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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

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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