THE POTENTIAL OF THE STERILE INSECT TECHNIQUE AND OTHER GENETIC METHODS FOR CONTROL OF MALARIA-TRANSMITTING MOSQUITOES

Report of a Consultants Meeting
organized by the
Joint FAO/IAEA Division of Nuclear Techniques Food and Agriculture
held in
Vienna, Austria, 29 July - 2 August 1996

Reproduced by the IAEA
Vienna, Austria, 1996

Contents

THE POTENTIAL OF THE STERILE INSECT TECHNIQUE AND OTHER GENETIC METHODS FOR CONTROL OF MALARIA-TRANSMITTING MOSQUITOES

An Update of a 1993 Consultants Report

Human malaria parasites of the genus *Plasmodium* are exclusively transmitted by mosquitoes of the genus *Anopheles*. Where these two groups co-exist, the transmission of the parasite to humans can create a major health problem. Malaria currently causes 2 million deaths world-wide and approximately 400 million clinical cases annually. There are ca. 15 major vector species and 30-40 vectors of lesser importance. This report considers the practicality of developing the sterile insect technique (SIT) or other genetic mechanisms in order to eradicate mosquito vectors from specific areas. This would interrupt transmission and eliminate malaria in those areas.

MAJOR POINTS

- **Recent advances in malaria control, including vaccines, insecticide treated nets and genetic manipulation of anopheline mosquitoes are promising but insufficient at this time to forgo improvement in vector control capability, although progress in this field over the next ten years is difficult to predict.**
- **In limited field trials SIT has been shown to be an effective mosquito control method, but propagation and distribution methods must be improved to justify large scale programs.**
- **A 5 year mosquito methods programme (MMP) on a major malaria vector should be initiated at Seibersdorf to develop automated mass rearing techniques, improve handling and release methods, and enhance genetic sexing capability. These improvements could serve many purposes as the implementation of most genetic methods would require these capabilities. This will require approximately US $6.5 million for staff, equipment and facility.**
- **A Coordinated Research Programme should be initiated to complement the MMP.**
- **If the MMP is successful, a 5 year mosquito field programme including the two essential phases (prior population suppression and sterile insect release) could be initiated in a location with a single vector; successful completion would eliminate the risk of malaria for 5-10 million people or more. This would require ca. US $13 million plus a facility and the costs of suppressing the indigenous mosquito population prior to releases.**

The programme could provide an example for more challenging area-wide projects against this and other anopheline vectors and locations with multiple species, including cryptic members of species complexes, throughout the tropics and some temperate zone areas.

This phase should be considered a relatively high risk programme with many challenges to be overcome to release these fragile insects in hardy, competitive condition.

- **These activities must be funded by extra-budgetary funds to ensure a manner which has no adverse effect on the highly successful and long term medfly and tsetse fly SIT programmes and activities of the Insect and Pest Control Section.**
- **The mandate of the Joint FAO/IAEA Division is food and agriculture. Therefore, should it embark on the MMP, it will should work cooperatively with WHO in the conduct of this programme.**

EXECUTIVE SUMMARY

This report updates information provided by a 1993 consultant group on the use of genetic methods for control of malaria-transmitting mosquitoes.

The results of recent intense research activity in three methods of malaria control, insecticide-treated nets (ITN), vaccination, and genetic manipulation of mosquitoes so that they cannot transmit malaria, have all been promising in many respects. Malaria vaccine research, in which progress has been escalating, now fully utilizes the resources of modern molecular biology and of rigorously controlled field testing methods. Expectations of success are rising. However, it seems unlikely that vaccination, ITN or genetically manipulated mosquitoes will solve the diverse malaria problems before SIT could be developed to the point at which it could make meaningful contributions.

Reduction by vector control in the estimated 400 million annual clinical cases of malaria presents an enormous challenge, exacerbated by the number of important vector species (ca. 15, plus 30-40 of lesser importance) distributed throughout the tropics and some temperate zone areas, including cryptic members of species complexes. The five anopheline species cited in 1993 retain their standing as the most suitable SIT candidates, *Anopheles albimanus, An. arabiensis, An. culicifacies, An. gambiae* and *An. stephensi.* Genetic sexing mechanisms were constructed in the past for four of these candidates, and improved or new versions of these manipulations could provide the potential to release only sterile males and very few or no vector females.

A five year mosquito methods programme (MMP) with one of the candidate species, focusing on genetic sexing, automated mass rearing and specialized handling for large scale distribution and release, will be required before serious field efforts could be considered. These improvements could serve many purposes as implementation of most genetic methods would require these capabilities. The staff and operating costs of the MMP are estimated to be US $5 million excluding the cost of a facility in which to conduct the studies, which could be US $1-1.5 million .

The most appropriate site for the MMP is the Entomology Unit at the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf. The work will require the recruitment of three scientists with appropriate staff and equipment, using extra-budgetary funds to ensure that the existing successful fruit fly and tsetse fly programs will not be adversely impacted by this endeavour.

It is recommended that a Coordinated Research Programme (CRP) be initiated as proposed by the 1993 consultants report to stimulate a renewal of international scientific effort on mosquito SIT for malaria control. This activity would be expected to provide positive impetus to the MMP and additional testing ground for the technology developed in the Entomology Unit.

If the combined results of the MMP and the CRP are sufficiently promising, the work could be taken to a second phase for large scale field implementation. The extension of the study to the field should be considered a relatively high risk programme and many

challenges would have to be overcome. Studies conducted 20-30 years ago have shown that mosquito SIT can be effective but the expenditure required for implementation of area-wide control has restricted interest in initiating practical operations. Also, the hardiness of fragile mosquitoes has not been tested after exposure to automated and mechanised systems such as would be considered for handling and large scale release. Other factors could affect competitiveness, e.g, the sterilization process or adverse selection during colonization, but methods used to minimize these effects with other species could be effective with mosquitoes.

To be as cost effective as possible this area-wide programme should be large and the initial releases conducted only after pre-suppression of the indigenous mosquito population, either by natural seasonal fluctuation, insecticides, or other density independent intervention methods. If the released mosquitoes are reasonably competitive the overall cost of eradication could be less using a combination of pre-suppression and SIT than the indefinite continuation of existing inadequate control methods.

Criteria to select a field location for the second phase of the programme would be based on isolation, vector species composition and potential for limiting the programme to one species, number of people at risk, and the local infrastructure for malaria and mosquito monitoring. These criteria point toward selection of an urban site either in south Asia in which *An. stephensi* is the vector or in west Africa in which *An. arabiensis* is the vector.

The actual pre-suppression (insecticidal or normal seasonal decline) and release (SIT) phase could take as little as 2 years to accomplish after the rearing facility has come on line. A minimum estimate for the entire second phase of the programme would be 5 years which, when added to the initial 5 year MMP would bring the minimum time requirement to 10 years. However, the advent of unforeseen technical or political problems could extend the time required.

A successful program of this scale would provide relief from malaria risk in one location for perhaps 5-10 million people at a probable cost of US $10-15 million plus the rearing facility and pre-suppression costs.

Repetition of the approach with the same species in other malarious areas with a single anopheline could further reduce the risk of malaria. In areas with more than one vector mosquito, releases or other eradication measures would be required with each vector. Eventually, releases to eliminate interzone infestations might be necessary to consolidate reclaimed regions. If successful, this type of programme could be an example for others to emulate until effective vaccines become generally available. A high proportion of Member States would benefit from vector eradication in selected locations or throughout their malarious zones.

