10557

WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR EUROPE

WELTGESUNDHEITSORGANISATION
REGIONALBÜRO FÜR EUROPA

ORGANISATION MONDIALE DE LA SANTÉ
BUREAU RÉGIONAL DE L'EUROPE

ВСЕМИРНАЯ ОРГАНИЗАЦИЯ ЗДРАВООХРАНЕНИЯ
ЕВРОПЕЙСКОЕ РЕГИОНАЛЬНОЕ БЮРО

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

EUR/ICP/CEH 054
ORIGINAL: ENGLISH
UNEDITED

HEALTH EFFECTS OF METHYLMERCURY IN THE MEDITERRANEAN AREA

BIBLIOTHÈQUE OMS
6 AVR. 1988
WHO LIBRARY

Report on a joint WHO/FAO/UNEP meeting

Athens
15-19 September 1986

Published under the joint sponsorhip of the United Nations Environment Programme, the Food and Agriculture Organization of the United Nations and the World Health Organization

1987

EUR/HFA target 20

The issue of this document does not constitute formal publication. It should not be reviewed, abstracted or quoted without the agreement of the World Health Organization Regional Office for Europe. Authors alone are responsible for views expressed in signed articles.

Ce document ne constitue pas une publication. Il ne doit faire l'objet d'aucun compte rendu ou résumé ni d'aucune citation sans l'autorisation du Bureau régional de l'Europe de l'Organisation mondiale de la santé. Les opinions exprimées dans les articles signés n'engagent que leurs auteurs.

Dieses Dokument erscheint nicht als formelle Veröffentlichung. Es darf nur mit Genehmigung des Regionalbüros für Europa der Weltgesundheitsorganisation besprochen, in Kurzfassung gebracht oder zitiert werden. Beiträge, die mit Namensunterschrift erscheinen, geben ausschließlich die Meinung des Autors wieder.

Настоящий документ не является официальной публикацией. Не разрешается рецензировать, аннотировать или цитировать этот документ без согласия Европейского регионального бюро Всемирной организации здравоохранения. Всю ответственность за взгляды, выраженные в подписанных авторами статьях, несут сами авторы.

TARGET 20

Water pollution

By 1990, all people of the Region should have adequate supplies of safe drinking-water, and by the year 1995 pollution of rivers, lakes and seas should no longer pose a threat to human health.

Index:

ENVIRONMENTAL MONITORING
FOOD CONTAMINATION %PC%
FISH PRODUCTS
MERCURY
WATER POLLUTION %AN%
MEDITERRANEAN SEA
EPIDEMIOLOGY
GREECE
ITALY
YUGOSLAVIA

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

HEALTH EFFECTS OF METHYLMERCURY IN THE MEDITERRANEAN AREA

Report on a joint WHO/FAO/UNEP meeting

Athens
15-19 September 1986

WORLD HEALTH ORGANIZATION
Regional Office for Europe
Copenhagen, 1987

ICP/CEH 054

CONTENTS

	Page
Foreword	1
1. Opening of the meeting	3
2. Scope and Purpose of the meeting	3
3. Election of Officers	4
4. Adoption of the Agenda	4
5. Organisation of the meeting	4
6. Review of the results of Phase I studies	4
7. Review of results of supporting studies	8
8. Review of revised draft protocol on clinical epidemiological studies on health effects of methylmercury	8
9. Institutional arrangements for possible extension of Phase I studies to other areas and for commencement of Phase II studies	10
10. Recommendations regarding interim environmental quality criteria for mercury in Mediterranean seafood	11
Annex 1: Report on the comparative mercury intake of sample populations in two Adriatic coastal areas, by Professor R. Buzina	13
Annex 2: Report on a dietary survey and mercury levels in hair in selected Greek population samples, by Professor A. Polychronopoulou-Trichopoulou and Dr S. Nakou	28
Annex 3: Report on mercury intake from seafood in sample populations from three coastal areas in the Tyrrhenian Sea, by Professor A. Renzoni	33
Annex 4: Analysis of total mercury, methylmercury and selenium in hair in Greek, Italian and Yugoslav sample populations, by Dr P. Stegnar	38
Annex 5: List of Participants	49

FOREWORD

The relatively high levels of mercury recorded in various species of Mediterranean seafood, and the potential health effects on the population of the region through excessive intake of mercury (particularly methylmercury) have led to considerable international activities, mainly organised 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 past decade in an effort to identify and quantify the problem.

An assessment of the state of pollution of the Mediterranean Sea by mercury, mainly based on the results of monitoring activities performed by laboratories in the region between 1976 and 1981 within the framework of the Joint Coordinated Mediterranean Pollution Monitoring and Research Programme (MED POL Phase I) was completed in 1983 by the United Nations Environment Programme (UNEP) in collaboration with FAO and WHO. From the point of view of actual and potential health hazards, it was concluded that, on the basis of mercury levels in seafood and seafood consumption data in the various countries, general populations could not be considered at risk. At the same time, however, certain populations in the Mediterranean region could have an intake of methylmercury through seafood in excess of tolerable levels.

A project on evaluation of methylmercury in Mediterranean populations and related health hazards was developed by WHO in collaboration with FAO and UNEP within the framework of the Long-term Programme of Pollution Monitoring and Research in the Mediterranean Sea (MED POL Phase II), and finalised at a Consultation Meeting in Athens in September 1982. The main components of this project consisted in:

- selection of sensitive pilot areas
- analysis of the mercury content of seafood within such areas
- dietary surveys on selected human population samples
- analysis of human hair for mercury on the same sample population

Apart from the above, which were considered to constitute the first phase of the project, it was also agreed to develop a comprehensive protocol for clinical epidemiological studies, which would be carried out on those individuals within the sample populations which showed positive indications on the basis of their hair mercury levels.

The first phase of the project became operational in Yugoslavia in 1984, and in Greece and Italy in 1985. In each case, pilot areas were selected where there appeared to be *prima facie* evidence of relatively high seafood consumption and/or relatively high levels of mercury in seafood consumed. During a Consultation meeting on biological monitoring of methylmercury in Mediterranean populations, jointly convened by WHO, FAO and UNEP in Zagreb in September 1984. The Consultation meeting, apart from reviewing progress achieved in the project, finalised a standard reference method for analysis of total mercury, methylmercury and selenium in human hair. A draft protocol for clinical epidemiological studies, which would constitute the second phase of the project, was also discussed, and the necessary elements for its revision identified.

Interim environmental quality criteria for mercury in Mediterranean seafood were adopted by Governments of the region at the Fourth Ordinary Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against pollution and its related protocols, held in Genoa in September 1985.

These criteria included the taking note by Mediterranean Governments of the interim criterion proposed by the Joint FAO/WHO Expert Committee on food additives, according to which the provisional tolerable weekly intake (PTWI) of 0.3 mg of mercury of which not more than 0.2 mg should be in the form of methylmercury, should not be exceeded; and the taking into consideration of this criterion to establish, if national circumstances so required, standards for maximum concentrations of mercury in seafood.

The same approved recommendation regarding mercury also included the continuation of the monitoring and research component of MED POL Phase II relevant to the assessment of the mercury content of Mediterranean seafood, and the risks affecting all sections of the population arising from seafood consumption, in particular:

- identification of population groups at risk;
- surveys on seafood consumption patterns among such populations;
- surveys on mercury levels in affected population groups;
- epidemiological studies to obtain the necessary information on the relationship between mercury intake and health effects;
- studies on the relationship between the total mercury and methylmercury content of seafood, and the effects of cooking on such content;
- studies on biogeochemical cycles of mercury in the Mediterranean;
- studies on the effects of selenium in decreasing mercury toxicity.

Within the framework of this programme, a Consultation meeting on health effects of methylmercury in the Mediterranean area was jointly convened by WHO, FAO and UNEP, with the following objectives:

- to review the results of studies carried out by participating institutions as part of Phase I of the project;
- to review and finalise the revised draft protocol on clinical epidemiological studies on health effects of methylmercury;
- to finalise institutional arrangements for Phase II of the project, as well as extension of Phase I to other areas;
- to make appropriate recommendations on any revisions necessary to the interim Environmental Quality Criteria for mercury adopted by the Fourth Ordinary Meeting of the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols (Genoa, 9-13 September 1985).

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: The Intergovernmental Oceanographic Commission (IOC), the United Nations Educational, Scientific and Cultural Organization (UNESCO), the World Meteorological Organization (WMO), the International Atomic Energy Agency (IAEA) and the Commission of European Communities (EEC)

1. Opening of the meeting (Agenda item 1)

The meeting took place in the offices of the Coordinating Unit for the Mediterranean Action Plan, Athens, from 15 to 19 September 1986. It was attended by 23 temporary advisers from seven Mediterranean countries and one from a non-Mediterranean country, one representative each from IOC, IAEA and FAO, two representatives from UNEP and four Staff members from WHO Headquarters and the Regional Office for Europe. A list of participants is given in Annex 5.

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 J.E. Asvall. He briefly outlined the events leading to the meeting, explained its context within the general framework of the Long-term Programme of Pollution monitoring and research in the Mediterranean Sea (MED POL Phase II).

Mr A. Manos, UNEP Coordinator of the Mediterranean Action Plan, welcomed participants on behalf of UNEP and the Coordinating Unit. He stressed the importance of the subject to be discussed, particularly with regard to the formulation of environmental quality criteria for seafood in the Mediterranean, extra to those already adopted by Governments of the region in Genoa. He augured all participants a fruitful meeting.

Mr 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 in matters concerning food consumption and health. In this particular subject area, the Provisional Tolerable Weekly Intake (PTWI) had been formulated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). He also made reference to the intercalibration exercises on the determination of mercury in seafood which were organised within the framework of MED POL, and stressed the importance of participants in the project taking part in such exercises.

2. Scope and purpose of the meeting (Agenda item 2)

Dr L.J. Saliba outlined the scope and purpose of the Consultation meeting. The first phase of the project was scheduled to end in 1987, by which time overall results of the pilot studies being performed in all the three countries (Greece, Italy and Yugoslavia) should be available. The general position would then be reviewed to determine the necessity or otherwise of carrying on with Phase II of the project (the clinical epidemiological studies), as the performance of these necessarily depended on the identification of individuals with sufficient levels of mercury in their hair to warrant clinical testing for health effects. Apart from this, however, one of the main purposes of the review of the current phase was to identify problems, and if necessary amend the work programme for 1986-1987 if this was considered necessary to obtain improved and/or more harmonious results.

3. Election of Officers (Agenda item 3)

Professor V. Silano was elected Chairman, Professor Antonia Polychronopoulou-Trichopoulou Vice-Chairman and Dr P. Stegnar Rapporteur. Dr L.J. Saliba as Secretary to the meeting.

4. Adoption of the agenda (Agenda item 4)

The provisional agenda was unanimously adopted

5. Organisation of the meeting (Agenda item 5)

The Chairman explained the detailed organisation of the work of the meeting, including the hours of sessions and other arrangements. It was agreed that the meeting would work in plenary, with the understanding that one or small working groups could be formed to tackle any specific topic.

6. Review of results of Phase I studies (seafood analysis, dietary survey, human hair analysis) (Agenda item 6)

The review of results obtained so far in the Phase I Studies was based on the progress reports of ongoing work since 1984 in Yugoslavia and 1985 in Greece and Italy. It was agreed that as the Consultation meeting was being held approximately mid-term through the current phase, which was scheduled for completion in late 1987, advantage would be taken to discuss any modifications necessary or advisable in the light of progress reviewed and problems encountered.

The progress reports of Principal Investigators are reproduced in annexes 1 to 4 of this report. The following account is based on the presentations and on the ensuing discussion.

6.1 Studies in Yugoslavia

The study in Yugoslavia, carried out by the Department of Nutrition, Institute of Public Health of Croatia, Zagreb (since mid-1976 within the Institute for Endocrinology, Metabolic Diseases and Diabetes "V. Vrhovac", Medical Faculty, University of Zagreb) utilised two areas on the Adriatic coast, one (the "experimental" area) shown by previous studies to contain relatively high concentrations of mercury in coastal waters and sediments as a result of industrial pollution in the locality, and the other (the "control" area), with no industries, and located at a distance of 80 km along the coast. Representative villages with similar lifestyles and fishing patterns were selected from each area. In each village, sample populations were selected for a dietary survey, selection being made on the basis of fishing activities, with full-time and part-time fishermen and their families being accorded priority. 79 families with 314 members were selected from the experimental area, and 63 families with 255 members from the control area.

