULV than with conventional spraying because of the small amounts used (WHO, 1973d).

Information about the impact of ULV formulations on nontarget animal life is less available than that for humans. Since ULV applications are made in the open, this is a very relevant concern.

Malathion has been found to be non-toxic to goldfish, mice and quail at the recommended ULV ground application rate of 1.5 fluid ounces per minute. It was also found non-toxic when the recommended rate was increased tenfold to 15 fluid ounces. Cholinesterase inhibition, which accompanies organophosphate intoxication, was not detected (Joseph et al., 1972).

The long safety record of pyrethrins is clearly a point in their favor for ULV uses, though their innocuous use in earlier applications was as an inside spray.

The very scanty use of ULV in malaria campaigns may account for the dearth of information on ULV toxicity to non-target animals in the less developed countries. This topic is discussed below under research requirements.

Specifications for insecticides and vector control equipment are published and revised by WHO (WHO, 1973c; WHO, 1974). Equipment for the older technologies, such as residual house spraying, is better developed than that for ULV.

Problems of ULV equipment extend to motorized knapsack mist blowers used in ULV operations, as well as vehicle-mounted aerosol generators. ULV vehicle-mounted equipment, which is gasoline-driven, has been said to constitute a hearing hazard (Nelson, Sachs, Johnson, Pennington, and Young, 1975).

The size of droplets dispensed by ULV equipment is an important determinant of insecticidal effectiveness; large droplets are both less effective and likely to spot automobile finish in urban areas. Product ratings for various makes of ground and air ULV equipment have been done (Mount, Pierce, and Baldwin, 1975a).

Rotary-cylinder atomizers tested against *Aedes taeniorhynchus* produced smaller and more uniform droplets than conventional flat fans and required smaller doses of insecticides (Mount, Pierce, Lofgren, and Salmela, 1971).

The basic question confronting malaria programs in respect to ULV is not, however, one of insecticides or equipment. The question is whether ULV has any place in an anti-malaria program except for rapid control of epidemics. High hopes have been expressed on this point by the WHO Expert Committee on Malaria:

> Ultra-low-volume (ULV) application is being developed for ground and aerial spraying. With new insecticides it is hoped that this type of application will give a reasonable persistence and that there will be less need for repeated application. ULV application is being tested for its suitability as a means of house spraying and may prove to be a rapid and economical imagicidal measure. (WHO, 1974b).

It has been suggested that ULV outdoor application may be indicated where the pressure of indoor residual spraying has forced the vector to adopt outside resting places (Schoof and Taylor, 1972).

The recent successful experiences with weekly pyrethrin ULV application from El Salvador have already been cited. The only other published report of ULV applications in malaria programs are from Haiti, the Solomon Islands, and Egypt.

In Haitian trials aimed at reducing epidemic transmission of malaria, aerial ULV application of malathion at 4.5 fluid ounces per acre every ten days led to reduction of A. albimanus populations and to reduction of malaria to preepidemic levels. Success was attributed in part to the large (20,000 acre) area covered and in part to favorable topographic and climatic conditions. Ecological assessment determined no significant impact on nontarget vertebrates. Malaria and vector density returned to earlier levels after the experiment was terminated.

The investigators noted that ULV application, being aimed at the entire mosquito application, was more likely to encourage development of anopheline resistance than house spraying. Moreover, ULV was about twice as expensive an epidemic control measure in this area as mass drug administration would have been (Eliason, Joseph, and Karam, 1975).

In the Solomon Islands, the malaria eradication program encountered operational problems in ULV operations with malathion against A. farauti. Vehicle-mounted Leco[R] and knapsack Fontan[R] equipment was used in accessible and inaccessible areas, respectively. Since the vector bites at night, the applications had to be done then, under difficult nocturnal conditions. Furthermore, the active biting period is only two hours long, so the application period had to be short. Moreover, villages were small and scattered, so that transport took up much time. It was concluded that ULV was suitable for eliminating foci of indigenous malaria when used in conjunction with other measures; it was not found suitable, under local conditions, for long-term vector reduction over a broad area (Turner, 1977).

In Egypt, application of a mixture of malathion and temephos in a 9:1 mixture at 8 ounces per acre over ten day intervals brought about great reductions in density of A. pharoensis in the Nile Delta. Vector populations rose to lower than baseline levels when spraying was stopped (Mahdi, Taha and el-Arab, 1967).

It may be too early to assess the feasibility of ULV applications against malaria. ULV use in malaria work requires a knowledge of when and where the vector is most commonly to be found in the area to be sprayed, as well as technical expertise in maintaining the equipment, especially the regulation of droplet size. However, the biggest hurdles in the way of regular ULV use in reducing vector density are not ones of technical know-how, but of cost and the need for frequent application.

In addition to ULV applications, space spraying can also be done by thermal or cold fogging, using different equipment and higher dosages and droplet sizes.

In thermal fogging trials against A. albimanus adults, highest kills were obtained with malathion and BAY 78182. None of the other compounds tested effected mortalities over 90 percent at 300 feet (Taylor and Schoof, 1969).

Recent comparisons of cold and thermal aerosols in Chatham County, Georgia showed operating costs (machines and vehicles) of 4.1 cents and 15.6 cents per acre, respectively. Moreover, nonthermal aerosol generators were reported to give higher public acceptance, better safety and higher efficiency. Thermal aerosols were more useful only in sites with dense ground cover, and then only marginally (Fultz, McDougal and Thrift, 1972).

Use of ULV ground equipment in other trials with low-volume concentrates of chlorpyrifos and naled (Dibrom [R]), gave high kills of culicine mosquitoes at distances of 10,000 feet over flatland. The unusually long range was attributed to small droplet size (Stains *et. al.*, 1969).

The bulk of recent experience with space sprays suggests that non-ULV applications have little advantage over ULV. The superior performance of ULV over high volume thermal aerosols has been demonstrated in the case of *Aedes taeniorhynchus* with malathion and naled (Mount, Lofgren, Pierce, and Husman, 1968). ULV gave kills of *A. albimanus* superior to those of thermal aerosols in cages in the open, but produced kills at lower rates with caged specimens in wooded areas at distances over 150 feet (Taylor and Schoof, 1971).

Anderson and Schulte found ULV application to be more effective than thermal aerosols, in addition to offering the advantages of simplicity, lower maintenance, much lower insecticide cost, and greater safety (Anderson and Schulte, 1970). In tests against caged *Aedes taeniorhynchus* and *Culex nigripalpus*, ground ULV application of malathion gave equal or higher kills than thermal aerosols at the same dosages (Rathburn and Boike, 1972).

2.2 Antilarval Measures

The rapid change in expert opinion on the usefulness of antilarval alternatives can be illustrated by the following quotations:

> Imagicidal control has in most circumstances proved immeasurably more satisfactory than control of breeding, which it totally supplants and does not merely supplement. . . [Larvicidal attack] has, therefore, greatly diminished in importance, and has relatively very little significance in any modern programme. (Russell *et al.*, 1963).

* * *

> Wherever DDT residual sprays are adequate to interrupt transmission of malaria, this method should be continued since it is still the most economical antimalarial measure for large rural areas. However, where DDT is unable to interrupt transmission other measures will be required. Experience has shown that in these latter situations (termed problem areas) the simple changing from DDT to a new insecticide or insecticides plus drug administration has seldom produced a complete solution to the problem of malaria transmission. Such situations need to be studied carefully in depth in order to ascertain the exact factors, epidemiological, entomological or human, which permit malaria transmission to persist. On the basis of the findings of these investigations, it is believed that a program of integrated control measures can be developed which will do much to solve the problems (Wright *et al.*, 1972).

Attitudes towards antilarval mesures have changed, and larval control is part of 20 national antimalaria programs (NAS, 1976).

Renewed interest in antilarval measures has sprung largely from the fact that many adult mosquitoes tend to prefer outdoors resting places (exophily), thus avoiding contact with the residual insecticide. However, antilarval measures are not suitable for all areas, or even for all areas where spraying alone has failed to achieve program objectives. A WHO working group has concluded that

> Antilarval measures are the methods of choice where urban housing is relatively dense, as the mosquito breeding places are usually accessible and not unduly extensive. Legislation is required to control the pits caused by the removal of earth for building which, when filled with rainwater, form excellent mosquito breeding places. Residual house spraying in urban areas can be prohibitively expensive and should only be applied in the slums on the outskirts of towns (WHO, 1974a).

The WHO's Division of Malaria and other Parasitic Diseases sees antilarval operations as worth considering:

a) when there are few breeding places;

b) when house spraying is impractical, or uneconomical, as when the area to be protected is a densely populated urban area;

c) as a preventive measure in areas receptive to malaria after transmission has stopped for several years; and

d) where for technical or operational reasons house spraying either alone or combined with mass drug administration fails to interrupt transmission or to effect adequate reduction of malaria cases or endemicity. (WHO, 1973b).

Mulla has suggested the appropriateness of antilarval measures for those arid and semiarid areas where the number of breeding places is small in relation to the number of houses (Mulla, 1970). In this connection, it is notable that oiling against exophilic A. sergenti in the Jordan Valley proved more efficient than spraying houses and led to local species eradication. Even in wet areas, the number of breeding places may be very much limited during the dry season, and mapping and larviciding at that time may be a feasible control measure (Mulla, 1970). This approach, coupled with residual spraying of HCH, is said to have reduced A. minimus-borne malaria in Yunnan, China (Yunnan Provincial Anti-Malaria Institute and Menghai Commune Health Center, 1976).

Larval control should not be undertaken without a careful prior assessment of its chances for success. This means determining whether the breeding places for larvae are few enough and accessible enough to permit effective control, as in the case of stream-breeding A. minimus. An example of a vector which cannot be easily controlled by larval measures is A. balabacensis, which breeds in hard to find sylvan puddles and pools. The undertaking of larvicidal measures requires, as MacDonald has pointed out

> an exact incrimination of the vector with exclusion of other species from suspicion, which requires a considerable programme of mosquito collection and dissection; a precise study of the breeding places of the vector, leading to an incrimination of a type of water in which it may be found breeding; an analysis of the frequency of this type of water, and information on the normal range of flight of the vector from its breeding places. (MacDonald, 1957).

Larvicidal measures are aimed at killing larvae of the malaria vector in those breeding places which are within flight range of human night time habitations. MacDonald suggests three quarters of a mile as a general rule for the radius around the area to be protected, though the wide variations in flight path between and among species justify local studies where possible.*

The need for intimate knowledge of vector habits is pointed up by the experience of the Bombay provincial authorities. In Bombay State, clean weeding of streams eliminated stream breeding A. fluviatilis, but declines in larval density were not matched by declines in adult density or in the prevalence of malaria. Investigation revealed that continuation of transmission was due to vector breeding in terraced rice fields; extension of control measures to rice fields was followed by a decline in malaria (Anon., 1948). The need for routine monitoring of antilarval impact is at least as great as with spray measures. Larviciding requires on-the-spot

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* It is hard to generalize about the flight range of anophelines. The only review of the literature shows very great variations between and within species (Eyles, 1944). Recent work on flight ranges as they relate to disease has been reviewed elsewhere (Johnson, 1969).

verification, through larval dips, to make sure that the larviciding was done and done properly (MacDonald, 1957; Russell et al., 1963). Moreover, assessing the ultimate impact of antilarval measures requires not only pre- and post-treatment data on larval levels, but also night catches to see whether there has been a commensurate reduction in the density of adults.

Anti-larval meaures must be costed out before being undertaken. The belief that they are more expensive than spraying is not always borne out in practice. The cost of larviciding will vary with the expanse of areas covered, the length of the larviciding season, the choice of larvicide, and the interval between applications. In sparsely populated parts of the northern Transvaal, larviciding was no more expensive than spraying with DDT and HCH. Larviciding was done during the winter, when breeding places were few (Brink, 1958).

Finally, antilarval operations, more than adulticiding, require a prior assessment of ecological impact. There is enough of a literature on the subject to make an informed judgement in many cases. Nevertheless, consultation with local ecologists is advisable, especially where introduction of fish or other biological agents into a foreign environment is contemplated.

Much of the following discussion is drawn from a recent WHO publication on antilarval operations to which persons interested in mounting an antilarval campaign are referred (WHO, 1973b).

2.2.1 Use of Oils

Oils have long been used as larvicides, giving the advantages of high toxicity and, for some formulations, a residual effect of several days. They do not, of course, produce resistance against chemical adulticides. Properly used, they are effective against larvae, but not specific for them; their environmental impact, especially against game fish, should be assessed before undertaking a program.

WHO concluded that "selection of a larvicidal oil is generally a matter of compromise between the factors of high toxicity and long residual effectiveness." (WHO, 1973). While the price of oils has risen in the last few years, the amount necessary to cover a hectare dropped from 45-90 liters in 1965 to 9-27 liters by 1973.

Flit[R] MLO is effective against larvae and pupae of *Anopheles* and *Culex* at 1.0 gal per acre. Like three other oils, it did not induce resistance in *C. fatigans* in 20 or more generations of selection pressure (NAS, 1976). American mosquito abatement districts have gone back to using oils like Flit[R] MLO because of vector larval resistance to chemical larvicides like DDT (NAS, 1976). Laboratory tests with FLIT[R] MLO at 0.75 gallons per acre showed larval mortality for *A. quadrimaculatus* of under 90 percent for all stages, but high mortality for pupae (Micks, Chambers, Jennings, and Barnes, 1968).

Despite its efficacy, the application of oils, alone or in mixtures with diluents or spreading agents, has many drawbacks: the oil slowly evaporates, requires frequent reapplication except in calm, enclosed breeding places, is heavy to transport, and makes the water unfit for domestic use (Sandosham, 1965). Because of its bulk, oil poses problems of storage, transport, and distribution. Moreover, farmers may object to its application on grounds of odor and contamination (Rafatjah, 1975a). What places oils outside the range of choices for many programs is their high cost in comparison to that of other larvicides. The only countries which may find a role for oiling in their antilarval campaigns are those with domestic supplies of cheap oil or those which choose to use waste oils. These latter present esthetic and environmental problems, especially in running water, and should in any event not be used without a spreading agent.

Of special relevance to oil producing countries are low cost mixtures of crude oils with spreading agents. Excellent results against larvae of *A. stephensi* have been reported with a 70:30 mixture of heavy furnace oil and light diesel oil, plus 0.5 percent Triton X-100 detergent. The low volumes of oil required mean lower costs for labor and transport. The published report does not discuss environmental impacts (WHO, 1973d).

2.2.2 Chemical Larviciding

The earliest chemical larvicide, and the only one to achieve area eradication of a malaria vector, is Paris green.

Paris green is an arsenate stomach poison which affects anopheline larvae because of their feeding method. Anopheline larvae sweep surface particles into the mouth with brushes which permit them to cover large areas of water. Since no other larvae feed this way, they do not ingest large enough quantities to do them harm (Russell *et al.*, 1963).

Paris green is an extremely effective poison responsible for both recorded instances of area vector species eradication (*A. gambiae* from Brazil in 1940 and Upper Egypt in 1945). While highly toxic to white mice, it has an excellent safety record for wildlife and man. Among the commonly used larvicides, it is cheaper in cost of coverage per hectare than all others except tempehos (Abate[R]) and fenthion (Baytex[R]) (WHO, 1973d). Stream breeding *A. darlingi* has been successfully controlled in the Rio Doce basin of Brazil through application of Paris green and occasional ditching and filling. Reinfestation, as measured by positive larval dips, occurred when operations were terminated, indicating the need for continuous application (Anon., 1948). Weekly aerial application of Paris green against *A. quadrimaculatus* achieved dramatic results against larvae and adults in 1943 controlled trials in the Potomac River and nearby creeks. Average application was 1 1/4 pounds per acre; heavy cover of water chestnut (*Trapa natans*) made this dosage necessary (Murray and Knutson, 1943).

In Florida trials, Paris green in granular form for buoyancy (oil with vermiculite) was applied by hand shaker in ditches infested with larvae of *Anopheles bradleyi*. Dosages of 0.15 pounds of Paris green per acre effected 92.3 percent mortality in anophelines after 24 hours; doubled doses effected 100 percent mortality (Rogers and Rathburn, 1958).

