

**FIRST PLANNING MEETING ON
DEVELOPMENT OF THE STERILE INSECT
TECHNIQUES FOR CONTROL OF MALARIA-
TRANSMITTING MOSQUITOES**

5-8 June 2001

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1. BACKGROUND

At the request of Member States a series of consultant's reports were commissioned over the past 10 years to assess the potential of developing and using the Sterile Insect Technique (SIT) for the control of vectors of malaria. The experts reports recommended that the Agency proceed with such an evaluation. The rationale for the possible inclusion of SIT into malaria vector control were detailed in these reports and will not be dealt with here. All the reports emphasized that significant R and D would be required to develop and evaluate the SIT technology for mosquitoes before operational pilot projects could be initiated. Following the last of these meetings a document was prepared in which the essential R and D components were identified. This plan also included the collection of baseline data from a potential field site in Africa and the proposal that the target species should be *Anopheles arabiensis*.

On the basis of these activities a Technical Co-operation (TC) project was developed which focussed on the identification of a potential field site and provided funds for initiation of the collection of epidemiological and entomological data from the site. The R and D requirements for mosquito SIT were addressed in two ways. Firstly by undertaking limited R and D activities at the Agency's Laboratories in Seibersdorf and secondly by elaborating a Co-ordinated Research Project (CRP).

This first planning meeting was thus held in Vienna from 5-8 June 2001 with representatives from Ethiopia, Mali, Namibia, Niger, Nigeria, Senegal, South Africa and Sudan; as well as experts from the UK and the USA; and a representative from the World Health Organisation (WHO) (see Appendix 1 and 2 for agenda of the meeting and participants list). The meeting provided a forum for the participants to summarize the current malaria situation, its control and the importance of *An. arabiensis* in their respective countries. The outside experts complemented these presentations by dealing with specific issues.

The objectives of the meeting were to:

- Review the status of the control of malaria-transmitting mosquitoes in Member States including Government policies.
- Review the state of art for possible use of SIT for the control of malaria-transmitting mosquitoes.
- Formulate long-term and short-term strategies and action plans for R and D aimed at possible use of SIT for the control of *A. arabiensis*.
- Identify international and regional partners and discuss modalities for co-operation.

Presentations were made on the first two days followed by break-up of the participants into three working groups looking into Research and Development (R&D), baseline data collection and formulation of draft project proposal.

2. SUMMARY OF REPORTS FROM THE COUNTRY REPRESENTATIVES

The full reports for each country are available on request and only the more important points will be highlighted here based on the written information provided.

2.1. Ethiopia

Geography: Ethiopia is located between 3⁰-15⁰ N latitude and 33⁰-48⁰E longitude. The total surface area is about 1.1M km². The highland plateau that ranges between 2000-3000 meters above sea level is dissected by the Great Rift Valley and many other river valleys and escarpments and covered by about a dozen of mountains rising above 4000 meters. Altitude is one of the important factors that influence the distribution of diseases in Ethiopia.

Malaria Situation: Malaria affects about 4-5 million people annually, and is prevalent in 75% of the country putting over 40 million people at risk. Generally, areas lying below 2000 meters altitude are malarious whilst the highlands are densely populated and over cultivated. In addition, transmissions of malaria in Ethiopia are closely linked with the rainy seasons. The major transmission season follows the June-September rains and occurs between September-December while the minor transmission season occurs between April-May following the February-March rains. Of the total 350 DLY's/1000 population lost annually, malaria accounts for 10.5%.

Epidemiology: All the four *Plasmodium* parasites are reported in Ethiopia. *P. falciparum* is the most important one and comprises 60% of all malaria cases in the country. *P. vivax* makes 40% of the cases. *P. malariae* and *P. ovale* constitute less than 1%. *P. falciparum* has been reported to be resistant to chloroquine. Malaria vectors in Ethiopia include *Anopheles arabiensis*, *An. pharoensis*, *An. funestus* and *An. nili*; the major vector being *An. arabiensis*. *An. arabiensis* is the major vector of malaria in Ethiopia. *An. gambiae* complex (in which *An. arabiensis* is a member) is known to be the most frequent and widely distributed species in the country. From the *An. gambiae* complex only two species, *An. arabiensis* and *An. quadriannulatus*, are reported to exist in Ethiopia. In a five year period (1984-1988) outdoor and indoor collections made at areas representing low, moderate and intense transmissions of malaria in different administrative regions; 75.5% of the total collection comprised of *An. gambiae*. Different cytogenetic studies have shown that *An. arabiensis* is more predominant species than *An. quadriannulatus*.

Malaria Control: The major vector control measure that is being used in the country is in-door residual insecticide (DDT) spraying. As a result *An. arabiensis* has developed resistance to DDT in some areas. In such cases malathion is used as an alternative. Other vector control measures such as source reduction, chemical larviciding and very recently use of insecticide treated nets (ITNs) are also used in selected areas.

2.2. Mali

Malaria: Malaria prevalence in Mali is predicted to be highest in the south west of the country and lowest in the north reflecting the different climatological zones. Malaria is transmitted by both *An. gambiae* and *An. arabiensis*. The two species are found throughout the country with *An. arabiensis* tending to be the sole vector close to the

border with Mauritania. There has been an intensive study of *An. gambiae s.s.* in Mali resulting in the identification of three chromosomally distinct forms which are distributed according to the climatological zones. The Savannah form is found predominantly in the south-west with the Mopti form being found in the drier north east.

Malaria Control: The national malaria control programme (PNLP) places much reliance on the large scale use of ITN and chloroquine is the first line drug. Special attention is paid to children under 5 and pregnant women. The Malaria Research and Training Centre is doing extensive work on the different chromosomal forms and is using microsatellites to characterize the population structure. Mark-release-recapture studies are also being undertaken to assess dispersion and population size.