A comparative study was made on mercury levels in selected seafood species in each area. 40 species were identified in the diet of families in the experimental area, and 38 in the diet of those in the control area. Both total mercury and methylmercury levels were determined in these species, those in the experimental area ranging from 0.070 to 1.370 mg/kg (total mercury) and 0.011 to 0.618 mg/kg (methylmercury). In the control area, the corresponding ranges of levels were 0.040 to 0.520 mg/kg and 0.016 to 0.508 mg/kg respectively. The mercury content of seafood was higher in the experimental area, the difference being particularly pronounced in the case of total mercury. A considerable degree of variation was recorded among the various species in the proportion of methylmercury to total mercury, ranging from 10.1 to 105.8% in the experimental area, and from 5.3 to 106.8% in the control area.

The dietary survey was essentially conducted on the basis of the protocol developed during the September 1982 Consultation meeting in Athens. A much higher rate of seafood consumption was recorded in the control area as compared to the experimental area. In the former, 27.0% of the families surveyed consumed seafood 5 to 7 times per week, and only 3.2% were found to be consuming no seafood at all during the period. On the other hand, the corresponding figures for the latter area were 13.9% and 11.4% respectively. This difference was ascribed to be the result of reluctance of the populations in industrialised areas to eat seafood because of industrial pollution (in the general sense, not specifically because of mercury).

On the basis of these results, it was calculated that the average weekly intake of total mercury ranged from 64.5 to 177.0 micrograms per person in the experimental area, and from 44.5 to 125.7 micrograms in the control area. In the case of methylmercury, calculated intakes were 34.5 to 90.8 micrograms in the experimental area, and 27.5 to 102.0 micrograms in the control area. 46 subjects in the experimental area (14.7% of the sample) and 22 in the control area (8.6% of the sample) were calculated to have a total mercury intake above the JECFA Provisional Tolerable Weekly Intake of 300 micrograms. In the case of methylmercury, 20 subjects (6.4%) in the experimental area and 43 (16.9%) in the control area were calculated to be exceeding the PTWI of 200 micrograms.

The only way in which these differences could be explained were that the higher methylmercury levels in the "control" area population were the result of a higher consumption of seafood, together with relatively high levels of methylmercury (as a proportion of total mercury) in the species consumed.

Hair samples were taken from 42 children and teenagers (age 1-16) from each area. The samples were analysed for total mercury and methylmercury by the Nuclear Chemistry Department, "Josef Stefan" Institute, Ljubljana, according to a reference method developed by the Institute for the project. Subjects from the experimental area had total mercury levels ranging from 0.10 to 5.30 ppm (with a mean of 1.75 ppm) and methylmercury levels ranging from 0.09 to 3.7 ppm (with a mean of 1.41). Corresponding levels for subjects in the control area were 0.41 to 3.8 ppm (mean : 1.73) and 0.28 to 3.6 ppm (mean : 1.37). There was very little overall difference, and no single subject had a hair methylmercury level of 4 ppm or above.

6.2 <u>Studies in Greece</u>

The study in Greece, which was of a preliminary nature, was carried out jointly by the Department of Nutrition and Biochemistry of the Athens School of Hygiene, and the Institute of Child Health, Aghia Sofia Children's Hospital, Athens. Two main areas were selected - an industrial coastal town near Athens, from which families with small children were selected as a sample population, and a small non-industrial town located approximately 20 km from the sea, from which schoolchildren were selected. A simplified dietary survey, modified from the original 1982 protocol to take account of local circumstances, was conducted on a total sample of 1,500 subjects from the two areas.

The survey identified 250 individuals who were found to have a seafood consumption of 2 meals or more per week. Out of these, it was only possible to take hair samples from 121 individuals, either because of the impossibility of collecting a sufficient quantity of hair, or because of other factors (mainly the use of selenium-containing hair lotions). 36 of these (21 adults and 15 children) were from the industrial area, and 87 from the non-industrial area. In addition, hair samples were also taken from 17 young men just drafted into the Greek Navy, all coming from fishing families from various parts of Greece.

As in the case of the Yugoslav populations, hair samples were analysed for total mercury and methylmercury by the Department of Nuclear Chemistry, "Josef Stefan" Institute, Ljübljana, Yugoslavia. Total mercury levels in the hair of the 21 adults (mostly pregnant women) from the industrialised area ranged from 0.72 to 2.6 ppm. Methylmercury levels ranged from 0.25 to 1.42 ppm. The corresponding figures for the 15 children (age 2-5) from the same area were 0.48 to 5.1 ppm and 0.37 to 1.59 ppm respectively. In the case of the non-industrial area, the 87 shoolchildren sampled had total mercury levels ranging from 0.38 to 4.0 ppm and methylmercury levels ranging from 0.09 to 4.2 ppm.

The 17 young adults from the Navy base had hair total mercury levels ranging from 0.56 to 37.51 ppm, and methylmercury levels ranging from 0.16 to 35.9 ppm. Both upper values were recorded in one individual consuming 20 seafood meals per week. None of the other subjects in this sub-sample had levels exceeding 5.0 ppm total mercury or 3.6 ppm methylmercury.

As in the case of the Yugoslav study, the levels of total mercury and methylmercury in hair were generally similar in both "exposed" and "non-exposed" population samples. In both areas, children had higher levels.

6.3 Studies in Italy

A number of studies on the health effects of mercury ingested through seafood consumption had been conducted in Italy on various sample populations during the last decade. The review, however, was conducted only on the current project study, which had only been in operation for a few months.

Three areas on the coast of the Tyrrhenian Sea had been selected, one with mercury pollution due to industry, another with high mercury levels due to volcanic activity in the vicinity, and the third with fishermen populations spending at least 7 months in the year at sea.

13 Seafood species were identified as being regularly consumed by fishing families in the first area. The total mercury content of these species ranged from 0.180 to 1.190 mg/kg. A preliminary dietary survey on 58 fishing families revealed that the heavy consumers were the fishermen themselves who consumed 4 to 8 seafood meals per week rather than the wives and children, who only consumed up to 3. Up to the time of the report, the hair of 7 fishermen from this station had been analysed by the Department of Nuclear Chemistry, "Josef Stefan" Institute, Ljubljana, Yugoslavia. Levels of total mercury ranged from

3.58 to 29.66 ppm total mercury (mean : 16.03 ppm) and from 3.45 to 25.31 ppm methylmercury (mean : 13.28 ppm).

The dietary survey and seafood analysis components in the studies on the other two areas were still in progress at the time of reporting, and no numerical results were therefore available. A number of people being interviewed in the area subject to mercury liberation into the sea as a result of volcanic activity were recorded as consuming 4 seafood meals or more per week. Similarly the ongoing survey of fishermen in the third area (spending 7 months a year on board their vessels) showed that these were consuming a minimum of 7 seafood meals per week.

As a comparison, the hair of 19 subjects living inland, and considered as average non-seafood-eating persons following a simplified dietary survey, was sampled and resulted in a range of 0.26 to 2.97 ppm (mean : 1.12 ppm) total mercury, and 0.17 to 2.10 ppm (mean : 0.77 ppm) methylmercury.

6.4 <u>Overall progress and results</u>

The data obtained provided the opportunity to compare the different approaches which, while conforming to the same overall plan, varied in a number of specific details depending on national conditions. In all these countries, selection of test areas was based on industrialisation and the consequent pollution of the sea by mercury. In Italy, a non-industrialised area, but with high mercury levels due to natural sources (volcanic activity) was also selected. The areas of comparison differed slightly in that the Yugoslav control area was non-industrialised and slightly less polluted, the Greek and Italian controls were both inland areas, with the Italian control being also based on non-consumption of seafood in the area.

Both in Italy and in Yugoslavia, analysis of seafood for total mercury and methylmercury content was commenced simultaneously with the other components of the project. In Greece, this was omitted from the initial pilot phase. Population samples for the dietary survey and hair analysis were all based on a highly selective approach, pinpointing fishermen and their families as the main target group, either in all areas or at least in the test area. The questionnaires used in the dietary survey varied somewhat in degree of elaboration and in the contents - in Greece, the same degree of emphasis was placed on consumption of non-seafood items to avoid misinterpretation of the situation leading to the performance of the project. Subjects for hair analysis (from within the dietary survey groups) were selected either (where consumption was combined with seafood mercury levels) on a calculated excess of intake over the PTWI, or on the basis of amounts of seafood consumed.

The overall averages of methylmercury levels in seafood were twice as high in the areas in Italy as those found for the areas in Yugoslavia. Regarding the proportion of methylmercury to total mercury in seafood, the studies in Italy and Yugoslavia confirmed that this varied to a considerable extent with particular species, and that risks were, to a not inconsiderable extent, associated with consumption of particular species not only with regard to bioaccumulation capacity, but also with regard to methylmercury proportions within the total mercury levels.

A total of 250 samples of human hair from all three countries were analysed for total mercury and methylmercury. In addition levels of selenium were determined in 25% of these samples. In general, methylmercury levels in the hair of Greek and Yugoslav subjects (irrespective of area) and of "non-exposed" Italian subjects were, with one exception, lower than 4 ppm. This appeared to indicate that the varying degrees of mercury pollution in Greece and Yugoslavia did not result in significant differences in concentrations found in the hair.

In the case of the two groups composed exclusively of fishermen, the hair samples from the seven Italian subjects showed high levels of methylmercury (two above 4 ppm and six above 10 ppm), but, with one exception (36 ppm in the person recorded as consuming 20 seafood meals per week), low levels were found in the samples from the seventeen Greek subjects. Although the differences between the two groups could not be explained at the time on the basis of the present interim data available, an extremely high consumption of seafood was the only common feature linked with high methylmercury levels in the hair, regardless of country.

The overall results are summarised in Table I. The figures for the different countries are not directly comparable, owing to the variation in exposure conditions (pollution levels, and amount of seafood consumed). Out of the total number of 250 hair samples analysed, only 5 were recorded as having methylmercury levels above 10 ppm, and all these were fishermen. It was however significant that, out of the 7 fishermen from Italy, 4 had levels above 10 ppm, and another 2 above 4 ppm.

The results obtained in the project so far revealed no other cases of high exposure. This confirmed that a highly selective approach to a large population was necessary to identify potential groups at risk in the Mediterranean area.

7. Review of results of supporting studies (Agenda item 7)

Supporting studies consisted mainly in the comparative analysis of total mercury and methylmercury levels in selected species of seafood, and were reviewed along with the main components of the project (see item 6 above).

8. Review of revised draft protocol on clinical epidemiological studies on health effects of methylmercury (Agenda item 8)

The meeting reviewed the main components of the revised draft protocol on clinical epidemiological studies on health effects of methylmercury, which had been prepared by an interdisciplinary team under the coordination of the High Institute of Public Health, Rome, in conformity with the recommendations of the 1984 Zagreb Consultation meeting. In this context, recent findings indicated that only relatively small groups of adult mean and of mothers of newborn infants whose hair had methylmercury levels exceeding 25 ppm and 6 ppm respectively were likely to be identified in the Mediterranean area as suitable for a clinical epidemiological study. In discussion of the draft protocol, therefore, the need was emphasised to have the most comprehensive methodology possible to detect relevant clinical and sub-clinical effects, as the prima facie evidence was that such studies would only have to be carried out on relatively small population numbers. The feasibility of eventual implementation was discussed, and agreement was reached on the elements to be incorporated in the final version.

Table I

Overall summary of hair mercury determinations as related to exposure

Country	Dietary survey	Exposure conditions		Mercury levels in hair				Risk groups	
						Concentration Range		No. of samples	
		Area	Seafood meals	Population sector	No. of samples	Total mercury	Methylmercury	4-10 ppm Me Hg	10 ppm Me Hg
Greece	n = 1500 simplified questionnaire	Non-polluted (fishermen)	4+/week	M	17	0.56 -38.0	0.16 -36.0	0	1
		Non-polluted	2 /week	F	-	-	-	-	-
				Ch.	87	0.38 - 4.0	0.09 - 4.15	1	0
		Polluted	2 /week	M	2	0.98 - 1.16	0.62 - 1.00	0	0
				F	19	0.69 - 2.60	0.25 - 1.42	0	0
				Ch.	15	0.48 - 5.10	0.37 - 1.59	0	0
Italy	n = 200 simplified questionnaire	Polluted (fishermen)	4+/week	M	7	3.58 -29.66	3.45 -25.31	2	4
		Polluted	2+/week	F	-	-	-	-	-
				Ch.	-	-	-	-	-
		Non-polluted control	0 /week	M	19	0.26 - 2.97	0.17 - 2.10	0	0
				F	-	-	-	-	-
				Ch.	-	-	-	-	-
Yugoslavia	n = 255 complete questionnaire	Non-polluted	0-7/week av.2-3	M	-	-	-	-	-
				F	-	-	-	-	-
				Ch.	42	0.41 - 3.80	0.28 - 3.60	0	0
	n = 314	Polluted	0-7/week av.3-5	M	-	-	-	-	-
				F	-	-	-	-	-
				Ch.	42	0.10 - 5.30	0.09 - 3.70	0	0
TOTAL	2269				250			3	5

9. Institutional arrangements for possible extension of Phase I studies to other areas and for commencement of Phase II studies (Agenda item 9)

On the basis of results obtained so far, the meeting recognised that one of the groups at highest risk in the region constitutes adult fishermen, mainly those who spend a significant proportion of their time at sea, and whose main source of food during this period is fish. Considering that mothers of newborn infants (particularly, but not necessarily, if they come from fishing families) constitute a recognised high risk group, the meeting agreed that immediate follow-up activities within the project should aim at the identification of individuals within these two target groups with high methylmercury levels in their hair. This would show whether such groups were large enough to establish a relationship between exposure to methylmercury and impairment of the central nervous system.