Paris green granules, developed in 1956, have proven effective against all mosquitoes (NAS, 1976). Pellets (granulated carriers) of Paris green offer several advantages including greater penetration of vegetative cover and less susceptibility than sprays and dusts to air currents. Hand casting and air drops are both convenient with pellets.. However, incidents of rash associated with skin exposure have been reported in field personnel (Fehn *et al.*, 1959).

Paris green can also be diluted with road dust (1:100, volume per volume) or mixed with kerosene as a carrier and is quite effective and safe (WHO, 1974a).

Paris green is an arsenical and increases the concentration of arsenates in soil and water above their natural level; however, that level has been shown to fall again within a few days, both for Paris green and other arsenicals. Paris green has an excellent safety record with vertebrates; among invertebrates, its effects appear to be limited to filter feeders. This virtually restricts its impact to mosquito larvae. (NAS, 1976). Experiments with granular Paris green in Florida found very low residual absorption of arsenic in sandy loam lining the bottom of impoundments (Rathburn, 1966).

Paris green offers two advantages over other chemical larvicides: 1) it is not related to house sprays, and, therefore, cannot promote resistance; and 2) it is not recorded to have produced resistance in anopheline larvae.

The chemical larvicides now in use are, for the most part, organophosphates. Natural larvicides of the pyrethrum group were formerly used, but their high cost makes them unattractive for mass campaigns. Chlorinated hydrocarbons, while effective alone and in combination with oils, are no longer considered suitable because of their impact on nontarget organisms (Pickering *et. al.*, 1962; WHO, 1973b; Newsom, 1967; Mallis, 1960; WHO, 1974a).

Among the organophosphates, and among larvicides in general, temephos (Abate^R) has received the widest acceptance. In laboratory trials with *A. labranchiae atroparvus*, larvicidal effect of the following insecticides was, in descending order, temephos, diazinon, fenthion, DDT, and dieldrin, malathion, and HCH (Duport, Sandescu, and Păun, 1971). Temephos (Abate^R) is an organophosphate of relatively recent date with low acute toxicity to animals except larvae. Its lack of toxicity towards fish makes it ideal for use in combination with larvivores like *Gambusia*. It is also safe for drinking water at concentrations not in excess of one part per million. After reviewing results in campaigns against *A. stephensi*, *A. claviger*, *A. pulcherrimus*, *A. sergenti*, and *A. superpictus* in a variety of situations, WHO concluded that temephos' "large margin of safety and the low dosage applied makes this compound the larvicide of choice for *Anopheles* control." (WHO, 1973d). Brown concluded that "Abate is considered to be the insecticide of choice against anopheline larvae in areas where surface water is liable to be used for all purposes, not only for washing but also for drinking." (Brown, 1972). The National Academy of Sciences found in 1976 that temephos is now the preferred mosquito larvicide in nearly all situations, not only against anopheline larvae for malaria control, but also against Aedes aegypti, often in indoor sites (NAS, 1976).

Temephos' most notable qualities are its slow release, permitting application at intervals of a month or longer, its low toxicity to man even in drinking water, and its action against larvae of both anophelines and Aedes aegypti, the vector of hemorrhagic dengue and yellow fever (WHO, 1967). Temephos emulsifiable concentrate (4 pounds per gallon) was compared to similar formulations of fenthion and malathion in Okinawa field trials against larvae of A. sinensis and two culicine species. Larval dips showed results for temephos which were superior to the other formulations against all species. Residual effect of temephos was two weeks (Armstrong, 1970). In trials against larvae of A. gambiae and other mosquitoes near Lagos, Nigeria, temephos proved to be the most effective larvicide among the compounds tested. Next highest in toxicity was parathion, whose toxicity to humans precludes its use in malaria programs. Fenthion and bromophos required concentrations ten times higher than that of temephos to produce the same 90 percent mortality. Fenitrothion, DDT, HCH, and dieldrin were even less effective (Self and Pant, 1966).

While temephos was not the most toxic larvicide against third instar A. gambiae in later Nigerian laboratory trials, it was chosen for field trials over fenthion, Ciba C-14814, and chlorpyrifos (Dursban^{R}) for field use on account of its remarkably low toxicity to mammals (LD_{50} of 13,000 milligrams per kilogram of weight in female white rats). Temephos had no appreciable residual effect in overflowing borrow pits during the rainy season. Trials toward the end of the rainy season showed suppression of second, third, and fourth instars for 24 days at concentrations as low as one part per million. Dry season trials showed almost complete suppression of later instars and pupae with 1 percent floating granules used at 1 part per million. The trials showed evidence of a sorption-release mechanism for temephos on mud and particulate matter in the water. The authors concluded that temephos granular formulations were suitable for larviciding during the transition period from the dry to the wet season (Fontaine and Rosen, 1973). Use of temephos against A. gambiae effected 90 percent reduction in adult densities from baseline levels in villages of the Comores Archipelago, where the vector breeds in seaside streams and puddles. It was concluded in this case that temephos larviciding should be associated with adulticiding during the rainy season but could be used alone at other times (Subra and Hebrard, 1973).

Successful control of A. minimus flavirostris in running water was achieved in San Pablo, Philippines by weekly application of temephos emulsifiable concentrate. Temephos was effective against all instars, and reductions in adult density and indigenous cases paralleled declines in larval catches. Labor costs and entomological assessment made larviciding two to three times as expensive as house spraying (Catangui et. al., 1972). Subsequent developments in drip can technology and use of slow-release formulations, discussed elsewhere, hold promise for reducing labor costs with temephos applications in running water.

While temephos has been generally found to be nontoxic to nontarget organisms, this is not the case with shrimp; temephos should be used in coastal marshes with great caution, according to Mulla (Mulla, 1970). Some mortality was observed in nontarget chironomids, cladocerans, and other aquatic life when Abate[R] 2G was applied in laboratory trials at a rate of 2.5 pounds per acre (Didia, LaSalle and Liem, 1975). Efforts to reproduce these results in the field did not succeed; temporary population losses were suffered, especially in cladocerans, but all nontarget organisms returned to their normal population levels after 48 hours (Liem and LaSalle, 1976).

Temephos' strong larvicidal activity against Aedes aegypti, a vector of dengue and yellow fever, has been noted above. In a built-up residential area of Bangkok, temephos larviciding of containers in houses against Aedes aegypti, requiring 13 minutes per house, was done on four occasions. One man could treat 40 to 50 houses daily. Considerable decreases in larval and adult densities were effected. Residual effect ranged from two to three months. In Thailand, where dengue hemorrhagic fever peaks during the rainy season, two yearly applications were considered sufficient for control of Aedes aegypti (Bang and Pant, 1972). One percent temephos in sand granules was effective against Aedes aegypti larvae in water jars in Bangkok. The residual effect of several months was shortened in direct proportion to the length of time that the granules were kept in empty jars (Bang and Tonn, 1969). Field trials with temephos larviciding carried out in Saudia Arabia from 1968 to 1972 showed it to be 20 percent cheaper than oiling and 36 percent cheaper than use of Paris green (Rafatjah, 1975a).

The only problem with temephos is resistance. Among temephos-resistant larvae, two compounds which show much promise are OMS-1653 and OMS-1657 (WHO, 1976).

Parathion and parathion-methyl are extremely toxic to man, and the safety measures necessary to avoid toxic incidents with these substances are very stringent and difficult of application in a mass campaign. The WHO does not recommend the use of these substances in malaria programs (WHO, 1973d).

Fenthion, malathion and chlorpyrifos (Dursban^{R}) are effective larvicides belonging to the same family with temephos. However, considerations of cost, acute toxicity and development of resistance make them less desirable for larviciding than temephos.

Results from use of chlorpyrifos (Dursban^{R}) have been equivocal, both from the standpoint of efficacy and toxicity.

Dursban^{R} in pelletized form has labeling approved by the Environmental Protection Agency for larviciding. Dursban^{R} 10CR pellets, spread by shakers and seed spreaders against a variety of non-anopheline larvae, achieved 100 percent kills for periods of 12 to 18 weeks in standing water (Keenan, 1978). California rice field trials of low volume chlorpyrifos sprays as larvicide gave equivocal results against _A. freeborni_ (Washino, Ahmed, Linn, and Whitesell, 1972). Such nontarget organisms as the mayfly (_Siphlonorus_), the diving beetle (_Laccophilus_), and the bluegill (_Lepomis macrochirus_) suffered adverse effects from .0125 to .0167 pounds of chlorpyrifos per acre (Washino, Ahmed, Linn and Whitesell, 1972). In rice field trials of chlorpyrifos at 0.0125 pound per acre against larvae of _A. freeborni_ and _Culex tarsalis_, rice fields treated with the chemical had higher larval densities than untreated controls, perhaps due to chemical effects on invertebrate predators of the larvae (Hoy, Kauffman and O'Berg, 1972).

From the ecological standpoint, New Jersey salt marsh trials with temephos and chlorpyrifos showed that both were highly selective, failing to effect significant changes in the richness or diversity of the nontarget aquatic insect commuity (Campbell and Denno, 1976).

Concern has been expressed about possible danger to work crews of cholinesterase inhibition associated with the use of chlorpyrifos. Field trials of cholorpyrifos as a 0.5 percent suspension or emulsion were conducted in the U.S. in 1968. Five of seven spraymen showed plasma cholinesterase depression greater than 50 percent within two weeks of the start of spraying. All spraymen were free of symptoms when the trials were terminated. The authors found chlorpyrifos in emulsion and suspension form unsuitable for larvicide spraying application against *Aedes aegypti* under current U.S. conditions, but did not close the door to its use in granular form, with monitoring of cholinesterase levels in work crews (Eliason *et al.*, 1969).

Chlorpyrifos has been found to produce asymptomatic depression of cholinesterase in man. No-effect levels have been found in man and some animals (WHO, 1973a).

The WHO Expert Committee on Insecticides concluded that fenthion and chlorpyrifos were safe for use in non-potable and grossly polluted water. These substances should not be used in outdoor water that is likely to be drunk. Malathion, temephos and OMS-1155 are safe when applied in the recommended manner. Only temephos was found safe for drinking water, and then at the target dose of 1 milligram per liter (WHO, 1973d).

Resistance to chlorpyrifos has been reported in some populations of *C. pipiens* in France (Sinègre, Jullien, and Crespo, 1975).

Other larvicides include pyrethroids, the aliphatic amines, and monomolecular organic surface films.

Synthetic pyrethroids have shown promise against culicine and *Aedes* larvae at very low dosages, but with some impact on nontarget aquatic species (Mulla, Darwazeh and Majori, 1975).

Even before the oil price rise, aliphatic amines were judged more economical than oils for larval control. Their spectrum of effect is as broad as that of oils, including eggs and pupae as well as larvae. Moreover, they do not require the large dosages of oils. Their environmental impact, which has already been determined in the case of certain crustaceans, needs further assessment (Mulla, 1970). Aliphatic amines have shown promising results with non-anopheline larvae.

Ground application of Alamine 11 and Duomeen L-15 has shown encouraging results against culicine larvae (Mulla and Darwazeh, 1975). While aliphatic amines have lower biological activity than organophosphates, they are superior to them in having a pupicidal effect and in not yet having shown resistance (Mulla, 1970).

A relatively recent addition to the family of chemical larvicides is the monomolecular organic surface films, which are applied in low volumes and are said to be ecologically desirable. Two such compounds, SMO 75/2EB and ISA-20E, achieved 100 percent kills of A. quadrimaculatus larvae after 24 hours in small pool trials (White and Garrett, 1977).

The ecological effects of larvicides vary with dosages and formulations, and assessment of a potential larvicide's ecological suitability requires study of the local conditions. Nonetheless, some generalizations are possible. Mulla has stated:

> Generally speaking, the organochlorine [chlorinated hydrocarbon] insecticides which persist and accumulate in the environment pose hazards of both acute and chronic manifestations. The nonpersistent organophosphorus and organocarbamate insecticides at larvicidal rates manifest some acute toxicity to fish and other vertebrates, but this problem can be minimized by employing materials which show little toxicity to game species. (Mulla, 1970).

Since organophosphates can cause acute toxicity to humans in their larvicidal as well as their adulticidal forms, work crews handling organophosphates should be subject to routine monitoring of cholinesterase levels (WHO, 1973b).

A number of improvements in formulation and application techniques have enhanced the effectiveness of chemical larvicides. Some techniques have been aimed at increasing convenience, spreadibility and penetrating power. Others, the "slow release" formulations, have sought to decrease the need for frequent reapplication. These techniques will be discussed in order.

Advances in drip technology now permit even, accurate application of liquid larvicides to flowing and impounded waters (Sjogren, Mulhern and Coplen, 1969).

A variety of spreading agents and emulsifiers is available which increase the efficiency of surface larvicides (WHO, 1973b). These mean a thinner surface film and lower dosages. Granular formulations, higher in price than their liquid counterparts, offer the advantages of faster application (with resulting reduced labor costs), better penetration through vegetation and greater safety. Temephos and chlorpyrifos applied as clay granules in order to penetrate vegetation and minimize drift effected high kills of Aedes larvae and pupae in ponds in Alberta, Canada. Application was done at two pounds of clay (0.05 pounds active ingredient) per acre (Tawfik and Gooding, 1970).

The largest expense in American larviciding programs has been the cost of labor and transportation which are required for frequent reapplication of liquid larvicides. The chemicals themselves represent only five to ten percent of the total cost (Keenan, 1978). Slow release formulations have been devised which reduce the labor and transportation requirements by making frequent reapplication unnecessary. Most research with plastic-based slow release formulations has been conducted at the U.S. Army Environmental Hygiene Agency, Edgewood Arsenal, Maryland. There, plastic briquettes and polyurethane pellets proved less successful than polyethylene pellets of chlorpyrifos (Cardarelli, 1976). Several larvicides are now produced in slow release formulations which effectively lengthen the residual effect. Paris green is available in a mixture with vermiculite and emulsifiable oil which is used for control of culicine mosquitoes. Plastic pellets of such organophosphates as chlorpyrifos and malathion have given good results (NAS, 1976).

Slow-release briquettes were used as long ago as 1949, giving residual effect in standing water for periods up to one year. A recent literature review shows that materials used for briquettes include cement, sand, and plaster of paris. Field trials at several U.S. sites with Dursban[R]-impregnated briquettes of plaster of paris showed good results with Aedes and culicine larvae in temporary pools, pot holes, and small swamps and salt marshes. The high dosage of 0.75 to 1.5 pounds per acre caused no apparent harm to aquatic wildlife, perhaps because the larvicide was released slowly. Negative findings from larval dips persisted up to five months, less in an area with heavy flooding (McDonald and Dickens, 1970).

Slow-release, solid-base formulations have been firmly established as efficacious for larval control in such confined habitats as ponds, containers, and cisterns, but are not thought to be adaptable to such large environments as rice fields, pastures, and marshes. Their efficacy in streams has not been determined. It is thought that the persistent effect of slow-release formulations will have more adverse effects on non-target organisms than conventional larvicides (Mulla, 1970).

Use of chlorpyrifos in the form of chlorinated polyethylene pellets gave excellent larval control in woodland pools at one part per million. Residual effect lasted 22 weeks. Organic matter was present in the water. Long duration of effect may have been related in part to low water temperatures (Evans _et. al._, 1975).

While polymer formulations of chlorpyrifos gave high kills against culicine larvae in field trials, there were damaging effects on a number of non-target organisms (Miller, Lawson, Nelson, and Young, 1973; Roberts _et. al._, 1973; Miller, Nelson, Young, Roberts, Roberts, and Wilkinson, 1973).

In laboratory tests, polyethylene pellets containing chlorpyrifos produced residues for 16 weeks at levels sufficient to justify field trials (Lawson _et. al._, 1973).

Laboratory bioassays of rubber impregnated with different larvicides showed that only Abate[R] in EPDM (floating rubber) affected over 90 percent mortality after 72 hours with culicine mosquitoes (Schultz and Webb, 1969).

In microencapsulation, droplets of insecticide are covered with a thin layer of water soluble polymer. Encapsulated formulations of malathion and temephos had poor residual effect against culicine larvae in Maryland field trials (Miller, Lawson, Nelson, and Young, 1973).

