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Report of the WHO *Informal Consultation*

Test Procedures *for* Insecticide Resistance Monitoring in Malaria Vectors, Bio-Efficacy and Persistence of Insecticides on Treated Surfaces

WHO, Geneva, Switzerland
(28-30 September 1998)

World Health Organization
Control of Communicable
Diseases (CDS)
Prevention and Control

CONTENTS

1 INTRODUCTION

consultative meeting was opened by Dr D L Heymann, the Executive Director of the Cluster of Communicable Diseases (CDS), in the new WHO structure. He welcomed the participants, opened the meeting, and presented an overview on the new structural arrangements being planned in WHO, specifically in relation to the CDS and malaria control. Dr K. Behbehani, Director, Division of Control of Tropical Diseases (CTD), and Dr A. V. Kondrachine, Chief, Malaria Unit, were present at the opening.

Dr Pushpa R. J. Herath, Scientist, Malaria Unit, presented the objectives of the meeting and the expected outcome of the discussions. She emphasised the importance of monitoring insecticide resistance in the malaria vectors, and pointed out that the reason for focusing the meeting on malaria vectors was because of an urgent need for updating a number of aspects of the test system for monitoring insecticide resistance and related aspects for malaria vectors.

Dr Herath also pointed out that there is a need for similar updating on the test procedures for the other disease vectors and pests, but that these could not be dealt with on this occasion.

2 BACKGROUND TO THE MEETING

Insecticides play an important role in vector borne disease control. In malaria control, they are used for the treatment of mosquito nets and other materials (ITMs), for indoor residual spraying, or as larvicides. Pyrethroid insecticides are increasingly important for these purposes, but have limited use as larvicides. This is in addition to their extensive use as agricultural and household pesticides. Development of vector resistance to these insecticides will obviously lead to problems in their uses, e.g. by making insecticide uses ineffective and limiting the available options. Despite limited monitoring of insects in the field, vector resistance, including multiple resistance covering all four major classes of insecticides (organochlorines, organophosphates, carbamates and pyrethroids) has been reported in some important malaria vectors. This is of great concern and demands improved monitoring for an overall assessment of the current status of vector resistance and related problems.

Knowledge of vector insecticide resistance status, changing trends of resistance in target vectors, and their operational implications are basic requirements to guide insecticide use in disease control programmes. This information provides a basis for selecting insecticide(s), for ascertaining continued susceptibility to and efficacy of insecticides in use, and for vector insecticide resistance management.

Appropriate monitoring of vector resistance to insecticides is an integral component of planning and evaluation of insecticide uses in malaria control programmes. Such monitoring should be standardised to ensure comparability of data from different sources, hence a standardised test system is a prerequisite. In this context, definition of standards and procedures, and ensuring access to quality assured test materials/kits by potential users, are among the functions of the WHO's Global Programme on Insecticide Resistance Monitoring (GPIRM). This meeting was convened to address some of the relevant issues.

WHO instructions on test procedures already exist for the detection and monitoring of resistance to the organochlorine, organophosphorous and carbamate insecticides. Instructions are also available for assessing the biological efficacy of insecticides sprayed e.g. on walls and other surfaces of houses; but not for the pyrethroid treated ITMs. The latest version of these instructions was prepared in 1981. An update on these instructions was urgently needed, especially to accommodate the current requirements for (a) the pyrethroids, which are being increasingly used in malaria control, and (b) insecticide-treated

mosquito nets and other materials (ITMs), which are currently an important component in malaria prevention and control.

For the pyrethroids, the discriminating concentrations, and other aspects of the test system needed clarification or establishment. In relation to the former, WHO commissioned a multi-centre study to establish the discriminating concentrations for 5 pyrethroid insecticides against major malaria vectors. The results of the study were reviewed by this consultative group, who made recommendations for discriminating concentrations. Equally important was a review of the current systems for assessing the biological efficacy and the persistence of pyrethroids on ITMs, and a revision of the formats for recording/reporting the results of insecticide susceptibility tests to accommodate the testing of the pyrethroids.

There have been queries (mainly from industry) concerning the use of three different solvents in the preparation of impregnated papers for different classes of insecticides i.e. Rissela oil for organochlorines, olive oil for organophosphates and carbamates, and Dow Corning 556 silicone fluid for the pyrethroids. These uses were developed over time i.e. as and when these insecticides became available. The possibility of using only one solvent for all classes of insecticides was considered by the consultative group.

The logistic arrangements for the test kits and components i.e. the preparation of insecticide impregnated papers and solutions, the procurement and storage of test kit components, assembling of test kits, and their global distribution (the latter in response to requests) has been a function of WHO Headquarters since 1958. Constraints at WHO compelled devolution of these functions to Universiti Sains Malaysia (USM), Penang, Malaysia, in late 1993. Some issues related to this were reviewed.

3 OBJECTIVES OF THE CONSULTATION

- To review results of a multi-centre study leading to recommendations for "discriminating dosages" for 5 pyrethroid insecticides: *permethrin, deltamethrin, lambda-cyhalothrin, cyfluthrin* and *etofenprox.*

- To review and update WHO test procedures for detecting and monitoring insecticide susceptibility/resistance status in malaria vectors, and for assessing bio-efficacy and persistence of insecticide treated surfaces.

- To review solvents used to prepare insecticide-impregnated papers and to make recommendations.

- To review criteria for defining *'resistance'* in the context of interpreting the results of the WHO test for measuring insecticide susceptibility/resistance status.

- To review the current arrangements for the supply of standardised test kits for monitoring insecticide resistance and of quality control of materials prepared at USM and to make appropriate recommendations for improvements, if necessary.

The agenda (Annex 1) and the list of participants(Annex 2) are annexed.

The updated test methods are to be made available for field use mainly in relation to malaria control, through a WHO document.

The issues that emerge and the recommendations from the meeting are to be communicated to the 20th WHO Expert Committee on Malaria, 19-27 October 1998.

4 PROCEEDINGS, OBSERVATIONS, RECOMMENDATIONS FROM THE MEETING

4.1 DISCRIMINATING CONCENTRATIONS OF INSECTICIDES AGAINST MALARIA VECTORS

The *discriminating concentrations* (or dosages) of insecticides are routinely used to detect and monitor insecticide resistance in mosquitoes. These concentrations are established under standardised laboratory conditions, using known "*susceptible*" strains or populations of a range of mosquito vector species.

4.1.1 Multi-centre study on discriminating concentrations of pyrethroids

The *discriminating concentrations* for adult mosquitoes are already established for the organochlorine, organophosphate and carbamate insecticides currently in use for malaria control. These concentrations now need to be clarified or established for different pyrethroids.

A multi-centre study was commissioned by WHO to establish the discriminating concentrations for five pyrethroid insecticides - *permethrin, deltamethrin, lambda-cyhalothrin, cyfluthrin* and *etofenprox* - against malaria vectors. Nine (9) Institutes (Annex 3) which had access to "susceptible" strains/populations of some of the major malaria vectors participated in the study.

Five concentrations were tested for each insecticide (Table 1) covering the concentrations (determined in consultation with relevant experts, some being the participating scientists) which were 'expected' to produce mortalities in a range of mosquito species, both below and above 50%. The concentrations tested were as follows:

Table 1

Concentrations of the insecticides tested in the multi-centre study

Insecticide	**Concentrations**				
Permethrin	0.1	0.25	0.5	0.75	1.0
Deltamethrin	0.005	0.0125	0.025	0.05	0.1
Lambda-cyhalothrin	0.01	0.025	0.05	0.1	0.2
Etofenprox	0.1	0.25	0.5	0.75	1.0
Cyfluthrin	0.005	0.0125	0.025	0.05	0.1

All the insecticide-impregnated papers used for the study were prepared at the same time, at the WHO collaborating centre, Universiti Sains Malaysia (USM), Penang, Malaysia and made available to the participating Institutes.

The papers were prepared with Dow Corning Silicone fluid 556 according to standard WHO specifications

Quality control under GLP conditions was undertaken on batches of these papers at all concentrations; by the University of Wales, Cardiff, UK, which is also a WHO collaborating centre.

It is to be noted that the permethrin used for preparing the impregnated papers was the 60:40 cis/trans isomer which has been used for the preparation of permethrin impregnated papers by WHO for more than a decade.

Twenty one (21) strains/populations of 9 different anopheline species were investigated. The anopheline species used were *Anopheles aconitus, An. albimanus, An. arabiensis, An. dirus, An. freeborni, An. gambiae s.s, An. maculatus, An. minimus* and *An. stephensi.* More than one strain or the same strain from more than one Institute, were used for *An. albimanus, An. dirus, An. gambiae* and *An. stephensi.* The choice of the anopheline species/malaria vectors investigated depended on the availability/access of "*susceptible*" populations. The anopheline species and the strains tested at each Institute are listed in Table 2. A total of 66991 adult anophelines were tested as follows; 13384 with permethrin, 14990 with deltamethrin, 13284 with lambda-cyhalothrin, 13418 with cyfluthrin, and 11915 with etofenprox. The numbers for each of the species, strain/population tested at each Institute are given in Table 3.