It was therefore agreed that the programme for the remainder of the first phase, which would involve 500 hair samples each from Greece, Italy and Yugoslavia, would include the following:

- Selection of areas with a high probability of population groups consuming seafood, such as islands and coastal areas;

- Selection of individuals consuming a high amount of seafood (at least three meals per week for mothers with newborn infants, and four meals per week for adult fishermen);

- Sampling of hair according to the procedure described in the reference method;

- Analysis of hair for total mercury, and identification of mothers with newborn infants with levels exceeding 6 ppm and adult men with levels exceeding 25 ppm;

- Analysis of hair for levels of methylmercury in the case of individuals exceeding these thresholds;

- Compilation of extensive information on food consumption patterns (particularly the amount and type of seafood consumed) in the case of the individuals so identified, together with data on methylmercury levels in seafood, in order to identify the source of methylmercury in groups at high risk;

- Assessment of the data obtained to evaluate the size of population groups with elevated mercury levels, and establishment of the feasibility of undertaking a clinical epidemiological study.

The centralisation of hair analysis in one laboratory precluded the need for interlaboratory intercalibration exercises, and the "central" laboratory had its own quality control programme. The importance of all participating institutions performing the sampling of hair conforming to the procedures laid down in the protocol was emphasised. Following a brief discussion of some problems involved, the section on hair sampling in the appropriate reference method was modified slightly to provide more exact clarification and ensure more complete adherence to it.

The possible expansion of the first phase studies to include other Mediterranean countries (in addition to the present three) was discussed. Although this was highly desirable on technical grounds, one particular problem was that of finance, since there was very little possibility of increasing the present total level of assistance to participating laboratories, either currently or in the near future. It was agreed that steps should be taken to attempt to increase the present financial allocation for the project.

After considering similar projects in other areas, the meeting concluded that coordination and cooperation with such projects was highly desirable. It also appeared feasible that the immediate follow-up work in the Mediterranean could share some basic components with related programmes in other areas.

The meeting made the following recommendations regarding future work in the implementation of the project:

1. The aim of the eventual epidemiological study in the Mediterranean, if feasible, should be to measure the occurrence of effects of the exposure of fishermen to methylmercury by means of a cross-sectional study on adults, and of the effects of prenatal exposure of children by means of a follow-up study.

2. The follow-up activities of the present pilot phase should, if possible, include testing, training and harmonisation of the examinations indicated in the protocol of the epidemiological study.

3. WHO, FAO and UNEP should ensure that an adequate financial allocation from the MED POL budget is made available for the continuation of the pilot phase of the project. This should include the geographical extension of the project through performance of the preliminary components in other Mediterranean countries.

4. Endeavours should be made to obtain other financial resources from both national and international sources to ensure the coverage of later stages of the project.

5. In view of the need to investigate further the problem of neurotoxicity in children, a working group should perhaps be convened to develop a core battery of neurodevelopmental tests for infants and preschool children for use in epidemiological studies.

10. Recommendations regarding interim environmental quality criteria for mercury in Mediterranean seafood (Agenda item 10)

The meeting took note of the interim environmental quality criteria for mercury in seafood approved by the Contracting Parties to the Convention for the Protection of the Mediterranean Sea against Pollution and its related Protocols, at their Fourth Ordinary Meeting in Genoa from 9 to 13 September 1985. In this regard, the Consultation meeting acknowledged that the JECFA Provisional Tolerable Weekly Intake (PTWI) of 0.3 mg total mercury, of which not more than 0.2 mg should be in the form of methylmercury for a person of 60 kg bodyweight remained a valid recommendation in the light of available data. In view of recent epidemiological data and the relatively small safety factor built into the PTWI, however, strict adherence to this limit was considered essential for women of child-bearing age.

Considering all the factors involved, the Consultation meeting felt that it was not in a position to propose any concrete modification to the measures already approved by the Contracting Parties (i.e. Mediterranean Governments). From results obtained so far in the project, it was evident that, from the viewpoint of human health, the earlier conclusion that the imposition of common regional upper limits for mercury concentrations in seafood did not appear to be justified could be confirmed, as the risk was not a general one, but one confined to specific population groups, constituting minorities, as a result of over-consumption of seafood. The eventual adoption of emission standards for mercury by Mediterranean Coastal States in terms of the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources would contribute to some reduction in the overall mercury load, which might be significant in some local areas presently affected by mercury-containing discharges. However, inasmuch as the greater part of the mercury in the Mediterranean sea was considered to be due to natural, as opposed to anthropogenic sources, the overall problem would remain.

It was therefore considered by the Consultation meeting that one of the most important activities in the region should be the identification of those population groups at risk as a result of excess consumption of mercury-contaminated seafood. Following evaluation of the problem, both qualitatively and quantitatively, the necessary measures aimed at reducing the seafood component of the diet of affected individuals and/or groups could be worked out. These measures would be expected to take the form of (a) biological monitoring of affected population sectors and, (b) dietary advice on consumption of specific fish species, fish caught from certain waters, or for certain population groups.

The meeting made the following recommendation concerning this item:

- The conclusions drawn at this meeting, as well as the results obtained from the continuation of the pilot studies, should be used when submitting recommendations on suitable measures to reduce both environmental levels of mercury and exposure of people to mercury through seafood to Mediterranean Governments at their next meeting in September 1987. If necessary, a small expert group should be convened to formulate such recommendations.

Annex 1

Report on the comparative mercury intake of sample populations in two Adriatic coastal areas

by

Professor R. Buzina
Institute for Endocrinology, Metabolic Diseases and Diabetes
"V. Vrhovac", Zagreb, Yugoslavia

1. INTRODUCTION

In recent years there has been considerable concern about the possibility of foods becoming contaminated by toxic chemicals through industrial accident or negligence. Among these chemicals, mercury represents a particularly toxic element, though small amounts up to 60 mcg/kg may be found as a natural constituent in a number of foodstuffs. However, the amount of mercury, particularly in is organometalic form, is usually higher in fish, reaching concentrations of 100-200 mcg/kg in smaller species, and up to 1,000-1,500 mcg/kg in larger carnivorous species. Although the intake of mercury from food in general has not been studied with adequate precision, it is estimated that the existing natural mercury content in the daily diet varies from 5 to 20 mcg and may reach up to 200-300 mcg in coastal areas. In these areas any further increase in food mercury content from environmental sources may be come critical.

Several activities not directly related to mercury production may account for substantial releases into the environment. They include the burning of fossil fuel, the production of steel, cement and phosphate, and the smelting of metals. As some of these industries have been set up along the Adriatic coast, it was of great interest to find out whether the existing situation had an impact on seafood contamination in these areas and, further, whether the present mercury content may be considered critical from the health point of view especially for population sectors consuming seafood in relatively large quantities.

2. OBJECTIVES OF THE STUDY

The present study was performed within the framework of the WHO/FAO/UNEP Project on "Methylmercury in Mediterranean populations and related health hazards". Its general objective was to evaluate possible health hazards arising from the methylmercury content of seafood, particularly in areas where relatively large consumption could lead to a mercury intake exceeding the JECFA Provisional Tolerable Weekly Intake. The specific objectives were (i) to select a population in the Adriatic having a relatively high mercury intake, (ii) to determine the total mercury and methylmercury content in the seafood consumed, (iii) to study the population's seafood consumption pattern including seasonal variations in order to determine the weekly dietary mercury intake, and (iv) to correlate such intake with levels of mercury in hair in the indicated segments of the population.

3. METHODS

Selection of populations

The study was carried out in an area located on the Adriatic coast where previous studies showed mercury to be present in relatively high concentrations in coastal waters and marine sediments as a result of mercury pollution from local industries. Another area, 80 kilometres distant from the experimental area, with no industries present, was selected as a control. In both areas, representative villages having similar lifestyles and fishing patterns were selected. The selection of families was based on a village roster with those engaged in fishing professionally or semi-professionally being accorded priority.

Dietary surveys

Dietary surveys were carried out in two phases. In the first "interview" phase, the composition of families and their seafood consumption habits (including every single species of fish and other seafood consumed throughout the year) was recorded, using the protocol developed for this part of the overall project in 1982.

The second phase consisted of registration of the seafood consumption pattern of the family, covering the type and quantity of seafood consumed during the survey period both at home and away from home. The surveys were carried out over a two-week period during each of the summer and winter seasons (The 1984 winter survey had to be repeated in 1985 because of a substantial reduction in fishing and fish consumption due to a very servere winter). Specially developed forms were used for the registration of seafood consumption, enabling the conversion of family seafood consumption figures into pertinent figures for individual members by using mathematical factors calculated on the basis of energy requirements for the main age groups in the population.

The forms were completed by the families under supervision of the project field staff. 79 families with 314 family members in the experimental area, and 63 families with 255 members in the control area, were included in the study.

Determination of mercury in seafood

1. Total mercury

The determination of total mercury was carried out on the basis of cold vapour technique by use of a Pye Unicam atomic absorption spectrophotometer SP 90 Series 2 fitted with a mercury cold vapour analyser kit, utilising the UNEP/FAO reference method developed for the MED POL Phase II studies.

A lyophilysed sample was oxidised in a 1:10 concentration of sulphuric and nitritic acid agent in a closed system. In this process, mercury was converted into its inorganic form. Following a complete organic substance decomposition total mercury was determined by measuring the amount of mercury reduced from its ionic form into atomic states by means of bivalent tin. The reduced mercury was then introduced on a stream of air into a quartz-made gas cell in which the absorption of characteristic mercury resonance radiation from a light source took place.

2. Methylmercury

An aliquot of freeze-drying sample prepared according to the UNEP/FAO/IAEA Reference method was homogenised, and the methylmercury in biological material was liberated with strong concentrated HBr. The methylmercury was then extracted with benzene and separated from interfering impurities by extraction with aqueous ethanolic solution of cysteine.

After liberating the methylmercury from the thiol-compound with strong HBr and extracting again with benzene, the extract was analysed by gas-liquid chromatography on a PERKIN ELMER SIGMA IB Gas Chromatographic System.

3. Results

During the seafood consumption pattern survey, 40 species of fish and other seafood were identified in the diet of examined families in the experimental area, and 38 species in the control area. Most of the seafood was caught locally and prepared either fresh, or after a couple of days' storage in home refrigerators. It was only occasionally that fish such as tuna or herring (Clupea haringus), coming from other parts of the Adriatic or from abroad, was brought to the market.

The results of the chemical analyses showed no significant differences in mercury content between seafood caught in summer and in winter, and mean concentrations of total mercury and methylmercury in the species analysed, along with the percentage number of families consuming each species during the course of the dietary surveys is given in tables 1 and 2.

In the experimental area the highest mercury content was found in Diplodus annularis (Annular Git head), Uranoscopus scaber (Star gazer), Mustellus mustellus (Dogfish), followed by Sepia officinalis (Cuttlefish) and Nephrops norwegicus (Norwegian lobster). In the control area the highest mercury content was found in Ozoena muscata (musky octapus) and among fish species in Scorpaena scrofa (scorpion fish). On comparing the two areas examined, the seafood in the experimental area generally had a higher mercury content for the same species as compared to the control area. A comparison of the total mercury and methylmercury content in the seafood samples examined in the two areas is shown in tables 3 and 4.

The seafood in the experimental area had higher levels of both total mercury and methylmercury levels, but the differences between the two areas were statistically significant only for total mercury.

The seafood consumption frequencies in the families examined showed a more frequent weekly intake of fish and other seafood by families in the control area, so that the two areas were not strictly comparable with regard to seafood consumption (table 5).

The average weekly mercury content in the diet of individual family members divided by age and sex is summarised according to season in tables 6 and 7 and the decile distribution of these data is given in tables 8 to 11.