2.3. *Namibia*

Geography: Namibia has a surface area of 824,295 km² with an estimated population of 15M with an annual population growth rate of 3.16%. The country has three major topographical regions namely: the Namib Desert, the Central Plateau and the Kalahari Desert.

Malaria Situation: There are about 400,000 cases and 300–500 deaths due to malaria each year. Malaria is mainly endemic in the northern regions of Namibia where about 60% of the population lives. Every year there are about 350,000 new outpatient cases and 26,000 new inpatient cases reported. Namibia is the driest country in Southern Africa with about 92% of the total surface area classified as arid. Rainfall in Namibia varies greatly in time and space. Rainfall varies from less than 50mm/year on the Skeleton coast and in the Namib and Kalahari deserts to more than 500mm/year on the Caprivi Strip in the extreme north east of the country. The rainy season is from November to April with peak rains in February-March and malaria transmission is expected to peak from February to April. The predominantly seasonal nature of malaria transmission in northern Namibia prevents individuals from acquiring strong immunity to malaria.

Epidemiology: *P. falciparum* is the most predominant and widely spread malaria parasite and is responsible for about 97% of all malaria infections. Despite chloroquine resistance to *P. falciparum* recorded throughout Africa, chloroquine still is and remains an effective first-line drug in Namibia. *An. arabiensis* is probably the major vector of malaria and is most predominant throughout Namibia. *An. funestus* is mainly found in the NE due to the permanent water bodies such as rivers. *An. gambiae s.l.* is the third vector but is not present in high numbers due to DDT residual house spraying.

Malaria Control: Vector control measures are of particular relevance in areas where transmission is seasonal and unstable and vector breeding places are restricted. Namibia opted for selective control consisting primarily of indoor residual house spraying with DDT. The coverage for 2000/01 ranged from 75-92% in two Directorates; in addition, residual house spraying is carried out in high risk areas of Central and South Health Directorates. Deltamethrin was used for the first time in Namibia during the recent epidemic in Katima Mulilo urban areas. There is so far no evidence of DDT resistance in the local vectors.

2.4. Niger

Malaria Situation: An average of 850 000 cases and 7500 deaths occur per year due to malaria. 25% of all motives for medical visits is due to malaria; this rises to about 70% in the rainy season. The lethality rate in hospital is greater than 20% and about 15 children die per day due to malaria. There is more and more evidence of therapeutic failure and resistance to insecticides in vectors. The consequences of malaria in Niger include an increase of morbi-mortality especially among the children between 0 to 10 years, pre and post natal complications and troubles in infant development. It is estimated that the estimated cost of treatment per year amounts to FCFA425.000.000 with an average monetary value loss of FCFA 6.000.000.000 per annum.

Epidemiology: There are between 13 to 20 species of Anopheles with a predominance of the *An. gambiae* complex. The highest density of vectors is in south and central areas especially during the rainy season. There is also evidence of colonization of oases and cities in the mining areas in the north. Malaria caused by *An. arabiensis* is present throughout the country.

Malaria Control: Vector control activities include sanitation measures, destruction of larval breeding sites, promotion of impregnated mosquito nets and house spraying. Since the launching of the RBM initiative, an average (over the past 3 years) of almost FCFA 250.000.000 per annum has been allocated to this programme with contributions from the Government (4.5%), WHO (30%), UNICEF (45.5%), World Bank (15%) and the European Community (5%).

2.5. Nigeria

Malaria Situation: Malaria is the major cause of mortality and morbidity in Nigeria and accounts for 25% of infant mortality and 30% of child mortality. 50% of the population has at least one malaria attack/year.

Malaria Control: Malaria is holoendemic in the rural areas and *P. falciparum* accounts for 90% of all malaria infections. The predominant vector in the savannah and cities is *An. arabiensis* with *An. funestus* in the forest and *An. gambiae* present where there is permanent water. Permethrin resistance has been found south of Benin but in the north the population is still susceptible. Pyrethroid treated bednets work effectively and are strongly promoted. The possible occurrence of urban populations of *An. arabiensis* in large towns could provide potential field sites for evaluation of SIT for *An. arabiensis* control.

2.6. South Africa

Malaria Situation: In South Africa, 10% of the population is at risk and transmission is seasonal. Reported cases peaked to 60,000 in 1999.

Epidemiology: The high risk areas are found in the NE part of the country and *An. arabiensis* is the major vector. *An. funestus* was eradicated using DDT; but it returned following the switch to pyrethroids as it was resistant to this class of insecticides.

Malaria Control: The mosquito control programme targets the parasite and the vector. Vector control uses residual house spraying and larviciding in winter or in emergencies. Insecticide treated bednets are also being used.

2.7. Senegal

Malaria Situation: Malaria is the main cause of morbidity in Senegal and accounts for 7,000 deaths/year. There has been an increase in the number of severe malaria cases as well as new epidemics in irrigated areas and in peri-urban situations. Drug resistance is extensive and increasing but chloroquine is still effective. Drug resistance has had a major impact on child mortality. The front-line drug is chloroquine which can be substituted by amodiaquine. The second line treatment is sulfadoxin-pyrimethamin (SP) and for severe cases quinine is used. There is some evidence that resistant gametocytes are more infectious than sensitive ones.

Epidemiology: In the Sahelian and Sahelo-sudanian areas *An. arabiensis* is the most abundant species but in the Sudanian region *An. funestus* and *An. gambiae* occur in larger numbers. The infection rate is generally lower in *An. arabiensis* where it co-exists with *An. gambiae*. There is fairly widespread resistance to DDT but permethrin and deltamethrin are still effective.

Malaria Control: There are 38 impregnated bednet centres giving 25% coverage and operated by 152 trained technicians. In Dakar and other urban areas house spraying is used.