- All investigators followed a standard protocol (copy in Annex 4) prepared for this purpose.
- **The insecticide exposure tubes were held vertically for all tests.**
- One to 3 day old non-blood fed adult females were used for all tests.
- Insecticide impregnated papers were not used more than 6 times for any test.

The susceptibility tests were run under optimum conditions of temperature and humidity with mosquitoes from laboratory 'susceptible' colonies maintained at the 9 Institutes.

Table 2

SPECIES, STRAINS OR POPULATIONS TESTED AT EACH INSTITUTE IN THE MULTI-CENTRE STUDY

Species	Strain or population	Institute
An. aconitus	Java	Vector Control Research Station, Indonesia (VCRS), Indonesia
An. albimanus	Teco	Centres for Disease Control, Atlanta, USA (CDC/USA)
	Panama	University of Wales, Cardiff, UK (Cardiff/UK)
	Mexico	London School of Hygiene & Tropical Medicine, UK (LSHTM/UK)
An. arabiensis	Sa	Cardiff/UK
An. dirus	—	CDC/USA
	B	Centres for Disease Control, Thailand (CDC/Thailand)
An. freeborni	F1	CDC/USA
An. gambiae	G3	CDC/USA
	G3	Cardiff/UK
	G3	LIN/ORSTOM, Montpellier Cedex, France (ORSTOM/France)
	Kwa	LSHTM/UK
	Mopti	Department of Epidemiology and Infectious Disease, (BIMSA) Bamako, Mali
	Kisumu	ORSTOM/France
An. maculatus	Java	VCRS, Indonesia
An. stephensi	Delhi	CDC/USA
	Beech	Cardiff/UK
	Beech	LSHTM/UK
	Delhi	Malaria Research Centre, New Delhi, India (MRC, India)
	Beech	School of Public Health, Iran (SPH, Iran)
An. minimus	CD 17	CDC/Thailand

Table 3

NUMBERS OF MOSQUITOES TESTED FOR DIFFERENT SPECIES/STRAINS AT EACH INSTITUTE IN THE MULTI-CENTRE STUDY

Species	Country/ Institute	Strain	Permethrin	Delta-methrin	Lambda-cyhalothrin	Cyfluthrin	Etofenprox	TOTAL
An. aconitus	Indonesia (VCRS)	Java	1000	1000	1000	1000	1000	**5000**
An. albimanus	USA (CDC)	Teco	500	500	500	500	500	**2500**
	UK (Cardiff)	Panama	160	160	200	150	200	**870**
	UK (LSHTM)	Mexico	504	562	509	490	527	**2592**
An. arabiensis	UK (Cardiff)	Sa	162	209	290	200	200	**1061**
An. dirus	USA (CDC)		500	500	500	500	500	**2500**
	Thailand (CDC)	B	-	500	300	-	300	**1100**
An. freeborni	USA (CDC)	FI	500	500	500	500	500	**2500**
An. gambiae	USA (CDC)	G3	500	500	500	500	500	**2500**
	UK (Cardiff)	G3	162	184	158	205	200	**909**
	UK (LSHTM)	Kwa	524	533	500	514	485	**2556**
	(BIMSA) Mali	Mopti	1425	1525	1500	1375	1500	**7325**
	France (ORSTOM)	G3 Kisumu	1500 1400	1100 1200	1300 1200	1300 1400	700 1000	**5900** **6200**
An. maculatus	Indonesia (VCRS)	Java	1000	1000	1000	1000	1000	**5000**
An. stephensi	USA (CDC)	Delhi	500	500	500	500	500	**2500**
	UK (Cardiff)	Beech St	162 160	169 200	202 200	200 202	192 200	**925** **962**
	UK (LSHTM)	Beech	522	524	528	524	523	**2621**
	India (MRC)	Delhi		525	500	628	350	**2003**
	Iran (SPH)	Beech	1063	999	897	790	1038	**4787**
An. minimus	Thailand (CDC)	CD17	1140	2100	500	940	-	**4680**
TOTAL			**13384**	**14990**	**13284**	**13418**	**11915**	**66991**

For each insecticide and the concentration tested, the following information was recorded:

- The laboratory involved.
- The species and strains tested.
- The insecticide concentration.
- The replicate number, and for each replicate the number of mosquitoes tested, those knocked down at the end of the exposure period, those dead at the end of the 24 hour holding period, and the % corrected mortality (adjusted by Abbott's formula, for control mortalities, where relevant).
- The minimum and maximum temperatures, and relative humidity during exposure to the insecticides, and at the beginning and end of the 24 hour holding period (before recording mortality).

The data that emerged from this study specifically (a) the details for each replicate, and b) the summaries for each insecticide, concentration, strain and species were presented to the group.

The data for the 5 different insecticides are summarised in Tables 4 - 8. Data for *An. albimanus*, *An. gambiae* and *An. stephensi* are presented separately (Annexes 5 to 9), as these species were tested on several strains and in a number of Institutes.

Table 4

SUMMARY OF DATA FROM MULTI-CENTRE STUDY
PERMETHRIN
(% mortality)

Concentration / Species	0.1%	0.25%	0.5%	0.75%	1.0%
An. aconitus	100	100	99.5	100	100
An. albimanus	61.3	100	92	100	100
An. arabiensis	38.1	97.5	-	100	100
An. dirus	21.0	87.0	100	100	100
An. freeborni	89.0	77	98	100	100
An. gambiae	76.1	94.8	94.5	99.8	99.8
An. maculatus	100	98.5	100	100	100
An. minimus	93.7	99.7	100	100	-
An. stephensi	74.7	98.6	95.4	100	100

Table 5

SUMMARY OF DATA FROM MULTI-CENTRE STUDY
DELTAMETHRIN
(% Mortality)

Concentration / Species	0.005%	0.0125%	0.025%	0.05%	0.1%
An. aconitus	100	100	100	100	100
An. albimanus	53.5	92.5	87.6	100	100
An. arabiensis	100	95.3	100	100	100
An. dirus	41.5	79	97.5	100	100
An. freeborni	9	65	93	100	100
An. gambiae	92.3	98.4	97.6	99.1	100
An. maculatus	99	100	100	100	100
An. minimus	39.5	100	100	100	100
An. stephensi	73.5	88.7	95.7	99.6	100

- = not tested

Table 6

SUMMARY OF DATA FROM MULTI-CENTRE STUDY
LAMBDA-CYHALOTHRIN
(% Mortality)

Concentration / Species	0.01%	0.025%	0.05%	0.1%	0.2%
An. aconitus	100	100	100	100	100
An. albimanus	61.9	84.2	100	100	-
An. arabiensis	4.4	77.5	100	100	100
An. dirus	95	99	100	100	100
An. freeborni	100	100	100	100	100
An. gambiae	93.5	95	99.8	100	100
An. maculatus	100	100	100	100	100
An. minimus	38	55.7	-	-	-
An. stephensi	74.2	94.6	97.6	99.3	100

Additional information for lambda-cyhalothrin provided from University of Wales, Cardiff, UK

	LD_{50}	LD_{90}	Extrapolated discriminating dosage
Anopheles sacharovi	0.027	0.039	0.1
Anopheles atroparvus	0.007	0.011	0.04

Table 7

SUMMARY OF DATA FROM MULTI-CENTRE STUDY
ETOFENPROX
% Mortality

Concentration / Species	0.1%	0.25%	0.5%	0.75%	1.0%
An. aconitus	89.5	99	100	99.5	100
An. albimanus	63.8	100	100	100	100
An. arabiensis	65	97.5	100	100	100
An. dirus	41	97	100	100	100
An. freeborni	34	85	100	100	100
An. gambiae	75	98.9	99.7	100	100
An. maculatus	96.5	100	100	100	100
An. stephensi	65.9	97.9	99.2	100	100

- = not tested

Table 8

SUMMARY OF DATA FROM MULTI-CENTRE STUDY
CYFLUTHRIN
% Mortality

Concentration / Species	0.005%	0.0125%	0.025%	0.05%	0.1%
An. aconitus	98.5	100	100	100	100
An. albimanus	58.9	55	68.7	78.9	98.6
An. arabiensis	95	100	100	100	100
An. dirus	23	35	58	85	94
An. freeborni	10	17	33	60	85
An. gambiae	40	74.7	85.4	88.1	95.7
An. maculatus	83	90.5	98.5	100	100
An. minimus	58.3	31.7	-	59	-
An. stephensi	39.8	62.1	80.7	71.8	95

- = not tested

The following data for cyfluthrin calculated from full log-dosage probit lines was available via a WHO Pesticide Evaluation Scheme (WHOPES)[1] funded trial, by the University of Wales, Cardiff, UK.

	LC_{50}	LC_{99}
An. albimanus	0.0036	0.0314
An. atroparvus	0.0039	0.062
An. gambiae	0.0021	0.062
An. sacharovi	0.0042	0.074
An. stephensi	0.0013	0.0159
Ae. aegypti	0.0037	0.042
Cx. quinquefasciatus	0.0044	0.053

Using the criteria that the WHO discriminating dose is double the extrapolated LC_{99} from the probit line the suggested discriminating concentration for cyfluthrin was 0.15%.