Table 1

Total mercury and methylmercury content of seafood and percentage of families consuming each of the listed species during the period of dietary surveys

Experimental area

Local name	Latin name	English name	Total mercury (mg/kg)	Methylmercury mg/kg	Consumption (% of families)
Bugva	Boops boops	Bogue	0.391	0.130	12.7
Cipal	Mugilidae (FAM)	Mullet	0.093	0.064	26.6
Gavun	Atherina heps.	Smelt	0.135	0.086	6.3
Spar	Diplodus annularis	Annular Git Head	0.628	0.321	15.2
Srdela	Sardina pilchardus	Pilchard	0.198	0.055	39.2
Sarun	Trachurus spp.	Scad	0.215	0.103	21.5
Mol	Merluccius merluccius	Hake	0.312	0.122	29.1
Glavoc	Gobius spp.	Goby	0.111	0.048	5.1
Incun	Engraulis encrasich.	Ancory	0.405	0.128	1.3
Ovcica	Lithognatus mormyr.	Striped bream	0.106	0.036	2.5
Fratar	Diplodus vulgaris	Bass	0.326	0.098	2.5
Salps	Salpa salpa	Goldfin	0.109	0.011	1.3
Macka	Scylliorhinus sie	Spotted dogfish	0.375	0.092	1.3
Lojka	Alosa falax nilotica	Mediterranean Shad	0.113	0.111	1.3
Trlja	Mullus spp.	Mullet	0.318	0.128	3.8
Pirka	Serranus scriba	Comber	0.270	0.074	3.8
Bezmek	Uranoscopus scaber	Star gazer	1.370	0.618	2.5
Volak	Murex spp.	Murex	-	0.231	2.5
List	Solea vulgaris	Common sole	0.390	0.170	1.3
Pas	Mustellus must.	Dogfish	0.810	0.477	3.8
Haringa	Clupea harengus	Herring	0.300	0.114	1.3
Sipa	Sepia officinalis	Cuttlefish	0.483	0.179	10.1
Rak	Portunus corrugatus	Crab	0.361	0.122	3.8
Lignja	Loligo vulgaris	Common squid	0.322	0.194	53.2
Kozica	Crustacea natantia	Shrimp	0.207	0.124	1.3
Girice	Spicara smaris	Pickerel	0.208	0.055	39.2
Jegulja	Anguilla anguilla	Common Eel	0.159	0.152	2.5
Skusa	Scomber scombrus	Mackerel	0.070	0.016	3.8
Tunj	Thunnus thynnus	Bluefin tuna	-	-	1.3
Muzgavac	Eledone spp.	Musky octopus	0.370	0.170	5.1
Skarpina	Scorpaena scrofa	Scorpion fish	0.276	0.230	5.1
Arbun	Pagellus erythrinus	Pandora	0.155	0.138	8.9
Komarca	Sparus aurata	Gilt head	0.119	0.031	1.3
Pic	Puntazzo puntazzo	Sharp snouted sparus	0.110	0.028	1.3
Iglica	Belone belone gracilis	Garfish	-	-	1.3
Lubin	Dicentrarchus labrax	European seabass	0.155	0.138	1.3
Skamp	Nephrops norvegicus	Norwegian lobster	0.540	0.508	1.3
Ugor	Conger conger	Sea eel	0.152	0.139	1.3
Tragalj	Spicara maena maena	Caockarel	0.326	0.098	1.3

Table 2

Total mercury and methylmercury content of seafood and percentage of families consuming each of the listed species during the period of dietary surveys

Control area

Local name	Latin name	English name	Total mercury (mg/kg)	Methylmercury mg/kg	Consumption (% of families)
Bugva	Boops boops	Bogue	0.096	0.018	69.8
Sarun	Trachurus spp.	Scad	0.091	0.063	20.6
Girica	Spicara spp.	Pickerel	0.102	0.082	20.6
Trlja	Mullus spp.	Mullet	0.166	0.180	17.5
Mol	Merluccius merluccius	Hake	0.210	0.211	20.6
Raza	Raja spp.	Ray	0.268	0.200	4.8
List	Solea vulgaris	Common sole	0.222	0.220	4.8
Skarpina	Scorpaena scrofa	Scorpion fish	0.276	0.383	14.3
Srdela	Sardina pilchardus	Pilchard	0.078	0.026	42.9
Gavun	Atherina spp.	Smelt	0.040	0.035	15.9
Salpa	Salpa salpa	Goldlin	0.054	-	17.5
Kokot	Trigla lyra	Piper	0.079	0.085	3.2
Vranja	Labrus merula	Brown wrasse	0.073	0.046	6.3
Traganj	Spicara maena maena	Caockarel	0.110	0.056	3.2
Kavala	Sciaena umbra	Brown meagre	0.067	0.059	1.6
Pirka	Serranus scriba	Comber	0.060	0.063	1.6
Fratar	Diplodus vulgaris	Bass	0.160	0.074	3.2
Luc	Euthynnus quadripunctatus	Little tunny	0.077	0.016	6.3
Kovac	Zeus faber	Dory	0.082	0.025	4.8
Ugor	Conger conger	Sea eel	0.152	0.159	14.2
Usata	Oblata melanura	Saddled bream	0.092	0.038	1.6
Kantar	Spondyliosoma cantharus	Black sea bream	0.063	0.054	3.2
Barbun	Mullus spp.	Mullet	0.222	0.126	1.6
Grdobina	Lophius spp.	Angel fish	-	0.146	3.2
Muzgavac	Ozoena muscata	Musky octopus	0.505	0.485	1.6
Arbun	Pagellus erythrinus	Pandora	0.155	0.138	6.3
Sipa	Sepia officinalis	Common cuttlefish	0.236	0.285	4.8
Skampi	Nephrops norvegicus	Norwegian lobster	-	0.508	11.1
Liganj	Loligo vulgaris	Common squid	0.255	0.160	54.0
Cipal	Mugilidae (FAM)	Mullet	0.067	0.023	1.6
Lokarda	Scomber japonicus	Spanish mackerel	0.080	0.022	9.5
Gof	Seriola dumerili	Greater amberjack	0.065	0.052	4.8
Iglica	Belone belone gracilis	Garfish	0.092	0.063	3.2
Spar	Diplodus annularis	Annular Git Head	0.077	0.021	11.6
Sarga	Diplodus sargus	White seabream	0.163	0.079	1.6
Zubatac	Dentex dentex	Dentex	0.150	0.135	1.6
Hobotnica	Octopus vulgaris	Common octopus	0.520	0.495	7.9

Table 3

Total mercury and methylmercury content in seafood (mg/kg)

N	Min.	Max.	Median	Mean	SD
		Total mercury			
I Experimental area	.043	1.910	.200	.330	.330
II Control area	.034	.505	.111	.149	.114
		Methylmercury			
I Experimental area	.011	.800	.105	.159	.170
II Control area	.008	.536	.074	.133	.134

Table 4

Percentile distribution of total and methylmercury content (mg/kg) in fish and seafood in the examined areas

		Percentiles								
		10	20	30	40	50	60	70	80	90
				Total mercury						
I	Experimental area	.071	.090	.113	.150	.200	.270	.350	.480	.786
II	Control area	.045	.060	.072	.079	.111	.152	.160	.222	.268
				Methylmercury						
III	Experimental area	.036	.047	.059	.091	.105	.120	.128	.212	.320
IV	Control area	.022	.032	.045	.059	.074	.118	.146	.210	.240
t-test	I:III = 2.770	p 0.05								
	II: IV = 0.820	p 0.05								

Table 5

Seafood consumption frequency in examined families
(times per week)

	0		1-2		3-4		5-7		Total	
	N	%	N	%	N	%	N	%	N	%
Experimental area	9	11.4	23	29.1	36	45.4	11	13.9	79	100.0
Control area	2	32	12	19.1	32	50.8	17	27.0	63	100.0

Table 6

Average weekly mercury intake by individual family members according to sex and age (Summer season survey)

		EXPERIMENTAL AREA		CONTROL AREA	
		Total mercury (mcg)	Methylmercury (mcg)	Total mercury (mcg)	Methylmercury (mcg)
		Mean SD	Mean SD	Mean SD	Mean SD
1 - 6		67.5 ± 39.8	39.9 ± 19.1	83.6 ± 80.6	69.4 ± 73.4
7 - 12	M	168.4 ± 144.4	77.6 ± 61.8	95.9 ± 84.8	54.4 ± 76.4
	F	137.6 ± 85.6	83.4 ± 53.8	130.8 ± 104.6	101.7 ± 94.4
13 - 19	M	207.9 ± 123.0	115.9 ± 73.2	122.4 ± 99.2	86.0 ± 76.3
	F	205.2 ± 132.4	106.8 ± 54.9	145.4 ± 72.9	103.1 ± 52.5
20 - 49	M	201.9 ± 139.3	105.6 ± 66.4	150.5 ± 126.6	115.4 ± 109.2
	F	135.5 ± 79.7	67.4 ± 38.2	124.9 ± 99.4	81.1 ± 72.9
50 - 69	M	180.1 ± 87.3	96.3 ± 53.9	146.7 ± 145.1	125.4 ± 138.8
	F	146.7 ± 98.5	72.1 ± 43.9	104.5 ± 112.2	96.6 ± 109.7
70	M	177.5 ± 90.5	94.1 ± 52.7	159.1 ± 144.7	135.4 ± 152.2
	F	59.0 ± 30.9	36.2 ± 27.0	88.0 ± 92.3	80.8 ± 104.9

Table 7

Average weekly mercury intake by individual family members according to sex and age (Winter season survey)

		EXPERIMENTAL AREA				CONTROL AREA			
		Total mercury (mcg)		Methylmercury (mcg)		Total mercury (mcg)		Methylmercury (mcg)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1 - 6		100.3 ±	70.1	48.2 ±	38.4	48.7 ±	36.1	35.0 ±	24.6
7 - 12	M	129.8 ±	84.2	59.1 ±	42.3	156.8 ±	151.6	80.8 ±	64.5
	F	162.4 ±	113.1	74.8 ±	59.2	125.6 ±	124.6	99.4 ±	106.1
13 - 19	M	187.3 ±	104.6	103.5 ±	75.4	138.0 ±	87.1	119.6 ±	80.3
	F	131.4 ±	106.7	65.4 ±	55.[illegible]	154.1 ±	126.5	132.7 ±	99.1
20 - 49	M	176.4 ±	118.9	92.6 ±	74.3	126.6 ±	66.5	101.5 ±	63.1
	F	123.6 ±	75.6	60.4 ±	42.9	96.3 ±	67.1	85.6 ±	64.1
50 - 69	M	162.9 ±	106.7	84.6 ±	75.9	137.6 ±	81.9	109.9 ±	81.9
	F	123.1 ±	84.1	67.4 ±	58.8	92.8 ±	52.2	76.6 ±	54.6
70	M	128.1 ±	67.4	57.0 ±	29.0	92.0 ±	59.9	90.9 ±	54.2
	F	151.5 ±	89.3	85.2 ±	57.3	85.1 ±	38.8	67.4 ±	37.7

Table 8

Percentile distribution of weekly mercury content in the diet of individual family members (Experimental area)

T o t a l m e r c u r y (mcg)

		Percentiles								
Age(yrs)		10	20	30	40	50	60	70	80	90
1 - 6		27.8	33.4	38.9	44.5	70.5	80.5	109.5	134.5	159.5
7 - 12	M	49.5	64.5	73.5	82.5	91.5	134.5	149.5	304.5	327.0
	F	58.5	71.4	79.1	86.8	124.5	178.5	208.5	250.5	310.5
13 - 19	M	69.5	94.5	109.5	124.5	177.0	224.5	249.5	304.5	349.5
	F	27.0	49.5	72.0	94.5	117.0	184.5	207.0	289.5	349.5
20 - 49	M	65.6	87.8	109.5	129.5	149.5	179.5	215.6	267.0	371.2
	F	43.5	64.9	81.0	96.7	110.3	123.9	155.4	195.3	237.6
50 - 69	M	62.2	87.4	110.9	130.4	150.0	173.9	199.2	242.3	311.2
	F	41.3	63.8	78.3	92.7	109.2	134.5	161.6	195.3	262.2
70	M	66.8	76.5	86.3	157.5	177.0	192.5	205.5	226.5	265.5
	F	34.5	42.0	49.5	57.0	64.5	94.5	124.5	214.5	244.5

Table 9

Percentile distribution of weekly mercury content in the diet of individual family members (Control area)

T o t a l   m e r c u r y (mcg)

Age (yrs)		Percentiles 10	20	30	40	50	60	70	80	90
1 - 6		12.0	19.5	27.0	34.5	44.5	54.5	64.5	124.5	194.5
7 - 12	M	18.7	32.8	47.0	94.5	119.5	136.5	153.5	181.5	269.5
	F	22.5	38.5	50.5	62.5	94.5	112.5	160.5	178.5	298.5
13 - 19	M	38.5	62.5	72.8	81.8	90.8	136.5	181.5	216.5	240.5
	F	67.5	85.5	99.0	108.0	117.0	130.5	166.5	202.5	238.5
20 - 49	M	29.5	54.5	75.3	96.2	118.6	142.0	167.0	193.8	236.6
	F	22.8	39.3	52.7	67.2	89.5	111.8	134.2	157.5	234.0
50 - 69	M	25.8	47.0	66.7	97.8	125.7	146.1	166.5	192.0	274.5
	F	18.8	33.1	47.5	62.9	79.5	96.1	115.6	138.1	175.8
70	M	19.5	34.5	45.8	57.0	79.5	124.5	147.0	214.5	319.5
	F	14.3	24.0	33.8	52.5	69.5	82.5	97.5	136.5	175.5

