

Long-term Programme for Pollution Monitoring and Research
in the Mediterranean Sea
(MED POL Phase II)

BIOLOGICAL MONITORING OF METHYLMERCURY IN MEDITERRANEAN POPULATIONS

Report on a joint WHO/FAO/UNEP meeting

Zagreb
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WORLD HEALTH ORGANIZATION
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CONTENTS

	<u>Page</u>
Preface	1
1. Opening of the meeting	2
2. Scope and purpose of the meeting	4
3. Election of officers	4
4. Adoption of the agenda	4
5. Organization of the Consultation meeting	4
6. Criteria for the identification of high-risk population groups	4
7. Review of draft protocol on determination of mercury and selenium in human hair	7
8. Review of draft protocol on clinical epidemiological studies	7
9. Review of progress in implementation of project on Evaluation of methylmercury in Mediterranean populations and related health hazards	9
10. Other matters	9
Annex 1 : Revised workplan, time-table and budget for project on evaluation of methylmercury in Mediterranean populations and related health hazards	10
Annex 2 : List of participants	14

PREFACE

The problem of methylmercury in Mediterranean seafood, together with the potential health effects on the population of the region, has been the subject of considerable international activities, mainly organized by the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) over the last decade. Up to 1982, studies carried out, revealed that, according to the best available data, most of the general Mediterranean population appeared to have a methylmercury intake through seafood below the Provisional Tolerable Weekly Intake (PTWI) established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA), and could therefore be considered not at risk. On the other hand, it appeared equally evident that some population groups in the Mediterranean region may have a methylmercury intake through seafood in excess of permissible levels. The gaps in current knowledge have so far made it difficult to undertake the proper assessment of hazards to health from consumption of methylmercury-contaminated Mediterranean seafood or to protect those sectors of the population that may be at risk.

A comprehensive project was formulated on the evaluation of methylmercury in Mediterranean populations and related health hazards, designed to fill, to the extent possible, gaps in existing knowledge, and to provide the basis for the finalization of common regional environmental quality criteria for mercury in Mediterranean seafood. This project is being implemented both within the framework of the research component of the Long-term Programme for Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II) and as part of the WHO Regional Office for Europe's programme on chemical safety.

A consultation meeting was convened by the WHO Regional Office for Europe in cooperation with UNEP in Athens from 13 to 17 September 1982 with the aim of finalizing the details of the project and securing its effective implementation. During this consultation meeting, agreement was reached on the following components :

- identification of "hot spots" and population groups at risk
- analysis of seafood for total mercury and methylmercury content
- dietary surveys to determine seafood consumption patterns in the selected areas
- analysis of human hair in the same sample populations as for the dietary surveys
- clinical epidemiological studies on health effects of methylmercury in the indicated sectors within the original population samples

The project became operational in Yugoslavia in early 1984, and efforts were continued to extend it to other Mediterranean countries, particularly Greece and Italy.

An assessment of the present state of pollution by mercury in the Mediterranean Sea, together with proposed control measures, was carried out by UNEP, FAO and WHO in late 1983. This assessment was mainly performed on the basis of results obtained during the pilot project on baseline studies and monitoring of metals (particularly mercury and cadmium) in marine organisms, organized jointly by FAO and UNEP within the framework of the Joint Coordinated programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase I) between 1975 and 1980. This study confirmed the previous conclusions that the general Mediterranean population did not appear to be at risk, and recommended that, for this reason, the imposition of upper limits for mercury concentrations in seafood on a common regional basis did not appear to be justified at the present stage.

On the other hand, the study also confirmed that certain population sectors in the Mediterranean region may have an intake of methylmercury through seafood in excess of permissible levels, and recommended that the studies being carried out as part of the methylmercury project should be accorded continued support within the framework of MED POL Phase II.

Within the framework of this programme, a consultation meeting on biological monitoring of methylmercury in Mediterranean populations was convened jointly by WHO, FAO and UNEP, with the following objectives :

- to discuss the criteria for identification of high-risk population groups
- to review the draft protocol on determination of mercury and selenium in human hair
- to review the draft protocol on monitoring and epidemiological studies on health effects of methylmercury
- to review progress in implementation of the project on evaluation of methylmercury in Mediterranean populations and related health hazards.