Most data from the multi-centre study did not give sufficient spread to allow an accurate calculation of the log-dose probit lines to determine the predicted LC_{99}. With the variability observed in the data, it was evident that there were some outliers. But given the large number of mosquitoes, species, strains/populations tested, more weight was given to samples of the same species which had all clustered round similar mortality values. Hence estimations were made with a reasonable margin of error (possibly overestimating the discriminating concentrations) on the LC_{100} values.

[1] WHO Pesticide Evaluation Scheme (WHOPES) Department of Prevention & Control, World Health Organization, Avenue Appia 20, CH 1211, Geneva 27, Switzerland

Based on the review of the overall data, the following discriminating concentrations were recommended :

- Permethrin 0.75%
- Deltamethrin 0.05%
- Lambda-cyhalothrin 0.05% (with the exception of *Anopheles sacharovi* where 0.1% should be used)
- Etofenprox 0.5
- Cyfluthrin 0.15

- These discriminating concentrations are valid for a range of major malaria vectors and will give a robust testing system, with dosages, which for some anophelines are higher than the observed LC_{100} values. However, they are all considered low enough or within the range to ensure that significant levels of resistance will be detected in the major malaria vectors. This point was illustrated by data from pyrethroid resistant *Anopheles gambiae s.s.* from the Ivory Coast.

4.1.2. Discriminating concentrations of insecticides used in malaria control

Based on the above data for the pyrethroids, and taking into consideration the discriminating concentrations already in use, the Table below summarises discriminating concentrations of insecticides commonly used in malaria control, for adult malaria vectors.

Insecticide Class	**Insecticide**	**Discriminating concentration** (period of exposure 60 minutes)
Organochlorines	DDT Dieldrin	4% 0.4%[1] 4%[2]
Organophosphates	Malathion Fenitrothion	5% 1%[3]
Carbamates	Propoxur Bendiocarb	0.1% 0.1%
Pyrethroids	Permethrin Deltamethrin Lambda cyhalothrin Cyfluthrin Etofenprox	0.75% (previously 0.25%) 0.05% (previously 0.025%) 0.05%[4] (previously 0.1%) 0.15% 0.5% (previously 0.25%)

Note [1] = kills susceptibles (ss) but not resistant heterozygotes (Rs)

[2] = kills heterozygotes (Rs) but not homozygous resistant (RR)

[3] = 2 hour exposure for *An sacharovi*

[4] = 0.1% for *An sacharovi*

4.1.3. Discriminating concentrations for larvicides

It was noted that:

- From the list of insecticides given in WHO documents as suitable larvicides for mosquito control, the discriminating concentrations are available for chlorpyrifos, fenitrothion, fenthion, malathion and temephos.

It is recommended that:

- Discriminating concentrations are established as a priority for:
  - Chlorpyrifos-methyl
  - Pirimiphos-methyl
  - Methoprene
  - Pyriproxyfen

  and for the following species:

  - *Aedes aegypti*
  - *Anopheles stephensi*
  - *Culex quinquefasciatus*

  This list reflects the current patterns of larvicide use.

4.2 INSECTICIDE SUSCEPTIBILITY TEST FOR ADULT MOSQUITOES

4.2.1 Equipment and supplies

4.2.1.1. Composition of the test kit

Baseline, and diagnostic test kits were used in the past. However, the WHO Expert Committee on Vector Resistance to Pesticides, 1992 (WHO Technical Report Series 818) recommended that only the diagnostic kit be used as the standard.

The Consultative Group suggested minor adjustments in the composition of the standard test kit and, based on this, the components of a test kit are now to be as follows:

- 12 plastic tubes (125 mm in length and 44 mm in diameter), with each tube fitted at one end with 16-mesh screen. The 12 tubes include:
  - Five (5) marked with a *red dot* for use as *exposure tubes,* i.e. for exposing mosquitoes to the insecticide impregnated papers.
  - Two (2), marked with a *green dot* for use as *control tubes,* for exposure of mosquitoes to the oil-treated control papers i.e. without insecticide.
  - Five (5) with a *green dot* for use as *holding tubes,* for pre-test sorting and post-exposure observation
- Seven (7) slide-units, each with a screw-cap on either side, and provided with a 20 mm filling hole.

- 40 sheets of clean paper (12 x 15 cm) for lining the holding tubes.

- 14 spring wire clips to hold the papers in position against the walls of the tubes. Of these, the 7 steel clips are for use only for the holding and the control exposure tubes, and the 7 copper clips are for use in the insecticide exposure tubes.

- Two (2) glass (or plastic) aspirator tubes of 12 mm internal diameter, together with 60 cm of tubing, and mouthpiece.

- One (1) roll of self-adhesive plastic tape.

- Instruction sheet, 20 copies of report forms.

4.2.1.2 Insecticide impregnated papers

The insecticide impregnated papers are currently prepared at University Sains Malaysia, Penang, Malaysia (on behalf of WHO). As a routine, the papers are prepared only with the discriminating concentrations of the relevant insecticides (indicated by WHO). Other concentrations, such as those which may be needed for establishing the baseline, may be obtained, on special request.

The impregnated papers with the discriminating concentration of a given insecticide are packed in plastic boxes; each box contains 8 papers.

The equipment and/or insecticide impregnated papers can be ordered separately.

The procedures and conditions for procuring these items are specified in the document "Supplies for Monitoring Insecticide Resistance in Disease Vectors: *Procedures and conditions* WHO/MAL/95.1073 WHO/CTD/VBC/95.998).

4.2.2 Test procedures and conditions

4.2.2.1 General conditions to be met

It was noted that:

- Sometimes it may be necessary to clarify the baseline susceptibility for local species.

- In establishing the baseline susceptibility for a mosquito population, for all insecticides (i.e. organochlorines, organophosphates, carbamates and pyrethroids), batches of mosquitoes are exposed to different concentrations of the relevant insecticide for 60 minutes. Mortality is determined after the 24 hour holding period. The concentrations should be chosen such that at least one concentration gives 100% mortality, some give 50-99% mortalities, and at least 2 concentrations give mortalities between 5 to 50%.

- Use of a discriminating concentration is the method of choice for routine monitoring of insecticide susceptibility/resistance status in mosquito vectors. When proper discriminating concentrations have been determined for a given insecticide (ideally also for a species), they can be used without preliminary determination of the baseline.

- The following general factors affect the WHO susceptibility test:

- *Age of the adult mosquitoes* is extremely important in determining the extent to which mosquitoes exhibit a resistant phenotype. The changes of resistance with age differ dramatically depending on the resistance mechanism involved, with insects sometimes becoming more susceptible over time.

- The *physiological status of adult females* (i.e. whether they are unfed, blood fed, semi-gravid or gravid) has a notable effect on susceptibility to insecticides and expression of the resistance phenotype.

- The *temperature at which insecticide exposure occurs* influences toxicity. With some insects it is reported that these are inversely correlated for pyrethroids. However, data were presented from one study which showed that for *An. gambiae* and *An. stephensi* mortality increased with temperature over the range of 22° to 35°C (M.H.Hodjati and C. F. Curtis, unpublished data). The temperature/toxicity correlation should be taken into account when performing several tests under different temperature conditions. The temperature and relative humidity should be recorded during both the exposure and the holding periods.

It is recommended that:

The WHO instructions should give the *ideal testing conditions* as:

- Use of non-blood fed adult females, 24-48 hours post emergence
- Temperature for testing 25± 2°C.

- Ideally the tests should be undertaken on non-blood fed adult females of known age (24- 28 hrs post emergence).

- Mosquitoes of known adult age can be obtained using larval collections or the F1 progeny from wild caught females.

- Where adults derived from larval collections are used, the type of breeding sites concerned (e.g. rice fields, rain water collections, irrigation channels, river beds, wells) should be specified since exposure to pesticides can differ with the type of water body.

- Where only field-collected adults can be used, their physiological status (i.e. unfed, blood fed, semi-gravid, gravid) should be carefully recorded.

- Comparative tests of field material with a known susceptible strain (which could even be from areas not exposed to any insecticide) should be undertaken, whenever possible.

- Although there is seldom a large difference in susceptibility between the sexes, female mosquitoes should be used exclusively in field tests, as they survive better and show lower control mortalities.

- Comparisons of susceptibility test data from a single place over time are useful to indicate resistance trends, as are comparisons at a single time from multiple locations to assess its distribution.

However, generalisations on the rate of resistance spread for different species of mosquito are not currently possible, e.g. resistance may or may not be highly focal.

It is recommended that:

- A minimum number of 100 mosquitoes should be tested for any insecticide at a given concentration, with 4-5 replicates of 20-25 mosquitoes per test tube.
- Where it is not possible to collect this number of mosquitoes on a single occasion, multiple tests over a few days should be undertaken to achieve this ideal.
- As far as possible, any locality surveyed should be monitored over time to examine the trends.

A note should be added to the instructions to alert field personnel to the possible consequences of deviating from the above procedures.