2.8. Sudan

Geography: Sudan is the largest country in Africa with a total area of 2.6M km² and malaria endemicity increases from north to south as the isohyets increase.

Malaria Situation: Malaria is hypoendemic in the north, meso-endemic in the central regions and some holoendemic transmission in the south where transmission is perennial.

Epidemiology: *P. falciparum* is the main parasite species and malaria prevalence was 12.75 in 1998. There are 31 species of *Anopheles* identified in Sudan, 18 of which are malaria infectious. *An. arabiensis* is the principal vector and is distributed throughout the country. *An. funestus* and *An. gambiae* are vectors in the south. *An. arabiensis* is highly endophilic except in the east and is strongly anthropophilic. The species is well adapted to dry conditions and in the seasonal breeding areas the vector density can be virtually zero. It is not clear how these areas become re-infested in the rainy season. There is little inversion polymorphism in Sudanese populations of *An. arabiensis*.

Malaria Control: Mosquito control activities focus mainly on residual house spraying but with some fogging activities in irrigated areas. Malathion house spraying is used in Khartoum but the use of impregnated bed nets is not popular. There is also some aerial spraying.

3. SUMMARY OF PRESENTATIONS BY OUTSIDE EXPERTS

3.1. *Methods for Control of Vector Mosquitoes and the Possible Role of SIT*

C. F. Curtis

a) China, Vietnam and Singapore

Approximately 10 million bednets are treated with insecticide in China and Vietnam annually. The nets are privately owned but treatment is provided by health authorities who also carry out house spraying in the same areas. Artesunate (from *Artemesia*) was shown to be effective against *P. falciparum* but it is resistant to other drugs; however, the combined use of artesunate and treated bednets has greatly reduced malaria burden in Vietnam in the last 10 years. Despite a sophisticated *Aedes* control programme, Singapore remains endemic for dengue. The use of SIT for routine control or to eradicate *Ae. aegypti* and *Ae. albipictus* from the island and nearby parts of Malaysia has been suggested.

b) Indian sub-continent

Though the number of malaria cases is less than what it used to be in the 1930s, it rose in the 1960s at the height of house spraying campaigns when India used 18,000 tonnes of DDT annually. Sri Lanka switched from DDT in 1970s and India has stated that it intends to do so. Rural malaria is mainly transmitted by *An. culicifacies* and other species whilst urban malaria is transmitted by *An. stephensi*. If *An. stephensi* exists as "urban islands" it should be possible to control or eradicate by SIT; however, this needs to be confirmed. In the 1970s SIT trials with *Culex* and *Aedes* showed that moderately competitive sterile males could be produced but village to village movement of *Culex* pointed to the fact that urban populations could be better targets. Sex separation in culicine mosquitoes can be done on the basis of pupal size.

c) *An. arabiensis* in north east Africa and Red Sea coast

It is the only man-biting member of the *An. gambiae* complex in central and northern Sudan, Ethiopia and the Arabian Red Sea coast. *An. arabiensis* invaded Upper Egypt in 1942 and caused a malaria epidemic as it is a much more efficient vector than *An. pharoensis* but it was eradicated using arsenical larvicide (and some DDT) by 1945. The creation of Lake Nasser by Aswan High Dam has not (yet) led to another invasion by *An. Arabiensis* into Egypt. There were successful house spraying programmes in the Gezira from the 1960s to 90s with switching of insecticides as resistances developed. There have been several research studies on the survival of *An. arabiensis* and malaria through the long dry season of eastern Sudan but the picture is far from clear. Filariasis transmitted by *Cx. pipiens* is a more important mosquito borne disease than malaria in Egypt at present.

d) Europe and Central Asia

Malaria disappeared from northern Europe in the first half of the 20th century. It was eradicated from southern Europe and USSR between 1940s and 60s but there has been a resurgence of major epidemics in Central Asia in the 1990s. The *An. maculipennis* complex is only susceptible to *P. vivax*, but other species are susceptible to *P. falciparum*. There are thousands of imported cases of malaria into Europe each year and global warming would increase the chances of any *Plasmodium* gametocytes ingested by a mosquito completing their development. The prompt treatment of imported malaria cases renders infection of mosquitoes by gametocytes very

improbable. Invading populations of *Aedes albopictus* in Italy and Albania are potential dengue vectors and have been considered as targets for eradication by SIT.

e) Tropical Africa

The *An. gambiae* complex and *An. funestus* are highly anthropophilic and are therefore efficient vectors. About 80% of the world's annual 400 million clinical malaria cases are in this region as well as about 90% of world's annual 1-2.5 million malaria deaths. The death rate is rising probably because of rising drug resistance. Extensive house spraying trials in 1950's, 60's and 70's reduced morbidity but did not eradicate the disease; currently there is almost no house spraying in tropical Africa. Vector control efforts are now concentrated on Social Marketing of Insecticide Treated Nets but free insecticide treatment gives better coverage, better reduction of vector populations and costs less. Filariasis is transmitted by *Culex quinquefasciatus* in East African towns, but by anophelines in rural East Africa and West Africa. Drug donations are now the basis of attempts to eliminate filariasis, but expanded bead layers can control *Culex* larvae and anti-malaria vector activities will probably also impact on filariasis transmission.

f) Southern Africa

DDT spraying has greatly reduced malaria burden compared to the 1930's. There was no DDT resistance and *An. funestus* was eradicated in South Africa and Madagascar highlands. The switch to pyrethroids in S. Africa in 1996 was followed by 4 fold increase in malaria and the re-appearance of *An. funestus* which was resistant to pyrethroids, but not to DDT. This resulted in a switch back to DDT in 2000. The resurgence of *An. funestus* in the Madagascar highlands caused a malaria epidemic that killed tens of thousands but the reinstatement of DDT spraying has controlled the epidemic and re-eradicated *An. funestus* in the plateau region. Negotiations in Johannesburg in December 2000 about the Treaty on Persistent Organic Pollutants, resulted in DDT being re-introduced for vector control.