The public relations aspects of mosquito SIT need to be taken very seriously as the mass release of mosquitoes might seem highly objectionable to many in the affected communities. It would be particularly necessary to demonstrate to the local population, the authorities and the media that the releases would be of non-biting males.

Finally, global malaria control by vector eradication is a huge task, involving several continents and many anopheline species. The vaccines that are being sought may offer complete protection in the future; if so, it will then no longer be necessary to control the vectors. But SIT may offer an important contribution to area-wide vector control programmes. Protection of urban populations followed by expansion of the effort to regional programs will also protect agricultural workers and enhance agricultural productivity.

The mandate of the Joint FAO/IAEA Division is food and agriculture. Therefore, should it embark on the MMP, it should work cooperatively with WHO in the conduct of this programme.

1. INTRODUCTION

1. 1. Review of the 1993 consultants report

The Joint FAO/IAEA Insect and Pest Control Sub-Programme has reviewed in depth the use of sterile insects and other genetic mechanisms for control of economically mosquitoes on several occasions:

- In 1982 a consultants meeting was convened to address the topic "Investigations in East Africa on the feasibility of the sterile insect technique (SIT) to control the anopheline mosquito".

- In 1984 the Agency sponsored a consultant group on "Research and development of the sterile insect technique (SIT) for use against the malaria vector, *Anopheles arabiensis*, in Mauritius".

- In 1987, in a review of "Genetic methods of insect control" an advisory group recommended that an effort be made for control of *An. arabiensis*.

- In 1993 a consultant group was asked to evaluate the use of genetic methods for control or eradication of malaria mosquitoes. In its report, "The potential for genetic control of malaria-transmitting mosquitoes", the group addressed the appropriateness of the strategy, the genetic options available, candidate species and possible locations for pilot studies.

In the concluding remarks of the 1993 report, the group emphasized the extraordinarily broad scope of past and present work with mosquitoes, ranging from field ecology through physiology and now molecular biology. Mosquitoes had been targeted for this type of effort because of their importance as vectors of human and animal disease, especially malaria which has recently been estimated by WHO to cause ca. 400 million clinical cases and 2 million deaths per year. The spread of drug resistance in malaria parasites and insecticide resistance in anopheline mosquitoes has affected malaria incidence, which can be expected to increase until adequate new methods of intervention become available.

As of 1993 the development of mosquito genetic techniques to control or reduce malaria transmission potential had not provided systems suitable for field assessment. The techniques studied included chromosomal aberrations, hybrid sterility, cytoplasmic incompatibility, parasite inhibiting genes, genome modification and population replacement or transformation. Rapid progress is now being made on genome mapping, transformation vectors and molecular understanding of specific physiological systems. These are all tools required for development of transgenic technology to provide new strategies for mosquito control. However, these methods will be dependent on mass production, quality control of mass-reared mosquitoes and methods for efficient distribution into the natural habitat.

A possible obstacle to full exploitation of these strategies, including SIT, is the existence of cryptic species within some major anopheline species complexes which prevents accurate species identification in the field. However, in many areas of Asia and Africa these species complexes are well understood.

The 1993 report recommended further research and development with *An. albimanus*, *An. arabiensis*, *An. culicifacies*, *An. gambiae* and *An. stephensi* because of their importance as malaria vectors, ability to be colonized and knowledge of their genetics. Also considered in this selection is the existence of a malaria programme and infrastructure, national scientific capabilities, and potential of there being isolated field sites for pilot studies..

The specific recommendations of the 1993 report were:

- Organize a Coordinated Research Programme for genetic control of anophelines, including population genetics and colonization.

- Initiate a 3-5 year research and development project with at least 2 scientists and space for production of 0.5 million mosquitoes per week at the Seibersdorf facility to develop automated procedures for mass-rearing, sterilization, and dispersal of anophelines.

- Contract a group of expert consultants to visit various locations and choose an experimental site for pilot projects to evaluate the behaviour and effectiveness of mass-produced males of a malaria vector.

- Maintain contact with scientists involved in genetics of mosquitoes and their respective organizations in the planning and execution of this project.

1. 2. Objectives of the current consultant review

The terms of reference for the current review are as follows:

- Assess the advances world-wide in all aspects related to malaria mosquito SIT since the last consultants meeting in 1993, as well as the progress made or expected in the medium term in alternative approaches, including vaccines and population replacement. In view of these developments, assess the current and future need of affected Member States for a supplementary genetic/autocidal vector intervention technique.

- Review the financial and human resources required to implement a long term laboratory and field malaria mosquito SIT project within the Insect and Pest Control Subprogramme for the Joint FAO/IAEA Division.

- Advise the Joint Division on the feasibility of successfully implementing a malaria mosquito SIT field project in relation to top current tsetse or fruit fly projects or other potential action projects, such as Lepidoptera, or other agricultural pests.

2. RECENT PROGRESS SINCE 1993 AND ITS IMPACT

The development of SIT and genetic methodology for anophelines will take several years and the question must be asked: will the world's malaria problems be solved in some other way before these vector control methods are ready? In this connection the following subsections update the information provided in the 1993 consultants report in those instances in which progress has been reported.

2. 1. Insecticide treated nets

Insecticide treated nets (ITN) integrate a chemical and a physical barrier against night biting mosquitoes. In China, millions of nets are treated annually with deltamethrin and clear reductions in the levels of malaria have been achieved, so far without detectable evolution of resistance in the two vector species involved (CHENG *et al.*, 1995). Trials in Africa, sponsored by WHO in four countries, of provision of permethrin impregnated nets have shown reductions in child mortality from all causes of 17-63% (ALONSO *et al.*, 1995; D'ALLESSANDRO *et al.*, 1995a; NEVILL *et al.*, 1996; BINKA *et al.*, 1996). It is likely that use of this method will expand greatly over the next decade by some combination of publicly provided or subsidised programs and individual self-help. A limitation to the universally successful use of ITNs would be with those vector species which bite at hours when people are not in bed.

Continued large scale use may select for insecticidal resistance or for behavioural resistance in the form of biting out of doors or at hours when people are not in bed. Biting female mosquitoes are attracted to ITNs by the odour of sleepers inside them. However, non-biting males are not attracted and would only rest on ITNs by chance. Thus, the concurrent use of ITNs and SIT could form an integrated approach.

2. 2. Malaria vaccines

Malaria vaccines have been the target of a major research effort in many laboratories, with field trials in all the tropical continents. There has been an acceleration of activity and a rapid increase in new information. The synthetic polymer vaccine, SPf66, developed by M.E. Patarroyo in Colombia for use against the sporozoites of *Plasmodium falciparum* and components of the stages which occur in the blood, has been satisfactorily tested for safety on thousands of people. It has given 31-34% protection from attacks of *falciparum* malaria in trials in children and adults above the age of 1 year in Colombia (VALERO *et al.*, 1993) and in children aged 1-4 in Tanzania (ALONSO *et al.*, 1994). However, in a trial with babies aged under 1 year in The Gambia no significant protection could be detected (D'ALLESSANDRO *et al.*, 1995b). The results of trials with the 1-15 year age group in Thailand and with babies in Tanzania are awaited. A clinical trial is planned in Papua New Guinea by R.F. Anders of a vaccine against another component of the stages of *P. falciparum* which occur in the blood.