4.2.2.2 WHO susceptibility test for pyrethroids

It was noted that:

- There was some discussion as to whether exposure tubes for pyrethroid testing should be held vertically or horizontally. Data were presented on a range of species. This demonstrated that there can be marked differences between results using the two tube positions, but that clear differences between resistant and susceptible insects are seen with both tube positions, using appropriate discriminating concentrations.
- Analysis of knock-down (KD) rates is facilitated by using the exposure tubes in the vertical position. Horizontal positioning of exposure tube avoids rapid knock down and subsequent recovery of mosquitoes but with a risk of over exposure to the insecticide.
- Data presented on *Anopheles gambiae s.l.* showed that changes in the knock-down rate of mosquitoes acted as a good indicator for early detection of resistance.
- The recent WHO commissioned multi-centre study to establish discriminating concentrations was undertaken with the exposure tubes in the vertical position. Moreover, in the tests with DDT (which also has a strong KD effect and acts similarly to pyrethroids) the exposure tubes are kept in a vertical position and emerging resistance to DDT had been successfully detected.

It is recommended that:

- While recognising that there are advantages and disadvantages to having the exposure tube in either vertical or horizontal positions, a vertical position should be used.
- For pyrethroids timed observations of the rate of knock-down of mosquitoes should be made at fixed intervals during the exposure period.
- Tests should be carried out ideally at 25°C (± 2° C) and 70- 80% relative humidity (RH), never at temperatures higher than 30° C.

When testing pyrethroids, timed observations of the rate of knock-down (KD) of mosquitoes should be made routinely after 10, 15, 20, 30, 40, 50 and 60 minutes of exposure, the latter observation being just before transfer to the holding tube. If less than 80% KD is observed after 60 minutes, then KD should also be noted after 80 minutes, i.e. another 20 minutes, in the holding tube. After 10 minutes exposure, the tubes are gently handled to count the number of KD mosquitoes on the bottom of the tube. Where knock down resistance (kdr) is involved, KD rate is a sensitive indicator for early detection of pyrethroid resistance. From KD counts at various intervals, a probit analysis by computer or a regression line on log-probit paper can be done and KD_{50}, as well as KD_{95}, calculated or graphically determined.

It was noted that:

- Adult mosquitoes often lose legs during pyrethroid testing, especially when exposed to high concentrations.

It is recommended that:

- Adults should be considered as live if they are able to fly, regardless of the number of legs remaining.

It was noted that:

- The WHO guidelines (1981) suggest insecticide impregnated papers can be used up to 20 times. Representations from industry and data presented at the meeting suggest that this value is too high for some insecticides.

It is recommended that:

- The WHO guidelines be altered to suggest that pyrethroid impregnated papers should be used not more than 5 times (in line with available data showing efficacy over this range).
- That further work be undertaken with a range of mosquito species to determine the maximum number of times such papers can be used without resulting in spurious indications of resistance.

4.2.3 Interpretation of susceptibility test results

- The WHO recommendation on the following is still valid:
  - 98-100% mortality indicates susceptibility
  - 80-97% mortality suggests the possibility of resistance that needs to be confirmed
  - <80% mortality suggests resistance.
- Where <95% mortality occurs in tests that have been conducted under optimum conditions with a sample size of >100 mosquitoes then resistance can be strongly suspected.

When control mortality is between 5% and 20%, the average observed mortality is corrected by Abbottt's formula:

$$\frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \times 100$$

4.2.4 Data recording format

Formats for recording the results of susceptibility tests have evolved over the years. The last version was updated to accommodate the specific requirements for pyrethroids.

This was recently reviewed during the "*Workshop on Monitoring Malaria Vector Susceptibility to Insecticides in the African Region", Bouake, Côte d'Ivoire, 22-24 September 1998*, to accommodate the requirements for the pyrethroids and in the context of the malaria vectors in the African region, mainly *An. gambiae*.

The draft from this meeting was further reviewed and adapted by the Consultative Group to meet the global needs.

The revised format is in Annex 10.

4.3 BIOLOGICAL EFFICACY OF PYRETHROIDS ON TREATED MOSQUITO NETS AND OTHER MATERIALS: BIOASSAY TEST

It was noted that:

- The bio-assay cones can be attached to bednets in the same way as they are attached to insecticide sprayed walls.

- The standard WHO bioassay method currently recommends a 3 minute exposure time to treated nets with mortality assessed after 24 hours. Exposure is by confining adult mosquitoes to the net under a WHO bio-assay cone. With pyrethroids which have strong repellent properties this may not be ideal; there is a risk of the mosquitoes resting on the cone and not on the net.

- An alternative method utilising a simple netting apparatus has been used. Here part of an intact bednet is wrapped round a frame consisting of two intersecting circles of wire about 15 cm in diameter. The netting is held round the frame in such a way that a "sleeve" is left through which mosquitoes can be introduced and removed with an aspirator (figure 1). Data obtained by this method suggests that median time of knock-down for mosquitoes exposed to impregnated nets may be more informative than 3 minute mortality data (CTD/WHOPES/IC/96.1).

- Using these testing systems the following assessments may be made for the insecticide-treated materials.

    - *Tests with WHO cones for mortality assessment:* After exposure to the net for 3 minutes (with 5 mosquitoes per assay), the mosquitoes are removed and held to observe mortality after 24 hours. Mortality is observed in controls exposed to untreated netting. Where control mortality is between 5% and 20%, the observed mortality is corrected by using Abbott's formula. In addition to mortality, attempts should be made to note KD rates after the 3 minute exposure period.

    - *Tests using the netting apparatus with the wire frame for mortality and knockdown assessment:* Listing the time for knockdown of each individual mosquito and reading off from the list the median time for knockdown (e.g. the sixth in the case of a sample of 11). As each mosquito is knocked down, it is sucked into an aspirator so as to avoid confusion with mosquitoes which recover and are knocked down subsequently. This method has been found to give a sensitive indication of the effect of washing and re-treating nets, whereas a standard 3 minute exposure of a susceptible strain/population to an alpha-cyano pyrethroid tends to give 100% mortality in all tests.

- The mortality and knock down assessments can be undertaken in parallel utilising the netting apparatus.

- The relationship between knock-down and mortality should be established for a large range of species before a positive recommendation for either test over the other is made.

- It was suggested that the WHO susceptibility test tubes lined with netting may be used, under laboratory conditions, for assessing both mortality and knockdown.

- For any test, adequate replicates are needed to examine the variations in bio-efficacy, on different parts of the same net and between nets. Ideally about 50 female mosquitoes should be tested by adequate replications.

Netting apparatus to assess biological efficacy of pyrethroids on treated mosquito nets and other materials

It is recommended that:

- Both mortality and knock-down assays are recorded in parallel when testing pyrethroid impregnated material.
- The WHO guidelines are modified to reflect the above recommendation.
- The relationship between knock-down and mortality should be established for a large range of species before a positive recommendation for either test over the other is made.

4.4 DETERMINATION OF INSECTICIDE CONTENT APPLIED PER UNIT AREA

This is carried out primarily to check the proper application of an insecticide on the treated surface. The information can also be related to, and support the interpretation of the bio-efficacy based on bioassay data.

4.4.1 Walls and other sprayed surfaces

To determine the insecticide content per unit area, a 100 cm^2 area on a sprayed mud-wall may be marked with the aid of a metal frame 10 x 10 cm and a clean, sharp pointed instrument. Samples of mud will be collected from the inscribed 100 cm^2 area to a uniform depth of 1mm with the aid of a clean chisel. A household dustpan held firmly against the wall immediately below the marked out area may be used for the collection of the samples. Similar 100 cm^2 samples can be cut from thatch and other surfaces. For those surfaces from which scrapings cannot be taken, Whatman No 1 filter papers may be attached to the wall prior to spraying, to sample what is sprayed. However, experience has shown that spray-men usually spray higher doses on such papers. Careful supervision is therefore needed.

The samples should be transferred to glass test tubes or to thick aluminium foil, and securely wrapped. Each sample should then be placed in a plastic bag which is firmly sealed.

Each sample should be marked with the name of the village and the household, date of insecticide application (if available), date of collection, location of the scraping of the wall (e.g. top, middle, lower part) and the surface area sampled. The latter is particularly important to relate the results of chemical analysis to the surface area. Samples may be sent to a relevant reference institute for analysis.

4.4.2 Insecticide-treated nets and other materials

Measured areas may be cut out of treated nets for chemical analysis or additional nets may be treated along with the others, for obtaining samples for the analysis. Here it is necessary to ensure that there is no preferential treatment of those nets from which samples are to be used for the analysis.

The samples for analysis should be wrapped in aluminium foil, placed in a plastic bag, securely wrapped, properly labelled and sent to an appropriate institute (e.g. WHO collaborating centres or others with facilities), for analysis e.g. by High Pressure Liquid Chromatography. WHOPES can provide guidance on where the analytical services are available.

Research is being conducted on non-destructive sampling by dissolving out the insecticide deposit from a measured area of netting, and the use of flame photometry or immunological analytical methods for pyrethroids. The intention here is to obtain more rapid feedback of data.