The current state of work in controlled release formulations is the subject of a recent book (Cardarelli, 1976). The requirements for slow-release polymers have been discussed elsewhere (Stockman *et. al.*, 1970).

One problem of larviciding against stream breeders is the need for continuous application. Use of drip cans in irrigated California pastures proved successful in controlling larvae when chlorpyrifos was applied at dosages of .14 pounds per acre and higher. Larval dips were negative at distances up to 2,000 feet from the drip can. Temephos at 0.15 pounds per acre was less effective than chlorpyrifos. Advantages of drip can over airplane application include savings in labor and aviation fuel costs, and elimination of the need for repeated applications over one transmission season (Mulla *et. al.*, 1969). Temephos has been used successfully at 0.001 part per million against anopheline and culicine larvae in the Philippines (Catangui *et al.*, 1972).

2.2.3 Use of larvivorous fish

Among predators and pathogens of mosquito larvae, larvivorous fish are the only ones currently in widespread field use. In addition to the better-known *Gambusia affinis* and *Poecilia reticulata* (formerly *Lebistes reticulatus*), which are used in permanent water collections, such annual fish as *Nothobranchius guentheri* and *Cynolebias bellotti* lay eggs which resist desiccation and offer promise for use in transitory pools. Such species as the carp (*Cyprinus carpio*) and *Tilapia* eat both larvae and vegetation, which is an advantage in places where water vegetation provides a harboring place for larvae (Chapman *et. al.*, 1972; Bay, 1967).

Larvivorous fish have many advantages, notably low cost, program capability to reduce or forego chemical larviciding, suitability for areas inaccessible to hand larviciding, and suitability, in the case of the larger species, as food (WHO, 1973b).

The best known of the larvivorous fish is the mosquitofish, *Gambusia affinis*. Mosquitofish are widely adaptable to a variety of environments (WHO, 1973b). The mosquitofish is a surface feeder which inhabits permanent waters. Its ability to live and propagate in confined waters makes it suitable for control of *Aedes aegypti* as well as anophelines (NAS, 1976). The field procedures for raising and release of gambusia fish have been described elsewhere (U.S. Navy, 1972). Recent descriptions explain the program of the U.S. Navy to achieve larval control through the use of *Gambusia* (Sholdt *et. al.*, 1972; U.S. Navy, 1972). Practical aspects of large-scale raising have been discussed by Davey and Meisch (Davey and Meisch, 1977).

The Iranian malaria program has used mosquitofish in areas where the vector rests outside houses and is little affected by residual house spraying. The fish have been placed in over 3,000 permanent water collections, such as stream edges, areas with standing vegetation, and rocky pools. Efficiency of the gambusia is reduced by insufficient light and oxygen, by rains and floods, and by changes in the water level. They are less effective in areas where temporary breeding places are created by rains (Tabibzadeh *et. al.*, 1970).

Despite a wealth of literature on mosquitofish programs, reviewed by Gerberich and Laird (Gerberich and Laird, 1966), there is not too much information on the efficacy of such measures. California rice field control of culicine and anopheline larvae by Gambusia was found to be directly correlated to density of fish. The authors found that reasonable larval control, yielding about 0.1 larva per dip, could be achieved with 300 fish per acre (Hoy et. al., 1971). In northeast Afghanistan, distribution of Gambusia affinis in rice field breeding places of A. pulcherrimus was associated with lower larval densities and adult biting activity than in control areas (Polevoj et. al., 1973). In the area around Thermopylae, Greece, introduction of Gambusia in 1936 was associated with a decline in larval density, spleen rates, and parasite rates. No other control measures were in force at that time. Gambusia were found effective in the permanent breeding places of A. sacharovi, but not in those of A. superpictus, where they were washed away after rains (Hadjinicolaou and Betzios, 1973). Introduction of Gambusia into the South Pacific for control of larvae in standing water was fully successful only when weeding was done at the water's edge (Boyd, 1949). An area of Istria, Italy where Gambusia was introduced saw a decline in the spleen index from 98 to 10 percent betwen 1924 and 1930; no other vector control measures were in force at the time (Hackett, 1931). Gambusia fish failed to have much impact on A. funestus in Madagascar or on A. sacharovi and A. labranchiae in Corsica, apparently because the larvae took shelter in dense water vegetation (PAHO, 1972). In such areas, cutting or herbiciding of vegetation is necessary to give the fish access to the larvae. Satisfactory control of larvae has been reported in a 35 acre lake in Hawaii where Tilapia mossambica proliferated in sufficient numbers to clear the water of a dense canopy of algae, permitting Gambusia access to the larvae (Nakagawa and Hirst, 1959).

The second most popular of the larvivorous fish, judging from the number of published reports, is Poecilia reticulata (formerly Lebistes reticulatus), the guppy.

Introduction of Poecilia reticulata into polluted standing waters in Bangkok effected little reduction in density of C. pipiens fatigans larvae. The authors suggested that obstruction by debris and vegetation or presence of organic matter as an alternate food source may have prevented the fish from being efficient larval predators, pointing to earlier, successful work with larval reduction through a combination of pond clearing and fish introduction (Mathis et. al., 1976).

A review of sites in Bangkok, Rangoon and Taipei where Poecilia reticulata was used for larval control showed that while some sites are unsuitable because they are too transitory, too anaerobic, or too obstructed with debris, others are extremely suitable for the guppies, regardless of their degree of pollution. Dangers to guppies include piscivorous fishes and human fishing (Bay and Self, 1972).

A large number of other larvivorous fish have been used for vector control. The quatic habitats of annual fishes thought suitable for larval control have been described by Hildemann and Walford (1963). Introduction of new fish may alter the balance in favor of eutrophication (Hurlbert et. al., 1972). Ecological considerations favor the breeding and augmentation of indigenous species over the introduction of foreign ones, unless a careful ecological assessment justifies the latter course.

Raising, transportation and distribution of larvivorous fish has become easy, and fish have proven adaptable to a wide variety of habitats, including marshes, swamps, ponds, streams, irrigation canals, rice fields, and wells. Maintenance of larvivorous fish at adequate density has proven hard in many areas with seasonal transmission (Anon., 1976).

Per capita annual cost of antilarval protection with fish has been estimated at $0.02 in Afghanistan (Karimzad, 1972). This places larvivorous fish operations within reach of even programs with low budgets.

Despite the large body of literature on program interventions with Gambusia and other larvivorous fishes, it is unfortunately true that "there is still no real body of careful research on the effect of such larvivorous fish as the mosquito-fish, Gambusia affinis, on the indigenous fish fauna in most of the many areas into which it has been introduced. . .The same can be said of introductions of the guppy, or 'millions fish' (Poecilia reticulata) outside its natural range" (Smith, 1973). This knowledge gap needs to be closed through field research. Also required is more study on the combined efficacy of fish and other antilarval measures. It appears that in environments where larvae take shelter in vegetation, application of herbicides will assist larvivorous fish in doing their work (Craven and Steelman, 1968). Clean weeding and promotion of large, herbivorous fish species will accomplish the same purpose.

Persons interested in undertaking larvivorous fish programs are referred to the annotated bibliography of Gerberich and Laird (1966).

2.2.4 Environmental engineering

The oldest anti-larval measures are those of environmental engineering, which was first employed on a large scale in reducing mosquito density in the Panama Canal Zone and in the Malayan peninsula. The methods of environmental engineering range in scale from filling of pools to water management on dams. They are described below with a listing of the conditions for which they are suitable.

The cheapest and simplest method of antilarval engineering is the filling of breeding places with mounded dirt which settles to the level of the surrounding ground after rain. It is useful for small water collections only, and care must be taken that filling does not create borrow pits which themselves become breeding places. Soil is only one of many materials available for filling; others include cinders, sawdust, timber shavings, rice husks, and, in closed impoundments, rubbish covered by compacted cover (Russell et. al., 1963).

Draining and ditching are in very common use against a variety of vectors. The value of drainage in terms of reducing disease was established as early as 1902, when deaths in two Malayan towns fell from 582 to 144 after drainage was installed (Boyd, 1949). The value of drainage was also demonstrated in Panama early in this century (Boyd, 1949).

The simplest way to drain water from land is vertical drainage, the cutting of a hole through an impervious surface to a pervious substratum. This cheap and simple method has been used to drain areas as large as a pond measuring 1800 by 50 feet (Russell et. al., 1963).

Horizontal drains can be either lined or unlined, and can be built on soil or in subsoil. While initial costs of lining ditches are higher, they are more durable, more easily cleaned, and need less maintenance than unlined ones. Lining is done weeks or months after the ditch is dug in case deepening proves necessary (Russell et. al., 1963).

Since permanent ditches are expensive, consideration may be given to making them serve more than one purpose, e.g., drainage for irrigation or highways as well as public health (Hinman, 1947). In Merced County, California, farmers contracted with the public authorities to undertake drainage activities because it resulted in higher yields of grass and beef (Smith, 1952). Wherever drainage against malaria serves some other purpose, such as prevention of waterlogging in irrigated fields, public support can be mobilized on a broader basis than would otherwise be possible.

The efficacy of drainage varies with terrain and vector habits. "Agricultural drainage in the United States has generally been a more effective antimalarial measure than it was in Italy, largely because the American vector A. quadrimaculatus has a strong preference for standing water and is not apt to proliferate in drainage ditches as does A. labranchiae in Italy" (NAS, 1976).

Coastal breeding places present special situations which require a different approach. In coastal waters, installation of tidal gates can control brackish water breeders like A. sundaicus, and freshwater breeders can be controlled by flooding with salt water (WHO, 1972). Effective control of A. gambiae melas was achieved in coastal swamp breeding grounds in Nigeria through a system of drainage. A seawall was built to separate the swampland from the tidal waters, and a sluice gate was built to be opened at low tide (Chwatt, 1949).

In Florida, salt marshes and ditches were built to follow the contours of the land, built deep enough always to have water, and connected to open waters, both natural streams and man-made canals. Savings are effected when ditches can be connected with natural streams. Adult collections of mosquitoes showed a 42 percent decline during the first four years of the ditching program (Carmichael, 1957).

The National Academy of Sciences has declared that

> Salt-marsh mosquitoes, the main problem in most districts along the Atlantic and Gulf coasts, can be eliminated by proper ditching or by impounding and flooding the

tidelands. These are major manipulations of the environment and as such are not viewed very enthusiastically by ecologists (NAS, 1976).

In the sea level swamp areas of the Panama Canal Zone, drainage is the method of choice. In areas which cannot be drained, oiling is done. Areas not accessible by land are dusted with Paris green from the air (NAS, 1976).

While dredging and filling controls salt marsh mosquitoes, it does serious damage to the shrimp industry by destroying the organic substrate on which the shrimp feed (NAS, 1976).

Most of the pioneering work in draining and ditching against malaria was done in the era before environmental concerns attained their present prominence. The elimination of water collections eliminates, of course, the aquatic life associated with them. No drainage project should be undertaken without a prior assessment of the impact on local ecology of the loss of the vector breeding place.

In this connection it is worth noting that exclusion of drainage on ecological or other grounds still leaves the planner a variety of choices.

Shading and sunning are two methods which take advantage of the fact that some vectors are sun-loving, while others prefer shade. Shading of A. gambiae breeding places through construction of hedges has been suggested as a larval control method for Africa (Anon., 1948). Clearing is a suitable, labor intensive method for such shade-loving species as A. umbrosus, and shading is useful against A. maculatus and other sun lovers (Gray, 1943). Program experience with these complementary strategies is scarce but generally encouraging. In Cuba, Ficus benjamina was planted to control A. albimanus with excellent effect (Russell et. al., 1963). It was suggested that bamboo might be equally suitable (Carr, 1938). Ficus benjamina is not suitable, however, for areas where frost occurs. Early studies of A. quadrimaculatus showed that shading of this sun-loving species does not prevent breeding, but more recent studies from the U.S. show that anopheline and culicine larvae are far less abundant in water shaded by cypress and tupelo (Taxodium distichum and Nyssa aquatica) than in nearby herbaceous shorelines exposed to sun (Smith et. al., 1969).

Shading and sunning, like all antilarval measures, must be undertaken with full knowledge of confirmed and potential vectors. They are appropriate larval control measures where there is only one vector, or where no vector will benefit from measures directed against others. Clearing of shady breeding places of A. bancrofti in northern Queensland, Australia made breeding conditions more favorable for the sun-loving A. punctulatus moluccensis (Boyd, 1949).

A method used rarely but successfully is the desiccation of breeding places by tree planting. Eucalyptus planting in South Africa is said to have served this purpose well. The trees must be spaced far enough apart to permit ground evaporation (Rafatjah, 1975; Home, 1926).

Naturalistic methods for control of stream breeders include automatic flushing. Flushing can be accomplished by building a small dam or sluice gate upstream from the breeding place which is triggered to open by a siphon or tipping bucket when a quantity of water builds up behind it. The water flushes out the downstream larvae for several hundred yards. Earth or wood dam construction reduces building costs (Gray, 1943). Flushing is suitable for such vectors as A. minimus, A. fluviatilis, A. superpictus, and A. culicifacies, which breed in slow running water or in streambed pools (Herms and Gray, 1944). It also proved effective against A. letifer in West Malaysia, where flushing and drainage brought about vector reduction in steep ravines where filling was impractical (Dugdale, 1947).

The siphon does not appear to pose a risk to fish because it reproduces in the dry months the intermittent flushes of water produced by flooding in the rainy season (Cochrane, 1943). Environmental concern about flushing centers around bank erosion in the stream immediately below the siphon; this can be minimized by timber or concrete siding. Other considerations concerning flushing, both by siphons and sluice gates, are discussed at length elsewhere (Ejercito, 1940 and 1943, Ejercito and Celis, 1943, Boyd, 1949). Techniques and methodology for siphoning are outlined by Blacklock (1939), by Williamson and Scharff (1936), and by WHO (1973b).

Pettmann has devised a method for estimating the required distance between flushing stations where more than one is required (Pettmann, 1947).

Packing of borrow pits and hollows with chopped up vegetation has been used with some success in Africa (Boyd, 1949). Application of thick grass to standing water collections achieved anopheline larval control for four to eight weeks in Papua New Guinea, but failed to control breeding of culicines (Boyd, 1949). Use of vegetation is at best a temporary measure, and, therefore, not preferable to filling. Moreover, care must be taken with this cheap measure lest it create a breeding place for any vectors which prefer overgrown ponds, such as the larvae of filariasis vectors (Travis, 1957).

A variety of strategies has been adopted to avoid the creation of man-made malaria in irrigation and other development projects. Man-made malaria produced by poor engineering can be "planned out" of a project before work is begun; this is a cheaper and simpler strategy than correcting the problem after construction is finished (Anon., 1947). Russell has listed the defects in irrigation works which create breeding places and pointed the way to their correction (Russell, 1938).

There are many cheap and simple engineering measures which can minimize malaria associated with irrigation. Removal of soil for construction of irrigation works should be from high ground to avoid creation of borrow pits; drawdown of water from impoundments should be rapid enough to assure that the shoreline larvae are left stranded. Banks should be kept clear of vegetation and debris which shelter the larvae. Small breeding places should be filled, and large ones drained or dewatered (Boyd, 1949). This listing is only illustrative. The most important measure in avoiding irrigation malaria is the training of irrigation engineers in antilarval engineering.

Water level management has been declared to be the most important single measure in prevention and control of anophelines on the Tennessee Valley Authority, with the preservation of a clean water surface being of secondary importance (Hall, 1972). A TVA publication gives detailed information on antimalarial measures to be taken in the construction of irrigation dams (TVA, 1947).

Perhaps the simplest anti-vector measure in development projects is site selection. If houses are placed far enough from breeding places to lie beyond the vector's flight range, malaria will be greatly reduced.