Some of the most promising lines of research on vaccines against pre-erythrocytic stages of *Plasmodium* appear to be vaccination with DNA which encodes the circumsporozoite protein and induces both an antibody and a cellular response (SEDEGAH *et al.*, 1994), and reverse immunogenetics based on strong protection against malaria symptoms associated with possession of certain natural HLA types in African populations (HILL *et al.*, 1992).

A transmission-blocking vaccine has been prepared by KASLOW *et al.* (1994) in genetically transformed yeast. This vaccine induces antibodies which are intended to be imbibed from vaccinated humans by mosquitoes, where the antibodies would interfere with the development of the ookinetes (zygotes) of *Plasmodium*. The vaccine has been approved for phase 1 clinical trials.

All vaccines are subject to doubts about whether antigenic variability in the parasite will prevent their universal applicability and/or whether vaccine-insensitive types will be selected by a successful vaccination program. Almost all the vaccine research and field trials on human malaria has been on *P. falciparum*, which is the lethal form of malaria. But in most parts of Asia and Latin America *P. vivax* is the more common cause of disease, for which candidate vaccines have not been available for field trial. Protection from *vivax* malaria thus seems to be further in the future than *falciparum* malaria.

In terms of administering vaccines, coverage of a sufficiently high proportion of the human population will also be difficult to organise. And, in the case of transmission-blocking vaccines which would benefit not the vaccinated individual but only the well vaccinated community, the level of voluntary compliance remains to be seen. As a solution to the problem of vaccine delivery to human populations, Crampton (ANON., 1996) has suggested the use of sterilised mosquitoes into which synthetic genes have been inserted for vaccine molecules expressed in the mosquito saliva. However, major problems can be foreseen of gaining universal consent to involuntary vaccination and of wide variability of the dose received, depending on local and individual variation in amount of mosquito biting.

In summary, based on the progress reported and in spite of the rapid occurrence of new approaches and the impossibility of predicting when the first acceptable vaccine will be registered, it seems unlikely that vaccines will have solved the world's malaria problems before the SIT could be developed to a point where it could make a contribution to the solution of these problems.

2. 3. Genetically engineered mosquitoes unable to transmit malaria

Obtaining the necessary molecular biological background to the production of such mosquitoes has been the subject of intensive research sponsored by WHO (COLLINS and PASKEWITZ, 1995). Conventional animal breeding methods have long been used to produce refractory strains of mosquitoes, i.e., those which are not susceptible to pathogen infection. However, the refractoriness has always proved to be under polygenic control and to be genetically at least partly recessive. Such forms of refractoriness would be almost impossible to drive into wild populations. A more hopeful suggestion appears to be the production of a

synthetic gene for a transmission-blocking antibody and incorporation of the gene into a mosquito's genome (CRAMPTON, 1996). It would be placed under the control of a promoter which would cause the gene to be expressed at the time of blood feeding, when *Plasmodium* gametes or ookinetes may be present in or on the mosquito's stomach.

Another genotype that could interfere with malaria transmission is that which causes *An. quadriannulatus*, a member of the *An. gambiae* complex, to bite animals rather than humans. It should be possible to backcross this genotype into *An. gambiae (sensu stricto)*, the principle disease vector of this species complex, making use of the recent discovery that this human biting species is specifically attracted to the odour of human feet. Rendering a mosquito population unable to bite humans would answer the objection, which is sometimes raised to genes which only block *Plasmodium*, that other mosquito borne diseases and mosquito nuisance would remain unaffected. It might be possible to use a strain of *An. gambiae* rendered harmless in this way for sterilisation and release in a SIT programme. This would provide insurance with this vector against any imperfections in the genetic sex separation system by preventing human biting by any inadvertently released females.

There has been speculation for 30 years about possible means of driving desirable genes into vector populations. The currently most promising driver candidates are transposable genetic elements and *Wolbachia* symbionts, to which the desirable genes might be linked (CURTIS, 1996). There are theoretical reasons and precedents from events in natural *Drosophila* populations to expect that such entities could spread through a wild population from a limited "seeding", without the need for a mass release program, with its attendant requirement for an expensive mass rearing facility. It has been proposed that, after population replacement in this manner, the new harmless population would occupy the ecological niche previously occupied by the vector. The replacement mosquito could then compete with immigrant vectors. This argument has been the main one in favour of this approach rather than an eradication method, such as the SIT, whose benefits might be rapidly reversed by immigration unless releases were resumed. However, the effective driving of a desirable genotype into a population depends crucially on exceedingly close genetic linkage of the driving mechanism and the gene which it is desired to drive. It remains open to question whether such levels of linkage could be achieved in practice.

Apart from these sophisticated alternatives to SIT, various genetic manipulations have been considered as means of improving SIT for malaria mosquitoes. However, development of practicable systems has not yet come to fruition. The systems mainly considered were chromosome aberrations such as compound chromosomes (SEAWRIGHT, 1993), natural hybrid sterility (DAVIDSON, 1970) and artificial cytoplasmic incompatibility created by transfer of the causative symbiont *Wolbachia* from other insects into anophelines (O'NEILL and SINKINS, in prep.). However, there were major problems with each of these approaches and no recent progress has been reported.

Thus, radiation or chemically induced sterility are the only currently available genetic options which have been demonstrated to be effective in field trials (LOFGREN *et al.*, 1974; PATTERSON *et al*, 1975; 1977). And, as noted in the 1993 consultants report, this approach still requires 3-5 more years for methods development before field trials can be realistically considered. Because SIT deals with the biology, physiology and ecology of individual species,

each new venture constitutes a fresh start. The development of the successful SIT programmes against screwworms and fruit flies required 10-20 years from conception to operational success. Malaria vector eradication in an isolated situation with a single mosquito species, if achieved, may still be another decade or more away.

The investment in the commitment with screwworms and medflies to such lengthy development periods has been returned many-fold in subsequent decades. Over 30 years after the initial releases in Curacao and Florida the screwworm programme is still active, having eradicated the insect from the entire United States and Mexico and over half of Central America and now pushing inexorably towards Panama where a permanent 200 mile barrier release zone is planned. Similar long range perspective is required for malaria vectors. An economic assessment of localized or regional malaria eradication would be likely to predict significant economic returns in terms of productivity and reduced health costs alone. The SIT is an eradication technique which, if it can be successfully employed, could eliminate transmission of the parasite in each sequential or parallel program.

2. 4. Malaria control in areas of intense transmission

In areas of intense malaria transmission in lowland tropical Africa, where 80-90% of the world's malaria problem resides, immunity is acquired as a result of repeated malaria attacks as a baby grows up; most African adolescents and adults suffer only mild disease from malaria infections. In such areas practically achievable levels of control of infective biting by the vector (e.g. by 90-95%) may produce short term benefits in an immune human population. However, it has been a matter of concern that in the long term the development of immunity might be delayed, thus setting back the occurrence of malaria attacks to a later age but not preventing them. Comparison of data from areas differing naturally in level of transmission, from the high level of 200-600 infective bites per person per year to lower levels of about 20 infective bites per person per year, does seem to show this delaying effect (SNOW, 1994; TRAPE and ROGIER, 1996). This might suggest that permanent reductions in biting rates by any vector control scheme would produce little long term benefit. However, it can be argued that delay of attacks until an age at which the immune system is more mature may make the attack less life-threatening, and that an effective vaccine could replace the missing naturally acquired immunity. These problems might be entirely avoided by an effective SIT program since this method is designed to achieve eradication. If a malaria vector population could be permanently eradicated, there would need to be no concern about whether this would lead to a loss of immunity to malaria. However, the practical difficulties, especially in terms of multiplicity of species and habitats, in achieving sustainable eradication over large areas of rural tropical Africa should not be underestimated.