Table 10

Percentile distribution of weekly mercury content in the diet of individual family members (Experimental area)

M e t h y l m e r c u r y (mcg)

Age (yrs)		Percentiles 10	20	30	40	50	60	70	80	90
1 - 6		10.5	16.5	22.5	28.5	34.5	45.6	56.8	72.5	100.0
7 - 12	M	10.9	17.4	23.8	30.2	49.5	72.0	83.3	124.5	139.5
	F	13.5	22.5	31.5	46.5	64.5	91.5	118.5	135.0	148.5
13 - 19	M	23.3	40.5	55.5	72.0	90.8	114.5	139.5	169.5	207.0
	F	12.0	19.5	27.0	64.5	79.5	94.5	128.5	139.5	150.8
20 - 49	M	24.5	40.2	51.6	63.1	82.0	101.4	119.9	147.0	194.5
	F	14.7	24.9	35.1	45.8	56.4	67.5	80.3	93.0	127.5
50 - 69	M	28.0	40.9	49.8	58.7	69.4	82.8	98.1	123.9	180.0
	F	14.5	24.5	34.5	44.1	53.7	63.3	80.7	104.5	148.5
70	M	17.5	30.5	43.5	56.5	69.5	82.5	96.0	115.5	145.5
	F	12.0	19.5	27.0	34.5	49.5	64.5	79.5	124.5	154.5

Table 11

Percentile distribution of weekly mercury content in the diet of individual family members (Control area)

M e t h y l m e r c u r y (mcg)

Age(yrs)		Percentiles 10	20	30	40	50	60	70	80	90
1 - 6		5.9	11.3	16.7	22.1	27.5	38.5	56.5	102.5	162.5
7 - 12	M	6.2	11.8	17.5	23.2	28.8	78.5	104.0	138.5	170.0
	F	16.5	28.5	40.5	52.5	64.5	82.5	136.5	172.5	238.5
13 - 19	M	14.4	24.2	34.1	51.0	72.0	132.5	155.3	172.5	205.5
	F	37.5	55.5	69.0	78.0	87.0	100.5	166.5	193.5	211.5
20 - 49	M	13.7	27.0	45.5	66.0	86.4	113.0	138.8	170.5	193.6
	F	11.7	22.8	34.7	48.1	62.6	91.2	113.5	137.8	185.0
50 - 69	M	18.0	35.5	53.4	73.4	93.4	115.5	138.8	176.8	272.2
	F	14.0	27.5	40.9	54.1	66.5	78.9	91.3	116.5	178.5
70	M	12.9	21.3	29.7	82.5	102.0	112.5	123.0	160.5	262.5
	F	12.9	21.3	29.7	40.5	54.5	68.5	82.5	130.5	202.5

Discussion

The results of this study showed that the mercury content in seafood consumed by a population living in a coastal area with a relatively large industrial concentration was higher than that of an area without industry. This difference was particularly pronounced with regard to total mercury content which was statistically significant at the 5 per cent level. The difference in methylmercury content was smaller, and did not reach the level of statistical significance (0.1 p 0.05). These results also indicate that the industrial pollution had primarily an impact on the total mercury content of the seafood consumed, and less on the methylmercury content.

On the average, methylmercury accounted for 52,5% of the total mercury in the seafood in the experimental area, and for 66.7% of the total mercury in the control area. There were, however, great variations in the proportion of methylmercury to total mercury, ranging from 10,1 to 105,8% in the experimental area, and from 5,3 to 106,6% in the control area. The linear regression between the methylmercury and total mercury contents of seafood in the examined areas was, nevertheless, statistically significant (Figs. 1 and 2).

There were no significant differences in mercury content within the same species of the seafood caught in the summer and winter seasons. The differences in the mercury content according to the size of fish, described by many authors, were also rather small and not statistically significant (Table 12). The probable reason for the absence of correlation between fish and mercury content is that the fish consumed in both areas was of rather small size ranging only between 40 - 250 g.

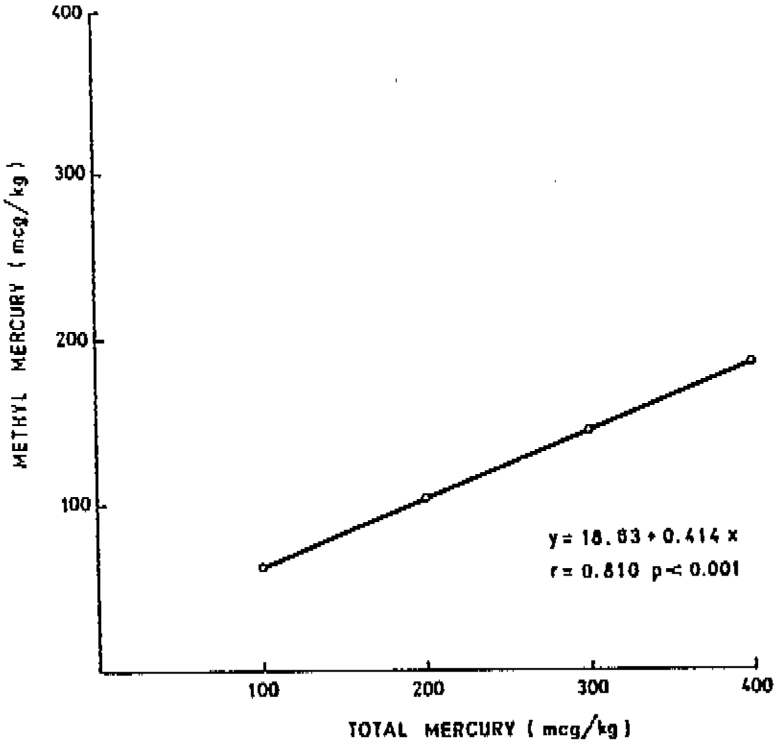

Fig.1 Relationship between total mercury and methylmercury in seafood
(Experimental area)

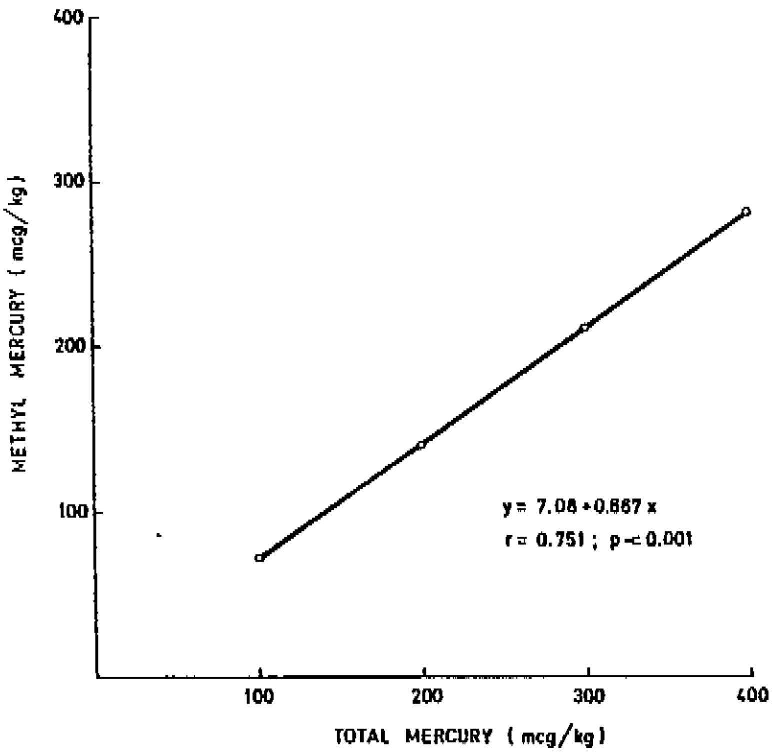

Fig.2 Relationship between total mercury and methylmercury in seafood
(Control area)

Table 12

Total and methylmercury levels in fish species analysed, divided according to weight of fish

Weight (g)	Total mercury				Methylmercury			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
50	.045	.845	.290	.250	.032	.320	.114	.090
50 - 99	.034	1.370	.252	.305	.019	.656	.161	.178
100-149	.054	.550	.258	.158	.038	.201	.109	.053
150-200	.073	1.180	.326	.364	.022	.583	.156	.192
200	.077	.820	.233	.290	.024	.316	.191	.103

As already described in the literature the crustaceans had a higher mercury content per unit of body mass than fish. However, this food was consumed by a smaller number of families, and rather infrequently. Species of seafood with a relatively high mercury content which was consumed more frequently were molluscs, primarily blackfish and squid.

As a result of the dietary study on seafood consumption patterns, it was estimated that, based on the mercury content of the species consumed, the weekly average intake of total mercury ranged between 64.5 mcg to 177.0 mcg in the experimental area, and between 44.5 and 125.7 mcg in the control area. On the same basis, the intake of methylmercury ranged from 34.5 to 90.8 mcg in the experimental area, and from 27.5 to 102.0 mcg in the control area. There was, however, less difference in the weekly intake of mercury in the higher percentiles of intake distribution between the two areas. In fact, the weekly methylmercury intake in higher percentiles of distribution was higher in the control area than in the experimental area.

The analysis of seafood consumption data showed that the higher methylmercury content of the diet of control area families was not a result of consumption of seafood with a higher mercury content, but primarily due to the more frequent consumption of seafood during the survey period. As already shown in Table 5, only 3.2% of control area families did not consume seafood during the survey period as compared to 11.4% of experimental area families. On the other hand, 27.0% of the control area families consumed seafood 5-7 times a week which is almost twice as much as in experimental area families (13.9%). These data show that despite a lower seafood mercury content (i.e. less contamination) in the control area, families consuming seafood more frequently could also ingest mercury in amounts exceeding the JECFA Provisional Tolerable Weekly Intake levels.

The number and percentage of individual family members ingesting both total mercury and methylmercury in excess of the PTWI are summarised according to age and sex in table 13. These data show the percentage of individual family members whose total mercury ingestion in the weekly diet exceeded the PTWI level was generally higher in the experimental than in the control area, despite a lower amount of seafood consumed. This is the result of a higher total mercury content (due to contamination) in the seafood in the experimental area. On the other hand, in the control area there was a higher percentage of individuals with a weekly methylmercury intake of over 200 mcg, resulting primarily from the higher level of seafood consumption.

The highest percentage of subjects with estimated intakes of both total and methylmercury above the PWTI was found in the 7-19 years age group.

Table 13

Number and percentage of subjects with estimated total and methylmercury dietary intake in excess of JECFA Provisional Tolerable Weekly Intake (PTWI) of 300 mcg. Total mercury including 200 mcg methylmercury

Age (yrs)		Experimental area				Control area			
		Total mercury		Methylmercury		Total mercury		Methylmercury	
		N	%	N	%	N	%	N	%
1 - 6		0	0.0	0	0.0	0	0.0	0	0.0
7 - 12	M	3	20.0	0	0.0	1	5.9	1	5.9
	F	2	11.1	0	0.0	1	8.3	2	15.4
13 - 19	M	5	20.0	4	16.0	1	4.2	3	13.0
	F	4	20.0	0	0.0	1	8.3	2	16.7
20 - 49	M	13	16.3	7	8.8	4	5.3	10	13.3
	F	5	5.8	0	0.0	3	4.5	5	7.5
50 - 69	M	9	10.5	7	8.1	7	8.2	13	15.5
	F	4	4.9	2	2.5	1	1.6	4	6.5
70	M	1	7.7	0	0.0	2	13.3	2	14.3
	F	0	0.0	0	0.0	0	0.0	1	7.1

Levels of mercury in hair

Following completion of the dietary surveys, hair samples were taken from 42 children and adolescents (age 1-16 years) from each area, and analysed for both total mercury and methylmercury. The analyses were performed by the Department of Nuclear Chemistry, "Josef Stefan" Institute, Ljubljana, by Gas Chromatography, utilising a reference method specifically developed for the overall project.

In the subjects from the experimental area, levels of total mercury ranged from 0.10 to 5.30 ppm (mean: 1.75 ± 1.33) and those of methylmercury from 0.09 to 3.70 ppm (mean: 1.41 ± 1.05). In the subjects from the control area, total mercury levels ranged from 0.41 to 3.80 ppm (mean: 1.73 ± 0.88). In both cases, the differences were not statistically significant.