Where the vector prefers to bite animals, erection of cowsheds and stables between breeding places and human settlements may effect declines in man-vector contact and consequent declines in human malaria (Sergeyev, 1946). Another barrier to man-vector contact, suitable only where affordable and comformable to local custom, is the use of house screening (Bacon, 1946). The impact of screening on malaria has been demonstrated in Northern Alabama (Boyd, 1949). The limitations of screening in tropical countries have to do with initial and maintenance costs, with house building practices in some areas, and with outdoor activity of the human population at night during the malaria transmission season. While none of these obstacles is insurmountable, they should be weighed in the balance before undertaking a screening program.

When the aims of health and farm production can be harmonized, benefits accrue from the agricultural as well as the malaria standpoint. Land reclamation projects with agricultural and health objectives may have much to learn from the Italian experience. An example of the dual purposes served by Italian land reclamation is the diversion of silt-laden water into low marshes and coastal lagoons, with simultaneous elimination of breeding places and creation of farmland (Boyd, 1949). In a combination irrigation and drainage project in Sonsonate, El Salvador, excess irrigation water was conveyed through a system of drainage ditches back into the irrigation system, with the result that an <u>A. albimanus</u> breeding site was converted to farm production (NAS, 1976).

The selection of engineering strategies will depend on local conditions. The following examples are meant only to be illustrative. Strategic planning requires the combined efforts of entomology, environmental engineering, and economics.

Filling is a suitable strategy for elimination of permanent breeding places. It is of limited usefulness in areas where the vector breeds in temporary or inaccessible breeding places.

Use of herbicides is expensive, though it was found effective in Trinidad in eliminating bromeliads, parasitic plants growing on forest trees which harbored <u>A. bellator</u>. The cost and possible ecological impacts make it less desirable than cutting of plants which harbor vectors in those situations where elimination of plants is desirable.

Intermittent irrigation of rice fields has also been shown to be an effective control measure against *A. pseudopunctipennis* in Mexico. Fields were left dry for periods long enough to kill the larvae. Farmer co-operation was, of course, essential to the success of this method (Hinman, 1947). In Portuguese trials using intermittent irrigation as a control measure in rice fields, larval density was reduced by over 80 percent, with incidental agricultural benefits in the form of slightly increased yields and declines in water usage (Hill and Cambournac, 1941). Intermittent irrigation was not successful in Nyanza Province, Kenya, where the ground failed to dry sufficiently in the intervals between irrigation (Grainger, 1947).

Drainage alone proved insufficient for malaria control in Italian marshlands, since the vector continued to breed in canals and ditches. Agricultural settlement schemes used a combination of drainage, larviciding, screening and drugs to achieve control (Russell, 1955).

In combatting malaria in Delhi borne by *A. culicifacies*, antilarval measures were undertaken during the dry season, when breeding places were limited. Flood control, filling and drainage were used in areas where water could be eliminated, and fish were introduced into such permanent water sources as ornamental ponds. Areas at the periphery of the control zone were subjected to house spraying (Afridi, 1954).

Anophelines in the Panama Canal Zone found breeding places in ponds, marshes, swamps, and standing water. The control of anophelines in the Zone was accomplished by a large number of measures, cited by Gorgas in declining order of importance as drainage, brush and grass cutting, oiling, use of soluble larvicide, use of quinine for prophylaxis, screening, and adulticiding in laborers' quarters. Use of these measures effected a decline in the malaria hospitalization rate from 821 per 1,000 population in 1906 to 76 per 1,000 in 1913 (Russell, 1955).

The costs of environmental engineering can be very low when the major expenditures are for labor, as in filling. Where epidemiological conditions permit, operations can be undertaken during the agricultural slack season, when labor is abundant. Filling is perhaps unique among vector control methods in that it entails no running costs.

While environmental engineering measures often incur high initial costs for materials, they may be cheaper than chemical larviciding over the long run. The Northern Salinas Valley Mosquito Abatement District estimates that drainage costs are offset by savings in chemicals within five to ten years (NAS, 1976).

Published data on the efficacy of source control measures are scanty, though a professional consensus has developed that they are generally effective for reducing, but not interrupting, transmission. During the era of time-limited eradication, they were grouped with other anti-larval measures among the supplementary attack measures useful as adjuvants to house spraying. The advent of revised strategies for control and eradication as a long-term goal makes them more relevant.

The foregoing description is intended only as an overview of methods. The techniques of engineering suitable for larval control have been set out at length elsewhere (Scharff, 1935; Herms and Gray, 1944; Tennessee Valley Authority, 1947; Covell, 1941; Herms and James, 1961). A current view of engineering methods suitable for urban malaria control is provided by Carmichael (Carmichael, 1972; WHO, 1973b; WHO, 1974a).

2.3 Combinations of measures

Many malaria programs have chosen to control the vector by a variety of methods. Israel's successful eradication effort, for example, put heavy emphasis on a large variety of antilarval measures aimed at the four vectors. These included larviciding, water regulation, use of larvivorous fish, control of vegetation, and drainage (Saliternik, 1977).

Beginning in 1944, a wide variety of anti-vector measures, such as siting, larviciding, drainage, filling, provision of pumped water, mosquito-proofing of houses, and mass drug administration was used to control malaria during the construction of the Sarda Hydel Dam in Uttar Pradesh, India. These measures were supplemented in 1946 by DDT house spraying. Spleen and parasite rates declined from baseline levels (Srivastava and Chand, 1951).

Efforts to interrupt transmission in sub-Sahara Africa by a combination of insecticide application and mass drug administration have usually failed (Hamon et. al., 1963). DDT spraying coupled with mass drug administration failed to interrupt savannah transmission by *A. gambiae* and *A. funestus* in northern Nigeria (Najera *et. al.*, 1973). In Dar-es-Salaam, Tanzania, however, a combination of drainage, oiling and residual spraying against urban *A. gambiae* was associated with steep declines in vector density. Control measures and changes in vector habits due to urbanization were credited with effecting the declines (Bang *et. al.*, 1973).

Some programs have undertaken to reduce transmission of malaria through mass drug administration, the periodic door-to-door distribution of antimalaria drugs. The operational constraints inherent in mass drug administration, both done alone and together with vector control measures, have been discussed by Gilroy (Gilroy, 1959). Mass drug administration of chloroquine used as a supplementary attack measure in DDT-sprayed areas of Syria was associated with a decline in the slide positivity rate. Transmission ceased after application of the joint measures, despite an increase in the density of *A. sacharovi* adults (Onori, 1972). Mass drug administration of amodiaquine, primaquine, and chloroquine was conducted in conjunction with DDT spraying in Zanzibar in 1968. It failed to effect a reduction in overall malaria incidence in Zanzibar, despite better than 80 percent coverage by mass drug administration and 98 percent coverage by spraying (Dola, 1974).

In a rural Haitian area where *A. albimanus* was unresponsive to a combination of DDT spraying and mass drug administration, larviciding at twice-monthly intervals and large-scale drainage succeeded in reducing malaria incidence and vector density (Schliessman *et. al.*, 1973).

Laboratory trials using *Poecilia reticulata* with a number of larvicides showed temephos to be the most effective agent for control of *Culex pipiens fatigans*. OMS-1210, OMS-1211, fenthion and fenitrothion were also effective and did not kill the larvivorous fish. Chlorpyrifos, while shown to be an effective larvicide, inhibited feeding by the larvivorous fish at low concentrations (Rongsriyam *et. al.*, 1968).

Combined use of chemicals and larvivorous fish has been defended on grounds that it requires weeks or months for the predator population to build up to the point where mosquito populations are suppressed, and that chemical larviciding during this build-up period, done at dosages not toxic to the predator, is necessary to maximize its effectiveness (Mulla, 1970).

From an ecological standpoint, there is an advantage in the combined use of fish and larvicides: the dosages for the larvicides can be reduced from the levels which would have been necessary if they had been used alone (Mulla, 1970).

One area for the useful application of combined measured is in rice fields, where environmental and chemical control measures can complement each other by respectively reducing the areas which favor mosquito breeding and controlling the larval populations which survive source reduction. The task is less daunting than it might appear when one remembers that only fields within flight range of night-time human habitations require treatment. Surtees has reviewed the methods suitable for rice fields (Surtees, 1971).

In urban areas, residual house spraying is very difficult because householders are usually reluctant to have their homes sprayed. Control of water sources through engineering should be an adequate method for control of urban malaria, but in practice the creation of man-made breeding places, especially in illegal squatter towns, may require antilarval measures, such as the use of fish, the imposition of requirements that water receptacles be covered or larvicided, and the establishment of adequate garbage collection services. In their comprehensive survey of urban vector control services in less developed countries (1975), Graham and Gratz have noted the following characteristics of many programs: 1) high per capita expenditures in many cases, sometimes as much as US$7.87 per year, 2) severe shortages of technical personnel, with the likelihood of errors in choice of vector control strategy, 3) the need for more attention to good sanitation as part of a combined approach to vector control, 4) frequent absence of such basic data as susceptibility of vectors to pesticides being used. Since running costs of urban vector control programs are so high, and since there are often several vector-borne diseases in one city, there is a clear need for entomological expertise as a cost-effective investment.

3. BIOLOGICAL CONTROL METHODS CURRENTLY UNDER DEVELOPMENT

The use of biological control agents for vector control has been described by Wright *et al.* (1972):

> "The use of insect predators, parasites, and microbial agents for mosquito control has been under investigation for more than 50 years. Some of these, particularly larvivorous fish, have shown effectiveness in regulating densities of mosquitoes, but only in limited areas. However, the full potential of known biological agents has never been fully investigated and demonstrated. A number of microbial agents have been discovered which are known to exert certain levels of control in nature. Others have been shown to be virulent in various degrees in the laboratory. Some of these natural biotic agents could be mass produced, particularly certain bacteria, viruses, and nematodes. Such pathogens and others that are shown to be potentially useful need to be evaluated under practical conditions.
>
> "The ideal biological control agent is one that becomes self-sustaining after release at a density that controls the pest at a level compatible with man's requirements. In general, however, biotic agents do not fulfill this requirement because, in order to survive, they may greatly reduce populations of their host or prey but must not entirely eliminate them. For this reason, biological control is most likely to succeed if numbers of the agents are artificially increased by inundative release. Larvirorous fish constitute exceptions to this generalization because, although feeding essentially on mosquito larvae, they can also sustain themselves on a diverse diet of other organisms when larvae are no longer present."

3.1 Genetic Control

Genetic control of mosquitoes is based on the release of sterile or partially sterile individuals into natural populations of the species to cause sterility or to alter or replace hereditary material. The F_1 sterile insects (predominantly male) can be produced by direct exposure to doses of chemical radiation that cause death in the female offspring or result in heritable recessive mutations that are maintained in special strains. Numerous theroretical approaches to the use of genetic mechanisms for mosquito control have been proposed. However, except for the sterile insect method (SIM), research on development of these mechanisms for practical mosquito control has been limited to the selection and characterization of strains and the determination of their suitability and behavior.

Genetic control of mosquitoes has certain advantages over traditional methods of control. Most genetic control techniques are not subject to increased biochemical or behavioral resistance and sterilized insects possess the ability to seek out fertile individuals. The latter advantage is particularly important in geographical areas that are inaccessible for traditional application control methods. Problems with natural selection and biochemical resistance to traditional insecticides is well known and has been discussed in section 2, of this paper.

Another kind of selection that occurs in traditional methods and is not so easily recognized, is selection for behavioral mutants. Mosquitoes which enter houses are killed, those which stay outside are not, a typical example of behavioral polymorphism in the population. Heavy selections of these types can lead to rapid changes in a population, especially in one of reduced size (Kitzmiller, 1972).

Due to the complex life cycle of mosquitoes, either larvae, adults, or both may be unreachable by insecticides because of vector behavior, house construction practices, or human behavior. Genetically sterilized insects seek out fertile individuals in environmental sites normally protected from insecticides; they also have an impact on several generations given the varying species' characteristics (Kitzmiller, 1972; and Lofgren, 1974a).

There are many possible methods of genetic control, but only the principal types -- sterile insect method (= sterile insect release method (SIRM) and sterile male technique), cytoplasmic incompatibility, hybrid sterility, chromosomal translocations, conditional lethal genes, gene replacement, chromosomal inversions, population replacement, meiotic drive, and compound chromosomes -- will be discussed in this paper.

3.1.1 Sterile Insect Method

"The sterile male technique is one of the most intriguing methods of insect control developed during the past several decades. Its practicality was demonstrated in the 1950s in the USA against the screw-worm fly, *Cochliomyia hominivorax* and it has since been evaluated against numerous other insects. However, it has only been in the past few years than an application to mosquito control has been successfully demonstrated (Lofgren, 1974a)."

The sterile male technique may be utilized in two different methods. One involves the mass rearing, sterilization and release of enough insects over a sustained period of time to overwhelm the natural population. The second method involves treatment of the natural population with a chemosterilant which will induce sterility in the female progeny. Due to possible hazards associated with direct application of chemosterilants to natural populations, environmental control, and the distinct lack of an efficient means of attracting mosquitoes to chemosterilant baits or treatments, "the latter method does not seem practical at the present (Weidhaas, 1972)."

The sterile male technique is not so simple a method as would seem. It requires that the scientist deal with natural, ever-changing populations over extended periods of time. It is also necessary that the scientist completely understand the biology, ecology, and dynamics of large insect populations as well as the epidemiology of the disease in question.

The successful utilization of the technique itself is dependent upon certain requirements (outlined by Knipling, 1968b) which include: 1) a method of rearing the insect in large numbers; 2) a sterilization method without serious adverse effects on mating behavior and competitiveness; 3) methods for dispersing sterile insects; 4) quantitative information on the density and growth rates of natural populations; 5) a practical means for reducing natural populations to levels manageable with sterile insects; and 6) costs that are favorable in relation to costs for current methods of control, and losses or economic damage from the insect. "However, if all these problems can be solved, the techniques can be very effective. Indeed, it is more effective and less expensive than insecticidal control when population densities are low (Lofgren, et al., 1974a)."

The two methods of employing the sterility principle have important differences in their effect on population trends as noted in the following tables first postulated by Knipling (1968a and b):

Table 1

Model of Population Trend in a Stable Insect Population Subjected to Continued Release of Sterile Males Each Generation

Generation	No. of Insects	No. of sterile insects released	Ratio	No. of insects reproducing
1	1,000,000	9,000,000	9:1	100,000
2	100,000	9,000,000	90:1	1,099
3	1,099	9,000,000	8000:1	0

Table 2

Model of Population Trend in a Stable Insect Population Subjected to a Treatment Which Sterilizes 90% of the Population Each Generation

Generation	Number of insects in population	Number fertile	Number sterile	Number of insects reproducing
1	1,000,000	100,000	900,000	10,000
2	10,000	1,000	9,000	100

3	100	10	90	1

Source: Knipling, 1968b.

It should be noted that the release technique (Table 1) gains more effectiveness as the population decreases and the sterile-fertile ratio increases. The chemosterilant treatment technique, however, carries the same effect per treatment, only its initial impact is much greater. The combined effect (assuming the steriles are equally competitive with the fertiles), is that reproduction rates are reduced to one percent. If an insecticide or similar biological activity were employed at the 90 percent efficiency level, the rate of surviving reproducing mosquitoes would only be 10 percent. Thus, according to Knipling (1968a and b), the chemosterilant method at the 90 percent efficiency level is potentially ten times as effective as conventional methods utilizing insecticides, in terms of number of reproducing mosquitoes.

Although ionizing radation (X-ray and gamma irradiation) was the first means of sterilizing insects for release, certain chemicals are also effective in causing sterility (Kitzmiller, 1972; Proverbs, 1969; and Weidhaas, 1972). A summary (LaBreque and Smith, 1968) of research on chemosterilants, a complex field that has been studied for many years, is available and includes: 1) methods by which chemosterilants could be used for control; 2) theories behind the use of chemosterilants; 3) use of chemosterilants as biological tools for studying population dynamics and behavior; 4) screening and laboratory evaluations of chemicals as sterilants; 5) types of compounds that sterilize insects; 6) mode of action, including uptake and persistence; 7) development of resistance to chemosterilants; 8) effects of chemosterilants on mating competitiveness, vigor, and survival; 9) field studies on the use of chemosterilants; 10) biological and ecological requirements; and 11) integration with other methods of control (Weidhaas, 1972).