Alternative methods for rapid analysis of pesticide residues are under investigation.

5 REVIEW AND UPDATE ON WHO INSTRUCTIONS

The instructions on test procedures (insecticide susceptibility test) for mosquitoes evolved over the years and are contained in several documents (Annex 11) and require consolidation. The last review of the test procedures for mosquitoes which was in 1981 addressed the organochlorines, organophosphates and the carbamates but not the pyrethroids. Updating was required to accommodate pyrethroid testing.

WHO instructions exist for assessing the biological efficacy of insecticides on sprayed surfaces e.g. walls of houses. These needed updating to incorporate issues pertaining to the pyrethroids, and pyrethroid treated materials such as mosquito nets and curtains.

WHO had prepared a draft document consolidating the available WHO instructions contained in the different WHO mimeographed documents for determining the insecticide susceptibility or resistance of vectors and pests of public health importance. The consultative group reviewed this document only in the context of mosquitoes. A revised version incorporating their suggestions in relation to the pyrethroids and ITMs is to be published separately.

It is recommended that:

- A single document updating the test procedures is produced which reflects the current testing procedures for mosquitoes, and for all vectors and pests of public health importance.

The consolidated draft document, prepared by WHO, referred to above, had also been presented to the participants of the "Workshop on Monitoring Malaria Vector Susceptibility to Insecticides in the African Region", Bouake, Côte d'Ivoire, 22-24 September 1998, which met immediately prior to this meeting. Here appropriate adjustments had been considered to meet the African Regional requirements, mainly in the context of *Anopheles gambiae s.l*, the main malaria vector in the region. An overview of the outcome of this workshop was presented to the Consultative Group for information.

It was noted here that:

- Resource Support Networks for monitoring insecticide resistance in malaria vectors and on ITMs are being initiated in Africa, linked to the Roll Back Malaria (RBM) initiative. The former is complemented by a Multilateral Initiative for Malaria (MIM) network on insecticide resistance already established in Southern Africa.

- Where practical, different networks should be appropriately integrated to avoid duplication of effort. For Africa this is being achieved by utilising groups and

collaborating centres which are already involved in one or more of these networks and act as external conduits for information flow between networks.

- Efficient resistance monitoring systems are dependent on adequately trained personnel. This is lacking to a large extent in most countries in Africa. Through the Resource Support Networks, the relevant core group expertise may serve as resource persons to provide training at country level, in addition to assisting in or undertaking the resistance monitoring itself.

- Training needs at country level should be identified and addressed.

- Methods for resistance testing at a mechanistic level (laboratory and field) are now available. These apply to most, but not for all resistance mechanisms. A separate WHO document "Techniques to detect insecticide resistance mechanisms (field and laboratory manual) CTD/MAL/98.6" (in press) addresses the currently available techniques.

6 LOGISTICAL ARRANGEMENTS: PRODUCTION AND SUPPLY OF TEST KITS

Ensuring and facilitating access to standardised test kits and other materials by potential users has been a function of the WHO's Global Programme for Monitoring Insecticide Resistance.

Since late 1993, many activities related to logistic aspects of the WHO Global Programme for Monitoring Insecticide Resistance are being undertaken (on behalf of WHO) by the Universiti Sains Malaysia (USM), Penang, Malaysia.

Dr Zairi Bin Jaal who represented USM at the meeting, provided an overview of the activities being undertaken at USM and issues of concern. The following indicates the arrangements - past and present - for the different activities related to this.

6.1 ACTIVITIES RELATED TO SUPPLIES: PAST AND PRESENT

Activity	Past	Present
✓ Preparation of:	ATESMO[1] (1956-1993) Zurich, Switzerland (private)	University Sains Malaysia (USM), Malaysia
❖ Insecticide impregnated papers (IPPs)		
❖ Insecticide solutions	Pharmacy/Laussane (private)	USM, Malaysia
❖ Bioassay Cones	Plassimpress, Switzerland (private)	Malaysia (private)
❖ Plastic tubes of test kits i.e. with red, green dots	Plassimpress, Switzerland (private)	Plassimpress, Switzerland (private)
❖ Aspirators/plastic, straight	Switzerland (private)	Switzerland (private)
❖ Aspirators/glass, straight	Germany (private)	Germany (private)
❖ Aspirators/glass, bent	Germany (private)	Germany (private)
❖ Packing boxes for IIPs	Switzerland (private)	Malaysia (private)
❖ Cartons for packing test kits	Switzerland (private)	Malaysia (private)
❖ Thumb tacks	Switzerland (private)	Malaysia (private)
❖ Sponge tape	Switzerland (private)	Switzerland (private)
❖ Adhesive tapes	WHO/HQ	Malaysia (private)
❖ Steel/copper clips	Switzerland (private)	Switzerland (private)
❖ Bottles for larval kits	Switzerland (private)	Malaysia (private)
✓ Procurement processing	WHO/HQ, assisted by SUP, BUD/FIN, G7 staff	USM, sometimes assisted by WHO/HQ/SUP
✓ Storage of supplies, assembling test kits	WHO/HQ	USM
✓ Processing requests, dispatches, payments	WHO/HQ	USM
✓ Cost recovery	WHO/HQ	USM
✓ Financial management	WHO/HQ	USM
✓ Maintenance of database on requests, dispatches, payments	WHO/HQ	USM

1 = ATESMO: a small commercial enterprise in Zurich, established through WHO in 1956.

6.2 SPRAY MACHINE FOR PREPARATION OF INSECTICIDE IMPREGNATED PAPERS (IIPS)

ATESMO used a spray machine (*designed through and provided by WHO)* for uniform spread of insecticide on filter papers. This could not be recovered by WHO when the work at ATESMO was discontinued. USM now prepares the insecticide impregnated papers manually, according to the training and guidance given through University of Cardiff, UK.

Thus the testing kits and impregnated papers are supplied via the WHO collaborating centre USM, in Penang, Malaysia.

It was noted that :

- The papers are impregnated manually.
- There is the possibility of implementing a mechanical paper impregnation system utilising a machine used to prepare impregnated papers for monitoring resistance in ticks. This needs to be investigated.

6.3 QUALITY CONTROL OF INSECTICIDE IMPREGNATED PAPERS (IIPs)

There had been no mechanism in the past for regular quality checks on IIPs prepared, at ATESMO. Quality checks only of defective IIPs, received from the field, were made through WHO collaborating centres. Replacements were made for any defective papers.

With the transfer of IIP preparation to USM, quality control was introduced on a regular basis on samples of IIPs prepared at USM, and analysed in the GLP analytical laboratory of the University of Wales, Cardiff, UK, a WHO collaborating centre.

6.4 SHELF LIFE OF IMPREGNATED PAPERS

It was noted that:

- The current recommendation on the shelf life of organophosphate impregnated papers is one year, and for permethrin and deltamethrin impregnated papers it is thought to be 6 months from the date of impregnation. Stability data for these, and other pyrethroids (for which discriminating concentrations are now being established) were not readily available.

It is recommended that:

- Insecticide impregnated papers, made over the last two years, and properly stored at the University of Wales, Cardiff, UK be assessed by bioassay for current and future viability to establish an accurate shelf life.
- Similar studies should be undertaken for the pyrethroids for which discriminating concentrations are now being established, so that eventually labels can incorporate values for accurate shelf life of impregnated papers at 4° C, and at room temperature.

6.5 THE SOLVENT SYSTEMS USED FOR PAPER IMPREGNATION

It was noted that:

- Organochlorine impregnated papers are prepared with Risella oil.
- Organophosphate and carbamate impregnated papers are prepared with olive oil (with the exception of pirimiphos-methyl which is prepared without an oil carrier).
- Pyrethroid impregnated papers are prepared with Dow Corning 556 silicone fluid.
- A suggestion for a change in solvents was prompted by compatibility problems between insecticides and the initial solvent systems used in some cases.

It is recommended that:

- Where compatible, Dow Corning 556 silicone fluid should be used as the solvent of choice for all new insecticides being added to the WHO system.
- Risella and olive oil be retained for the organochlorines, organophosphates and carbamates to avoid problems of having to establish new discriminating dosages for these insecticides with the new solvents. This will also maintain comparability with the large volume of data already available for these insecticides.

6.6 REPORTED DELAYS ON DISPATCHES / RECEIPT OF ITEMS

It was noted that :

- Delays between ordering and receipt of test kits and insecticide impregnated papers tubes have occurred in several cases. There have been a number of reasons for this including constraints in the payment system and incorrect or incomplete information provided on order forms by the consignee.
- Initially there was a shortage of kit components which are still procured from Europe e.g. due to delays in financial allocations/ transactions. This problem has been overcome by ensuring > one year's backup of stocks on imports from Europe.
- Despite clear instructions given in the order form:
  - Problems/delays in receipt of payments have been experienced. Reasons include stipulated reference numbers not indicated, specified instructions in format designed to facilitate requests/dispatches not followed by consignee when forwarding payments. These problems mean that when USM receives reimbursements it cannot easily identify the source of payments (i.e. who is paying for what).
  - The suggestion that a separate bank account be established, or an identity reference for this activity be assigned is being considered by USM.
  - The desired mode of transport (which affects costs) is invariably not specified in the form; clarification on this takes time.
- Delays occur in import clearance at ports of entry or excessive import duties are imposed in some countries receiving supplies.
- Difficulties occur in delivery to some destinations including areas affected by civil disturbances.
- There were delays in processing at USM.
- The document "Supplies for monitoring insecticide resistance in disease vectors: Procedures and conditions: WHO/MAL/95.1073; WHO/CTD/VBC/95.998" was not widely available to end users, therefore the related processes to be followed in requesting were not known to some.