g) Latin America

As the intensity of the national DDT campaigns has waned, malaria cases have increased and this has been associated with an increase in the colonization of the Brazilian Amazon. Multiple insecticide resistance in *An. albimanus* in Central America in the 1970s was due to pressure from agricultural insecticides; with introduction of IPM and partial abandonment of cotton growing; however, this resistance has now declined. In 1970s there were successful field trials of SIT using chemosterilization against *An. albimanus* in El Salvador where genetic sexing based on Y translocation of insecticide resistance gene was used. *Ae. aegypti* was eradicated from most of the Americas (but not USA) in the 1950s as a measure to control yellow fever but there has been subsequent re-infestation resulting in dengue epidemics. Effective control has only been maintained in Cuba.

3.2. Population Structure of *Anopheles arabiensis* and its implications for SIT

H. Townson

An. arabiensis is the most widely distributed malaria vector in Africa and is also found in the Arabian peninsula and there are distinct differences in genetic variation throughout its range. There is less chromosomal polymorphism and greater zoophily and exophily east of the Rift valley with distinctive populations in Namibia and

Madagascar. There are now powerful tools to analyse DNA and they are being used to try to understand gene flow and genetic drift in field populations of *An. arabiensis*. Microsatellite analysis was used to analyse populations of *An. arabiensis* in Tanzania and Mozambique, a distance of some 2000 km. There were significant differences in allele frequencies between the two populations but high rates of gene flow. This suggests recent population separation and not large-scale present day migrations.

A second study was made of populations from Sudan (2), Ethiopia, Tanzania, Malawi (2) and Mozambique (3) with 8 microsatellite loci. The two populations studied in both Malawi and Ethiopia were not different from each other but populations separated by more than 2000 km showed highly significant differences with extensive barriers to gene flow. These data again suggest that the present distribution of *An. arabiensis* is due to a recent range expansion rather than contemporary gene flow. This recent range expansion is probably closely related to human activities. The information on population genetics is important for SIT as it indicates that there are probably no pre-mating isolation barriers between the different populations of *An. arabiensis*.

3.3. History of Mosquito Releases for Control and Potential of New Molecular Capabilities

M. Benedict

Ten different field trials, of varying sizes, have been carried out with sterile mosquitoes, the majority being in the 1970's. The major trials were in India, Burma and El Salvador. The major biological problems encountered were the failure of the sterile males to mate with the wild females and density dependent larval survival. There were also several problems associated with the technology, e.g. failures in mass rearing, inappropriate release technologies and immigration into the treatment area.

New transgenic developments may offer some improvements but the above constraints will still need to be solved. Systems to genetically sterilize insects in the field are being evaluated in mosquitoes and have been shown to be successful in *Drosophila*. The genetic constructs used in *Drosophila* may well function in *Anopheles*.

3.4. A Review of the SIT Programme for *Anopheles albimanus* in El Salvador

P. Kaiser

Two field trials were carried out with SIT in El Salvador, the first at Lake Apastepeque in 1971-72 and the second at La Libertad in 1975-79. In Lake Apastepeque, using mechanical separation, about 32,000 males were released/day for 14 weeks and 97% sterility was monitored in the wild population but the ratio of sterile to normal males was not estimated. The mosquitoes were chemosterilized and cost US\$280/million. The target area in La Libertad was 150 km² and about 50% of the local residents were infected with malaria. Initially sexing of males was carried out by membrane feeding with blood containing 0.025% malathion and 200,000 males/day were produced. In a test the release of sterilized male pupae was better than the release of adults. Males from a genetic sexing strain were field tested in 1977 and resulted in significant reduction in population increase. In a larger field trial, up to 1 million males were released/day and in 1979, 95% sterility was achieved in the field. Immigration of fertilized females into the area was a problem as well as breeding of mosquitoes in unexpected areas during the dry season.

4. OUTPUTS OF THE MEETING

After the first two days of presentations, participants were divided into three working groups. The three working groups were tasked to prepare a concise report on three important areas: short- and long-term R&D, baseline data collection from potential field sites and a project proposal. These reports were presented on the final day of the meeting during the plenary session where they were discussed and reviewed by all the participants.

4.1. Working Group 1. Short term and long term R and D

This group was tasked with identifying R and D that would be required to evaluate the feasibility of using SIT for the integrated control of *A. arabiensis*. The discussions covered both laboratory and field activities and were ranked in order of priority [**1-4 in square brackets**]. The group also identified two additional concerns that deserve attention during project development and these are summarized at the end of the list.

Release Methods

- a) [2] Devise appropriate techniques for dispersal of sterile adult males in consultation with a contractor using aerial or ground-based systems.
- b) [3] Study and consider alternative dispersal methods. Summarize methods that have been previously used in mosquito trials.