3. TARGET SPECIES AND SITE SELECTION

Malaria endemic areas abound in tropical Africa, Asia, Central America and South America and Melanesia. One or more model species recommended by the 1993 consultants report are found in each of these geographical areas except Melanesia. *Anopheles arabiensis* and *An. gambiae* are found throughout much of tropical Africa. *Anopheles stephensi* and *An. culicifacies* are widespread in south Asia. *Anopheles albimanus* is found in Central America and along the Pacific Coast in South America.

Each of these species exhibits behavioral characteristics that are dependent to some degree upon habitat and climatological circumstances. *Anopheles stephensi* and *An. arabiensis* are strongly related to urban situations in certain parts of their ranges and in many of these areas they are probably the only malaria vector species. Control of urban malaria transmission by either of these two species presents a highly desirable objective for mosquito SIT for two reasons:

- A tropical city represents a human population concentration in which the number of malaria cases per unit land area may be high. Thus, control of malaria in this situation provides a maximum return on effort in terms of the numbers of cases prevented.

- An urban anopheline focus may be surrounded by habitats beyond the city perimeter which are unfavourable to the urban vector species and thus act as a barrier. However, unless it can be established that the city anopheline population is completely isolated from any rural population of the same species in some manner, it would be necessary to continue preventative perimeter releases after reaching the objective of local eradication to assure continued protection against the threat of reinvasion.

Anopheles gambiae, An. arabiensis, An. culicifacies, and *An. albimanus* are plentiful in rural agricultural areas. Malaria control in these areas would have a significant impact on the cost of food production because of its effect on agricultural workers. In these situations, SIT operations could be expected to require releases over much broader areas to achieve the desired objective and, in some instances, be compromised by the presence of other important anopheline vectors.

Of the two options presented here, urban vs. rural, a greater impact on malaria prevalence would most likely be achieved in urban anopheline control. Also, an urban location could present fewer logistical challenges than a rural area. In either case, regional eradication of malaria by SIT or other genetic methods of mosquito control would, in the long run, depend on conducting sequential series of area-wide release programmes throughout existing malaria transmission areas against the resident vector species.

Previous experiences have emphasised that the benefits of eradication techniques such as SIT could be threatened by immigration of fertile mated females from outside the release area. In locations not isolated from mosquito populations that can re-infest the eradication zone, the likely solution to this problem is release the sterile insects over such an extensive area that the central part of the area is protected from fertile immigration by

continued releases in the border areas. Implementation of such a policy would involve a large and continued financial commitment for an indefinite period.

3. 1. Inspection of alternative sites

Prior to selecting a specific location it will be necessary to compile a list of potential cooperating Member States and/or agencies that can make the long term commitment required for a program of this nature. When this list has been culled and the best candidates have been identified, a multi-disciplinary team should be dispatched to visit and thoroughly inspect each location. The objective of the site visits would be to review the epidemiology of local malaria transmission, infrastructure quality and stability, physical and manpower resources, level of interest, long term commitment of financial support, local availability of necessary goods and services, and probable return in terms of human health.

3. 2. Clearances and project agreements

Studies at Seibersdorf related to this activity would require permits for anopheline introduction and colonization. The level of security required to prevent their escape and possible temporary establishment in the natural habitat would be subject to guidelines established by the Austrian government and the European Union. The cold Austrian winter can be relied on to prevent the risk of any permanent accidental establishment of these tropical species.

Project agreements relating to the initial methods development studies should identify the target anopheline species. This is an essential requirement as each target species differs biologically and 1-3 years may be required just to develop specific sexing methods. Subsequent site inspections would be directed to malarious zones infested with this vector.

4. REQUIREMENTS FOR A SIT PROGRAM FOR CONTROL OF MALARIA-TRANSMITTING MOSQUITOES

Information provided above in Section 2.3 and below in Appendix A outlines the general principles related to mosquito SIT for malaria control. This section describes in more specific terms the research and methods development required before embarking on small scale feasibility and large scale operational programmes.

4. 1. Research on target species and methods development

Extensive preliminary studies are required before embarking on a dispersal program for control of malaria with factory-produced mosquitoes. Once the target species and general location of a field study have been determined, 300-500 indigenous females could be collected for initiating a founder colony. As all five of the species recommended in the 1993 consultants report have been selected because of their potential for colonization, requirements

for mating in confinement should be well understood and egg production should be adequate to initiate the strain using small scale techniques. From this start, it should be possible to expand the colony enough to supply insects for general testing.

The experimentation at this stage would be directed toward automated mass-rearing, sterilization, sexing methods, and transport and dispersal methodology. These are areas in which the Entomology Unit of the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf has many years experience on other insect species. If studies are initiated, extra-budgetary funding should be provided for a 5 year period of methods development at Seibersdorf in a facility separate from the existing successful programmes on medfly and tsetse fly.

After completing this methods development phase and if initiation of a field phase is warranted, protocols for field assessment should be established and monitoring of target mosquito population trends should be initiated. After 18-24 months of monitoring and when production and dispersal capabilities are ready, releases could be initiated immediately after suppression of the indigenous anopheline population by insecticides, other density independent methods or by seasonal decline in density.

The specific requirements would be as follows.

4. 1. 1. Automated mass-rearing

Within 3-4 generations the founder stock can be increased dramatically because of the high fecundity of mosquito females. Thus, all stages would be available for testing special handling requirements for eggs, larvae, pupae and adults. There are several major objectives in this phase.

Egg handling routines must be simplified so that it is possible to manipulate eggs volumetrically. In some anophelines eggs can be dried and stored temporarily. By relying on volumetric techniques, not only is efficiency increased in setting larval rearing units but also the success of larval rearing is enhanced by systematic utilization of specific larval density in relation to known quantities of nutrients.

Larval nutrient requirements, which change from stage to stage, must be determined in order to provide high quality insects at optimal larval density. Once determined, the specifications can be adopted in an automated procedure. Purchase of dietary components, including yeast, liver powder and pulverised dog food, must be confined to close specifications, as variations in these commodities can have a major negative impact on production. Proper density and nutrient relationships encourage uniform larval developmental rates, which assure that a high percentage of the larvae are harvested. This is essential to minimizing the potential impact of genetic selection, which may occur if sufficient numbers of larvae suffer delayed development.

Ideally, pupal harvest could be confined to a single operation in each larval unit, but historically it has been necessary to harvest pupae from each unit on two or more consecutive days. An important objective of the automated rearing procedure is to minimize the number of daily harvests required per larval unit without increasing the probability of genetic

selection through excessive discard of immature larvae. Pupae can be treated and released or the resulting adults may be held for further manipulation prior to release. In adult cages stocked for colony maintenance and egg production, sugar water is provided for both sexes and females are provided a daily bloodmeal by membrane.