6.7 REPORTS ON OTHER ASPECTS

- Defective papers were reported on different occasions but quality checks did not substantiate this.

- Plastic cones for bioassay were reported as being too thin. These were from Plassimpress, Switzerland, the same source as in the past. The composition of the plastic used had been changed by Plasimpress with no reference to WHO. Cones are now manufactured in Malaysia; samples of these have been compared with and quality checked through WHO collaborating centre, against samples of two consignments from Plassimpress.

- Plastic tubes (with green and red dots) in susceptibility tests were reported to differ in height from the earlier tubes. Queries from Plassimpress revealed that a standard mould is in use.

- Reports that the slide units of susceptibility test kits disintegrated. Plassimpress had mistakenly used a glue of poor quality.

- Reports were received on unsuitability of a packing device using aluminium foil and cardboard boxes for packaging of IIPs. This was tried on a trial basis following recommendations from industry, and in consultation with the Insecticide Resistance Action Committee of Global Crop Protection Federation, because the source in Switzerland for the plastic box used in the past had not been located. When found unsuitable for field use, it was discontinued. New packing boxes (similar to the originals from Switzerland) have been developed through a small commercial enterprise in Malaysia.

- Initially, most USM staff on the project were "temporary" research staff, resulting in high turn over thus necessitating (re)training. USM has now assigned permanent staff to assist in the work.

The panel acknowledged their appreciation of USM, Penang, Malaysia for undertaking, the production and supply of the material for monitoring insecticide resistance.

7. GENERAL RECOMMENDATIONS

- Establishment of an e-mail contact at the Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, to facilitate communication.

- Strengthening of the procurement system for test kits and insecticide impregnated papers.

- Streamlining the system for production of insecticide impregnated papers by introduction of a mechanical device for the preparation of insecticide impregnated papers.

- Continuation of quality control as an integral part of this system.

- Establishment of discriminating concentrations for all insecticides cleared through WHOPES.

ANNEXES

INFORMAL CONSULTATION ON TEST PROCEDURES FOR INSECTICIDE RESISTANCE MONITORING IN MALARIA VECTORS, BIO-EFFICACY & PERSISTENCE OF INSECTICIDES ON TREATED SURFACES

Geneva, Switzerland
28-30 September 1998

AGENDA

Monday, 28 September 1998

09:00–09: 20	Opening and address by Executive Director, Cluster on Communicable Diseases	Dr D L Heymann
09:20-09:45	Objectives and expected outcome of the meeting	Dr P R J Herath
09:45-10:00	Administrative matters	Dr P R J Herath
10:00-10:15	*Coffee Break*	
10:15-11:00	Agenda item 1 *Test procedures: issues and concerns on current test Procedures with special focus on pyrethroids*	
	Susceptibility test e.g. vertical or horizontal positioning of exposure tubes during mosquito exposure to pyrethroid insecticides, and other issues	Dr P. Guillet
11:00-11:45	Bioassay test e.g. time of exposure, test equipment	Prof. C F Curtis
11:45-12:30	Review of draft protocol for pyrethroids, prepared during AFR 'Workshop on the standardization of a protocol for Testing *Anopheles gambiae* susceptibility to insecticides In the African Region', Bouake, Côte d'Ivoire, 22-24.9.1998	Dr P. Guillet
12:30-14:00	*Lunch Break*	
14:00-15:30	Agenda item 2 *Discriminating dosages for pyrethroids (permethrin, Deltamethrin, lambdacychalothrin, cyfluthrin, etofenprox)*	
	Presentation of data from the multi-centre study Discussion Finalization and recommendations on discriminating dosages	Dr P R J Herath
15:30-15:45	*Coffee Break*	
15:45-16:30	Finalization and recommendations on discriminating dosages (continued)	
16:30-17.30	Agenda item 3 *Criteria for defining 'resistance': interpretation of susceptibility/resistance test results* Discussion	Dr P R J Herath

Tuesday, 29 September 1998

08:30-10:15	Agenda item 4 *Review of test procedures contained in WHO mimeograph documents and updating with inclusions e.g. for pyrethroids and other relevant issues – Working Groups*	
10:15-10:30	*Coffee Break*	
10:30-11:15	Agenda item 5 *Solvents for preparation of insecticide impregnated papers*	
	Review of research data Discussion Recommendations	Prof. J Hemingway & IRAC representative
11:15-12:30	New chemicals for larval test kits Discussion	Dr M Zaim
12:30 – 14:00	*Lunch Break*	
14:00-15:30	Agenda item 6 *Logistic arrangements for the Global Programme on Insecticide Resistance Monitoring (GPIRM)*	
	Current procedures, issues of concern Discussion	Dr Zairi Bin Jaal
	How to make a difference – Working Groups	
15:30-15:45	*Coffee break*	
15:45-17:00	Updating WHO catalogue (WHO/VBC/81.828/Rev.2) Including pricing of test kits and components Discussion	Dr Zairi Bin Jaal

Wednesday 30 September 1998

08:30-9:30	Quality control on test kit components Discussion	Prof J Hemingway & Dr Zairi Bin Jaal
09:30-10:30	Agenda item 7 Issues for further action: Working Groups	
10:30-10:45	*Coffee Break*	
10:45-12:30	Agenda item 8 Recommendations	
12:30-14:00	*Lunch Break*	
14:00-15:30	Agenda item 9 Finalization of draft report	
15:30-15:45	*Coffee Break*	
15:45-17:00	Finalization of draft report (continued)	
17:00-17:30	Closure of meeting	

INFORMAL CONSULTATION ON TEST PROCEDURES FOR INSECTICIDE RESISTANCE MONITORING IN MALARIA VECTORS, BIO-EFFICACY & PERSISTENCE OF INSECTICIDES ON TREATED SURFACES

Geneva, Switzerland
28-30 September 1998

LIST OF PARTICIPANTS

Dr William Brogdon
Entomology Branch F22
Division of Parasitic Diseases
Centres for Disease Control
Atlanta, Georgia 3033
USA

Professor Chris Curtis
Department of Medical Parasitology
London School of Hygiene & Tropical Medicine
Keppel Street
London WC1E 7HT
UK

Dr Pierre Guillet (CHAIRMAN)
LIN/ORSTOM
BP 5045-3402
Montpellier Cedex 1
France

Professor Janet Hemingway (RAPPORTEUR)
School of Bio-sciences
University of Wales Cardiff
P O Box 915
Cardiff CF1 3TL
UK

Dr Hossein Ladonni
Medical Entomology Department
Tehran University of Medical Sciences
School of Public Health
P O Box 4664
Tehran 14155
Iran

Dr Sarala Subbarao
Malaria Research Centre
22 Sham Nath Marg
Delhi 110054
India

Dr Zairi Bin Jaal
Vector Control Research Unit
School of Biological Sciences
11800 Universiti Sains Malaysia
Penang
Malaysia

Note: Dr G Hesse, Insecticide Resistance Action Committee of the Global Crop Protection Federation was unable to attend

Annex 2

INFORMAL CONSULTATION ON TEST PROCEDURES FOR INSECTICIDE RESISTANCE MONITORING IN MALARIA VECTORS, BIO-EFFICACY & PERSISTENCE OF INSECTICIDES ON TREATED SURFACES

Geneva, Switzerland
28-30 September 1998-12-03

WHO Secretariat

Dr M Cham, Scientist
Roll Back Malaria[1]

Dr P R J Herath, Scientist (SECRETARY)
Malaria Team[1]

Dr M Nathan, Scientist[2]

Dr M Zaim, Scientist
WHOPES[3]

Ms Annette Peters[1]
(Secretarial assistance)

[1] Prevention & Control Department
Communicable Diseases Cluster
(formerly Malaria Unit, Division of Control of Tropical Diseases)
World Health Organization, Geneva
Switzerland

[2] Prevention & Control Department
Communicable Diseases Cluster
(formerly Filariasis Unit, Division of Control of Tropical Diseases)
World Health Organization, Geneva
Switzerland

[3] Prevention & Control Department
Communicable Diseases Cluster
(WHOPES Unit, Division of Control of Tropical Diseases)
World Health Organization, Geneva
Switzerland

Annex 3

INFORMAL CONSULTATION ON TEST PROCEDURES FOR INSECTICIDE RESISTANCE MONITORING IN MALARIA VECTORS, BIO-EFFICACY & PERSISTENCE OF INSECTICIDES ON TREATED SURFACES