Release Material

- a) [1] Inventory extant laboratory strains and catalog pertinent information for each. Place importance on place and date of origin and insecticide resistance.
- b) [1] Improve mass-rearing technology. Develop technology that is consistent with available labor and produces consistent quality material. Improve colony maintenance. Improve membrane blood feeding.
- c) [2] Develop methods to reduce loss of fitness and competitiveness of sterilized males. Develop improved larval diet, pupa separation, sterilization-specific handling, packaging, transportation, dispersal.
- d) [2] Develop sterilization techniques that minimize loss of competitiveness. Consider radiation, chemicals and molecular methods.
- e) [1] Develop classical genetic sexing system. Colony will be developed from the release site and introduction of fresh genetic material will be performed before releases begin. The system must be stable when mass-reared.
- f) [2] Develop molecular strategies (i.e. genetic transformation/RIDL) as a future replacement for classical sexing system.
- g) [3] Develop factory quality control (QC) protocols to monitor quality of sterile males (elements including e.g. longevity, pupa-yield per unit, pupa weight, cage mating frequency). In addition, develop methods to assess the post-release quality of males including dispersal, adult survival, and longevity.
- h) [3] Determine effect of quantitative factory QC elements and procedures (e.g. density, pupa size, development rate, sugar source) on male competitiveness.
- i) [2] Develop protocols for periodic measurement of competitiveness in field trials. An outdoor facility (e.g. net material) at the release site should be constructed that simulates mating site.

Field Site Information (this will require substantial resources)

- a) [1] Review and update Africa-wide distribution map for *An. arabiensis*.
- b) [1] Create a detailed spatial and seasonal release site map of *An. arabiensis* distribution (relative density) and relevant features (e.g. GIS, satellite, adult resting sites, larval breeding, domestic animal locations, water bodies, landscape). Develop techniques to carry out mark-release experiments to estimate absolute population size.
- c) [2] Confirm whether urban mosquito populations in western Africa constitute island populations of a member of the *gambiae* complex species, specifically *An. arabiensis*. Prof. M. Coluzzi (U. Roma) has previously published information indicating that populations in some cities in Nigeria consist only of this species and that the frequency of *An. arabiensis* is much lower, if not absent, from surrounding rural areas.
- c) [1] Develop protocols to measure vector population distribution, size and density. These protocols will be used for collection of baseline data preceding release and to measure the effect of releases on vector populations. Use wild-caught female sterility, larval surveys and adult collections and trapping. Collect information relevant to transmission including landing biting rates, sporozoite rate and clinical case rate (febrile illness with slide positivity).
- d) [1] Evaluate immigration from surrounding breeding areas before intervention. The level of effort required to determine this will depend on the exact site selected.
- e) [2] Assess population structure of wild *An. arabiensis* at release site. A representative sample over the release range should be performed along a transect covering the extremes of the release area. Microsatellite analysis will be performed with emphasis given to extensive sampling. The goal is to confirm that evidence of mating substructure that would interfere with effectiveness of releases does not exist.
- f) [2] Determine if insecticide resistance alleles occur at the site that could be utilized to create a classical sexing strain.
- g) [1] Confirm that *An. arabiensis* is the sole primary vector in the release area(s). Specifically, the absence of *An. gambiae s.s* and *An. funestus* should be confirmed beginning as soon as feasible and continuing until releases begin. This will require an extensive survey of *Anopheles* species present in the release area.
- h) [1] Test and apply methods to artificially suppress mosquito populations in the release area for the purpose of establishing barriers using adulticides/larvacides or other methods. Using capture, mark, release, recapture or other appropriate methods, establish how wide the barrier must be to prevent immigration.
- i) [1] A control area that is comparable to the release area will be designated. The measures of effectiveness will be the same as applied in the release area. As a last resort, if a suitable control area cannot be designated, historical data will be used.
- j) [3] Consider adult male attractants as a means to increase capture of released and wild males.

Biosecurity, biosafety, ethical and legal procedures

- a) [3] Develop bio-security protocols to prevent escape of experimental and factory material. As a model, follow quarantine protocols developed for other species that are appropriate in the country in which the activities are conducted.
- b) [4] Develop biosafety protocols for protection of factory workers.
- c) [4] Determine legal, ethical and regulatory requirements for production and release of mosquitoes.

Measures of effectiveness of the release programme must be determined. These may include entomological indicators such as wild-caught female sterility, larval surveys, adult collections and trapping. These may also include epidemiological indicators such as landing biting, sporozoite, clinical cases (febrile illness with slide positivity).

The group also recognizes the need to: (1) develop an educational and public relations programme that effectively communicates the purpose of the programme and engages the support of the public, politicians, and journalists during site development and preceding releases, and (2) strengthen research capabilities in disease endemic countries in vector biology and control (including training, equipment and facilities) in the host country and other participants.

4.2. Working Group 2: Baseline data collection from potential field sites

The group was tasked with identifying criteria that have to be used in the selection of a potential field site to evaluate the SIT technology for African malaria vectors.

1. Isolation of the mosquito vector population to avoid immigration from outside the release area of females already inseminated with fertile sperm which would continue to lay fertile eggs and thus prevent a major impact of the SIT on the vector population.
2. Substantial indigenous malaria transmission so that, if the mosquito population is successfully suppressed, a demonstrable effect on malaria incidence and prevalence could be expected.
3. A single local vector species so that, by release of sterile males of that one species, a substantial impact on malaria could be demonstrated.
4. A vector population small enough to allow a feasible rate of production and release of sterile males to induce sufficient egg sterility to initiate a downward vector population trend.

It seems unlikely that populations could be found of *An. gambiae s.s.* or *An. funestus* on the mainland of Africa which would satisfy both conditions 1 and 4. Experience in Zimbabwe and South Africa indicates that these endophilic species can be eradicated by conventional insecticidal means. Secondary vector species are unlikely to satisfy conditions 2 and 3. It seems therefore that, in Africa, ***An. arabiensis* should be chosen as the target for the proposed field trial(s).**

Rough estimates can be made of the maximum size of human communities, which should be chosen for baseline studies with a view to selection of site(s), assuming that:

- (a) a rearing facility and a genetic sexing system could be created comparable to those used in El Salvador in the 1970s which produced 1 million sterile males per day;

- (b) a 20:1 ratio of males released to males emerging per day would be sufficient to initiate a downward population trend, thus the emergence rate per day should not exceed 50,000 wild males and 50,000 wild females;
- (c) releases are initiated at a season of low population density when a vector population satisfying conditions 2 and 3 and it is assumed that such a vector population would inflict two bites per person per night and half as many bites on non-human hosts, i.e. 3 bites occur per night per human in the community;
- (d) each female bites once every two nights, i.e. there is a standing female mosquito population 6 times that of the human population;
- (e) the mean survival time of females is 8 days, i.e. the emergence of 50,000 females per day yields a standing population of 400,000.