Adults are extremely sensitive to environmental conditions, e.g., temperature and relative humidity, confinement in cages, excessive density and disturbance. Automated handling procedures might reduce detrimental effects caused by these and other factors. Research will be required to minimize these effects for mosquitoes held as adults prior to distribution.

Unique requirements for back-up or complementary colonies are dependent upon the genetic mechanism utilized. With genetic sexing schemes in which recombination occurs, it may be necessary to maintain not only a "purged" strain but also an indigenous strain for reintroduction of the wild genome after subsequent purging operations.

4. 1. 2. Induction of sterility or other desired characteristics

The advantages of any mechanisms available to induce sterility in anophelines must be weighed carefully in terms of stability, cost, sustainability and environmental impact in addition to production factors. Radiation sterilization is a well known procedure with no environmental hazards, but somatic damage to the irradiated mosquitoes needs to be re-evaluated, especially if pupae are treated, which would have the advantage of allowing transport on damp substrate to release sites without risk of damage. After chemosterilization, minute but detectable residues may exist for a short time and the possible environmental consequences on non-target organisms (BRACKEN and DONDALE, 1972) should be investigated before any large scale use of this approach.

4. 1. 3. Sexing

Some type of automated system is required to separate male and female mosquitoes, as it is impractical to manually handle the quantities of insects required. In the past, four of the recommended target anopheline species have been genetically manipulated to provide selective response to some conditional lethal factor. This has been done by translocation to the Y-chromosome of a gene for resistance to an insecticide (SEAWRIGHT *et al.*, 1978; CURTIS *et al.*, 1976; CURTIS, 1978; ROBINSON, 1986). In this situation the females succumb to the exposure and the males survive. The sexing could be accomplished at any developmental stage during which the conditional lethal factor is operative, e.g., in the *An. albimanus* SIT project eggs were treated so that only males were reared for release (DAME *et al.*, 1981). Genetic sexing strains prohibit the release of the biting sex (females) and also may provide the opportunity to produce almost twice as many male mosquitoes as could be reared without the mechanism. Usually, there is a significant reduction in fecundity of genetic sexing strains compared to normal strains but this factor is not expected to be important in maintaining rearing efficiency. For mass release programs very close linkage of the resistance gene to the translocation breakpoint on the Y chromosome is required. This was achieved by introduction of an inversion to suppress crossing over around the translocation breakpoint in *An. albimanus*

by SEAWRIGHT *et al.* (1978). Very close linkage has also been achieved in strains of the Mediterranean fruit fly at Seibersdorf, and the methods used in the construction of those strains would probably be helpful in the production of an improved genetic sexing strain of the chosen anopheline species.

4. 1. 4. Distribution techniques

Aerial release methodology may be required to effectively employ sterile insect strategies for malaria mosquito control. To achieve this capability it would be necessary to develop handling and packaging methods compatible with existing aerial release devices and to determine the extent to which the fragile mosquitoes can be released from aircraft without detriment to behaviour and longevity.

Ground release methods have been developed in several research programs, including several by the WHO/ICMR Unit in New Delhi in the 1970s (SINGH and BROOKS, 1975). Yet, improvement of ground release methodology is also an important objective in order to assure that mosquitoes can be distributed to areas not serviced by aerial delivery.

It may be necessary to conduct preliminary studies with an alternate species because of the restrictions against releasing the vector species in Austria, but these studies must be repeated with the vector species when an operations location phase has been authorized.

4. 1. 5. Assessment methods

Mosquito density trends must be monitored prior to initiation of releases at an operations location to determine the impact of releases and compare population dynamics during release periods with normal trends for the same areas and with untreated control areas. Thus, development of data for test and control areas should be initiated 18-24 months prior to initiation of a release effort. Standard SIT criteria for monitoring mosquitoes include trapping, human landing rate counts, mark-release-recapture studies, house resting indices, larval surveys, etc., (SERVICE, 1993) as well as determination of the ratio of released to indigenous individuals and fertility assessment of females. The methods most appropriate for the situation should be implemented in all areas to be monitored in order to assure compatibility of the data records. Monitoring should be continued after the active release program for a period sufficient to assess the full impact of the release program.

Malaria incidence and/or prevalence must also be monitored to be able to interpret the impact of the releases on malaria transmission and morbidity. Active and/or passive case monitoring deemed most appropriate for the population under study should be initiated in both test and control areas 18-24 months prior to initiation of release efforts and continued for a sufficient period to assess the full impact of the release program. Project staff should work closely with local health agencies and WHO in the planning stages and in collecting and interpreting data on malaria incidence.

4. 1. 6. Quality control

During the methods development phase in Seibersdorf routine quality control standards should be developed for relevant phases of the rearing, handling, sterilization, distribution, and monitoring activities. At an operations location a quality control team assigned to the director's staff would be responsible for conducting these assessments. The results of their findings would be routinely transmitted directly to the appropriate work stations and to the director as soon after completion of the assessment as possible. Supervisory staff would use this information to determine what corrective action is required if certain parameters fall outside the control limits.

4. 1. 7. Prior suppression

Suppression of the indigenous anopheline population should not be required prior to any preliminary small scale releases for feasibility or methods assessment. In these cases recovery of marked insects or monitoring the induction of sterility in the indigenous females could be the primary parameters for assessment.

However, prior to the main programme releases it would be necessary to suppress the indigenous anophelines by insecticides or other density independent methods or by taking advantage of natural seasonal decline. This phase of the programme should be coordinated by the programme director and carried out by the host government. The variety of options available would depend on the target species and location.

4. 2. Physical resources (preliminary rough estimates)

4. 2. 1. Seibersdorf

The initial development studies should be done at Seibersdorf, where a facility must be constructed, if not available, with office and laboratory space adequate for producing 0.5 million mosquitoes per week (for preliminary cost estimates see Appendix B).

4. 2. 2. Operations location

The following resource projections are based on the subsequent field operations having a production capability of 5-10 million release insects per day (for preliminary cost estimates see Appendix C). It could be housed in existing facilities (not previously utilized in conjunction with insecticides or products toxic to mosquitoes) or in new temporary or permanent structures. In addition to administrative and supervisory offices, space would be required for all phases of the rearing, sterilization, holding, packaging and pre-distribution activities as well as maintenance, repair, storage and vehicles.

Standard office and laboratory furniture would be required, and also laboratory equipment including but not limited to balances, compound and dissecting microscopes, glassware, dissecting tools, etc.

Rearing unit production estimates of 5-10 million mosquitoes for release per day were chosen based on one of the very few absolute estimates available for natural mosquito productivity over an extensive area. In Pondicherry, India mark-release-recapture studies by the Vector Control Research Centre (MENON and RAJAGOPALAN, 1980) provided an estimate for the entire city of a maximum of 9 million *Culex quinquefasciatus* adults of both sexes emerging in one day and a minimum of 173,000. Urban anopheline population densities are likely to be at least one order of magnitude less than those of *Cx. quinquefasciatus*, i.e., maximums in Pondicherry of ca. 900,000 emergees (450,000 males) or fewer per day.

Specialized equipment to support the laboratory and rearing activities would include, but not necessarily be limited to: computer network, GIS software and supporting hardware, security devices, transmitter-receivers, irradiator(s), backup generators, water holding tanks, rearing trays, holding cages, industrial level climate control, specialised tracks and trolleys, overhead hoists, refrigeration units, water storage towers, aerial refrigerated release devices, containerised refrigerated mobile transport containers, industrial humidifiers, tray and dishwashing equipment, etc.