A number of experts from Mediterranean Institutions which were either already participating in the project or had shown a positive interest in participating, together with a number of other Mediterranean and non-Mediterranean experts, were invited to attend the Consultation meeting. In addition, the following International organizations were invited to send representatives: IOC, UNESCO, WMO and IAEA.

Opening of the meeting (Agenda item 1)

The meeting took place at the Institute of Public Health of Croatia, Zagreb, from 17 to 21 September 1984. It was attended by 19 participants from six Mediterranean countries, one participant from a non-Mediterranean country. There was one representative of FAO, and two staff members of WHO headquarters and the Regional Office for Europe. A list of participants is given in Annex 2.

Dr L.J. Saliba, Senior Scientist, Mediterranean Action Plan, WHO Regional Office for Europe, opened the Meeting and welcomed participants on behalf of the Regional Director, Dr L.A. Kaprio. He briefly outlined the events leading to the meeting and stressed its importance within the Mediterranean programme. He also expressed the Organization's thanks and appreciation to the Yugoslav authorities, particularly the Institute of Public Health of Croatia, for hosting the meeting, and for the facilities and hospitality provided.

Dr Saliba also welcomed participants on behalf of Mr A. Manos, UNEP Coordinator of the Mediterranean Action Plan. He briefly outlined the components of the plan, which had now been operational since 1975, and which was a significant example of cooperation between Mediterranean governments.

Professor Ante Hrabar, Director of the Institute of Public Health of Croatia, welcomed participants on behalf of the Institute. He expressed his appreciation at the initiative of the WHO Regional Office for Europe for organizing the meeting at the Institute, which had a long tradition of public health activity since its foundation in 1927. He also stated that he considered the topic of the meeting as greatly important, as mercury contamination had become of great concern to Health Authorities in Mediterranean countries, and the health significance should therefore be better defined. Besides, the topic was also important in view of economic repercussions on the fishing industry. He stressed the importance of effective international cooperation and augured the meeting every success, expressing his belief that it would not only contribute to the advancement of research methodology, but would also be of great help to public health services. Finally he augured participants a pleasant stay in Zagreb.

Dr L. Jeftic, Director, Department of Environmental Protection, Committee for Building, Housing, Public Works and Environmental Protection of the Socialist Republic of Croatia, welcomed participants in his capacity of Yugoslav National Coordinator for MED POL. He expressed the interest of Yugoslavia in this particular aspect of the research component of MED POL, and recalled that Yugoslav Institutes had been extremely active in all the components of the MED POL programme ever since its inception in 1975. He was glad to see that this project had now become operational in Yugoslavia, as the mercury problem was of great importance. Finally, he augured all participants a fruitful meeting, and expressed the hope that they would also have the opportunity of seeing as much as possible the historic city of Zagreb.

Dr G.P. Gabrielides, FAO Senior Fishery Officer (Marine Pollution), welcomed participants on behalf of FAO and referred to his organization's long-standing collaboration with WHO on matters concerning food consumption and health. He also explained FAO's participation in the programme of biological monitoring, stressing the need for reliable and comparable analytical data, and urged the participants to use the Reference methods and take part in the intercalibration exercises organized within the framework of MED POL activities.

Scope and Purpose of the meeting (Agenda item 2)

Dr Saliba outlined the scope and purpose of the Consultation meeting, and its place within the overall project. He stressed the importance of finalizing (a) the identification of high-risk groups, (b) the protocol on determination of mercury and selenium in human hair, and (c) the protocol on epidemiological studies on health effects of mercury, as well as that of revising and updating the project's workplan, time-table and budget.

Election of Officers (Agenda item 3)

Professor R. Buzina was elected Chairman, Professor Antonia Polychronopoulou-Trichopoulou Vice-Chairman and Dr N. Egoz Rapporteur. Dr L.J. Saliba acted as Secretary to the meeting.

Adoption of the agenda (Agenda item 4)

The provisional agenda was unanimously adopted.

Organization of the meeting (Agenda item 5)

The Chairman explained the detailed organization of the work of the meeting, including the timing of sessions and other arrangements.

Criteria for the identification of high-risk population groups (Agenda item 6)

Dr Saliba briefly outlined document RCP/CEH 001 m02/6.