Past and present research has shown the use of alkylating agents to be a promising method of chemosterilization of mosquitoes for control purposes (Das, 1967; Dame *et al.*, 1974; Eddy *et al.*, 1965; Labreque and Fye, 1978; Lofgren *et al.*, 1973; Seawright *et al.*, 1973 and 1974; Smittle, *et al.*, 1968;

Weidhaas, 1962; Weidhaas *et al.*, 1963 and Wijeyaratne *et al.*, 1977). In a review by La Breque and Fye (1978) and research by Glancey (1965), it was found that hempa (hexamethyl melamine), in addition to tepa (tris(1-aziridinyl)phosphine oxide), metepa (tris(2-methyl-1-aziridinyl)phosphine oxide), and aopholate (2,2,4,4,6,6-hexakis(1-aziridinyl)-2,2,4,4,6,6-hexahydro-1,3,5,2,4,6-triazatriphosphorine), also accomplished sterilization in over 65 species of insects.

The first successful demonstration of the use of chemosterilized males for the control of a mosquito species was made in 1969 (Patterson *et al.*, 1970a and b). The test was conducted on Seahorse Key, a small island 2 miles off western Florida by the Entomology Research Division, Agricultural Research Service, USDA. The *Culex* population was controlled and almost eliminated by the release of thiotepa-sterilized males for a period of about 10 weeks. With the release of an average of 8,400 to 18,000 sterile (fully competitive with fertile) males per day during the second thru sixth generation, "a significant suppression of the adult population (was obtained) as evidenced by a 96 percent decrease in egg rafts."

In 1972 teams from Insects Affecting Man Laboratory, Agricultural Research Service, USDA, and the Central America Research Station (CARS), CDC, USDHEW, conducted a joint study in El Salvador with *Anopheles albimanus* (Lofgren *et al.*, 1974a and b). Releases of chemically-sterilized males obtained from a colony derived from females collected (Dame *et al.*, 1974) from the shores of Lake Apastepeque (Breeland *et al.*, 1974) were made between 19 April and 15 September around the lake and in the low, marshy area adjacent to it. The total number released for the period was 4.3 million males; the average per day ranged from 15,000 to 40,000. The results of this study showed that no biting female mosquitoes were collected in September, and only a few were collected in the succeeding two months. The previous year averaged 103 man-biting mosquito collections per person/hour in that same time frame. This study clearly illustrates a high degree of effectiveness of sterile males in reducing the population of *Anopheles albimanus*.

In Delhi, 1971, Sharma *et al.* (1975b) made three field release trials utilizing chemosterilized males (thiotepa) of *Culex pipiens fatigans*. The sterile males were released as pupae or adults. They found that adult release was superior to pupal release because initial pupal and early adult mortality (from natural hazards and parasites/predators) could be avoided. In the three releases, mosquito sterility was found to range from 39-95 percent after a six to eight week period; the expected sterility was 75 percent.

The largest research effort concerning mosquito control by genetics was conducted by WHO's Research Unit on Genetic Control in New Delhi which investigated only *Aedes aegypti* and *Culex pipiens fatigans* (Lofgren, 1974a; Rao, 1974; and WHO/UNEP, 1976). The research emphasized various methods of introducing sterility in males, development of mass-rearing methods, and effects of the release of the sterile males into natural populations. However, due to unforseen circumstances at the Unit, the research yielded no quantifiable results.

As the El Salvador study (Dame *et al.*, 1974) only illustrated the reduction in *Anopheles albimanus* and provided no data on the effect of mosquito reduction on malaria transmission, another study is presently being conducted in El Salvador by the Agricultural Research Service and the Center for Disease Control. The sterile male technique will be integrated with insecticidal and sanitation control to assist in reducing and maintaining the population at a very low level (Lofgren, 1974a).

3.1.2 Ionizing Radiation

The other leading sterilizing technique utilized in the sterile insect method is ionizing radiation, generally x-rays or gamma irradiation (Ali *et al.*, 1972; Darrow, 1968; Patterson, *et al.*, 1975; Patterson *et al.*, 1977; Smittle *et al.*, 1968; and Smittle *et al.*, 1971).

Although the future possibilities of utilizing chemosterilants are encouraging, there is some concern over environmental contamination (Hayes, 1968) from possible chemosterilant on the released mosquitos even though Seawright *et al.*, (1971) have shown that released chemosterilized males contained very little residue three days after eclosion. The use of radiation for sterilization would eliminate this residue problem (Patterson *et al.*, 1977). However, problems do occur with irradiation in that early studies (Davidson and Kitzmiller, 1970; Davis *et al.*, 1959; Morlan *et al.*, 1962; Schmidt *et al.*, 1964; Ramakrishnan *et al.*, 1962; and Smittle *et* al., 1968) have shown a noticeable decrease in sexual competitiveness of the sterilized males. Most of these studies were carried out with pupae irradiated at an unknown age. Subsequently, Patterson et al. (1975 and 1977) have demonstrated an improvement in the competitiveness of sterile males when they are irradiated as adults or older pupae. And, Wijeyaratne et al. (1977), by altering dosage levels and competitiveness, were able to determine a theoretical irradiation dose level which would be most effective against a given number of insects in the population with a known growth rate.

The last three studies were completed using Culex mosquitoes, not Anopheles, in field-cages only; their theory and applicability for field use with Anopheles are unknown.

One final point should be emphasized concerning the differences between chemosterilization and irradiation. As in the case of insecticides, the reduction of the number of insects by means of chemicals may result in the development of resistance to them by natural selections and pre-adaptations. Hazard et al. (1964) demonstrated the increased resistance of Aedes aegypti to apholate, a chemosterilant, beginning with the F_4 generation in a controlled laboratory study. Klasser and Matsumura (1966) also showed a tendency for increasingly resistant Aedes aegypti in the F_4 generation in a laboratory study utilizing metepa as a chemosterilant. Both studies acknowledge that field populations or other application techniques might produce different results.

3.1.3 Cytoplasmic Incompatibility

A field study in Burma (Laven, 1967) has shown that the crossing of allopatric strains of Culex pipiens fatigans would result in the production of embryos that will not hatch. Laven has attributed the lack of offspring to an inherited cytoplasmic incompatibility.* In an incompatible cross, the

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* Yen and Barr (1971) have postulated one cytoplasmic incompatibility theory based on an inherited infectious agent -- a "rickettsia-like organism...(possibly Wolbachia pipientits)." "It is possible," they stated, "that the microorganisms have a deleterious effect on presumably 'compatible' sperm so that they are unable to participate with the oocyte nucleus in the

sperm is blocked before it can fuse with the haploid egg nucleus. If the embryos develop, they do so from the haploid egg nucleus and die before hatching.

Following that demonstration, Laven and Aslamkhan (1970) proposed the use of cytoplasmic incompatibility combined with a male-linked translocation complex to form an integrated strain as a genetic control agent. Research on that proposal was conducted and assisted by the WHO/ICMR (World Health Organization/Indian Council of Medical Research) Unit on Genetic Control of Mosquitoes in Delhi (Brooks, et al., 1976; Curtis, 1975; Krishnamurthy, 1974; Krishamurthy et al 1975b; Rao, 1974; Subbarao, 1975; and Wright et al., 1972). The idea was to develop males that were cytoplasmically incompatible with Indian populations and strains that would require a male-linked translocation causing partial sterility in matings. This would prevent build-up of a large population of the released strain after elimination of the indigenous population. The strain, *Culex* 14-31B, was successfully developed (Krishnamurthy and Laven, 1974) and it has proven to be competitive (WHO, 1975) and effective (Rao, 1974) in causing a distinct population decline in field testing.

The principle of the cytoplasmic incompatibility method is comparable to that of the sterile male method used in programs with radiation treatment, with the difference, however, that the incapacitating effects of irradiation are avoided. The possibility of utilizing chemosterilants to effect incompatibility-translocation type changes in mosquitoes has also been discussed in the literature; however, "the logistical processes inherent in (such a)... technique appear to make it impractical for anopheline control" (Wright et al., 1972). Also, it should be noted, this method requires complete separation out of all females as a few released females could rapidly begin a new strain.

3.1.4 Hybrids

Frequently, when closely related species of mosquitoes mate, their chromosomes fail to pair and the hybrid offspring are sterile. The effect is usually greater in the male than in the female hybrid offspring (National Academy of Sciences, 1973). Indeed, Davidson et al., (1970), Oguma (1978), and the

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formation of a zygote."

WHO Scientific Group on the Genetics of Vectors and Insecticide Resistance (1964) have well documented this potential control technqiue with cage studies. However, attempts to demonstrate this technique by Fukuda and Woodard (1974) with Aedes, and by Davidson et al. (1970) with Anopheles have elucidated a possible ethological factor -- a mating barrier preventing mating between the introduced sterile males and natural females. Nevertheless, the hybrid technique of genetic control is still considered promising by those in the field.

3.1.5 Chromosome Translocations

Another possible method of genetic control is the use of chromosome translocations, rearrangements in which reciprocal exchanges take place between different chromosomes of a complement. Radiation can be used to encourage breaks at indiscriminate points along two of the three mosquito chromosomes, which, if the pieces rejoin on different chromosomes, will cause an abnormal arrangement. The rearranged chromosomes still contain all the genetic information required for survival and growth. However, irregular pairings occur during meiosis. This, in turn, results in 35-80 percent sterility in the offspring of a translocated male and normal female (Asman, 1976; Kitzmiller, 1972; Laven, 1969; Rabbani and Kitzmiller, 1972; and Seawright and Kaiser, 1976). It has also been postulated that 100 percent offspring sterility could result in the mating if all three chromosomes were translocated (Pal and La Chance, 1969).

Pal (1974) and Wright et al. (1972) are of the belief that translocations which are self-propagating and are not naturally derived are the most promising of the genetic control methods. The advantages of this method are (a) it is unnecessary to separate the females from the males before release, and (b) there is a sterilizing effect on subsequent generations. Another advantage, according to Whitten (1971), is that the release of several translocation strains in sterile heterozygotes would appear to be more effective with less effort than the sterile male method and may therefore prove economical for some species. He points out that a single release of four synthetic strains, in equal frequency to the native population, can be shown to cause a population reduction equivalent to a 20:1 sterile-to-normal-male release repeated for five generations.

Relatively few pilot experiments have been conducted utilizing this technique of genetic control. However, a field trial performed with *Culex pipiens fatigans* in southern France achieved a virtually sterile population within five weeks (Pal, 1974); another study by Seawright *et al.* (1976) has demonstrated translocated heterozygous *Aedes aegypti* to be competitive with males of a mutant strain in the laboratory and in outdoor cages.

The WHO/IMCR Unit had investigated translocated strains of *Culex pipiens fatigans* (Krishnamutthy *et al.*, 1975a), *Aedes aegypti* (Uppal *et al.*, 1975) and *Aedes stephensi* as control vectors, but it is clear that a number of ecological and biological problems will need to be solved before its application is feasible on a large scale (Pal, 1974; and Wright *et al.*, 1972).

3.1.6 Other Methods of Genetic Control

Other genetic methods of control, which are still in the developmental stages, includes the following: conditional lethal genes; gene replacement; chromosomal inversions; population replacement; meiotic drive; and compound chromosomes.

Conditional Lethal Genes

Manipulation of genes which cause behavior that under certain conditions is lethal for the organism is a promising mosquito regulatory technique. Examples of such conditions are temperature sensitivity, inability to diapause and inability to fly. One advantage of this technique is that individuals with the lethal character continue to breed for some time after their release so the lethal gene is transmitted to future generations (Knipling *et al.*, 1968; National Academy of Sciences, 1973; Pal, 1974; Wehrman and Klassen, 1971; and WHO, 1964).

Gene Replacement

A vector species need not be eliminated to control the disease it carries. Deliberately replacing vectors with strains that are of the same species but do not transmit the disease might accomplish disease control without reducing the overall number of mosquitoes. Already, genetic differences that render mosquitoes refractory to malaria or filaria parasites -- or change their choice of host -- have been demonstrated. These findings suggest that practical methods to replace vectors by harmless strains or species may be developed (National Academy of Sciences, 1973).

Chromosomal Inversions

Inversions are known to be common in mosquito populations. When homozygous, pairing is easily accomplished. Heterozygotes survive well as individuals, but have problems at meiosis. Crossing over takes place within the inversion (proportional to the length of the inversion) and produces dicentric chromosomes and acentric fragments. Cells in which this happens produce reduced numbers of viable gametes as only two of four chromatids survive after pairing. Release of males homozygous for inversion would result in an F_1 generation which would produce up to 50 percent viable gametes (Kitzmiller, 1972).

Much of the current research on chromosomal inversions is being conducted by Rabbani *et al.* (1976 and 1977).

Population Replacement

If circumstances arise in which the elimination of a vector population leaves a vacuum which might be filled by a dangerous replacement, innocuous strains could be substituted to fill the ecological niche. Before this approach becomes feasibile, further investigation on the genetics of vector ability to transmit disease is required (Knipling *et al.*, 1968; and WHO, 1964).

Meiotic Drive

Meiotic drive concerns the unequal distribution of a pair of homologous chromosomes to the functional products of meiosis. Several meiotic drive mechanisms have been described (Hickey and Craig, 1966; Suguna *et al.*, 1975; and Zimmering *et al.*, 1970). Once introduced into a population, the drive factors should theoretically continue to increase in frequency until they are fixed in the population (Hamilton, 1967; and Suguna *et al.*, 1976). Meiotic drive might also be used to cause sex ratio distortion, an increase in the frequency of some other deleterious factor, or utilized in conjunction with translocations (see 3.1.5). Further discussions of meiotic drive for insect control can be found by Waterhouse et al. (1973). Strains of Culex pipiens fatigans and Aedes aegypti with meiotic drive were investigated by the WHO/IMCR Unit (Pal, 1974; and Pal and La Chance, 1974). Some results have indicated that there might be some resistance to meiotic drive inherent in Aedes aegypti (Suguna et al., 1976).

Compound Chromosomes

The synthesis of compound chromosome stocks for insect control has been proposed by Foster et al. (1972). A rather extensive study of the formal genetics of the target species is required before such stocks can be developed. Compound chromosome stocks are characterized by low fertility inter se and complete sterility in all out-crosses. They have potential use as they combine the features of sterility with those of population replacement by a strain with desired characteristics. The only species where compound chromosomes have been developed is Drosophila melanogaster, the fruit fly, but research is ongoing in Phaenicia (=Lucilia) cuprina and in Musca domestica, the housefly (Pal, 1974; and Pal and La Chance, 1974).

3.2 Attractants

The use of attractants -- both natural and synthetic -- for the control of insect pests was noted as one of the "new, imaginative, and creative approaches to the problem of sharing our earth with other creatures (Carson, 1962)." While many concerned with environmental quality applaud this approach, its practicality for mosquitoes remains to be demonstrated (Beroza, 1972).

Mosquitoes have developed highly efficient olfactory organs as a result of evolutionary pressures; they rely heavily on this sense of perception to secure blood-meals and mate. Many of the odorous chemicals that attract mosquitoes are available only in minute amounts. Pheromones are examples of such chemicals; they are compounds emitted by one member of a species to influence the behavior or development of others of that species. Phermones can be sex attractants, aphrodisiacs, assembling scents, masking scents, or chemicals inducing alarm, foraging, recruitment, trail following, and swarming (Beroza, 1972).

Host attraction for mosquitoes, explained as heat, moisture, carbon dioxide, odor, visual factors and combinations thereof (Clements, 1963), is so complex that workers in the field are unable to postulate a unifed theory for its use (personal communication, ARS, USDA). Some researchers believe that warmth and moisture are the only important attractants (Wright, 1975), whereas others think highly of other chemicals as well as L-lactic acid as attractants, or, at the very least, main components (Acree et al., and 1968; and Smith et al., 1970).

Many of the problems in studying mosquitoes' attraction to man are caused by the number of variables and need to monitor each within very narrow limits. Other problems are relative to the time and cost necessary to procure and/or synthesize a new compound, including a satisfactory bioassay of the mosquito species; species differences due to normal biological variations; and trap or field application and dissemination design. A means of measuring the response of the insect to candidate chemicals must also be designed. As noted by Beroza (1972), "testing for an attractant is much more difficult and less certain that testing for an insecticide."