The correlations between hair mercury content and some of the examined variables in the experimental and control areas are shown in Tables 14 and 15 respectively.

Table 14

Correlation between hair mercury content and examined variables in 42 subjects from experimental area

Variable	Correlation with hair mercury content	
	Total Hg	MeHg
Age	-.325	-.224
Dietary Total Hg (Winter)	.152	.104
Dietary MeHg (Winter)	.133	.045
Dietary Total Hg (Autumn)	.168	.247
Dietary MeHg (Autumn)	.024	.101
Hair selenium	.557	.673

Table 15

Correlation between hair mercury content and examined variables in 42 subjects in control area

Variable	Correlation with hair mercury content	
	Total Hg	MeHg
Age	-.217	-.135
Dietary Total Hg (Winter)	.190	.139
Dietary MeHg (Winter)	.165	.113
Dietary Total Hg (Autumn)	.068	.016
Dietary MeHg (Autumn)	-.007	.057
Hair selenium	.495	.495

The above data show that hair mercury content was positively and significantly correlated with hair selenium, and negatively with age. There was also a positive correlation with the total dietary mercury intake, but the coefficients of correlation were of a much lower order.

In order to assess the contribution of some of the independent variables to the hair mercury content, multiple correlations were calculated. The results showed that age and dietary mercury contribute significantly to the variation in hair mercury content (R = .638; P 0.001), but if the age variable is removed, the contribution of dietary mercury alone is much lower (R = .473; P 0.05). This shows that only 22% of the variation of the hair mercury content could be explained by the dietary mercury intake.

4. CONCLUSIONS

The results of this study showed that in an Adriatic locality with increased coastal water and marine sediment, mercury levels due to local industrial pollution, there was an increase in the mercury content in the majority of seafood species consumed as compared to a control area with no local industries. Industrial pollution resulted in a significant increase in the total mercury content of seafood in the former locality, whereas the corresponding increase in the methylmercury content was smaller and not statistically significant. The results of dietary studies also indicated that the average weekly dietary intake of total mercury was higher in the industrially polluted area. The dietary intake of methylmercury was, however, higher in the control area, probably due to a higher fish consumption in that area. Nevertheless, when the examined subjects from both areas were matched by weekly seafood consumption frequency, both total and methylmercury intake was higher in the industrially polluted area. It can therefore be concluded that local industrial pollution of coastal water may play a significant role in raining the population's dietary mercury intake to a level above the current Provisional Tolerable Weekly Intake. On the other hand, however, these results also shown that in a locality with no industrial pollution, consumption of seafood more than five times per week may also increase the mercury intake to levels above the PWTI in a number of individuals. In no case did levels of hair mercury in the 84 subjects sampled indicate the advisability of clinical studies, and though a positive correlation was found between hair mercury levels and total dietary mercury intakes, such correlation had a coefficient of relatively low order.

Annex 2

Report on a dietary survey and mercury levels in hair in selected Greek population samples

by

Professor Antonia Polychronopoulou-Trichopoulou
Department of Nutritian and Biochemistry
Athens School of Hygiene
Athens, Greece

and

Dr Sheena Nakou
Institute of Child Health
Aghia Sofia Children's Hospital
Athens, Greece

1. INTRODUCTION

Concentrations of mercury in Mediterranean seafood have been recorded as generally higher than those found in other seas, and concern has been expressed with regard to potential health hazards in the region arising from consumption of mercury-contaminated seafood. The need has therefore been felt to attempt to identify those population sectors, mainly from coastal areas, suspected of being at risk, and to determine the extent and degree of the problem.

The present study was commenced in 1985 as part of the WHO/FAO/UNEP project on "Methylmercury in Mediterranean populations and related health hazards". Its purpose was to explore the effective exposure of Greek subjects to total mercury and methylmercury according to food consumption patterns and other factors, and to investigate the possible effects of low concentrations of methylmercury on human health. The components of the study were (1) selection of pilot areas along the Greek coast where marine pollution by industrial effluents and regular consumption of fresh, locally caught, seafood appeared to provide an indication to potential exposure, (2) the conduction of a dietary survey among selected populations samples in these areas, to determine the type and amount of seafood consumed, and (3) analysis of the hair of indicated subjects within these population samples for its mercury content.

2. SELECTION OF AREAS AND POPULATION SAMPLES

During the first year, two pilot areas in the Attica region of Greece were selected: The industrial seaside town of Elefsina, where the sea is normally subject to pollution by effluents from Athens and neighbouring industrial towns, and the non-industrial town of Paiania, located approximately 20 km from the coast. Population samples were selected from among families with small children in the former area, and from schoolchildren in the latter. In addition, a further group was selected from among young men newly drafted into the Greek Navy at the port of Skaramanga, all from fishing families from various parts of Greece. A total of 1500 subjects from these three groups were checked for amount and type of seafood consumption by a dietary survey.

Table 1

Questionnaire used in dietary survey on Greek population samples

ATHENS SCHOOL OF PUBLIC HEALTH

DEPT. OF BIOCHEMISTRY AND NUTRITION

CODE ....

DATE ....

NAME .... AGE ....

ADDRESS .... TEL .... PROFESSION ....

During the last six months how often, on the average, did you eat the following foods?

Kind of food	Never	Rarely	At least once a month	Once a week	At least twice a week	Daily or almost. If daily, how many times.
1. Pork						
2. Beef						
3. Lamb						
4. Liver						
5. Chicken						
6. Fresh fish						
7. Frozen fish						
8. Canned fish						
9. Dried salt codfish						
10. Octopus						
11. Sepia						
12. Cuttlefish						
13. Shrimps, Crawfish						
14. Shellfish						
15. Sausage, salami, etc.						
16. Eggs						
17. Milk						
18. Cheese						
19. Yoghurt						
20. Butter						
21. Margarine						
22. Fruit						
23. Vegetables						
24. Bread						
25. Pasta						
26. Rice						
27. Potatoes						
28. Pulses						
29. Ice cream						
30. Sweets						

3. DIETARY SURVEY

The dietary survey was conducted on the basis of the questionnaire reproduced in Table 1, which was essentially a simplified version of the protocol developed for the overall project in 1982. In the questionnaire format, and in the interviews, equal attention was devoted to all items (whether seafood or non-seafood) to avoid any possible misinterpretation of the project. From the original sample of 1500, a total of 250 individuals were identified as consuming two or more seafood meals per week.

4. HAIR SAMPLING AND ANALYSIS

Samples of hair were taken from 140 individuals, from within the sub-sample of 250, divided as follows: 36 from the "exposed" area (Elefsina), consisting of 2 adult males, 19 adult females (most of whom were pregnant) and 15 children, aged 2-4; 87 schoolchildren from the "non-exposed" area (Paiania) and 17 naval inductees from the port of Skaramanga (considered as exposed because of their recent fishing background). One individual from this last group was recorded as consuming 20 seafood meals per week.

Sampling was performed as described in the reference method for determination of total mercury, methylmercury and selenium in human hair, developed by the "Josef Stefan" Institute, Ljubljana, Yugoslavia for the project. Analysis was performed by that Institute.

In the Elefsina population, the range of total mercury hair levels was 0.48 to 0.51 ppm in the 15 children and 0.72 to 2.6 ppm in the 21 adults. Corresponding figures for methylmercury hair levels were 0.37 to 1.16 ppm and 0.25 to 1.4 ppm respectively. The ranges of total mercury and methylmercury hair levels in the 87 schoolchildren from Paiania were 0.38 to 4.0 ppm and 0.09 to 4.2 ppm respectively. Among the 17 young naval inductees (fishermen) from Skaramanga base, ranges of total mercury and methylmercury hair levels were 0.56 to 38.0 ppm and 0.16 to 35.96 ppm respectively. In this last group, apart from the individual at the extreme end of the ranges (identified as consuming 20 seafood meals per week), all the rest had levels under 5 ppm total mercury and 4 ppm methylmercury. The ranges, means and standard deviations of hair methylmercury levels are given in Table 2.

<u>Table 2</u>

Hair Methylmercury levels in Greek population samples

1.	Elefsina (industrial town)	adults	(21)	range : 0.25 - 1.42 ppm mean : 0.66 ± 0.58
		children	(15)	range : 0.37 - 1.59 ppm mean : 0.72 ± 0.6
2.	Paiania (non-industrial town)	children	(87)	range : 0.09 - 4.15 ppm mean : 0.63 ± 0.5
3.	Skaramanga (fishing families)	adults	(17)	range : 0.16 - 35.96 ppm mean : 3.9 ± 2.11
	TOTAL:		140	range : 0.09 - 35.96 ppm mean : 1.31 ± 1.06

Results for both methylmercury and total mercury were analysed by multiple regression, taking into account age, sex, place of origin and reported frequency of seafood consumption. The findings are summarised in Tables 3 and 4.

Table 3

Partial regression coefficients (2), Standard error of partial regression (3), t-values (4) and significance levels P (5) of the multiple linear regression of methylmercury on a set of independent variables (1)

Independent variables (1)	Partial regression coefficients (2)	Standard errors (3)	t-values (4)	P values (5)
Sex	- 0.1130	0.1210	- 0.93	0.35
Age	- 0.0022	0.0075	- 0.30	0.76
Fish consumption	- 0.0680	0.2164	- 0.31	0.76
Place of origin	- 0.8090	0.5153	- 1.57	0.12
Fish consumption X Place of origin	0.5462	0.2393	2.28	0.12

Table 4

Partial regression coefficients (2), Standard error of partial regression (3), t-values (4) and significance levels P (5) of the multiple linear regression of total mercury on a set of independent variables (1)

Independent variables (1)	Partial regression coefficients (2)	Standard errors (3)	t-values (4)	P values (5)
Sex	- 0.2171	0.1476	1.47	0.14
Age	- 0.0251	0.0092	- 2.74	0.006
Fish consumption	- 0.1851	0.2639	- 0.70	0.48
Place of origin	- 0.6581	0.6285	- 1.05	0.29
Fish consumption X Place of origin	0.6965	0.2918	2.39	0.02

The interaction term dominates the regression model of methylmercury (Table 1). At moderate and high frequencies of fish consumption the concentration of methylmercury is higher among residents of seaside villages and among them it increases with increasing frequency of fish consumption. No significant associations were found between methylmercury concentration in the hair on the one hand and age or sex on the other. In all but one of the individuals concentrations were within acceptable limits.

For total mercury the multiple regression model has some elements similar to those characterising the methylmercury model but in addition there is a strong and statistically highly significant negative association with age.

These results point to the need for continuing the study on a larger scale, and to correlation of the dietary survey with examination of the sources of fish and other seafood consumed and analysis of the methylmercury content of the various species.

Annex 3

Mercury intake from seafood in sample populations from three coastal areas in the Tyrrhenian Sea

by

Professor A. Renzoni
Department of Environmental Biology
University of Siena
Siena, Italy

1. INTRODUCTION

The high levels of mercury in various edible species of the marine fauna in the Mediterranean sea, as recorded in the literature and confirmed by the results of the FAO/UNEP pilot project on baseline studies and monitoring of heavy metals (particularly mercury and cadmium) in marine organisms, carried out between 1975 and 1981, indicate that the risk for the human population living around this basin could be higher than in other parts of the world.

Within the Mediterranean Sea, one area is particularly interesting. This is the Central Tyrrhenian Sea (Southern Tuscany) where inland cinnabar deposits abound and where, until recently, mercury has been refined for centuries. The marine fauna from this area has been analysed on a regular basis during the last ten years, revealing mercury levels higher than in any other part of the Mediterranean, including other areas within the Tyrrhenian Sea itself.

The present study was commenced in late 1985 within the framework of the research component of MED POL Phase II, as part of the WHO/FAO/UNEP project on Methylmercury in Mediterranean populations and related health hazards. It deals essentially with analysis of mercury in edible marine fauna in selected pilot zones, investigation of the diet of selected fishermen and their families in indicated sectors of the above sample populations, to find out as much detail as possible about the current situation with regard to methylmercury intakes as compared with acceptable or tolerable levels, and potential health effects.

2. SELECTION OF STATIONS AND POPULATION SAMPLES

Three stations were selected (vide Figure 1). Station A represents an area affected by industrial pollution and other anthropogenic sources of mercury, Station B an area with no anthropogenic source of mercury in the vicinity but with volcanic activity nearby, and Station C an area where fishermen spend almost seven months of the year aboard their fishing boats, and are therefore exposed to a high mercury intake through the excessive amount of fish consumed, rather than through the particular level of mercury in the fish themselves.