During an extensive discussion, the meeting recognized the fact that while a proper assessment of high-risk population groups could only be made on the basis of relevant data, for the purposes of the project, a preliminary assessment would have to be made in the light of existing knowledge. In this regard, the data obtained during the course of MED POL Phase I on mercury levels in Mediterranean seafood could only be treated as indicative, for the following reasons :

- in the majority of cases, only a relatively small number of seafood species were analyzed for mercury, and the overall picture from the geographical point of view was far from complete.
- an enormous concentration-discrepancy was encountered between different individuals of the same species, in many cases uncorrelated to age or size.
- one major unknown factor was the extent to which Mediterranean consumers included seafood (in fresh, frozen or canned form) imported from outside the region in their diet).

The meeting agreed that much more information was required on mercury and selenium levels in Mediterranean seafood, as an integral part of the project, and that there was a need for intercalibration exercises to ensure comparability of results.

With regard to consumer groups, the meeting recognized four major categories as being apparent :

- general populations within the region, consuming average or below average amounts of seafood, mainly caught in areas where mercury concentrations were average. This group would not be expected to exceed the maximum permissible intake of 0.2 mg methylmercury per week.
- general populations, also consuming average or below average amounts of seafood (approximately 0.5 to 0.7 kg per capita per week) in areas where high mercury concentrations have been recorded. Here, an average concentration of 1.2 mg/kg of mercury in seafood would result in an approximate weekly intake of 0.6 to 0.8 mg.
- population groups consuming higher than average amounts of seafood, or exhibiting species-selectivity in their seafood diet. Depending on the particular area, mercury concentrations in seafood within the area and the type of species consumed, these groups could exceed the maximum permissible weekly intake.
- population groups consuming higher than average amounts of seafood in areas where high mercury concentrations are found. These groups would be expected to exceed the maximum permissible weekly intake.

Apart from the first group, which could be said to constitute the large majority, all the rest could be considered to be at various degrees of risk. The actual degree of such risk could however only be assessed following the performance of dietary surveys and analytical determinations for mercury.

The meeting agreed that the best groups to concentrate on during the preliminary studies would be those known to have a high Mediterranean seafood consumption (e.g. fishermen and their families, particularly those known to consume seafood contaminated by mercury from specific industrial sources). Similarly, as the pre-natal stage is the most sensitive to methylmercury in the human life-cycle, it was confirmed that any studies undertaken should pay particular attention to women of child-bearing age.

The meeting also recognized that although exposure to methylmercury in the Mediterranean region was mainly through consumption of contaminated seafood, other sources, including both general and occupational exposure and the consumption of food and drugs containing methylmercury, should not be neglected.

It was agreed that the first step towards the identification of high-risk population groups with regard to methylmercury exposure was the selection of areas for pilot study and representative population samples within these areas. Such selection could be made on the basis of the following criteria :

1. Selection of area: This should be such as to provide the environmental conditions conducive to a potentially high mercury exposure. As the study was specifically designed to afford an indication of such exposure through consumption of contaminated seafood, the main criterion for area selection would have to be the methylmercury concentration in seafood caught, processed and

subject to the effects of an industrial or other source of mercury pollution (resulting in elevated levels in all or most species) or contain species of seafood normally accumulating more mercury than others as a main catch.

2. Selection of population sample: Determination of the population sample would necessarily have to be a compromise between statistical significance requirements and resource availability. With the limited financial resources available for implementation of the project, the actual size of the sample would have to be the minimum compatible with technical needs. The question would then arise as to whether the sample should be drawn completely at random from within the population inhabiting the area or whether it should be restricted a priori to those groups more likely to be exposed than others. In a mixed community, a random sample drawn at cross-sectoral level would afford an indication of the general situation prevailing in the areas as a whole. It would however result in the "positive" sub-sample on which the second phase (clinical studies for health effects) would be undertaken being too small for statistical significance. Conversely, a sample drawn exclusively from those groups known to be more exposed than others would provide valuable data on the population sector requiring most attention, afford a larger sample for the clinical studies, but at the same time would only provide a partial picture of the general situation.

Taking all factors into consideration, the meeting agreed that the second alternative would be more advisable as an initial phase. Furthermore, if the sample consisted of the entire population of a small fishing village, several disadvantages, including the statistical significance requirements would be automatically removed. It was also agreed that, in addition to the "potentially high-risk sample", a control group should also be similarly investigated.