Attractants have proven to be invaluable in survey applications. Traps are baited with an attractant and responding insects that are caught signal their presence in that area. This information can be used to direct (or modify) control or eradication operations (Beroza, 1972).

The development of attractants for vector control is still at an early stage. In most instances, the attractant will need to be combined with a powerful insecticide to be effective. In others, it is postulated, it would be possible to permeate the area with an attractant, either by spraying, airplane dispersal, or strategically located evaporators (Birch, 1974). With attractant everywhere, the males should not be able to locate the females' scent, or their odor receptors may simply become fatigued or saturated, thereby preventing them from responding and mating (Beroza, 1972; and McLaughlin, et al., 1975.

3.3 Insect Growth Regulators

Two new groups of chemicals which act by interfering with insect development have been synthesized and studied in recent years as potential insecticides. One group (juvenoids), represented by methoprene (Altosid^R) and R-20458, mimics the action of insect juvenile hormones. Thus it can cause death by preventing the transformation from larva to pupa if applied at a critical period of sensitivity, the second half of the last larval instar. A second group of development inhibitors, OMS-1804 (Dimilin^R), block the chitinization process during metamorphosis of immature insects, and thus, can cause mortality at any instar or during the pupa-to-adult transformation (Julin and Sanders, 1978; and WHO, 1977).

Juvenile hormones (JH) can also influence adult mosquito development and maturation. For example, if the mosquito is exposed to JH, then ovarian development, yolk formation, egg maturation in the females, and development of the accessory glands in the males may be influenced (Craig, 1970; National Academy of Sciences, 1973; and Riddiford, 1970).

The process of JH activity has been described by Bowers (1971) and Spielman (1970). During the moulting process, the presence of JH prevents cellular differentiation and, therefore, maturation. In the absence of JH, larval development, growth, and maturation towards the adult ensues. The application of a JH chemical to a stage of development that is due to undergo maturation results in the formation of an intermediate that is incapable of further development and dies. For ultimate sensitivity, the hormone treatment must be applied during the early phase of pupal development since JHs appear to interrupt normal physiological processes associated with genetic transmission and metamorphosis (Bowers, 1971; National Academy of Sciences, 1973; Spielman, 1970; and Staal, 1975).

Bowers (1971) further states that large doses of JH cannot reverse the cellular differentiation process. Hence, the use of JH for control purposes must be restricted to situations in which they can be applied during the most sensitive period of larval development, or whenever continuous contact throughout the life cycle is possible.

Given their high selectivity and unique modes of action, these chemicals raised hopes that their efficacy might not be seriously affected by resistance (WHO, 1976). However, it has been shown that intensive selection of houseflies, *Culex tarsalis* and *Culex pipiens* with methoprene leads to direct resistance to methoprene and related compounds (WHO, 1976).

Despite the demonstration of resistance to development inhibitors, it is noteworthy that the level of resistance achieved in mosquitoes toward methoprene is relatively low and that the chemical continues to exhibit a high degree of activity against selected strains. Further, under field conditions, natural selection by juvenoids may be expected to be relatively weak, due to asynchrony in most natural populations (except with the slow release formulations). This advantage may be pronounced in the case of chitinization inhibitors as they repeatedly affect insects in the course of larval development (Hsieh, 1974; and WHO, 1976).

Juvenile hormones are used in the same way as conventional larvicides with the exception of the timing of applications. Thus the equipment and manpower needs are similar (National Academy of Sciences, 1973).

Some of the advantages of the utilization of JHs as a vector control measure are: JH chemicals readily penetrate the insect integument and are biologically active at concentrations well below those of conventional insecticies; they have greater specificity for mosquitoes than conventional insecticies; they can be applied with standard ground or aircraft equipment; workers with elementary training can be employed; and control methods by JH can probably be used in conjunction with other control methods (National Academy of Sciences, 1973; and Robbins, 1972).

Most of the past and current research concerning the use of IGRs for mosquito control has focused on _Culex_ mosquitoes (Sales and Hervy, 1977; and Self _et al._, 1976); _Anopheles_ studies that were reviewed are inconclusive.

In a most recent study, OMS-1696 (methoprene) and OMS-1697 (diflubenzuron) were tested against _Culex quinquefasciatus_ (Self et _al._, 1978). Treatments were made in areas of one square kilometer in Jakarta with an applied dosage of approximately 1 ppm. A female landing population reduction of 85 percent was noted from the 2nd to 5th week after spraying a 0.5 percent suspension of methoprene on the study areas. The larval control demonstrated with diflubenzuron was shorter, however, as it only reduced the population by 54 percent up to ten days after spraying.

Case and Washino (1978), in a similar study with methoprene only, demonstrated a 50 percent reduction of adult

Culex tarsalis immediately after spraying. However, by the fourth day the rate had dropped to 10 percent; by the seventh day the treatment showed no significant difference. They also examined the kill rate for the 10F charcoal formulation of methoprene in the same study. It demonstrated a 50 percent reduction after spraying, 30 percent at the fourth day, and no significant reduction on the seventh day. Although, *Anopheles freeborni* was not the test organism, it was present in the study area, and it showed a significant increase during this time. This was attributed to natural seasonal growth.

Mathis *et al.* (1975) completed a field trial of both OMS-1697 (Altosid[R]) and OMS-1804 (Dimilim[R]) against *Culex pipiens* in Seoul. At 200 gm/ha in a liquid suspension, the charcoal formulation of OMS-1697 (10F) demonstrated a *Culex* mortality of greater than 70 percent for one week. (Their laboratory findings show greater than 70 percent mortality for 16 days with treatment 1000 gm/ha of OMS-1697 (10F) in liquid suspension.) Mathis *et al.* also demonstrated a *Culex* field mortality of 70 percent at 50 gm/ha of OMS-1804 for two days as shown from larval collections; at dosage rates of 100 and 200 gm/ha, 70 percent mortality was demonstrated at 7-10 days.

In other studies, Sharma *et al.* (1975a) achieved an 80 to 100 percent control of *Culex pipiens fatigans* breeding in polluted drains by application of target dosages of 0.5 ppm and 1.0 ppm of OMS-1804 (Dililin[R]), an insect growth regulator; Steelman and Schilling (1972) demonstrated a 100, 96 and 84 percent overall mortality resulting from application of 2.0, 1.0 and 0.5 ppm of Mon-585, a hormone-like compound, in the laboratory; and Jakob and Shoof (1972) completed a simulated field test involving six species of mosquitoes, including *Anopheles albimanus.* Their results have indicated that a higher anopheline larval mortality results from treatments of Mon-585 to the fourth-instar as opposed to the third.

"The effect of diflubenzuron on target organisms seems to be well documented. However, information for evaluating its safety to fish and fish food organisms is lacking" (Julin and Sanders, 1978). In a laboratory study, Julin and Sanders (1978) examined potential toxicological effects of dibflubenzuron and three of its degradation products to freshwater fish and invertebrates. Their findings indicate that diflubenzuron, applied in general mosquito control rates of 0.02-0.04 lb. AI/acre results in initial concentrations* that are well below those that would affect fish populations. They do report, however, that such concentrations would adversly affect populations of daphnids and scuds. They conclude that more in-depth studies concerning the accumulation or chronic toxicity to diflubenzuron after repeated applications are necessary.

3.4 Pathogenic Bacteria

Jenkins (1964) has indicated that as many as 22 species of bacteria have been found naturally occurring in mosquitoes. However, this group has not been well studied, though some species of bacillus do lend themselves well to mass production (Chapman, 1974).

Spore forming bacteria include species that produce toxins that act as poisons in insects. A crystalliferous, spore-forming bacterium, Bacillus thuringiensis has been reviewed by Heimpel (1967). Various strains of this microorganism have been commercially prepared for use in agricultural production for the past 15 years (Wright et al. 1972). Recent studies have shown limited Aedes and Culex control using Bacillus BA-068 which was isolated from dead Culex tarsalis larvae (Chapman, 1974; Reeves, 1974; and St. Julian et al. 1973).

Many species of Culex, Culiseta, Aedes, and Orthopodomyia show varying degrees of susceptibility to Bacillus sphaericus in the laboratory (Goldberg et al., 1977). However, it was only recently that a successful pilot-test has shown the possible effectiveness of this organism. Ramoska et al. (1978) demonstrated a reduction of approximately 90 percent in Culex nigripalpus and Psorophora columbiae with Bacillus sphaericus at three test sites -- a ditch, and two field depressions -- using undiluted bacterial suspensions in a 12-liter hand compression portable sprayer.

One of the advantages of spore-forming bacteria is that they produce dormant, desiccation- and heat-resistant, thick walled, refractile spores, and thus can survive outside the host. One disadvantage is that they need periodic

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* 0.1 and 0.2 mg liter at depths of 10 cm in natural waters.

applications, which, depending on the amount required, could result in excessive costs (National Academy of Sciences, 1973).

There is no indication of any environmental problems related to crystalliferous species of bacillus (National Academy of Sciences, 1973).

3.5 Fungi

Investigations of fungi for control of mosquito larvae has been actively pursued; Jenkins (1964) identified 47 fungal species as pathogens of mosquitoes (Chapman, 1974), and Roberts (1970 and 1974) has summarized some of the later information on *Beauveria*, *Coelomomyces*, *Entomophthora*, and *Metarrhizium* (Chapman, 1974; and Wright *et al.*, 1972). Of all the fungi available for control, *Coelomomyces* has been the most extensively studied and seems to hold the most promise as a control agent (Couch, 1972).

Fungi kill their larva hosts by infecting them and destroying the tissues (fat body) essential for the development of adults. Despite the infection, a few larvae do pupate and produce infected adults that disseminate the fungus to other water habitats (National Academy of Sciences, 1973).

The fungus produces infective stages (sporangia) within the larvae. The mode of infection, which involves the release of motile spores from the sporangia, is still unknown. When larvae die or are destroyed by predators, sporangia are liberated to infect other larvae. The spores and sporangia may be distributed to new sites in dust from dessicated pools. Amphibious animals may also transport sporangia to new pools on their skins (Chapman, 1974; and National Academy of Sciences, 1973).

The following discussion on fungi has been previously outlined by Chapman (1974):

> *Beauveria bassiana* - a summary by Clark *et al.* (1971) found that this fungus was effective against larvae of *Anopheles* and *Culex* but not *Aedes* when the agent was applied as a conidial dust to the surface of the water. The dose required, however, for effective laval control was considered impractical. This fungus was also found to be ineffective against adults of three genera in

laboratory tests. This strain has been reported to induce allergic responses to workers (Roberts, 1973).

Beauveria tenella - in nature this pathogen, found in treeholes, has only been found to infect *Aedes sierrensis* at a rate of 26 to 91 percent. Laboratory tests indicate it may have potential as a control for other species of *Aedes*, *Culex* and *Culiseta* (Vinson, 1972).

Entomophthora - in nature *Entomophthora aulicis* has been known to kill hibernating mosquitoes, and *Entomophthora aquatica* was noted in larvae of *Aedes canadensis* and *Culiseta morsitans*. Laboratory studies have demonstrated that *Aedes taeniorhynchus* and *Culex pipiens quinquefasciatus* (Lowe, 1972) could be infected by *Entomophthora* and that many species of *Entomophthora* can easily be cultured. However, *Entomophthora coronata* has also been identified as the possible cause of mycoses in man and horses (Roberts, 1973).

Metarrhizium - mosquitoes are not natural hosts of *Metarrhizium anosopliae* though it affects more than 200 other species in nature. However, spores and toxins of this fungus have been shown to be lethal to larvae of *Aedes*, *Anopheles*, (Sweeney *et al.*, 1973) and *Culex*, and can be easily cultured. There have been no reports of allergic responses or overt infection of vertebrates by this strain (Roberts, 1970 and 1973).

Lagenidium - a report by McCray *et al.* (1973) demonstrated high larval kills in *Culex pipiens quinquefasciatus*, *Culex fatigans*, *Culex tarsalis*, and *Culex nigripalpus*; and limited larval mortality in *Aedes aegypti*, *Aedes triseriatus*, *Aedes mediovittatus*, *Aedes taeniorhynchus*, and *Aedes sollicitans* with this pathogen in laboratory tests. However, the four anophelines tested exhibited no infections. In a later study, Glenn and Chapman (1978) found a "natural epizootic of *Lagenidium giganteum* occuring in larval populations of *Culex territans*" in a swamp in Louisiana. Their surveillance of the swamp (1975-77) and collections made after the fungus first appeared each year, showed that 61% of the larval population was infected and eliminated over the three year period. Although no environmental hazards have been noted, this strain has a low production of zoospores in an artificial medium, germinates resting spores, and brackish water inhibits growth and development (Roberts, 1973).

Coelomomyces - the most extensively studied of all the fungi, this pathogen occurs primarily in mosquitoes. As many as 70 mosquito species have been noted as hosts to approximately 24 species of *Coelomomyces*. Frequent collections in three locations for two to four years demonstrated that *Coelomomyces* was exerting an almost constant pressure on anopheline populations with larval infection levels above 50 percent (Chapman and Glenn, 1972; and Umphlett, 1969). Larval infection levels in other field surveys have ranged from 10 - 90 percent. This strain has exhibited no environmental hazards. The lack of successful laboratory larvae infection procedures with this fungus should be noted.

3.6 Parasitic Viruses

As there has been no field- or pilot-studies involving the use of viruses in anopheline mosquito control, the discussion here will be based on other mosquito vectors.

The first virus (said to be a cytoplasmic polyhedrosis) reported from mosquitoes was from *Culex tarsalis* in 1963. Since then, other cytoplasmic polyhedrosis viruses (near reovirus), nuclear polyhedrosis (baculovirus) and mosquito iridescent viruses (iridovirus) have been reported infecting mosquitoes (Chapman, 1974; Chapman *et al.*, 1978; and David, 1975).

Iridescent Viruses

The details concerning the uptake of mosquito iridescent virus (MIV) from the gut of the mosquito larvae to the cells and larval body have not been elucidated (David, 1975). The primary tissues that seem to be involved in the infection and replication of the virion are the larval fat body, epidermis and imaginal discs (Chapman, 1974; and David, 1975).

There are two strains of MIV, regular mosquito iridescent virus (RMIV) and turquoise mosquito iridescent virus (TMIV). RMIV has been successfully transmitted to

Aedes taeniorhyhchus per os in the laboratory at an average rate of 16 percent. The highest levels of peroral transmission were found when early-instar larvae were exposed for 24-48 hours to substantial amounts of RMIV (Chapman, 1974; and Williams, 1957). Infected larvae, as defined by the characteristic iridescent hue, normally die in the fourth-instar. However, the larvae of *Aedes stimulans* and *Aedes fulvus pallens* seem to be an exception as deaths were observed shortly before or during ecdysis into the fourth-instar (Anderson, 1970).

Cross-transmission of MIV between carrying hosts has been low, less than 1 percent according to Chapman (1974) and Woodard and Chapman (1968). All attempts to transmit MIV in the laboratory to species of *Anopheles* and *Culex* have failed; only cross-transmission in *Aedes* and *Psorophora* has been successfully demonstrated (Chapman, 1974).

Transovarian transmission has been demonstrated in laboratories with RMIV in *Aedes taeniorhynchus* (Chapman, 1974; Hall, 1971; Linley and Nielsen, 1968; and Woodard and Chapman, 1968) and with TMIV in *Aedes taeniorhynchus* and MIV in *Psorophora ferox* (Woodard and Chapman, 1968). Chapman (1974) believes transovarian transmission occurs in all hosts of MIV. In laboratory tests, RMIV in *Aedes taeniorhynchus* showed high levels of infection (20 percent) due to transovarian transmission when late third- to early fourth-instar larvae were exposed to MIV. The larvae demonstrated no observable infections and developed into adults, but these adults passed the MIV via the egg to their larval progeny which developed normal infections in the fourth-instar (Chapman, 1974; and Woodard and Chapman, 1968).