Thus the estimated **maximum size of human community in an area which might be chosen for a trial should be 400,000/6, i.e. approximately 67,000**. Such estimates should be refined by mark-release-recapture studies; to carry these out a pilot rearing facility would probably be required in the area.

There appear to be four types of ecological situation in which malaria vector populations could satisfy the above criteria:-

- (i) Populations confined along the banks of rivers by very arid country on each side inimical to mosquitoes; the *An. arabiensis* population along the Nile in northern Sudan north of the Fourth Cataract appears to fit the above criteria but would have to be attacked piecemeal because of the above constraints on a feasible target population size.
- (ii) Urban populations in areas where the surrounding rural areas are inhabited by other species (e.g. *An. gambiae s.s.*) which seems unlikely to be able to spread into the urban area in the event of suppression of the urban *An. arabiensis* population. Cytogenetic data of Coluzzi *et al* (1979 Trans. R. Soc. Trop. Med Hyg 73: 483-497) from Benin City and Sapele, Nigeria, suggest that such situations exist but, in view of major environmental changes in recent years, this requires to be re-checked using the PCR technique (Scott *et al* 1993 Am. J. Trop. Med. Hyg. 49: 520-529) now available for identifying members of the *An. gambiae* complex. In addition further studies in the Forest belt of West Africa of smaller urban communities of a size consistent with the above estimate need to be carried out.
- (iii) Areas which have been artificially isolated by insecticidal spraying such as on the northern fringes of South Africa.
- (iv) An offshore or oceanic island; the island of São Tomé has frequently been suggested as a possible target but the vector population there is *An. gambiae s.s.* Forest form (Pinto *et al*, 2000, Acta Tropica 76: 185-193).

If possible, two separate areas or communities should be designated for the field trial, one for the integrated SIT intervention and one to remain as a control. A minimum of a year's pre-intervention baseline data should be collected in each.

Entomological data:

1. Adult populations of *An. arabiensis* are customarily monitored by different methods in different countries depending on the extent to which the vectors rest in houses, whether most of the houses are sprayed and whether use of human landing catches is considered ethically acceptable. The monitoring methods which may be chosen in any

given area include: pyrethrum spray catches, light traps set beside occupied bednets, human landing catches, exit traps and pit traps. Where most houses are sprayed designated unsprayed sentinel houses may be needed. If not already known, the species composition of members of the *An. gambiae s.l.* should be determined by the PCR method. The ELISA or PCR method may be applied to confirm that sporozoite positive mosquitoes exist in the supposed vector population. Monitoring over all seasons (with contemporary meteorological data) and extensive replication is needed to obtain a reliable check that the population and malaria transmission intensity fit the above criteria and as a basis for comparison with the situation after the SIT intervention.

2. Breeding sites should be located with a view to:

- (i) making releases at these sites;
- (ii) possible need for larviciding as a supplementary control method.

3. Collection of sufficient males to allow determination of the ratio of marked released males to wild males may be a serious problem in *An. arabiensis* whose males tend not to rest in houses. The possibility should be explored of locating mating swarms and sweep netting samples of males from these swarms for determination of marked: unmarked ratios.

4. The success of sterile males in mating with wild females will be determined by catching samples of blood fed or gravid females and tubing them individually for oviposition and determination of percentage of egg batches which hatch. Baseline data should be collected to determine the natural rate of infertility. Spermathecae of females which produce infertile egg batches should be checked for insemination.

Malariological data:

Data on prevalence of malaria parasites and on incidence of clinical disease should be collected in the baseline year and in the SIT intervention and control areas. In each case a questionnaire should be used to ascertain whether each positive human case has spent one or more nights away from the area in the previous month so that their infection may have been acquired outside the area.

Reliable data on malaria infection require well-trained microscopists with a system of cross-checking of their data, and/or use of the newly available test kits. It should be noted that in South Africa these test kits have been extensively validated and are used routinely and economically at all health facilities to record all malaria cases occurring in the country.

Prevalence of infection may be monitored in samples of schoolchildren at the low and high malaria seasons. Very low malaria prevalence may not be detectable among children present at school and sampling of younger children by home visits may be needed.

Diagnosis at health facilities by clinical criteria alone is insufficient and, if necessary equipment, supplies and training should be supplied to a sample of health facilities (including those in the private sector) to allow fever associated with malaria parasitaemia to be positively identified.

4.3. Working Group 3: Project proposal.

This group was tasked with developing a draft document that will eventually be used to attract extrabudgetary funds for this programme. The draft document follows.

FEASIBILITY OF STERILE INSECT TECHNIQUE FOR CONTROL OF AN. ARABIENSIS

The Sterile Insect Technique (SIT) is an exciting development in the integrated control of insect pests and vectors that has proved remarkably successful in the control of important economic pest species including screwworm, tsetse and fruitflies. SIT is integrated with other pest management measures and relies on mass rearing and release of sterilized male insects. The released males mate with wild females, preventing production of offspring and thus suppressing or eventually eradicating the insect pest/vector population. This is a proposal to develop and assess the feasibility of SIT for the control of An. arabiensis in selected field sites in Africa.