Standard equipment for laboratory and rearing would include various quantities of unbreakable bottles, trays, drums, buckets, measuring devices, protective clothing, clean-up and sanitizing gear, etc.

Standard field monitoring equipment includes, but is not limited to: hand-held and large aspirators, traps of various design, carbon-dioxide dispensing apparatus, GPS devices, data loggers, communications devices, battery powered lights, dippers, protective clothing, etc.

Vehicles for transporting goods, equipment, staff and insects would be required at the onset and replaced on a fixed pre-determined schedule. The following vehicles (air-conditioned in passenger and cargo areas) would be required for multi-purpose use, with bi-annual replacement of 4-wheel drive units: station wagon, 4-wheel drive [9]; truck, 3-ton [2], 3/4-ton [2]. Also , 4-wheeler run-arounds [6] and front-end loaders [2].

Contract aircraft may be required on a regular basis for aerial release activities [1200 hr or more per yr].

The following air-conditioned space [m^2] would be required: administration [50], director [20], laboratories [100], staff offices (entomologists, epidemiologist) [75], section leader offices [75], conference [20], office utility [20], office storage [40], and diet and perishable goods storage [2500],

The following climate-controlled space [m^2] would be required for each 5-10 million release mosquitoes produced per day: eggs [30], larvae [3000], adult colony [600], sterilization [75], holding and packaging [700] and cold rooms [40].

An irradiator(s) or a constant supply of chemosterilant would be required. The sterilization process should be able to treat 5-10 million release mosquitoes daily (8 h/day). Production above this level may require additional work shifts to complete the irradiation if only one irradiator is available.

4. 3. Manpower resources

4. 3. 1. Seibersdorf

The initial methods development phase would include the laboratory-based research activities described above in Section 4.1. to be carried out by the Entomology Unit at the Joint FAO/IAEA Agriculture and Biotechnology Laboratory in Seibersdorf (for costs see Appendix B). To implement these studies three new scientist positions (geneticist, entomologist [2]) and support staff would be required. Extra-budgetary funds to support this initial activity are estimated at ca. US $1 million per year; total US $5 million. As already emphasised, provision of this funding should be made in a manner that assures no adverse impact on the important existing work programmed at Seibersdorf.

4. 3. 2. Operations location

Implementation of activities at an operations location would be dependent on the progress achieved during the MMP at Seibersdorf and the CRP.

The required personnel at the operations location include staff for each of the following levels of operation to produce and release 5-10 million sterile males daily, 7 days a week, including holidays: director, office manager, bookkeeper, supervisory entomologist [5], epidemiologist, section leaders [12], maintenance and repairs [4], and assistants [69]. (for costs see Appendix C).

5. SUPPORTING AGENCIES

The Joint FAO/IAEA Division, with its sucessful history in SIT development and operations, has a mandate which encompasses food and agriculture. Agricultural productivity and rural economics would be major benefactors of a successful programme as a result of relief from the burden of malaria.

Should the Joint Division embark on the MMP, it should enlist the participation of WHO, which has extensive technical resources related to malaria incidence, species distribution, and conventional vector control methodology. WHO, with a mandate for health initiative, could be expected to provide advice on species and potential operational sites. WHO could provide operational support in on-site vector and disease monitoring activities in cooperation with the host government(s).

6. TIMETABLE AND COST ESTIMATES

Estimates of possible programme schedules, recruitment, procurement and costs are provided in Appendix B for Seibersdorf and Appendix C for an operational programme.

7. LIST OF CONSULTANTS

Dr. C. F. Curtis
London School of Hygiene and Tropical Medicine
Keppel Street
London, WC1E 7HT, UK

Dr. D. A. Dame
Entomological Services
4729 NW 18th Place
Gainesville, FL 32605, USA

STATUS OF STERILE INSECT TECHNOLOGY FOR MALARIA CONTROL

The SIT is a management method designed to eradicate the target insect population. Eradication of all of the mosquito vectors of malaria would eliminate malaria as a human disease. As described in detail in the 1993 consultants report, SIT studies have been conducted with several species of mosquitoes. Most of the research efforts have been relatively small scale, but release rates exceeding one million sterile males daily for extended periods have been achieved. Sterile anophelines have been successfully utilized for control of indigenous malaria mosquitoes (DAME *et al.*, 1980), but no programs have been yet been conducted to actually control malaria.

Several major components of SIT remain to be developed before feasibility studies and operational efforts can be initiated for malaria control. These requirements are outlined above in Section 4.

Anopheline mosquitoes are very fragile and require more careful handling, especially in the adult stage, than other Diptera which are current targets of SIT programs (fruit flies, tsetse and screwworms).

Unlike the genus *Plasmodium* which includes four species that cause malaria in humans, there are some 400 species of *Anopheles* in different parts of the world of which 10-15% are human biting and transmit the parasite to humans. These vector species inhabit tropical and temperate zones, often coexisting with other anophelines, in which case it would be necessary to control each of the vectors in order to prevent malaria transmission. Conventional methods may in some instances be effective. For example, in tropical Africa *An. gambiae* and *An. funestus* often co-exist as vectors but experience from the 1950s shows that the latter species can be eradicated by house spraying, thus narrowing the problem to a single species.

Several important vectors are members of species complexes in which it is difficult or impossible to identify the species by use of morphological characters. Some of these complexes have been clarified, whereas others have not yet been resolved. In such situations extended study is required to determine which members of the complex actually transmit the disease to humans and need to be targeted for SIT. Field identification of cryptic species is not currently feasible although laboratory methods include polytene chromosomes, allozymes, and when developed for specific anophelines, DNA restriction enzyme polymorphism.

Only the females can transmit the disease because the males are not capable of taking bloodmeals. Thus, it is necessary to take special precautions to minimize the numbers of females released in SIT programs. The genome of several anophelines has been manipulated in the laboratory to provide a method of eliminating the females from releases. A genetically sexed strain of chemically sterilized *An. albimanus* induced a high level of sterility in the indigenous study population in El Salvador. For each species to be considered for SIT it is necessary to develop this capability in order to prevent the risk of malaria transmission by the released insects.