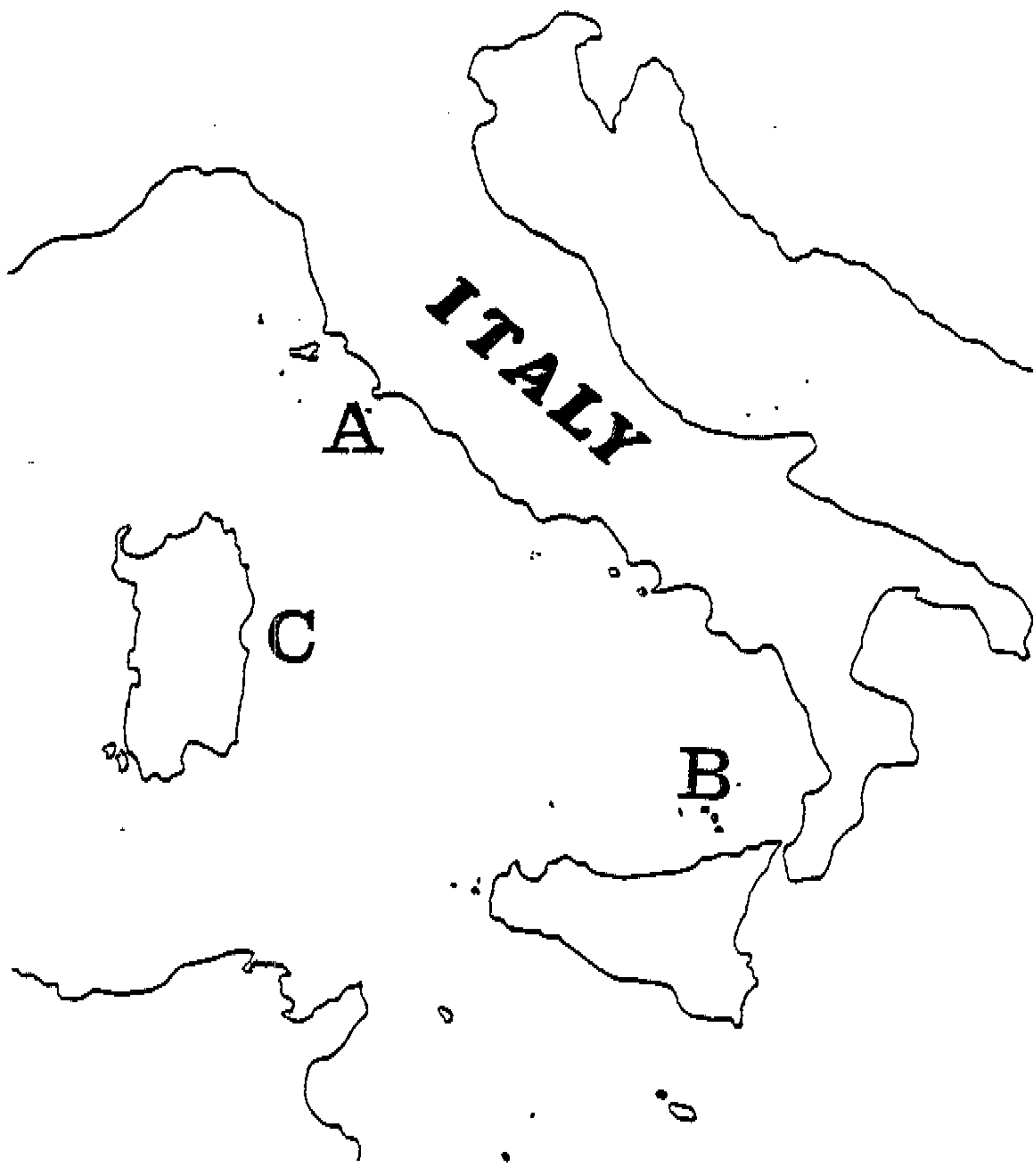

Fig.1 Stations in the central Tyrrhenian sea selected for the mercury study

3. CONCENTRATIONS OF MERCURY IN SEAFOOD

Data collected during the 1975-1980 MED POL project resulted in levels of mercury in marine species from the same area as Station A ranging from a minimum of 160 ppb (in anchovies) to a maximum of 1280 ppb (in striped mullet). Both figures represent averages of the various seasons in which sampling and analysis were performed. Recent analyses carried out as part of the present project (Table 1) would appear to indicate a slight reduction in mercury levels, at least in the three species most regularly analysed during the previous project (Table 2), though no valid conclusions can be reached at this stage. Collection of samples from the other two stations (B and C) are under way at the time of reporting. Previous studies in the area of Station B gave a range of total mercury concentration from 110 to 530 ppb with an average of 230 ppb. In Station C, 1975 figures for total mercury concentrations in two benthic species were 400-1100 ppb (average : 720 ppb) for Norwegian lobster, and 800-1500 ppb (average : 1140 ppb) in rockfish. Results to date from the present project in Station C regarding one of these species (Norwegian lobster) and Deep water pink shrimp are given in Table 3.

Table 1

Concentrations of total mercury in the most common species of seafood consumed by fishermen in Station A (expressed in parts per billion (ppb) fresh weight)

Species	X
Red shrimp	1190
Deep water pink shrimp	1120
Turbot	650
European hake	180
Squid	310
Cuttlefish	300
Red gurnard	190
Common pandora	535
Horse mackerel	260
Bogue	440
Common octopus	550
Striped mullet	700
Norwegian lobster	835

Table 2

Concentrations of total mercury in three seafood species from Station A (a) in 1975-1980 studies, and (b) during the present study (expressed as ppb fresh weight)

	1975-80		1986	
	Number of samples	Average concentration	Number of samples	Average concentration
Engraulis encrasicolus	190	160	19	140
Nephrops norvegicus	156	960	29	834
Mullus barbatus	153	1280	13	700

Table 3

Concentrations of total mercury in two benthic seafood species in Project Station C (coastal waters of Sardinia) in parts per billion (ppb)

Species	n	X	SD
Deep water pink shrimp	18	632	245
Norwegian lobster	16	460	180

During the present project, all analyses of total mercury in edible marine organisms are being performed by the standard reference methods utilising cold vapour atomic absorption spectrophotometry developed for the MED POL Programme, and used throughout the overall mercury project. All values are expressed in parts per billion (ppb) wet weight.

4. DIETARY SURVEY

In view of the preliminary nature of this phase of the project, an initial dietary survey was conducted mainly on the basis of oral interview. Sample populations were pre-selected for interview following the acquisition of general data regarding seafood consumption.

A total of 220 individuals were interviewed. In Station A (the two towns of P. San Stefano and P. Ercole on the Agentarro promontory) adult fishermen were recorded as consuming 4 to 8 seafood meals per week. Table 4 gives the equivalents in Kg/week among the 58 surveyed. On the other hand, other members of their families were consuming a lower amount (up to 3 meals per week, totalling approximately 0.4 Kg per capita). The most common species in the diet consisted of hake, shrimp (2-3 species), horse mackerel, striped mullet, squid, octopus, cuttlefish, rockfish, common pandora, scorpion fish, norwegian lobster and turbot.

Table 4

Seafood consumption recorded among 58 fishermen from Station A (Porto San Stefano)

Marine Food (kg/week)	Number of Fishermen
1	7
2	30
3	16
4	3
6	1
7	1

Most common species: hake, shrimp, horse mackerel, striped mullet, squid, octopus, rockfish, turbot, cuttlefish, norwegian lobster.

In Station B (the Island of Salina), both fishermen obtaining their catch from around the Aeolian Archipelago and non-fishermen working in related fields (fish-vendors, employees in fish restaurants, etc.) were recorded as consuming 4 or more seafood meals per week. The most common species were red mullet, swordfish, tuna, amberjack, anchovy, common pandora, lobster, shrimp, octopus and cuttlefish.

In Station C (the Sardinian coast) attention was mainly directed at fishermen who spend at least seven months of the year at sea, fishing in their own waters in Spring and Summer, and in the waters around Station A in Autumn and Winter. The main species of seafood consumed was the same as in Station A.

5. HAIR ANALYSIS

Analysis of hair for total mercury and methylmercury was performed by the "Josef Stefan" Institute, Ljubljana, Yugoslavia. Sampling was undertaken after the dietary survey. By the date of reporting, results were available for 7 fishermen from Station A. As a control, hair was also sampled from 19 individuals living in inland towns near the Station area, who consumed seafood only once a month or less. Levels recorded ranged from 3.58 to 29.66 ppm (total mercury) and 3.45 to 25.3 ppm (methylmercury) among the fishermen. Corresponding ranges in the "control" group were 0.35 to 2.97 ppm and 0.17 to 1.64 ppm respectively. Statistical analysis of the results is given in Table 5.

Table 5

Comparison of hair mercury levels in (a) 7 fishermen consuming 4 or more seafood meals per week and 19 non-seafood consumers

Sample population	Total Hg (ppm)	Me Hg (ppm)
A (no seafood) (n=19)	X = 1.12, SD = 0.7	X = 0.77, SD = 0.53
B (4+ meals/week) (n=7)	X = 16.03, SD = 9.93	X = 13.28, SD = 8.31
	Methylmercury / Total mercury	
A (n = 19)	Y = 0.03 + 0.65 X	r = 0.94
B (n = 7)	Y = 0.38 + 0.80 X	r = 0.96

6. CONCLUSION

Even though the present study has only been operational for less than one year, there is already evidence that adult fishermen constitute a particular high-risk group because of the amount of seafood consumed together with the relatively high mercury levels in such seafood.

Annex 4

Analysis of total mercury, methylmercury and selenium in hair in Greek, Italian and Yugoslav sample populations

by

Dr P. Stegnar
Department of Nuclear Chemistry
"Josef Stefan" Institute
Ljubljana, Yugoslavia

1. INTRODUCTION

The present study was performed within the framework of the WHO/FAO/UNEP project on "Methylmercury in Mediterranean populations and related health hazards", as part of which, hair samples collected from among selected population sectors in coastal areas in Greece, Italy and Yugoslavia, were analysed for their total mercury, methylmercury and (in a number of cases) selenium content. Up to the date of reporting, a total of 250 hair samples were received from participating institutions (140 from Greece, 26 from Italy and 84 from Yugoslavia). All were analysed for total mercury and methylmercury. 60 of the samples (42 from Gree and 18 from Yugoslavia) were also analysed for selenium.

2. METHODOLOGY

Hair samples were collected by participating institutions in accordance with a specially developed protocol to ensure standardisation. The code number, name, age of donor and date of sampling were recorded with a marker on a transparent plastic sampling bag, 12 x 5 cm. Hair was cut as close as possible to the scalp with a scissors, in an amount of approximately 0.5 g. It was recommended that hair should be preferably cut from the occipital area. The hair was collected on a sheet of white paper with proximal ends on the same side, and subsequently either transferred into the bag with tweezers or the paper folded and the hair slid into the bag. Proximal ends of the hair were identified. A recording form (Table 1) was also provided.

Total mercury was determined by cold vapour atomic absorption spectrophotometry after complete mineralisation of the sample in a reflux system. Methylmercury was determined by gas liquid chromatography. After disintegration of the sample in a solution of sodium hydroxide, methylmercury was extracted from an aliquot of the solution into toluene and, after purification, a small volume was injected into a chromatographic column filled with polyethylene glycol succinate on Diatomite AW. Methylmercury in the gaseous mixture was detected with an election capture detection, and its amount determined by comparing the peak height with those of appropriate standards. For determination of selenium, the samples were first ashed with magnesium nitrate. The principle involved the reaction of 4-nitro-1,2-diaminobenzene with Selenium IV to give 5-nitro-2,1,3-benzoselenodiazole extractable into toluene or benzene. Selenium in the soluent phase was determined by gas liquid chromatography using an electron capture detector.

All three determinations are described in detail in a reference method specially developed for use in the project.

Table 1

RECORDING FORM FOR HAIR SAMPLING

To be completed by the person collecting hair samples:

1. Sample code: Sampling date: .... Sample
....
taken by: ....
Location: ....
Institution: ....

2. Data on donor:
Name: ....
Date of birth: ....
Sex: ....
Occupation: ....
Employment: ....

3. Data on hair:
Colour: ....
Type: ....
Treatment[x]:
....

4. Data on exposure[xx]:
Type of seafood: ....
Origin: ....
Amounts consumed: daily: .... weekly: ....

x Enquire about, and record any special treatment applied to the scalp/hair during recent months such as medicated shampoos (Selsun!), dyes, ointments, perm.

xx Data on exposure may be taken from the dietary survey if available, or obtained by interviewing the donor during sampling.

To be completed by the analyst:

Sample received on: ....
Quantity grams: ....
Analysed for: ....
Analysis completed on: ....
Results: MeHg: .... Hg: .... Se: ....

Signature of the responsible analyst: ....

3. RESULTS

The results of the analyses were transmitted back to the principal investigators in institutes participating in the project, for correlation with other data (seafood analysis and dietary surveys). Detailed individual results (levels of total mercury, methylmercury and selenium) for the Greek, Italian and Yugoslav population groups are given in Tables 2, 3 and 4 respectively.