1. BACKGROUND

Malaria has a devastating effect on health and development in sub-Saharan Africa, and is the largest single cause of mortality in children. Over 90% of the World's malaria cases occur in Africa, and in many countries it consumes a major portion of the national health budget. The disease constitutes a major obstacle to poverty reduction in Africa; according to some estimates, it has slowed economic growth in African countries by 1.3% per year. As a result of the compounded effect over 35 years, the GDP for African countries is now up to 32% lower than it would have been in the absence of malaria.

The burden that malaria places on societies and economies was recognized when 48 African Heads of State and Government met in Nigeria in April 2000 and adopted the Abuja Declaration on Roll Back Malaria in Africa. This declaration stresses the regional commitment to reduce malaria mortality in Africa by half by 2010 and calls upon development partners to allocate substantial new resources of at least \$1 billion per year to combat the disease. The Abuja Summit also recorded the governments' commitment to increase support for research to facilitate the development of new investigative and control tools and to improve existing ones. The adopted Plan of Action emphasizes the importance of human resources development and calls for intensified research on issues of direct significance to national control programmes.

The most important elements in the Roll Back Malaria (RBM) initiatives are:

- 1) Bringing reliable, sustainable malaria prevention and early treatment, to affected populations;*
- 2) Investing in research and development of effective, affordable tools for epidemiology and control;*
- 3) Evaluating achievements against clearly defined goals, and*
- 4) Building human and institutional resources.*

Early treatment requires affordable and effective drugs but there are growing problems of drug resistance, which will increasingly limit the efficacy of the current first-line drugs and necessitate a switch to more expensive alternatives. Insecticide-treated bed nets (ITNs) have proved a valuable control tool but resistance to pyrethroid insecticides, already reported from several countries in Africa, could limit the efficacy of ITNs if it were to become more widespread. Whilst a malaria

vaccine has often been regarded as the 'magic bullet' against malaria, in recent trials of candidate vaccines, none have proved sufficiently protective against malaria to warrant use in malaria control and it is increasingly unlikely that an effective vaccine can be deployed before 2011 at the earliest. Even when one is available, the belief is that a vaccine should be deployed alongside other control methodologies.

2. STERILE INSECT TECHNIQUE FOR MOSQUITO VECTORS

These factors have led to renewed interest in the potential of the SIT for the suppression of mosquito vectors in suitable areas. The remarkable success of area-wide SIT programmes against screwworm, tsetse, and fruit flies provides a sound basis for contemplating the prospects for SIT intervention to suppress populations of malaria vectors. It is envisaged that SIT would be used under specific conditions as an adjunct to other, more orthodox technologies. This would conform with the current RBM strategy of not relying on any single approach to control.

Compared to the initial trials of using SIT for malaria control in the early 1970s, the technology has developed enormously for other insect pests in terms of delivery mechanisms of sterile males, the molecular basis to develop sexing strains and in sterilizing the males themselves. In addition, the regulatory framework of SIT has been developed, quality assurance mechanisms have become more refined and links with the *Anopheles* genome project have also been established improving development of transgenic mosquitoes. Technology such as GIS/GPS has facilitated the field evaluation of SIT.

Any mosquito SIT feasibility study will require a substantial R&D component which will address the following issues:

- *Developing methods of mass rearing: Research will be conducted on critical aspects of large-scale Anopheles rearing including adult holding, egg handling, larval rearing and pupal collection. This will include improving membrane feeding of adult females and improvements to blood diet or substitutes.*
- *Improving sterilization, handling and release methodology: Radiation procedures will be evaluated to enable pupae to be efficiently sterilized and thus reduce the need for excessive handling of the more sensitive adults. Techniques for aerial release will also have to be investigated.*
- *Devising genetic and molecular methods for the production of males: Any SIT programme for mosquitoes will require the release of males only. Genetic sexing methods will need to be developed.*
- *Improving field evaluation of release mosquitoes: this will entail developing methods for assessing male competitiveness, migration and monitoring of released mosquitoes.*

3. AIMS AND OBJECTIVES

Overall Objective

To assess the feasibility of the SIT for the suppression of *An. arabiensis* populations of malaria vectors in selected areas in Sub-saharan Africa.

Specific Objectives:

1. **Development of tools for rearing, sterilizing and releasing large numbers of male**

mosquitoes.

2. *Evaluation of the impact of the releases on field populations and, where appropriate, on malaria transmission.*

3. *Establishment of an improved network of centres in Africa, together with strengthened collaboration with centres of excellence elsewhere.*

4. PROJECT PLAN

Plans will be made for developing mass-rearing technology, including handling and transport of sterile males as detailed in the report of Working Group 1. As technology becomes available, it will be evaluated in the field sites, and its impact on the vector population and malaria transmission will be monitored. Criteria for the selection of field sites together with the collection of base line entomological and epidemiological data are presented in the report of Working Group 2.

5. NATIONAL COMMITMENT AND PARTNERS

Member States in Africa where malaria is a problem are the main stakeholders. Appropriate Government Agencies will provide staff, facilities infrastructure and logistics for field work as well as results of national field surveys, aerial photographs and existing relevant data. The national malaria control programmes, research institutes and universities in these countries would be the main government counterparts.

The WHO as the UN Organization responsible for the RBM initiatives is the logical partner for this project. Its specific contribution will be to provide, where needed, expert advice and operational and technical support to recipient countries in Africa. In particular the programme will exchange relevant scientific and technical information with the molecular entomology component of the TDR programme. In addition, centres of expertise in other parts of the world would be closely involved in providing technical support to the programme.

6. AGENCY INPUT

Promotion of regional co-operation through joint training courses and workshops, participation in conferences and meetings, and by providing documentation.

Development and evaluation of radiation sterilization strategies, genetic sexing techniques, and automated rearing, handling, and release systems.

Special services through subcontracts, including mosquito strain adaptation and methods development, including the design and testing of essential equipment and facility components.