In connection with this agenda item, the meeting also discussed the protocol for the dietary surveys (document ICP/RCE 211(1), annex 1). In this context, the following opinions were expressed:

1. The protocol should be retained in its present form. This opinion was shared by the Yugoslav institution currently working on the project.
2. That the protocol should contain a much shorter questionnaire, and cover a wider spectrum of dietary patterns, thus enabling a larger population to be sampled. In this regard, the inclusion of other food-groups and non-food habits would increase the validity of data on seafood consumption through comparison with other components.
3. That other food sources through which mercury in seafood could find its way to man be included. In this regard, one main example quoted was the situation in some parts of Egypt where mercury-contaminated fish were ground into meal and used as poultry-feed. Mercury in seafood could therefore reach man through an intermediate food-source.

4. That the questionnaire could be modified to cater for interviews 3 times a year, and would be on a more general basis, covering a range of other foods.
5. That the questionnaire could be divided into (a) common mandatory parameters, and (b) optional parameters which would be selected according to the prevailing situation in each particular area.

It was concluded that more information on food consumption patterns was required. The current protocol could be used as a basis for acquiring this information, and other questions could be added according to the particular requirements of the country or countries concerned.

Review of draft protocol on determination of mercury and selenium in human hair (Agenda item 7)

The meeting discussed the draft protocol on determination of (a) methylmercury, (b) total mercury and (c) selenium in human hair. A discussion took place as to whether the particular methodology recommended (gas liquid chromatography) for (a) and particularly (c) above were actually the best ones, and it was considered that methylmercury and selenium could also be determined by the use of atomic absorption spectrophotometry. It was explained to participants that the specific methods recommended had been agreed on at the first consultation on the project in September 1982, and that their use would not preclude (a) the use of any other method in parallel, or (b) their eventual updating and possible eventual substitution at some future date if circumstances so required.

The meeting established a small working group to look into the protocol in detail, and to recommend any necessary amendments. These amendments would be incorporated into a revised version of the protocol, which would then be circulated to selected Mediterranean laboratories for further comments prior to formal issue.

Review of protocol on clinical epidemiological studies (Agenda item 8)

The meeting reviewed the draft protocol for monitoring and epidemiological studies on health effects of methylmercury (document ICP/CEH 001 m02/8). During the discussion, the following general comments were made by a number of participants:

1. It appeared doubtful whether the actual exposure to methylmercury found during the initial stages of the project (dietary survey and human hair analysis) would result in the necessity of carrying out such a large-scale epidemiological study as proposed in the document.
2. Most of the health effects covered in the document were not specific to methylmercury poisoning. It was important therefore, that, as stated in the document, causes other than methylmercury exposure be taken into consideration, as well as factors that interact with the susceptibility of the examined persons. In the case of adults, psychiatric investigation did not appear to be necessary for the specific purpose of the project in the light of recently acquired knowledge.

3. Selenium levels should be one of the factors to be taken into special account. This element had been found to interact with methylmercury in animal experiments. No data have been recorded so far on its effects on humans. If such effects were to exist, the selenium content could also constitute a confounding factor.
4. Considerable improvement could be made in the distinction between the epidemiological studies to be applied to adults and those to children.

The meeting established a small working group to study the document in detail and advise on possible amendment and revision. The working group's recommendations which were accepted by the meeting were the following:

1. The introduction should be expanded to include details of recent up-to-date knowledge of experience gained from studies in Canada, Iraq and Japan. Considering the mercury levels expected in the Mediterranean, the symptom most likely to be found in adults would be ataxia, as experienced in the Canadian studies.
2. The purpose of the study should be modified to emphasize that the main purpose was to detect, by the most sensitive methods available, the signs of methylmercury poisoning in exposed populations, and whenever possible to establish dose-response relationships.
3. The establishment of a detailed protocol for epidemiological studies on effects on children (which appear to be 2 to 10 times more sensitive to methylmercury than adults) exposed in utero required information on exposure levels in the presence of confounding factors (such as nutritional status, alcohol intake, smoking habits, and socio-economic conditions). Information about these factors would only be available after the first phase. However, from past experience, the basic requirement for an epidemiological study to produce the necessary information could be identified. The study population should include an exposed group of babies born in an area where the median concentration of methylmercury in hair is 5 ppm or above, as defined by local monitoring, to be compared with a control group with lower exposure levels. The exact design of the study would have to depend on local conditions and the presence of confounding factors. The number of children to be studied should depend on the level of exposure and could be estimated on past experience, mainly studies in Iraq. The parameters to be tested would include psychomotor development of children and the presence of seizures. Specific neurological examination could also be considered. Detailed standard methods for examination would have to be updated. The children studied should be followed up from birth until at least age 5, and further if indicated.
4. In adults, exposure to methylmercury resulting in a minimum concentration of 30 ppm in hair is necessary to produce clinically-detectable effects. Therefore the population of adults to be studied should be exposed populations in which the median concentration of methylmercury in hair found in local monitoring is