Geneva, Switzerland, 28-30 September 1998

INSTITUTES WHICH PARTICIPATED IN THE MULTI-CENTRE STUDY TO ESTABLISH DISCRIMINATING CONCENTRATIONS FOR PYRETHROIDS

Department d'epidemiologie des Affections Parasitaires
Ecole Nationale de Medecine et de Pharmacie, B P 1805 Bamako
Mali

Vector Control Research Station
Ministry of Health
Jl. Hazanudin 123, P O Box 200, Salatiga, Central Java
Indonesia

Department of Medical Parasitology,
London School of Hygiene & Tropical Medicine
Keppel Street, London WC1E 7HT
UK

School of Public Health
P O Box 4664, Teheran 14155
Iran

Malaria Research Centre
22, Sham Nath Marg, Delhi - 110054
India

LIN/ORSTOM
B P 5045- 34032 Montpellier Cedex 1
France

School of Pure and Applied Biology, University of Wales Cardiff,
P O Box 923, Cardiff CFI 3TL
England

Applied Research Branch,
Malaria Division & Centre for Disease Vector Control
Department of Communicable Disease Control
Ministry of Public Health, Nonthaburi 11000
Thailand

Entomology Branch F 22, Division of Parasitic Diseases
Centres for Disease Control , Atlanta , Georgia 30333
USA

Vector Control Research Unit, School of Biological Sciences
11800 Universiti Sains Malaysia
Penang
Malaysia
(Supply of insecticide impregnated papers)

STANDARD PROTOCOL FOR THE ESTABLISHMENT OF DISCRIMINATING DOSAGES IN ADULT MALARIA VECTORS

1. *Test sample and conditions*

1.1 *Mosquito sample*:
Anopheline species (*malaria vectors*) which you have indicated to us as available in your laboratory, and already clarified/established for their susceptibility to the pyrethroid insecticides, and *1 to 3 day old, unfed, adult females* from these laboratory maintained colonies.

1.2 *Sample size per concentration/dosage of an insecticide*
Minimum of 100 mosquitoes (used in 4 replicates with 20-25 females per tube/ replicate) for each insecticide concentration/dosage. Each test should have controls. The tests to be repeated 2-3 times[1] (depending on mosquito availability) so that a given dosage is tested on samples of 200-300 mosquitoes.

1.3 *Exposure period* : 60 minutes

1.4 *Temperature and relative humidity: standard laboratory conditions (to be recorded in reporting)*

1.5 *Positioning of tubes during mosquito exposure to insecticide* : vertical

2. *Insecticides and concentrations to be tested in the impregnated papers*:

permethrin	0.1	0.25	0.5	0.75	1.0
deltamethrin	0.005	0.0125	0.025	0.05	0.1
lambda cyhalothrin	0.01	0.025	0.05	0.1	0.2
cyfluthrin	0.005	0.0125	0.025	0.05	0.1
etofenprox	0.1	0.25	0.5	0.75	1.0

Note: Each insecticide paper should not be used more than 6 times.

All tests to be completed within 2 months of receiving the insecticide impregnated papers.

If possible the anopheline populations to be tested with DDT 4% 60 minute exposure.

For further clarification, please contact Dr P R J Herath, Malaria Unit, Division of Control of Tropical Diseases, World Health Organization, 1211 Geneva 27, Switzerland. Fax (41 22) 791 47 47 or (41 22) 791 07 46. . E-mail: herath@who.ch

3. *Sample data recording format to be used for each concentration/dosage of an insecticide.*

3.1. Test no: .....(see section 2[1])

3.2. Insecticide.....

3.3. Concentration/dosage *(%)*.....

3.4. Anopheline species tested: ..... Strain: .....
Age (days) : ..... History of test colony: .....
(e.g. origin, period of laboratory maintenance)

3.5 Date of test: *Day*.....*Month*.....*Year*.....

3.6 Temperature range Humidity range
Exposure period *from* ..... *to* ..... *from* ..... *to* .....
Holding period *from* ..... *to* ..... *from* ..... *to* .....

3.7 Test results and other information

Test	Test Replicate 1	Test Replicate 2	Test Replicate 3	Test Replicate 4	Total Test	Total Control
Times impregnated paper used					*NA*	
No. mosquitoes exposed						
no. mosquitoes knocked down at end of exposure period						
No. mosquitoes dead at end of holding period						
Mortality observed (%)						
Mortality corrected (%)						

NA = not applicable

Abbott's formula to be applied when control mortality is between 5-20%.

$$\frac{(100 - \%\ \text{test mortality})}{(100 - \%\ \text{control mortality})} \times 100$$

Name of investigator : .....

Name & address of institution : .....
.....
.....
.....

Fax no : ..... E-mail address : ..... Tel No : .....
Comments : .....
.....
.....
.....

Annex 5

DATA FROM MULTI-CENTRE STUDY : DIFFERENT STRAINS TESTED

PERMETHRIN

Institute	Strain	Insecticide concentrate				
		0.1%	0.25%	0.5%	0.75%	1.0%
An. Albimanus						
CDC/USA	teco	8 (100)	100 (100)	84 (100)	100 (100)	100 (100)
Cardiff/UK	Panama	95 (40)	100 (40)	-	100 (40)	100 (40)
LSHTM/UK	mexico	100 (103)	100 (102)	100 (100)	100 (99)	100 (100)
An. Gambiae						
CDC/USA	G3	33 (100)	84 (100)	100 (100)	100 (100)	100 (100)
Cardiff/UK	G3	45.2 (42)	95 (40)	-	100 (40)	100 (40)
LIN/ORSTOM	G3	48.8 (400)	88.3 (400)	100 (300)	-	100 (400)
LSHTM/UK	Kwa	78.8 (104)	95.2 (105)	84.7 (111)	100 (102)	100 (102)
Mali	Mopti	99.4 (350)	100 (300)	76.8 (250)	99.6 (275)	99.2 (250)
LIN/ORSTOM	Kisumu	96.3 (400)	100 (400)	100 (300)	-	100 (300)
An. Stephensi						
CDC/USA	Delhi	17 (100)	94 (100)	100 (100)	100 (100)	100 (100)
Cardiff/UK	St	60 (40)	100 (40)	-	100 (40)	100 (40)
Cardiff/UK	Beech	40 (40)	90.5 (42)	-	100 (40)	100 (40)
LSHTM/UK	Beech	92.6 (108)	98.3 (118)	82 (100)	100 (96)	100 (100)
Iran	Beech	88.5 (243)	100 (248)	100 (194)	100 (184)	100 (194)
India	Delhi	87 (200)	100 (300)	-	-	-

- = not done ( ) = sample size

DATA FROM MULTI-CENTRE STUDY : DIFFERENT STRAINS TESTED

DELTAMETHRIN

Institute	Strain	Insecticide concentrate				
		0.005%	0.0125%	0.025%	0.05%	0.1%
An. Albimanus						
CDC/USA	teco	13 (100)	81 (100)	70 (100)	100 (100)	100 (100)
Cardiff/UK	Panama	37.5 (40)	-	100 (40)	100 (40)	100 (40)
LSHTM/UK	mexico	100 (101)	100 (155)	100 (102)	100 (101)	100 (103)
An. Gambiae						
CDC/USA	G3	91 (100)	100 (100)	100 (100)	100 (100)	100 (100)
Cardiff/UK	G3	40.9 (22)	100 (40)	90.5 (42)	100 (40)	100 (40)
LIN/ORSTOM	G3	95 (400)	100 (300)	99.5 (200)	-	-
LSHTM/UK	Kwa	97.2 (109)	100 (98)	100 (111)	100 (115)	100 (100)
Mali	Mopti	81.7 (300)	92.7 (300)	93.3 (300)	97.8 (325)	100 (300)
LIN/ORSTOM	Kisumu	99.5 (400)	100 (500)	100 (300)	100 (200)	-
An. Stephensi						
CDC/USA	Delhi	17 (100)	74 (100)	100 (100)		
Cardiff/UK	St	60.4 (48)	37.5 (32)	100 (40)		
Cardiff/UK	Beech	4.3 (46)	28.6 (42)	95 (40)		
LSHTM/UK	Beech	80 (100)	87.6 (105)	84.8 (112)		
Iran	Beech	95.1 (203)	99 (200)	100 (201)		
India	Delhi	95.5 (200)	100 (325)	-		