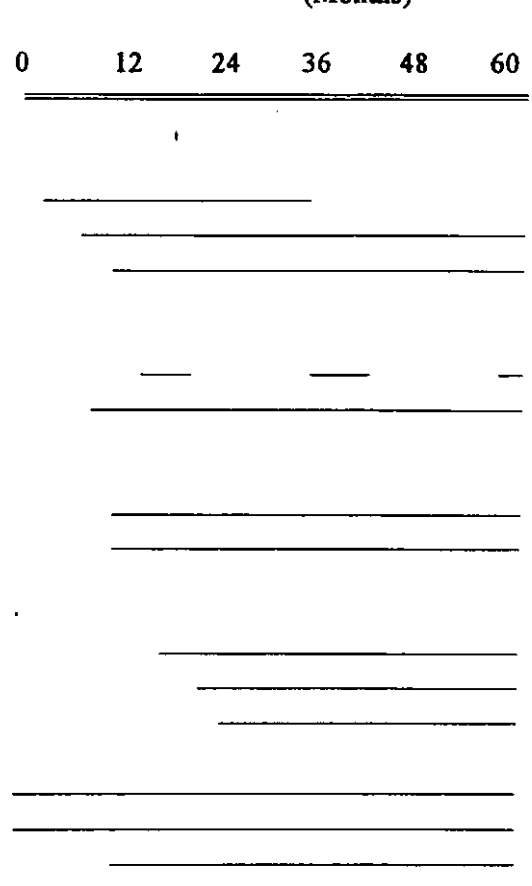
The use of sterile insects for population control is a density dependent method in which the requirement for release insects is dependent on the density of the natural population. In order to minimize SIT costs, natural seasonal declines are exploited and/or density independent suppression methods are employed to reduce the density of the target population. This can be accomplished with conventional insecticides, removal of breeding sites, etc. The suppression effort that precedes the release program can represent a major fiscal commitment. For this reason the entire integrated program is carefully planned to assure the most economical combination of the density independent control method and sterile insect releases. If multiple vectors are present the density independent control method is likely to suppress each species, but in most instances would have to be followed with repeated sterile releases of each vector species until eradication has been achieved.

Unfortunate experiences 20 years ago emphasised that very thorough public information programs are an important facet of mosquito SIT because people know that mosquitoes bite and transmit disease. It is necessary to demonstrate to the local population, medical and political authorities and the media that few if any biting females are being released and to convince them that there is a good chance that the program will reduce and eventually eliminate both the indigenous mosquito population and malaria. It is also important to demonstrate that measures are being taken to minimize the probability of reinvasion of the vectors from sources outside the eradication zone.

SEIBERSDORF MOSQUITO PROGRAMME FLOWCHART (AND BUDGET OUTLINE)

(Rough Estimates)

1. Activities

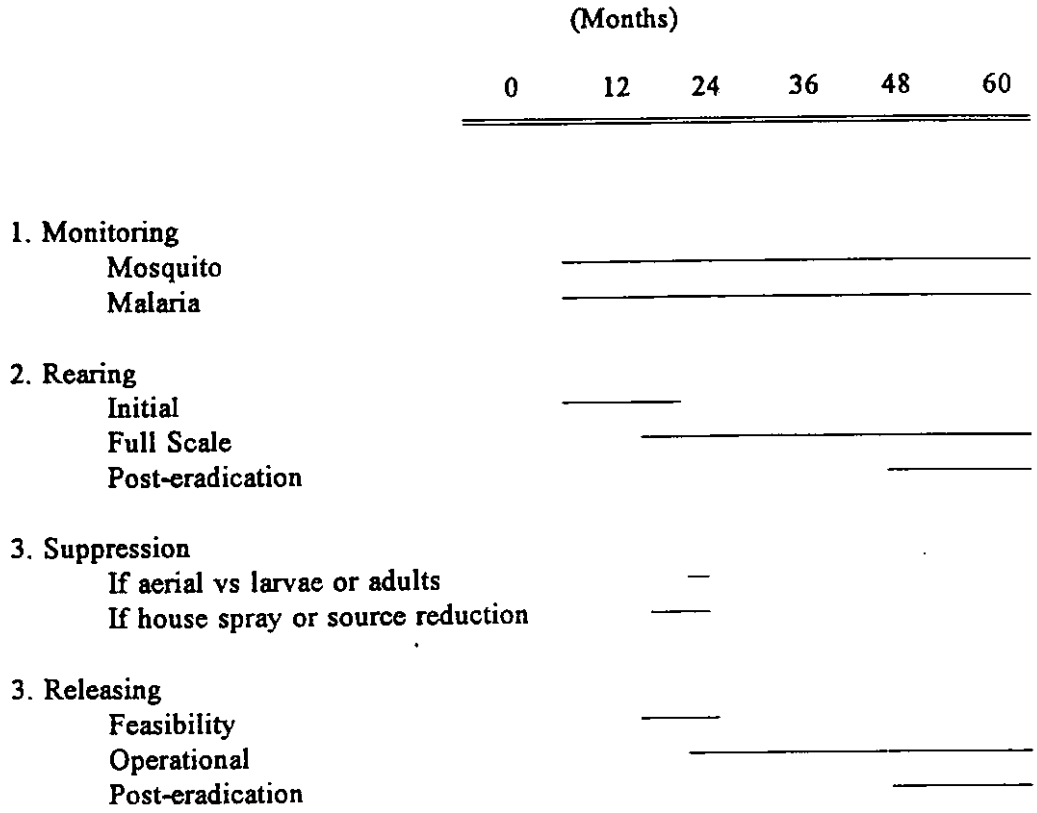


2. Budget

	US $1000					
Year	0	1	2	3	4	5
Programme						
Staff		480	494	505	520	536
Equipment, supplies		320	306	295	280	264
Overhead		200	200	200	200	200
Facility						
Construction	1500					
Total	1500	1000	1000	1000	1000	1000

OPERATIONS LOCATION PROGRAMME FLOWCHART

(Rough Estimates)



Operations Location Budget (US $; Rough Estimate)

		Year	1	2	3	4	5
Equipment	No.	US$/item					
Computers	15	1,800	27,000	2,000	2,000	2,000	2,000
Communications			25,000	2,000	2,000	2,000	2,000
Office			15,000	2,000	2,000	2,000	2,000
Generators	3	60,000	180,000				
Rearing			120,000	30,000	30,000	30,000	30,000
Distribution			55,000	15,000	15,000	15,000	15,000
Irradiator	2	160,000	320,000				
Vehicles							
Truck, 3-ton	2	35,000	70,000				
Truck, 3/4 ton	2	22,000	44,000				
Station wagon, 4WD	18	18,000	108,000	54,000	54,162	54,324	54,487
Front-ent loader	2	22,000	44,000				
4-wheeler	6	3,500	21,000				
Total Equipment			1,029,000	105,000	105,162	105,324	105,487
Supplies			220,000	300,000	300,000	300,000	300,000
Services Aircraft			40,000	160,000	480,000	480,000	480,000
Personnel							
Office							
Director	1	150,000	150,000	150,450	150,901	151,354	151,808
Office Manager	1	22,000	22,000	22,066	22,132	22,199	22,265
Bookeeper	1	15,000	15,000	15,045	15,090	15,135	15,181
Assistant	3	5,000	15,000	15,045	15,090	15,135	15,181
Rearing, Sterilization							
Entomologist	1	140,000	140,000	140,420	140,841	141,264	141,688
Section Leader	3	18,000	54,000	54,162	54,324	54,487	54,651
Assistants	30	5,000	150,000	150,450	150,901	151,354	151,808
Holding, Distribution							
Entomologist	1	140,000	140,000	140,420	140,841	141,264	141,688
Section Leader	2	18,000	36,000	36,108	36,216	36,325	36,434
Assistants	6	5,000	30,000	30,090	30,180	30,271	30,362
Release							
Entomologist	1	140,000	140,000	140,420	140,841	141,264	141,68?
Section Leader	2	18,000	36,000	36,108	36,216	36,325	36,4?
Assistants	6	5,000	30,000	30,090	30,180	30,271	30,362
Monitoring							
Entomologist	1	140,000	140,000	140,420	140,841	141,264	141,688
Epidemiologist	1	140,000	140,000	140,420	140,841	141,264	141,688
Section Leader	3	18,000	54,000	54,162	54,324	54,487	54,651
Assistants	12	5,000	60,000	60,180	60,361	60,542	60,723
Quality Control							
Entomologist	1	140,000	140,000	140,420	140,841	141,264	141,688
Section Leader	2	18,000	36,000	36,108	36,216	36,325	36,434
Assistants	6	5,000	30,000	30,090	30,180	30,271	30,362
Maintenance							
Supervisor	1	20,000	20,000	20,060	20,120	20,181	20,241
Electrician	1	16,000	16,000	16,048	16,096	16,144	16,193
Carpenter	1	16,000	16,000	16,048	16,096	16,144	16,193
Mechanic	1	16,000	16,000	16,048	16,096	16,144	16,193
Assistants	6	5,000	30,000	30,090	30,180	30,271	30,362
Total Personnel			1,656,000	1,660,968	1,665,951	1,670,949	1,675,962
TOTAL			**2,945,000**	**2,225,968**	**2,551,113**	**2,556,273**	**2,561,449**