30 ppm or above. The parameters to be tested would be clinical neurological examinations, including assessment of vibration-sense, hearing ability and visual fields by objective methods. If possible, tests on visual and auditory-evoked responses should be included. It was important to ensure that the relevant appendices to the revised protocol included all the necessary details.

5. In redesigning the studies, the importance of taking into account the possible confounding factors, such as age, alcohol consumption and smoking habits, and occupational exposure, was confirmed by the group.

The meeting recognized that because of (a) the time-factor, and (b) the advisability of utilizing more specific experience in certain fields, it was not possible to develop and finalize the revised version of the protocol at this time. It was agreed that WHO/EURO would undertake this task as early as possible.

Review of progress in implementation of the project on evaluation of methylmercury in Mediterranean populations and related health hazards (Agenda item 9)

Dr Saliba presented document ICP/CEH 001 m02/9 in combination with reference document ICP/RCE 211(1) and explained the proposed revisions in the workplan, time-table and budget. The latter was dependent on funds made available by Mediterranean Governments for the respective component of the Mediterranean Action Plan, and the figures should only be treated as indicative.

During the discussion, the Egyptian participant informed the meeting that his institution had a positive interest in taking part in the project. It was agreed that the necessary arrangements for such participation would be made.

The meeting noted the document, and expressed the hope that the necessary funds would be made available. The revised workplan and budget, as amended, are given at annex 1.

Other matters (Item 10 of the agenda)

In addition to France and Italy, Egypt and Yugoslavia also expressed their desire to participate in the supporting studies on the correlation between total mercury and methylmercury levels in seafood. It was agreed that the International Atomic Energy Agency Laboratory in Monaco should be requested to arrange for an intercalibration exercise at the appropriate time.

Annex 1

REVISED WORKPLAN AND BUDGET FOR THE PROJECT
ON EVALUATION OF METHYLMERCURY IN
MEDITERRANEAN POPULATIONS AND RELATED HEALTH HAZARDS

Introduction and Background

1. The original project document on evaluation of methylmercury in Mediterranean populations and related health hazards was finalized by a consultation meeting bearing the same title jointly convened by WHO and UNEP in Athens from 13 to 17 September 1982 (WHO document ICP/RCE 211 (1), Annex 2).
2. The project is composed of four main components :
 - analysis of seafood for methylmercury in selected hot-spots in the Mediterranean
 - a dietary survey on sample populations in the above areas
 - analysis of mercury (and selenium) in human hair, using the same sample populations
 - at a later stage, clinical epidemiological studies on the indicated groups within the original sample populations.
3. The first three components were commenced in selected areas in Yugoslavia in early 1984. It is the intention to extend the project to selected areas in Egypt, Greece and Italy during 1985.
4. A document on assessment of the present state of pollution by mercury in the Mediterranean sea and proposed control measures was issued by UNEP/WHO/FAO in November 1983. This assessment confirms the original view that while on the basis of (a) recorded data on mercury levels in Mediterranean seafood and (b) seafood consumption in the region, the general population cannot be considered at risk, on the other hand there is evidence that certain population sectors may have a mercury intake through seafood above the JECFA Provisional Tolerable Weekly Intake (PTWI).
5. The Second meeting of the Working Group on Scientific and Technical Cooperation (WGSTC) for MED POL, held in Athens from 21 to 25 November 1983, endorsed the recommendations contained in the assessment document regarding future research, including all the components of the methylmercury project document. The extraordinary meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea and its related Protocols (Athens, 10-13 April 1984) recommended, inter alia, that, in the case of mercury levels in seafood, "efforts should be continued and intensified to obtain the additional epidemiological knowledge needed for the full understanding and interpretation of data obtained up until now" (UNEP/IG.49/5, Appendix 3, page 21, para 9).