Laboratory studies have shown that only a portion of the larvae exposed to MIV develop typically (Chapman, 1974; and Linley and Nielsen, 1968). The reasons for this are not clear,although it may be related to high virus degradation in the midgut (Chapman, 1974; Stoltz and Summers, 1971; and Wright *et al.*, 1972). Another study suggested that the peritrophic membrane was responsible for the lack of infection of many larvae (Chapman, 1974).

MIV levels, as seen in naturally occurring larval populations, have never exceeded one percent (Anderson, 1970; Chapman, 1974; and Chapman *et al.*, 1966). Chapman *et al.* (1966), however, reported a 500-fold increase in infection levels of RMIV in *Aedes taeniorhynchus* when the breeding site was flooded six times in an eight week period and was not allowed to dry. The infection began in eggs that produced fourth-instar larvae infected with RMIV. Other healthy, similar-sized larvae in this species became infected by feeding on or near the infected larvae or cadavers. They subsequently pupated, and as adults, produced infected eggs. These eggs, in turn, could hatch when flooded to carry on the cycle. "However, often only one generation of even-sized larvae is present in a population of floodwater mosquitoes. Thus, the only patently infected larvae present are those from infected eggs, and transovarian transmission is then the key to future infections (Chapman, 1974)."

Fukuda (1971) demonstrated peroral transmission of chilo iridescent virus (CIV), a lepidopteran virus, to 13 species of *Aedes*, *Culex*, *Psorophora*, *Culiseta* and *Anopheles*. The larval fat body was the principal site of viral replication. The dose required for infection usually exceeded the yield of virus material from the test; however, Chapman (1974) notes large amounts of CIV can be produced *in vivo* by inoculating larvae of *Galleria mellonella*. Chapman makes further note of the fact that *Culex*, *Culiseta* and *Anopheles* developed laboratory infections of this iridescent virus of a moth, though no one has been able to infect larvae of these genera with MIV.

Cytoplasmic Polyhedrosis Viruses

Owing to their small size and other difficulties, the exact process of CPV formation has not been fully determined (David, 1975). CPVs chiefly infect the epithelial cells of the midgut. David (1975) postulates that intact virions pass through the peritrophic membrane. CPVs tend to act slowly on their host. The infected larvae are noticeably backward and may survive for a long time. However, these larvae excrete considerable quantities of virus which can infect other larvae. Also, some species do not produce normal adults with full reproductive capacity so the effect of the virus on an insect population may be much greater than would be expected by a study of the rate and level of larval mortality alone (David, 1974).

Although larvae of 18 species of Aedes, Anopheles, Culex, Culiseta, and Psorophora have shown symptoms of a CPV infection, the virus has only been confirmed in several of the cases by electron microscopy (Chapman, 1974). Levels of infection of CPV are very low, except in Aedes sollicitans. CPV of the gut cells and gastric caeca is usually not lethal to infected larvae according to Chapman (1974). A CPV was reported in the midgut of an adult Anopheles stephensi, but no evidence of the virus was found in the larvae (Bird et al., 1972).

Nuclear Polyhedrosis Virus

The first confirmed NPV from mosquitoes was reported by Clark et al. (1969) and the disease was characterized by infections of the nuclei in cells throughout the midgut, cardia, and gastric caeca in Aedes sollicitans (Chapman, 1974). Clark and Fukuda (1971) demonstrated that the NPV in Aedes sollicitans was lethal and transmission could be peroral or transovarian. They also recorded a combined CPV and NPV infection rate of 78 percent of Aedes solicitans from a survey in Louisiana. The NPV was cross-transmitted perorally to other Aedes species, but not Anopheles or Culex (Chapman, 1974; and Clark et al., 1969).

NPV infections have now been noted in field collected larvae of Aedes, Psorophora, Culex, and Anopheles crucians with most infections being confirmed by electron microscopy (Chapman, 1974). Due to the limited amount of material available, transmission tests were not attempted according to Chapman. However, Chapman (1974) has perorally trasmitted an NPV in Culex pipiens quinquefasciatus to early instar larvae, though levels of infection were extremely low.

3.7 Parasitic Nematodes

Chapman et al. (1970) listed 16 species of Anopheles mosquitoes that are known hosts of nematodes -- all in Merithidae. Of those species of Anopheles, only three of the 16 were found to be parasitized in the adult stage.

The life cycles of two of the nematodes, Romanomermis sp. and Gastromermis, that have been identified as infecting anophelines have been described by Chapman (1974), Chapman et al. (1970), and Petersen and Chapman (1970). In Romanomermis sp. and Gastromermis sp., the pre-parasitic stage hatches from the egg, penetrates the cuticle and enters the hemocoele of an early-instar larva. The nematode grows rapidly to the post-parasitic stage and emerges from the mosquito larva just before it would normally pupate. Emergence, according to Chapman (1970) and Petersen et al. (1968), has always resulted in death of the larva as the mosquito body fluids leak from the hole left by the exiting parasite. The nematodes then molt and mate and the female lays several thousand eggs. The eggs hatch in approximately four weeks to repeat the cycle (National Academy of Sciences, 1973).

Nematodes may be applied to target areas by using the same knapsack sprayers already available for use with chemical insecticides (National Academy of Sciences, 1973; Petersen et al., 1978; and Woodward, 1978), and once applied, will maintain themselves. The nematodes can be disseminated in either of three forms: eggs, pre-parasitic nemas, or post-parasitic nemas (National Academy of Sciences, 1973).

Another advantage of the use of nematodes as a control agent is that they remain at an established site and do not spread unless the water is accidentally moved to another aquatic habit. Indeed, Chapman et al. (1970) still find infected larvae at the pools where Romanomeris sp. and Gastromermis sp. were originally found in 1966 and 1967 respectively. Petersen and Willis (1975) had similar findings of sites they had previously infected in 1971, though the actual parasitism rates in Anopheles ranged from 0-85 percent, depending on how many treatments it had received over a three year period, 1-4 treatments respectively. Woodward (1978), in a study not unlike that of Peterson and Willis, also found infected larvae (Anopheles quadrimaculatus and Anopheles crucians) over a year after pond treatment was ceased. In fact, the level of parasitism actually increased -- from 15-42% -- during the study. This characteristic of remaining in the original habitat is advantageous as it reduces size and frequency of further treatments.

One possible disadvantage with the use of nematodes for mosquito control is that of accidental parasitism in man. A review by Poinar (1975) addresses this issue as he discusses all the cases of alleged parasitism in man that could be found, in relation to nematode identification and evidence of actual parasitism. He concludes that the cases reviewed are inconclusive, and that human infection by parasitism should not be accepted as fact until such time as it is experimentally proven or when parasites are found developing *in situ.*

Studies regarding the establishment of mermithids in mice and rats have also been conducted. Ignoffo *et al.* (1974) subjected them to *Reesimermis nielseni per os*, intransal and intraperitoneal. Body weight gain and histological examinations showed no differences between the treated animals and the control group.

Many of the field studies that have been completed in the past few years concerning anopheline control with nematodes are discussed below.

Petersen and Willis (1972) showed a 94 percent parasitism in second instar *Anopheles* larvae and 64 percent parasitism rate in all *Anopheles* sampled as a result of 20 treatments of 1000 pre-parasitic nemas *(Reesimeris nielseni)* per sq. meter of water in 10 natural sites.

Petersen *et al.* (1973) exposed first-instar *Anopheles quadrimaculatus* Say, to varying ranges of *Reesimermis nielseni* in a Louisiana rice field. They concluded that an estimated 1300 pre-parasitic *Reesimermis nielseni* per sq. yard would be required to produce greater than 95 percent parasitism in this species.

Petersen and Willis (1974) released *Reesimermis nielseni* 30 times (15 at rates of 1000 and 15 at rates of 2000 per sq. yard of surface area) to control *Anopheles crucians* larvae. At the lower rate, 76 percent of the hosts were infected and parasitism averaged 60, 80, 96, and 71 percent in the 1st- through 4th-instar hosts, respectively. At the higher rate, 85 percent of the larvae were infected and 10 of the 15 treatments resulted in levels of parasitism in excess of 90 percent.

Petersen and Willis (1976) introduced cultures of Reesimermis nielseni into habitats of floodwater mosquitoes. In a small sample, the estimated parasitism found was 83 percent after treatment with an eight-week old culture containing post-parasites, adults, and eggs.

Brown et al. (1977) demonstrated an 87.5 percent parasitism rate of Anopheles franciscanus after treatment of three ponds with 1000 preparasites per sq. meter of surface area of Romanomermis culicivorax. They also showed that anophelines were more susceptible than culicines to parasitism in mixed populations, 87.5 to 62 percent respectively.

Woodard and Fukuda (1977) observed a decline in nematode production in the course of routine culturing of the mermithid nematode Diximermis peterseni in the mosquito host Anopheles quadrimaculatus. The nature of the resistance was not known. However, the mechanism, avoidance of attack by larvae when nematode larvae were present, was noted.

Petersen et al. (1978), in a study in El Salvador, treated the mosquito breeding area (see 3.1.1) 11 times over a seven week period with Romanomermis culicivorax for the control of Anopheles albimanus and Anopheles p. pseudopunctipennis. Parasitism averaged 58 percent, but varied greatly (25-92 percent). This study also showed no correlation between the dosage of pre-parasites applied and levels of parasitism, nor between the levels of parasitism and the density of Anopheles larvae within a site. There was, however, a problem at the study site created by strong variable winds. When an attempt was made to correct for this influencing factor, treatments resulted in an 86 percent parasitism rate (as compared with 39 percent rate in earlier treatments). A 19-liter backpack sprayer was used to disseminate the 104 liters of solution which were required to cover the entire area of 10,700 sq. meters with 2400-4800 pre-parasites per sq. meter.

3.8 Parasitic Protozoans

Although Jenkins (1964) reported on 83 protozoan species known from mosquitoes, many of the species, especially the ciliates, flagellates, schizogregarines and eugregarines, have been insufficiently studied due to their low pathogenicity, or because they are commensal or facultative parasites (Anthony *et al.*, 1978; Chapman, 1974; Chapman *et al.*, 1970; and Chapman *et al.*, 1972). Most studies concentrate on Microsporida and protozoans because of their wide distribution and their occurrence in, and apparent pathogenicity to, many species of mosquitoes. Of the Microsporida, four genera, *Nosema, Pleisophora, Stempellia,* and *Thelohania* are the most common parasites to cause disease in mosquitoes. Of those genera, *Thelohania* has been found in more species of *Anopheles* than all other genera combined.

The most common method of microsporidial infection is peroral, but there are reported instances of transovarian protozoa transmission and by other parasites. Infected insects exhibit symptoms according to the degree to which they are diseased (Kellen, 1962). Due to the infection, host species can remain dwarfed, have impaired activity, or produce larvae that are unable to complete metamorphosis (Pramer and Al-Rabani, 1973).

Transovarian transmission has been found to be very common in nature in many mosquito species of *Aedes, Anopheles, Culex,* and *Culiseta* (Chapman, 1974; Chapman *et al.*, 1966; Chapman *et al.*, 1970; Chapman *et al.*, 1972; Kellen, 1962; Kellen *et al.*, 1966; and Kellen and Wills, 1962). Due to this trait, *Thelohania legeri* in *Anopheles quadrimaculatus,* among others, have been infected and maintained in the laboratory, although not consistently (Chapman, *et al.*, 1970). Transovarian transmission has also been noted in *Pleistophora* and *Stempellia* in culicines. No such trait has been evident in *Nosema.*

According to Chapman (1974), peroral transmission of *Thelohania* has been observed twice in the field, with *Thelohania campbelli* in *Culiseta incidens,* and *Thelohania inimica* in *Culiseta inornata.* In both successful attempts, larvae were exposed in screened plastic containers to ponds with past histories of *Thelohania* occurrence.

Most investigators that have reported levels of microsporidian infections in the field have noted infection levels of about one percent according to Chapman (1974). However, larval infection levels as high as 50 percent were reported for *Thelohania* in *Aedes communis* and *Aedes sticticus*, and as high as 80 percent for *Culiseta incidens* with *Thelohania campbelli*.

The only release of a microsporidian in a field population for mosquito vector control was completed by Reynolds (1972) with *Pleistophora culicis* against *Culex pipiens fatigans* on an island. Although the pathogen was still parasitizing mosquito population after two years, the level of infection was shown to be very low (Chapman, 1974).

3.9 Invertebrate Predators

Several kinds of invertebrates that prey in nature on mosquito larvae and pupae offer potential for control of vector mosquitoes. One predator, *Toxorhynchites*, which can be continuously mass-reared in the laboratory or field, is worthy of note and has been discussed by Chapman *et al.* (1972), and National Academy of Sciences (1973). Their findings are summarized below.

Toxorhynchites is a largely circumtropical genus containing about 60 species of unusually large mosquitoes. They breed in treeholes and artificial containers and are predacious both on their own larvae, and on those of other mosquitoes. All of the species are non-biters as adults. The larva, however, is an active, voracious predator, feeding on the larvae of such mosquitoes as *Aedes aegypti*, *Aedes simpsoni*, and *Aedes albopictus*. No information has been found on *Toxorynchites* predation of *Anopheles* larvae.

Little information is available concerning the biology of *Toxorynchites*. However, many species have been colonized, with *Toxorhynchites brevipalpis* from East Africa, appearing to be the most promising (Trpis and Gerber, 1973). During development, one larva of *Toxorhynchites brevipalpis* will consume about 250 larvae *Aedes aegypti*. About two days before pupation the larva kills, but does not consume, all other inhabitants in its container.

Toxorhynchites brevipalpis has been shown in Caribbean trials to be an effective predator for mosquito larvae (*Aedes aegypti*) in small containers (Gerberg and Visser, 1978), such as bottles, cans, tires, vases, household water jars, and treeholes. Such places are especially hard to control because the larval habitats are small, dispersed, and inaccessible. It has been theorized, that with repeated applications, female *Toxorynchites* could find these containers more readily and more frequently than could a mosquito control worker; therein lies the potential of this method -- its capability for dispersion.

Moreover, *Toxorhynchites* can be mass-reared, transported, and released as adults; costs of control should be competitive with chemical insectidies; and the animal could be distributed by unskilled workers.

4. REQUIREMENTS FOR PLANNING ORGANIZATION AND EVALUATION OF MALARIA VECTOR CONTROL CAMPAIGNS

Few generalizations can be made about the vector control methods which are suitable for individual countries or areas. Some general statements can be made, however, about the considerations which should be taken into account in choosing methods.

The methods chosen must be affordable. It is not wise to set up a pilot control project, however successful, if there is no likelihood that it will prove replicable due to budgetary constraints.

An assessment must be made of whether the vector control campaign is to be time-limited or long-term. A time-limited campaign will avoid methods with high initial and low running costs, such as engineering works. A long-term campaign will be more favorably disposed towards such methods.

Where vector control methods serve some other useful purpose, such as the reduction of waterlogging, support and cooperation should be sought from the beneficiaries of those measures.

The methods must be consistent with what is known about local vector and human ecology.

An ecological assessment must be made about the possible impact of the methods on non-target organisms and on each other.

An adequate logistical system must be set up to insure the efficient flow of supplies and information. In this connection, the usefulness of two-way radios for field operations has been demonstrated in U.S. programs (Geib, 1960).

A suitable evaluation system must be put in place to see whether the methods employed are achieving their entomological and epidemiological objectives. Wherever possible, such systems should rely on monitoring data from existing sources. The need for pre- and post-implementation data needs to be stressed.

It may well be that there is no single method or set of methods suitable for an entire country. Where this is so, the country should be divided into homogeneous zones. Landscape and geographical epidemiology are useful tools in this activity. Each country must make its own decisions on the basis of its own criteria.

The beginnings of a quantified stratification strategy can be seen in the establishment by the Indian malaria program of a standard that all areas with an annual parasite incidence of two per 1,000 be sprayed (Pattanayak, 1978). Tactical planning for antimalarial measures in the USSR has long been based on the landscape epidemiology of malaria (Pampana, 1969).

Stratification of areas along the lines laid out above requires more, not less, epidemiological and entomological expertise than conventional eradication strategies. What is required is simple standards for deciding what measures are suitable to what conditions, as measured by existing epidemiological and entomological data.