REFERENCES

ALONSO, P.L. *et al.* (1995) A control trial using insecticide treated bednets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa, 6. The impact of the interventions on mortality and morbidity from malaria. Trans. Roy. Soc. Med. Hyg. 87 (suppl. 2): 37-44.

ALONSO, P.L. *et al.* (1994) Randomised trial of efficacy of SPf66 vaccine against *Plasmodium falciparum* malaria in children in southern Tanzania. Lancet 344: 1175-1181.

ANONYMOUS. (1966) Might spread of malaria be halted by genetically engineered mosquitoes? [News item quoting J.M. Crampton]. Pharmaceutical J. 257: 19.

BINKA, F.N. *et al.* (1996) Impact of impregnated bednets on child mortality in Kassena-Nankana, Ghana: a randomized controlled trial. Trop. Med. Intl. Hlth. 1: 147-154.

BRACKEN, G.K. and DONDALE, C.D. (1972) Fertility and survival of *Achaesanea tepidariorum* on a diet of chemosterilized mosquitoes. Can. Ent. 104: 1709-1712.

CHENG HAILU, YANG WEN, KANG WANMIN and LIU CHONGYi. (1995) Large-scale spraying of bednets to control mosquito vectors and malaria in Sichuan, China. Bull. Wld. Hlth. Org. 73: 321-328.

COLLINS, F.H. and PASKEWITZ, S.M. (1995) Malaria: current and future prospects for control. Ann. Rev. Ent. 40: 195-219.

CRAMPTON, J.M. (1996) Prospects for genetic manipulation of insect vectors as a strategy for control of insect vectors of disease, Proc. 2nd Int. Conf. Insect Pests in the Urban Environment, ed. Wiley, K.B., Edinburgh, pp. 1-9.

CURTIS, C.F. (1978) Genetic sex separation in *Anopheles arabiensis* and the production of sterile hybrids. Bull. Wld. Hlth. Org. 56: 453-454.

CURTIS, C.F. (1996) Insect vectors of human diseases, in "Biotechnology and insect pest management". ed. Persley, G.J. CABI, U.K. pp. 327-339.

CURTIS, C.F., AKIYAMA, J. and DAVIDSON, G. (1976) A genetic sexing system in *Anopheles gambiae* species A. Mosq. News 36: 493-498.

D'ALESSANDRO, U. *et al.* (1995a) Reduction in mortality and morbidity from malaria in Gambian children following introduction of a national insecticide inpregnated bednet programme. Lancet 345: 479-483.

D'ALESSANDRO, U. *et al.* (1995b) Efficacy trial of malaria vaccine SPF66 in Gambian infants. Lancet 346: 462-467.

DAME, D.A., LOWE, R.E. and WILLIAMSON, D.L. (1981) Assessment of released sterile *Anopheles albimanus* and *Glossina morsitans*, in "Cytogenetics and genetics of vectors", Proc. XVI Int. Congr. Entomol., Kyoto, 1980, ed. Kitzmiller, J.B. and Kanda, T., Elsevier Biological, Amsterdam., pp. 231-241.

DAVIDSON, G., ODETOYINBO, J.A., COLUSSA, J.A. and COZ, J. (1970) A field attempt to assess the mating competitiveness of sterile males produced by crossing two members of the *Anopheles gambiae* species complex. Bull. Wld. Hlth. Org. 42: 55.

HILL, A.V.S. *et al.*, (1992) Molecular analysis of HLA-B53 and resistance to severe malaria. Nature 360: 434-439.

KASLOW, D.C. *et al.* (1994) *Saccaromyces cerevisiae* recombinant Pfs25 absorbed to alum elicits antibodies that block transmission of *Plasmodium falciparum*. Infection and Immunity 62: 5576-5580.

LOFGREN, C.S. *et al.* (1974) Release of chemosterilized males for control of *Anopheles albimanus* in El Salvador, 3. Field methods and population control. Am. J. Trop. Med. Hyg. 23: 287-297.

MENON, P.K.B. and RAJAGOPALAN, P.K. (1980) Relative importance of different types of breeding habitats in contributing to the population of *Culex quinquefasciatus* in Pondicherry. Ind. J. Med. Sci. 71:725-733.

NEVILL, C. *et al.* (1996) Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. Trop. Med. Intl. Hlth. 1: 139-146.

PATTERSON, R.S. *et al.* (1975) Use of radiosterilized males to control indigenous populations of *Culex pipiens quinquefasciatus*: laboratory and field studies. Mosq. News 35: 1-7.

PATTERSON, R.S. *et al.* (1977) Release of radiosterilised males to control *Culex pipiens quinquefasciatus*. J. Med. Entomol. 14: 299-304.

ROBINSON, A.S. (1986) Genetic sexing in *Anopheles stephensi* using dieldrin resistance. J. Am. Mosq. Contr. Assoc. 2: 93-95.

SEAWRIGHT, J.A. (1993) Genetic methods for control of mosquitoes, in "The potential for genetic control of malaria-transmitting mosquitoes", report of a consultants group meeting, Joint FAO/IAEA Division, Vienna. pp. 19-28.

SEAWRIGHT, J.A., KAISER, P.E., DAME, D.A. and LOFGREN, C.S. (1978) Genetic method for preferential elimination of females of *Anopheles albimanus*. Science 200: 1303.

SEDEGAH, M., HEDSTROM, R., HOBART, P. and HOFFMAN, S.L. (1994) Protection against malaria by immunization with plasmid DNA encoding circumsporozoite protein. Proc. Nat. Acad. Sci. USA 91: 9866-9870.

SERVICE, M.W. (1993) "Mosquito ecology: field sampling methods". 2nd Edition, Elsevier Applied Science, London and New York.

SINGH, K.R.P. and BROOKS, G.D. (1975) Semi-automatic release system for the distribution of mosquitoes during genetic control operations. J. Communic. Dis. 7:288-293.

SNOW, R.W. (1994) Severe childhood malaria in two areas of markedly different *falciparum* transmission in East Africa. Acta Trop. 57: 289-300.

TRAPE, J-F. and ROGIER, C. (1996) Combating malaria morbidity and mortality by reducing transmission. Parasit. Today 12: 236-240.

VALERO, M.V. *et al.* (1993) Vaccination with SPf66, a chemically synthesised vaccine against *Plasmodium falciparum* malaria in Colombia. Lancet 341: 705-710.