- Apart from the four main components listed in paragraph 2 above, the following supporting components are also envisaged :
 - studies on the correlation between total mercury and methylmercury in selected seafood species
 - studies on the effects of cooking on mercury concentrations in selected seafood species

Workplan

7. The following revised workplan and timetable is envisaged :

<u>Activity</u>	<u>Period</u>	<u>Remarks</u>
(1) Preparation of background documentation	30 June 1982	completed
(2) Preliminary identification of participants	30 June 1982	completed
(3) First consultation meeting	13-17 September 1982	completed
(4) Finalization of protocol for dietary survey	17 September 1982	completed
(5) Development of draft reference method for determination of total mercury, methylmercury and selenium in human hair	31 December 1982	completed
(6) Development of draft protocol for epidemiological studies	31 December 1982	completed
(7) Negotiations with Institutions	1983 - 1984	
(8) Formalization of arrangements for institutional participation (Yugoslavia)	31 December 1983	completed
(9) Performance of dietary survey (Yugoslavia)	January 84-December 86	operational
(10) Performance of seafood analysis (Yugoslavia)	January 84-December 86	operational
(11) Performance of human hair analysis (Yugoslavia)	January 84-December 86	operational

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|--|---------------------------|-----------|
| (12) Preparation of document on identification of high-risk population groups | August 1984 | completed |
| (13) Consultation meeting | 17-21 September 1984 | |
| (14) Finalization of protocol for human hair analysis | 21 September 1984 | |
| (15) Further development of protocol for epidemiological studies | 31 December 1984 | |
| (16) Finalization of arrangements for institutional participation (Egypt, Greece, Italy) | 31 December 1984 | |
| (17) Performance of dietary survey (Egypt, Greece, Italy) | January 85-December 87 | |
| (18) Performance of seafood analysis (Egypt, Greece, Italy) | January 85-December 87 | |
| (19) Studies on Hg/MeHg correlation in seafood (Egypt, France, Italy, Yugoslavia) | September 84-September 86 | |
| (20) Performance of human hair analysis (Egypt, Greece, Italy) | January 85-December 87 | |
| (21) Commencement of epidemiological studies (Egypt, Yugoslavia) | January 1986 | |
| (22) Commencement of epidemiological studies (Greece, Italy) | July 1986 | |

8. The above workplan is based on the fact that so far positive response to participation has been received from Egypt, Greece, Italy and Yugoslavia. It will be extended to cover other countries as appropriate.

Tentative budget (covering UN contribution)

9. The following tentative budget covering the period 1984 to 1987 is proposed. Expenditure in 1982 and 1983 is also shown. All items cover training to the degree appropriate. It is also envisaged that at least part of the expenditure under seafood analysis will be eventually covered through the monitoring component of the MED POL programme.

	1982 \$	1983 \$	1984 \$	1985 \$	1986 \$	1987 \$
(1) Preparation of protocols	2000	-	-	-	-	-
(2) Consultation meetings	13000	-	12500	-	16000	-
(3) Seafood analysis	-	3500	7000	10000	10000	10000
(4) Dietary surveys	-	8000	15000	15000	10000	10000
(5) Human hair analysis	-	8500	15000	15000	10000	10000
(6) Epidemiological studies	-	-	-	10000	20000	20000
(7) Supporting studies	-	-	5000	5000	-	-
(8) Miscellaneous	3000	3000	3000	2000	2000	2000
	<u>18000</u>	<u>23000</u>	<u>57500</u>	<u>57000</u>	<u>68000</u>	<u>52000</u>
<u>Recapitulation</u>	1982	1983	1984	1985	1986	1987
Experts	2000	-	-	-	-	-
Subcontracts*	-	20000	42000	55000	50000	50000
	<u>2000</u>	<u>20000</u>	<u>42000</u>	<u>55000</u>	<u>50000</u>	<u>50000</u>
Direct assistance	2000	20000	42000	55000	50000	50000
Meetings	13000	-	12500	-	16000	-
Miscellaneous	3000	3000	3000	2000	2000	2000
	<u>18000</u>	<u>23000</u>	<u>57500</u>	<u>57000</u>	<u>68000</u>	<u>52000</u>
TOTAL	18000	23000	57500	57000	68000	52000
WHO contribution in services	4000	5000	8000	8000	9000	7000

* including equipment and training

Annex 2

LIST OF PARTICIPANTS

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