Some countries desire integration of the malaria program into the general health services at the operational level. Those which do should consider whether this can be done with dual- or multipurpose field workers performing sanitary and vector control measures against more than one disease. Obvious examples are larviciding against Aedes and culicine vectors and environmental engineering against waterborne diseases.

5. POSSIBLE RESEARCH TOPICS

There are many lacunae in our understanding of vector control methods. What follows is not a review, but a nonjudgmental listing of possible topics for research in methods of malaria vector control. Where a source is not given, the topic has been suggested from the reading of the present writers. Topics related to vector control methods, such as vector bionomics, are not discussed here. Inclusion or exclusion of a particular topic does not signify approval or disapproval. Inclusion of overlapping research topics has been necessary to preserve the sense of authors' individual ideas.

5.1 Measures Against Adults in Past and Current Use

- comparative study of DDT and other insecticides (RAO, 1978)
- study of effective dosages and timing for DDT spraying (Rao, 1978)
- experimentatiton with varying doses and cycles of residual spraying to determine those best suited to attainment of epidemiological objectives
- partial appication of insecticides (Lepes, 1977)
- studies on optimum use of adulticides, such as 1) effect of the sprayed surface of the insecticide, 2) length of residual effect (with attention paid to the vector, insecticide formulation and dosage, vector ecology and susceptibility, and human behavior), 3) alternate use of different chemicals, 4) toxicity to men and animals, and 5) logistics of application (Pull, 1978)
- evaluation of the abrasiveness of water dispersible powders used in pesticide formulations (Anon., 1977)
- investigation of the pattern of the development of insecticide resistance under selective spraying
- controlled experiments to test the feasibility of slowing down the development of resistance through changes and coordination in malaria and agricultural pesticide

practices

- observations of effects on man of fenitrothion (WHO, 1975)
- effect of house spraying with fenitrothion on persistent transmission by *A. balabacensis* and *A. farauti* (Anon., 1977b)
- studies on the optimum size of ULV droplets for maximum kill in different types of work (Lofgren, 1970)
- use of bed nets and screening as a community protection measures
- development of slow-release adulticides
- studies of cost-effective methods of space spraying (PAHO, 1977)
- experiments in the use of space sprays (ICMR, 1978; Pattanayak, 1978)
- improvements in equipment used for ground and aerial space spraying (Lepes, 1977)
- use of ULV for eliminating foci of malaria in special situations, such as consolidation areas
- development of simple field techniques for determining mass median diameter of ULV droplets
- use of rotary-cylinder atomizers in ULV applications against anophelines
- improvement of ULV ground equipment to reduce noise and leakage hazards to operators
- study of the effectiveness of repellents in interrupting transmission in rubber plantation areas (Rao, 1978)
- appraisal of the repellent action of local products on human-vector contact and on malaria morbidity (Anon., 1977b)

- trials with animal barriers

5.2 Antilarval Measures in Past or Current Use

- studies on the use of antilarval measures in rural and semi-arid zones where *A. culicifacies* is the vector (ICMR, 1978; Pattanayak, 1978)

- studies on the use of antilarval measures in rural areas with resistance to residual house spraying with DDT, HCH and malathion (Pattanayak, 1978)

- studies on optimum use of larvicides, such as 1) type and formulations suitable to specific situations, 2) interval between applications, 3) toxicity to non-target fauna, and 4) methods of application (Pull, 1978)

- systematic studies on field efficacy of promising larvicides against key species in broad categories of breeding habitats (Mulla, 1970)

- studies on applicability of various formulations of larvicides in aquatic habitats (Mulla, 1970)

- development of larvicidal formulations with minimal toxicity to work crews and to non-targett organisms (Hamon, 1977)

- the use of larvicides in flowing water though such devices as drip cans

- temephos trials during the dry season

- trials of slow-release formulations in standing water collections

- trials of slow-release larvicides in rice fields and in running water

- application to anopheline larval control of slow-release technologies used against other organisms

- studies on egg-laying habits of anophelines, with a view to using larvicides, chemosterilants or biotic control agents in selected sources (Mulla, 1970)

- screening of compounds and improved formulations for ovicides (Mulla, 1970)

- ecological studies of impact on non-target organisms of slow-release larvicides

- entomological and epidemiological evaluation of larvivorous fish

- identification of local potentially larvivorous fish (SEARO, 1977; Anon., 1977b)

- feasibility studies on larvivorous fish (Pull, 1978; Rao, 1978)

- field trials with indigenous and introduced larvivorous fish, with ecological and economic as well as entomological and epidemiological assessment

- local ecological studies on impact of introduced larvivorous fish on nontarget organisms

- experimentation with different combinations of liquid larvidicing and larvivorous fish application

- field trials of monomolecular film

- trials of sunning, shading, siphoning, and desiccation by use of trees

- studies on use of intermittent irrigation (SEARO, 1977)

- covering of wells combined with chemical larviciding where the vector has become domesticated (Boschi, 1978)

- afforestation for control of *A. subpictus* (Boschi, 1978)

- feasibility studies on clean weeding (Pull, 1978)

- studies on efficiency of source reduction methods in urban, semiurban, and rural areas (Lepes, 1972b)

5.3 Combined measures in past or current use

- studies in biological control and use of combined measures (ICMR, 1978; Pattanayak, 1978, Anon., 1977b)

- joint studies with development project planners on development and evaluation of combined control measures

- use of combined measures in rice fields, along the lines described by Dukhanina and Quadeer

- efficient combination of antimalaria drugs with environmental measures in multipurpose land reclamation ("bonification")

- use of larvicides alone or in combination with adulticiding, chemotherapeutic measures, or both (Rao, 1978)

- studies on use of combined measures in areas of vector resistance (Pattanayak, 1978)

- combination of control measures with community involvement (Pattanayak, 1978)

- studies on environmental changes, such as drainage, land reclamation, water management, house screening, afforestation, and agricultural practice, with attention to their effect on the malaria situation (Pull, 1978)

- combined applicatiton of engineering, other antilarval and barrier spraying measures for control of urban malaria

5.4 Biological Control Methods Currently Under Development

In addition to a continuation and furtherance of the research mentioned in section 3, the following topics are proposed:

- extensive field trials utilizing all of the proposed biological control methods contained herein

- development of efficient and effective dispersal methods for possible control agents

- development of efficient mass-culture methods to supply parasites, predators, and mosquito larvae in large quantities on a continuing basis

- studies on the bionomics, population dynamics, ecology and efficacy of possible control agents and target organisms

- toxicological studies on all control methods for possible bioenvironmental contamination of non-target organisms

- studies on the possibility and practicality of integration of various control methods

- a practical means for reducing natural populations to manageable levels (Knipling, 1968)

- examination of new program costs that are favorable in relation to costs for current methods of control, and losses or economic damage from the insect (Knipling, 1968)

5.5 <u>Planning, organization, and evaluation</u>

- cost/benefit study of different interventions necessary to reduce malaria produced from borrow pits (Boschi, 1978)

- development of a standard methodlogy (formula, parameters) for measurement of cost-benefit (cost-effectiveness) in relation to malaria control (SEARO, 1977)

- development of unit costing for standard vector methtods (NAS, 1976)

- studies on cost and impact of multipurpose health workers

- studies on techniques for stratification of geographical areas, using existing data

- studies on impact, if any, on costs and field efficiency of using two-way radios

- study of the impact of agricultural pesticides on malaria transmission (MCPI, 1978) parasites, predators, and mosquito larvae in large quantities on a continuing basis

- studies on the bionomics, population dynamics, ecology and efficacy of possible control agents and target organisms

- toxicological studies on all control methods for possible bioenvironmental contamination of non-target organisms

- studies on the possibility and practicality of integration of various control methods

- a practical means for reducing natural populations to manageable levels (Knipling, 1968)

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- studies on cost and impact of multipurpose health workers

- studies on techniques for stratification of geographical areas, using existing data

- studies on impact, if any, on costs and field efficiency of using two-way radios

- study of the impact of agricultural pesticides on malaria transmission (MCPI, 1978)

- trials on adjustment of insecticidal application practice to account for the impact on vector larvae of agricultural applications (Mason and Hobbs, 1977)

- trials on slowing the development of insecticide resistance through control of agricultural applications (WHO, 1976)

- operational research to find more efficient and economical methods for source reduction, particularly in urban and semi-urban areas

- studies on resistance in field populations in order to characterize the type of resistance mechanism present, with a view to providing indications concerning suitable replacement insecticides and other countermeasures, such as synergists (WHO, 1976)

- studies on the role of species complexes in the epidemiology and control of malaria (Anon., 1977a)

- studies on bed bug problems as they affect malaria spraying operations (ICMR, 1978)

- studies on measures to delay the development of vector resistance (Pal, 1978; Anon., 1977b)

- studies on control methods against multi-resistant *A. culicifacies* in semi-arid and arid zones (Anon., 1977b)

- comparative studies of the cost of engineering and other antilarval measures in terms of long-term per capita expenditure for disease prevention

- development of methodology for prediction and containment of epidemics (Anon., 1977b)

- epidemiological evaluation to assure the most efficient and economical way of applying combined measures

- use of modeling through mathematical epidemiology for choosing control programs (Anon., 1977b)

- use of simulation techniques to select those malaria control methods which would produce the best acceptable reduction in mortality and morbidity under each epidemiological and socioeconomic situation (Pull and Gramiccia, 1976)

- use of cytogenetic studies as a complement to entomological surveys, to permit the selection of the best control methods and predict their effect (Anon., 1974)

- quantitative studies on factors influencing the rate of development of resistance in vector control programs, entailing developing and use of mathematical models incorporatitng genetic, operational, and genetic factors (WHO, 1976)

- use of computer modeling and simulation to aid in conducting anopheline research and formulating strategies for anopheline control (Ward and Scanlon, 1970)

Appendix I

Current WHO Estimates of Vector Control Costs

Several attempts have been made to estimate the costs of various types of malaria vector control. The most recent of these, which is reproduced in full below, is contained in the 1978 report of the WHO Director-General (Anon., 1978).

<u>Information of the Cost of Malaria Control</u>

The cost of a programme depends on the control methods selected. It includes expenditures on insecticides, drugs and transport, and also on the application of measures and their evaluation. Costs, particularly those of application, evaluation and maintenance, may vary tremendously from one country to another. The basic cost of antimalaria measures has been greatly affected during recent years by worldwide inflation and the increased price of petrol, which is vital for the manufacture of petroleum-derived chemical compounds. In 1972, DDT was still the insecticide of choice in the majority of the malaria programmes. However, since then the evolution of resistance in many anopheline populations has necessitated a change to other more expensive insecticides, such as malathion, fenitrothion or propoxur.

The following estimates of the cost of some antimalaria measures for the protection of one million inhabitants, excluding application costs since these are greatly influenced by local conditions (wages and salaries, transport, equipment, etc.) have only an indicative value and are based on actual cost estimates in an ongoing control programme. They may help health administrators when planning antimalaria activities. However, it is not intended to suggest that the regimens mentioned can be directly substituted one for the other; nor that effectiveness is similar for each method.

- One round of indoor residual spraying, using DDT 75% wdp

  requirements - 147 tonnes

  price per unit - US$ 950/t

  total cost of the insecticide = US$ 140 000

  cost per protected inhabitant = US$ 0.14

- One round of indoor residual spraying, using malathion 50% wdp

  requirements - 220 tonnes

  price per unit - US$ 1570/t

  total cost of the insecticide = US$ 345 000

  cost per protected inhabitant = US$ 0.35

- One round of fogging with malathion 57% and kerosene

  requirements - 4044 litres of malathion and of kerosene

  price per unit - malathion US$ 1.57/litre
  - kerosene US$ 0.12/litre

  total cost of the insecticide = US$ 7683

  cost per protected inhabitant = US$ 0.01

- One round of larviciding using kerosene and Triton X-100

  requirements - 57 tonnes

  price per unit - US$ 120/t

  total cost of the larvicide = US$ 6840

  cost per protected inhabitant = US$ 0.007

- Single dose treatment of fever cases under tropical African conditions (10 mg/kg body weight) using chloroquine tablets (100 mg base)

  annual requirements - 4 500 000 tablets

  price of 1000 tablets - US$ 10

  total cost of the drug = US$ 45 000

  cost per protected inhabitant = US$ 0.05

- One round of mass chemoprophylaxis covering 30% of total number of inhabitants using pyrimethamine tablets (0.25 mg base)

  requirements - 600 000 tablets

  price of 1000 tablets - US$ 6

  total cost of the drug - US$ 3600

  average cost per protected inhabitant = US$ 0.012

The cost for environmental measures such as biological control, drainage, filling, land reclamation and house improvement have not been included, in view of the numerous cost factors involved and types of equipment used. Nor have the costs for application and evaluation, as these largely depend on labour and transport costs which vary from place to place. However, the following ratios may serve as an indication.

RELATIVE COST VALUE OF MAIN COMPONENTS OF THE ANTIMALARIA PROGRAMME

Type of programme	Insecticides %	Labour and supervision (wages and salaries) %	Transport %	Equipment and other costs %
Malaria eradication (DDT)[a]	26.0	52.0	18.5	3.5
Central American malaria eradication (DDT)[b]	33.0	50.0	10.0	7.5
Large-scale control tropical Africa (DDT)[c]	30.0-50.0	25.0-40.0	10.0-40.0	2.0-5.0

[a] Manual of planning for malaria eradication and malaria control programmes (1972) (document ME/72.10, p. 165).

[b] Farid, M.A. & Rafatjah, H.A. (1973) Comparative effectiveness and cost of various antimalaria measures directed against malaria vectors under different situations (document WHO/MAL/EC16/73.8).

[c] Bruce-Chwatt, L.J. (1972) Malaria, demography and socioeconomic development in Africa (document MAL/IRC/72.2).

It therefore seems quite reasonable to estimate the average cost of antimalaria measures as around 30% of the total cost of the programme; 65% should be spend on the application of the measures; and 5% on other costs, such as office equipment and maintenance, stationery.

Appendix II

Acute Oral Toxicity of Pesticides Used in Malaria Control

One useful method of gauging toxicity is use of values reflecting the dosage required to kill 50 percent of laboratory animals exposed to individual insecticides. According to Ben-Dyke and colleagues, "the acute toxicity of a chemical gives the most useful rapid indication of the main potential hazard to human users, and probably also to domestic animals" (Ben-Dyke, Sanderson and Noakes, 1970). The following tables, reproduced from a WHO report, give LD_{50} values for some well known insecticides. Values are given in milligrams of pesticide per kilogram of body weight; a low LD_{50} reflects high toxicity (WHO, 1976).

TABLE OF TOXICITY OF SOME SELECTED CHLORINATED HYDROCARBON INSECTICIDES TO FEMALE LABORATORY RATS

Insecticide	Acute dermal LD_{50} (mg/kg)	Acute oral LD_{50} (mg/kg)
DDT SERIES		
DDT	2510	118
TDE	slightly irritating	2500
Methoxychlor	-	6000
HEXACHLOROCYCLOHEXANE SERIES		
Lindane (not less than 99% HCH)	900	91
CHLORDANE SERIES		
Chlordane	530	430
Heptachlor	250	162
Aldrin	98	60
Dieldrin (not less than 85% HEOD)	60	46
Endrin	15	7.5

Source: WHO, 1976

ACUTE ORAL AND DERMAL LD_{50} VALUES FOR FEMALE WHITE RATS OF 15 Pesticides

Compound	Type [a]	Oral LD_{50} (mg/kg)	Dermal LD_{50} (mg/kg)
carbaryl	C	500	> 4000
chlorpyrifos	OP	82	-
diazinon	OP	285	455
dichlorvos	OP	56	75
dimethoate	OP	245	610
fenchlorphos	OP	2630	> 5000
fenitrothion	OP	570	350
fenthion	OP	245	330
malathion	OP	1000	> 1444
naled	OP	250 [b]	800 [b]
parathion	OP	3.0	6.8
parathion-methyl	OP	21	67
temephos	OP	13000	> 4000
tetrachlorvinphos	OP	1125	> 4000
trichlorfon	OP	560	> 2000

Source: WHO 1976

[a] C = carbamate; OP = organophosphorus compound

[b] For male rats

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