- = not done ( ) = sample size

Annex 7

DATA FROM MULTI-CENTRE STUDY : DIFFERENT STRAINS TESTED

LAMBDA-CYHALOTHRIN

Institute	Strain	Insecticide concentrate				
		0.01%	0.025%	0.05%	0.1%	0.2%
An. Albimanus						
CDC/USA	teco	7 (100)	62 (100)	100 (100)	100 (100)	100 (100)
Cardiff/UK	Panama	100 (40)	100 (40)	100 (40)	100 (40)	100 (40)
LSHTM/UK	mexico	99.1 (107)	100 (101)	100 (95)	100 (100)	100 (106)
An. Gambiae						
CDC/USA	G3	100 (100)	98 (100)	100 (100)	100 (100)	100 (100)
Cardiff/UK	G3	100 (18)	100 (20)	100 (40)	100 (40)	100 (40)
LIN/ORSTOM	G3	97.3 (400)	97.8 (400)	100 (300)	-	-
LSHTM/UK	Kwa	100 (103)	99.1 (106)	100 (99)	100 (101)	100 (91)
Mali	Mopti	76.7 (300)	80 (300)	99.3 (300)	100 (300)	100 (300)
LIN/ORSTOM	Kisumu	98.8 (400)	100 (500)	100 (300)	100 (200)	-
An. Stephensi						
CDC/USA	Delhi	50 (100)	95 (100)	100 (100)	100 (100)	
Cardiff/UK	St	5 (40)	92.5 (40)	100 (40)	100 (40)	
Cardiff/UK	Beech	0 (37)	52 (50)	100 (45)	100 (40)	
LSHTM/UK	Beech	63.8 (105)	91.2 (113)	88.2 (102)	97.2 (106))	
Iran	Beech	93.5 (200)	100 (197)	100 (204)	100 (150)	
India	Delhi	100 (200)	99.7 (300)	-	-	

- = not done ( ) = sample size

DATA FROM MULTI-CENTRE STUDY : DIFFERENT STRAINS TESTED

ETOFENPROX

Institute	Strain	Insecticide concentrate				
		0.1%	0.25%	0.5%	0.75%	1.0%
An. Albimanus						
CDC/USA	teco	23 (100)	100 (100)	100 (100)	100 (100)	100 (100)
Cardiff/UK	Panama	65 (40)	100 (40)	100 (40)	100 (40)	100 (40)
LSHTM/UK	mexico	99.1 (44)	100 (98)	100 (111)	100 (102)	100 (102)
An. Gambiae						
CDC/USA	G3	100 (100)	100 (100)	100 (100)	100 (100)	100 (100)
Cardiff/UK	G3	10 (40)	95 (40)	100 (40)	100 (40)	100 (40)
LIN/ORSTOM	G3	88 (200)	100 (200)	100 (300)	-	-
LSHTM/UK	Kwa	85.7 (98)	99 (101)	101 (98)	100 (94)	100 (94)
Mali	Mopti	39 (300)	97 (300)	99 (300)	100 (300)	100 (300)
LIN/ORSTOM	Kisumu	99.3 (300)	100 (400)	100 (300)		
An. Stephensi						
CDC/USA	Delhi	50 (100)	100 (100)	100 (100)	100 (100)	100 (100)
Cardiff/UK	St	25 (40)	100 (40)	100 (40)	100 (40)	100 (40)
Cardiff/UK	Beech	6.3 (32)	95 (40)	100 (40)	100 (40)	100 (40)
LSHTM/UK	Beech	50.5 (101)	94.1 (101)	99 (102)	100 (115)	100 (104)
Iran	Beech	80.2 (202)	97.6 (247)	98.5 (200)	100 (193)	100 (196)
India	Delhi	91.3 (150)	99.5 (200)	-	-	-

- = not done ( ) = sample size

Annex 9

DATA FROM MULTI-CENTRE STUDY : DIFFERENT STRAINS TESTED

CYFLUTHRIN

An. Albimanus						
Institute	Strain	Insecticide concentrate				
		0.005%	0.0125%	0.025%	0.05%	0.1%
CDC/USA	teco	3 (100)	6 (100)	25 (100)	52 (100)	97 (100)
Cardiff/UK	Panama	97.61 (42)	100 (10)	100 (48)	100 (30)	100 (20)
LSHTM/UK	mexico	99 (99)	100 (99)	98.9 (95)	100 (97)	100 (100)
An. Gambiae						
CDC/USA	G3	40 (100)	48 (100)	75 (100)	93 (100)	97 (100)
Cardiff/UK	G3	54.5 (44)	94.3 (35)	76.1 (46)	97.5 (40)	100 (40)
LIN/ORSTOM	G3	-	53 (200)	85.7 (300)	89.7 (300)	95.8 (500)
LSHTM/UK	Kwa	74 (104)	95.4 (108)	100 (107)	100 (94)	100 (101)
Mali	Mopti	26 (300)	68.9 (225)	69.3 (300)	71 (300)	87.2 (250)
LIN/ORSTOM	Kisumu	-	92.7 (300)	97 (400)	96.3 (400)	100 (300)
An. Stephensi						
CDC/USA	Delhi	18 (100)	27 (100)	43 (100)	71 (100)	90 (100)
Cardiff/UK	St	5 (40)	53.8 (39)	97.7(43)	100 (40)	100 (40)
Cardiff/UK	Beech	25 (36)	27.5 (40)	63.6 (44)	77.5 (40)	100 (40)
LSHTM/UK	Beech	18.9 (111)	34.7 (101)	56.3 (103)	45 (109)	86 (100)
Iran	Beech	65 (200)	76.4 (203)	96.4 (192)	80 (200)	100 (198)
India	Delhi	45.2 (250)	75 (396)	99.4 (175)	-	

- = not done ( ) = sample size

FORMAT FOR RECORDING RESULTS OF SUSCEPTIBILITY TESTS ON ADULT MOSQUITOES

Investigator : .....
Name & address of Institute: .....

Area information

Country :.....*Region*..... *District :*.....

Locality/Village :.....*Geographical coordinates :* N/S.....E/W..... (if available)

Brief area description : .....

Elevation : .....Main crop(s) : .....

Specify insecticides used in the area sampled

Public health :
Insecticide-treated mosquito nets : ..... & for how long (years) .....
Indoor residual spraying : ..... & for how long (years) .....
Larviciding: : ..... & for how long (years) .....
Agriculture : ..... & for how long (years) .....

Sample information

Species tested : ..... *Member of complex* ..... *(if relevant)*

Sex : ..... *Age* (days) : .....(where applicable)

Physiological stage : non-blood fed ☐ blood fed ☐ semi-gravid ☐ gravid ☐

Sampling technique : from larval collection ☐ ; F1 progeny ☐ ; human landing catch ☐ ; animal baited trap ☐ ; indoor resting ☐ others (specify) ☐ .....

If sample is from larval collection, type of breeding site: rice field ☐ ; river bed ☐ ; irrigation channel ☐ ; rain water pools ☐ ; wells ☐ ; water storage container ☐ ; others ☐ (specify) .....

Insecticide information

Insecticide tested : ..... *Concentration :* .....

Impregnated papers prepared by :..... *Date of impregnation :* .....

Date of expiry :..... *Date paper removed from pack :*.....

Number times previously used : .....
Storage conditions: Room temperature at ....°C; Refrigerated at .....°C

Test conditions

Date of testing : ..... *Period of exposure (minutes)*.....

	Temperature		Relative humidity	
	From	to	from	To
Exposure period				
Holding period				

Annex 10

TEST RESULTS

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Total test	Total control
No. exposed						
No. killed End of holding period						
Observed mortality (%)						
Corrected mortality (%)						
No. knocked down after exposure* for minutes :						
10						
15						
20						
30						
40						
50						
60						
80						
Species identified*						
Resistance mechanism(s) **						

* = applicable to DDT and pyrethroids only ** = where identified if member of a complex

Annex 10

HISTORY OF PESTICIDE USE

Where insecticide resistance is encountered the following information is to be obtained

Region : ..... Province : .....

District : ..... Locality : .....

For method of application criteria to be used.

a = Indoor residual spraying ; b = insecticide-treated mosquito nets ;
c = space spraying/fogging; d = larviciding; e= agricultural use

Name of pesticide	*Method of application*	*Year introduced*	*Formulation*	*Dosage*	*Frequency*	*Rounds per year*	*Year terminated*
DDT	e.g. **a**		e.g. **wdp**	e.g. **2gm/ m^2**	e.g. **6 monthly**	e.g. **2**	

Source of information:.....

Investigator:..... Signature: .....

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF MOSQUITOES TO INSECTICIDES

Adult Mosquitoes

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOES TO ORGANOCHLORINE INSECTICIDES
WHO/VBC/75.581

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOES TO ORGANOPHOSPHORUS AND CARBAMATE INSECTICIDES
WHO/VBC/75.582

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOES TO ORGANOCHLORINE, ORGANOPHOSPHATE AND CARBAMATE INSECTICIDES : ESTABLISHMENT OF THE BASE-LINE
WHO/VBC/81.805
Supercedes Annex 1B TRS 443 (1970) & WHO/VBC/75.581 & 582

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF ADULT MOSQUITOES TO ORGANOCHLORINE, ORGANOPHOSPHATE AND CARBAMATE INSECTICIDES - DIAGNOSTIC TEST
WHO/VBC/81.806

Mosquito Larvae

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF MOSQUITO LARVAE TO INSECTICIDES
WHO/VBC/81.807
Supercedes WHO/VBC/75.583

Bioassay Test

WHO/VBC/81.812
Supercedes WHO/VBC/76.1 & VBC/EC/75.21
INSTRUCTIONS FOR THE BIO-ASSAY OF INSECTICIDAL DEPOSITS ON WALL SURFACES
VBC/81.5

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF MOSQUITO LARVAE TO INSECT DEVELOPMENT INHIBITORS