Establishment of a Co-ordinated Research Project (CRP) to create a network of researchers in Africa and elsewhere to improve the SIT technology for mosquitoes.

7. ACTIVITIES AND OUTPUTS

1. *Improved knowledge of the distribution of species of the Anopheles gambiae complex and identification of laboratory colonies.*

2. *Enhanced national capacity and improved regional collaboration and identification of centres of excellence in Africa where any future SIT programme can be implemented.*

3. *Improved tools for monitoring Anopheles population in the field and identification of field sites.*
4. *Methods of mass rearing and release of male mosquitoes,*

8. PROJECT IMPACT

The long term objective would be to have a significant impact on malaria transmission in sub-Saharan Africa. Even if this goal is not realised there are substantial benefits to be gained. A significant contribution will be made to the global effort for capacity building in the technical and managerial aspects of malaria vector control programmes in sub-Saharan Africa. R&D for assessing the feasibility of the application of SIT will have a long-term positive impact on developing research capabilities for combating malaria in sub-Saharan Africa. Universities and national and regional public health organizations will benefit from project activities by improving expertise in genetic, molecular, ecological and behavioural studies of mosquitoes, The advantages of integrated area-wide malaria intervention approaches and their relevance to demographic movements and settlements will be more widely recognized. The mass rearing technology will also be of importance for other methods of mosquito control being developed especially the use of transgenic insects that are refractory to malaria transmission.

9. BUDGETARY CONSIDERATIONS

5. RECOMMENDATIONS AND FOLLOW-UP ACTIONS

There was a strong recommendation from the group that a feasibility study of the use of the SIT for control/eradication of *An. arabiensis* be carried out. The essential R and D was identified together with the criteria to be used for the selection of a field site.

The information presented and discussed during the meeting will be used to prepare a project document including a general workplan and budget. This document will include all activities related to the project and will be used to solicit donor funds. Secondly a detailed work plan for the R and D and baseline data studies will be prepared that falls within the scope of the resources already assured. These two documents will be prepared by the Agency by the end of July and sent to all participants for comments.

Appendix 1.

**FIRST PLANNING MEETING ON
DEVELOPMENT OF THE STERILE INSECT TECHNIQUES
FOR CONTROL OF MALARIA-TRANSMITTING MOSQUITOES**

5-8 June 2001

**Vienna International Centre
Vienna, Austria**

Room B-0522

PROVISIONAL AGENDA

Meeting Objectives:

- To review the status of the control of malaria-transmitting mosquitoes in Member States including Government policies.
- To review the state of art for possible use of SIT for the control of malaria-transmitting mosquitoes.
- To formulate long-term and short-term strategies and action plans for R&D aimed at possible use of SIT for the control of malaria-transmitting mosquitoes.
- To identify international and regional partners and discuss modalities for co-operation.

Tuesday, June 5, 2001		
09:00	Opening	DDG-TC
09:30	Discussion and Adoption of the Meeting Agenda	All Participants
09:45	Administrative Information Area-wide SIT for Insect Control and Eradication	J. Hendrichs
10:15	Coffee Break	
10:30	Country Reports	Ethiopia Mali Namibia Niger
12:30	Lunch	
14:30	Country Reports	Nigeria South Africa Senegal Sudan
16:30	Coffee Break	
16:40	Presentation of the objectives of the meeting	A. Robinson
17:10	Discussion	All Participants
18:00	Cocktail Party	

Wednesday, 6 June 2001		
09:00	Methods for Control of Vector Mosquitoes and the Possible Role of SIT	C. Curtis
09:40	Population Structure of <i>Anopheles arabiensis</i> and its Implications for SIT	H. Townson
10:20	Coffee Break	
10:35	History of Mosquito Releases for Control and Potential of New Molecular Capabilities	M. Benedict
11:15	A Review of the SIT Programme for <i>Anopheles albimanus</i> in El Salvador	P. Kaiser
12:00	Lunch Break	
13:30	Discussion	
14:10	Constraints and Opportunities in the Use of SIT for Mosquito Control/Eradiation	Chairperson: C. Curtis Rapporteur: H. Townson
15:30	Coffee Break	
15:45	Current and Proposed Agency Activities for Mosquito SIT	Chairperson: A. Robinson Rapporteur: P. Kaiser

17:15	Mechanisms and Modalities for IAEA supported projects (CRPs and TCPs)	J. Hendrichs (CRPs) A. Boussaha (TCPs)
17:35	Discussion on the Outputs of the Meeting and Assignment of Tasks to Working Groups*	A. Boussaha A. Robinson
18:00	Preparatory Meeting of the Working Groups	All Participants
18:45	Close for the Day	

Thursday, 7 June 2001

08:30	Working Group Discussions and Drafting of the Report	Working Groups
12:00	Lunch Break	
13:30	Plenary Session: Reporting by Working Groups and Discussion on Major Findings and Recommendations	Rapporteurs of Working Groups
15:00 15:15	Coffee Break Working Group Discussions and Drafting of Working Group Reports	Working Groups
18:00	Plenary Session: Presentation and Discussion of Working Group Reports	All Participants
18:30	Close for the Day	

Friday, 8 June 2001

09:00	Finalization of Working Group Reports	Working Groups
12:00	Lunch Break	
14:00	Plenary Session: Presentation of Working Group Reports	Rapporteurs of Working Groups
15:00	Conclusions and Closing	All Participants

* Working Group 1: Short term and long term R & D
 Working Group 2: Baseline data collection from potential field sites
 Working Group 3: Project proposal with milestones and required resources

Appendix 2.

LIST OF PARTICIPANTS

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Techniques for Control of Malaria-Transmitting Mosquitoes
05-08 June 2001, Vienna, Austria**

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