Frequency distributions of hair mercury levels (total mercury and methylmercury) for the three groups are shown in Figures 1, 2 and 3, and frequency distributions of the methylmercury percentage of the total mercury hair content in each group are shown in Figures 4, 5 and 6.

Statistical analysis of the results, including correlation with data obtained from other components of the project, were performed by the Institutions providing the hair samples.

Table 2

Methylmercury, total mercury and selenium hair levels in Greek population group (N = 140)

Sample No.	CH_3Hg^+ (ug g^{-1})	Hg total (ug g^{-1})	Se (ug g^{-1})	Sample No.	CH_3Hg^+ (ug g^{-1})	Hg total (ug g^{-1})	Se (ug g^{-1})
1	0.57	0.89		71	0.65	1.25	
2	0.60	0.88		72	0.20	0.94	
3	0.60	0.92		73	0.33	0.83	
4	1.05	1.19	0.50	74	0.55	0.95	
5	0.44	0.92		75	0.15	0.55	
6	0.62	0.98		76	0.40	0.90	
7	0.59	1.59		77	0.44	1.53	
8	0.39	0.85		78	0.15	0.51	
9	1.59	2.02	0.43	79	0.23	0.90	
10	1.28	1.30	0.27	80	0.54	0.72	
11	0.90	1.68	0.46	81	0.50	1.10	
12	0.79	1.70	0.36	82	1.73	2.35	
13	0.25	0.69	0.31	83	0.17	0.60	
14	1.42	2.62	0.59	84	0.26	1.40	
15	0.61	1.85	0.36	85	0.09	1.60	
16	0.49	1.50	0.38	86	0.42	0.76	
17	1.00	1.16	0.44	87	0.25	1.08	
18	0.47	0.82		88	0.51	1.19	
19	0.37	0.48		89	0.56	2.15	
20	0.39	0.72		90	0.27	0.90	
21	0.39	0.78		91	0.10	1.17	
22	1.00	1.28	0.51	92	0.45	1.50	
23	0.89	5.09	0.46	93	4.15*	3.82	0.42
24	0.99	2.54	0.33	94	0.75	1.19	0.50
25	0.56	0.92		95	0.50	0.86	
26	0.85	1.07		96	1.81	3.00	0.44
27	0.57	4.03	0.48	97	0.54	1.38	

Table 2 (Continued)

Sample No.	CH_3Hg^+ (ug g^{-1}	Hg total (ug g^{-1})	Se (ug g^{-1})	Sample No.	CH_3Hg^+ (ug g^{-1}	Hg total (ug g^{-1})	Se (ug g^{-1})
28	0.60	0.80		98	0.25	0.47	
29	0.60	0.62		99	0.38	0.68	
30	0.58	0.70		100	0.34	0.85	
31	0.83	1.12	0.42	101	0.32	0.56	
32	0.93	1.58	0.33	102	0.15	0.50	
33	0.24	0.45		103	0.61	1.06	
34	1.04	1.10	0.42	104	0.84	0.91	
35	0.24	1.05		105	0.46	0.78	
36	0.37	0.77		106	0.19	0.40	
37	0.54	0.75		107	0.43	0.67	
38	0.43	0.75		108	0.90	0.93	0.37
39	0.32	0.45		109	1.01*	0.95	
40	0.62	0.79		110	0.33	0.71	
41	1.65	1.67	0.42	111	0.29	1.13	
42	0.69	0.78		112	0.81*	0.52.42	
43	1.31	1.45		113	0.88	1.06	0.41
44	1.14	1.14	0.39	114	3.21	3.80	
45	0.23	0.43		115	2.69	2.90	0.35
46	0.27	0.43		116	0.16	0.56	
47	0.44	0.81		117	0.55	1.90	
48	0.43	0.63		118	0.42	0.45	
49	0.48	0.91		119	1.11	1.40	0.56
50	1.11	1.41	0.47	120	0.85	1.60	
51	1.42	1.78	0.54	121	4.38*	4.29	0.51
52	0.59	1.24		122	3.59	4.20	0.56
53	0.36	0.53		123	35.96	37.51	0.74
54	0.20	0.38		124	0.55	0.73	
55	0.33	0.56		125	2.30	2.74	0.52
56	0.63	0.83		126	1.12	1.98	0.43
57	0.67	0.92		127	2.92	3.04	0.52
58	0.53	0.98		128	2.53	2.59	0.47
59	1.43	1.43	0.38	129	1.98	2.07	0.42
60	1.19	1.13	0.42	130	2.11	2.23	0.48
61	0.73	1.10		131	0.85	1.29	
62	0.50	0.69		132	0.45	4.71	
63	1.06	1.40	0.40	133	0.37	0.71	
64	0.49	0.64		134	0.41	0.85	
65	0.37	0.45		135	0.83	0.87	
66	0.52	0.66		136	0.32	1.13	
67	2.17*	1.91	0.45	137	0.42	0.86	
68	0.49	1.01	0.32	138	0.55	1.42	
69	0.55	0.77		139	0.70	2.86	
70	0.30	0.40		140	0.55	1.97	

* Variation in the results was shown to be mainly due to the unhomogeneity of the sample

Table 3

Methylmercury, total mercury and selenium hair levels in Italian population group (N = 140). OMS = non-seafood eating controls, SA = adult fishermen

Sample No.	Hg total ugHg g^{-1}	MeHg ugMeHg g^{-1}
OMS 1	2.97	2.10
OMS 2	0.74	0.48
OMS 3	1.22	0.84
OMS 4	0.58	0.44
OMS 5	0.58	0.34
0,S 6	1.53	0.86*
OMS 7	0.26	0.20
OMS 8	0.76	0.72
OMS 9	2.07	1.64
OMS 10	0.76	0.51
OMS 11	0.35	0.17
OMS 12	1.01	0.85
OMS 13	1.84	1.53*
OMS 14	0.81	0.81*
OMS 15	0.91	0.43
OMS 16	2.59	1.31
OMS 17	0.84	0.50
OMS 18	1.15	0.68
OMS 19	0.45	0.30

Sample No.	Hg total ugHg g^{-1}	MeHg ugMeHg g^{-1}
SA 1	6.82	6.17*
SA 2	3.58	3.45
SA 3	29.66	25.31
SA 4	24.76	15.78*
SA 5	8.99	6.34
SA 6	16.04	14.09
SA 7	22.42	21.79

* Too small an amount of hair to permit replicate determination in independent aliquots

Table 4

Methylmercury, total mercury and selenium hair levels in Yugoslav population group (N = 84)

Sample No.	Hg Total ugHg g^{-1}	MeHg ugMeHg g^{-1}	Selenium ugSe g^{-1}	Sample No.	Hg Total ugHg g^{-1}	MeHg ugMeHg g^{-1}	Selenium ugSe g^{-1}
1	1.67	1.36	0.41	43	2.27	2.00	0.34
2	3.61	3.24	0.38	44	0.78	0.64	
3	3.24	2.40		45	0.68	0.58	
4	1.43	1.17		46	0.85	0.64	
5	1.17	1.07	0.42	47	0.58	0.28	
6	2.74	2.58	0.42	48	0.53	0.41	
7	1.19	1.09	0.38	49	0.41	0.37	
8	0.66	0.56		50	2.58	2.28	
9	1.31	1.04	0.37	51	0.81	0.54	
10	1.44	1.31		52	1.38	1.35	
11	1.08	0.97		53	1.38	1.35	
12	0.33	0.32		54	1.01	0.76	
13	0.10	0.09		55	3.04	2.48	0.33
14	0.50	0.49		56	1.78	1.52	
15	1.37	0.77		57	0.58	0.31	
16	1.29	0.93		58	1.57	0.94	
17	0.54	0.50		59	2.62	1.89	0.29
18	0.82	0.66		60	1.52	1.00	
19	1.18	1.04		61	2.36	1.76	
20	0.32	0.22		62	2.52	2.02	
21	0.10	0.10		63	2.13	1.02	
22	0.66	0.53		64	3.51	2.32	
23	0.81	0.72		65	3.75	3.35	
24	1.10	0.99		66	1.65	1.40	
25	0.46	0.24		67	2.08	1.74	
26	4.90	1.34		68	1.84	1.75	
27	3.61	2.63	0.36	69	2.59	2.03	
28	0.52	0.44		70	0.83	0.58	
29	5.30	3.90	0.35	71	1.34	1.33	
30	1.68	1.58		72	0.57	0.45	
31	2.45	2.35	0.42	73	2.32	1.95	
32	1.85	1.22		74	4.72	3.14	
33	0.82	0.66		75	2.18	1.86	
34	1.65	1.48		76	7.40	7.09	
35	2.26	2.06	0.45	77	4.60	3.71	
36	1.73	1.70		78	5.50	4.46	
37	3.75	3.62	0.33	79	2.79	1.95	
38	3.85	3.47	0.34	80	2.94	2.36	
39	1.58	1.56		81	5.10	4.52	
40	3.06	2.30	0.33	82	1.75	1.36	
41	1.21	0.93		83	4.80	4.75	
42	4.11	3.70	0.35	84	2.30	1.90	

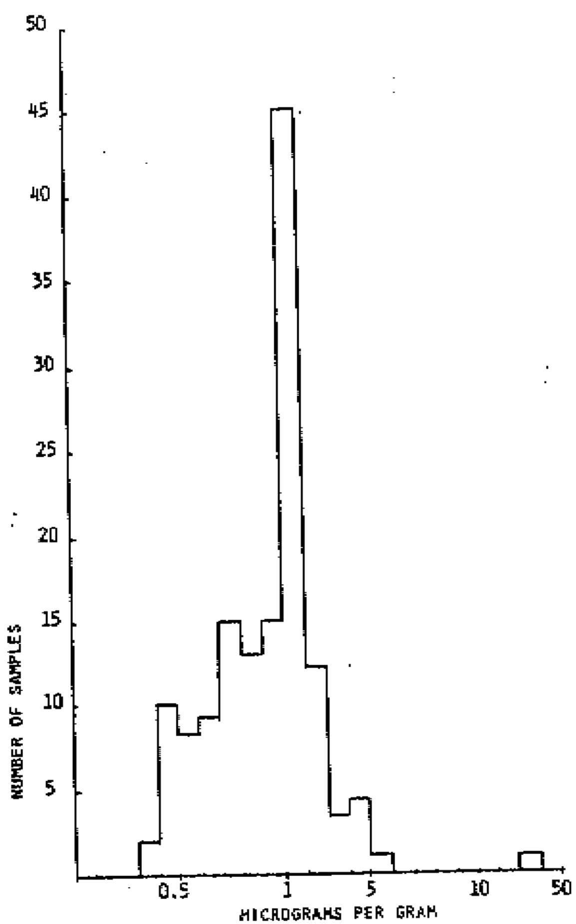

Fig.1(A) Frequency of hair mercury levels in Greek population group (N = 140). Total Mercury.

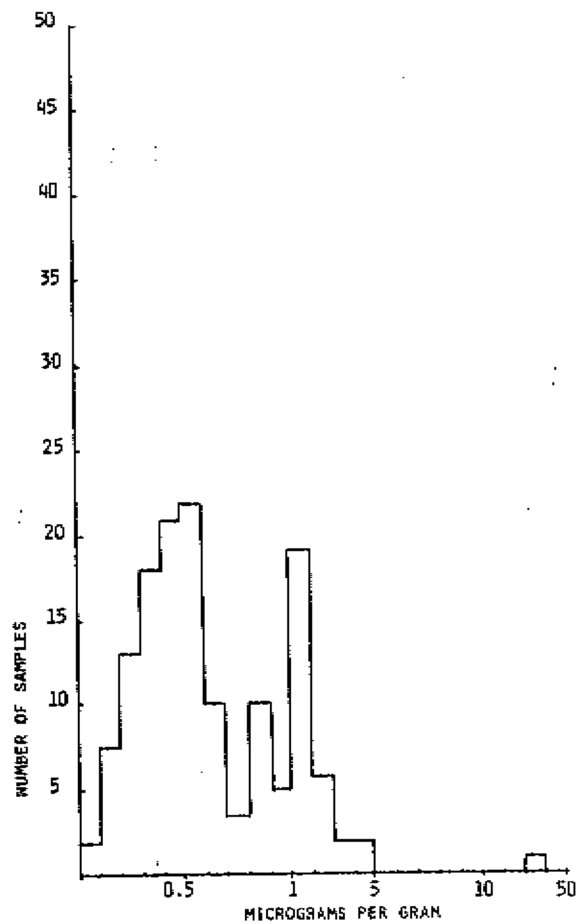

Fig.1(B) Frequency of hair mercury levels in Greek population group (N = 140). Methylmercury.

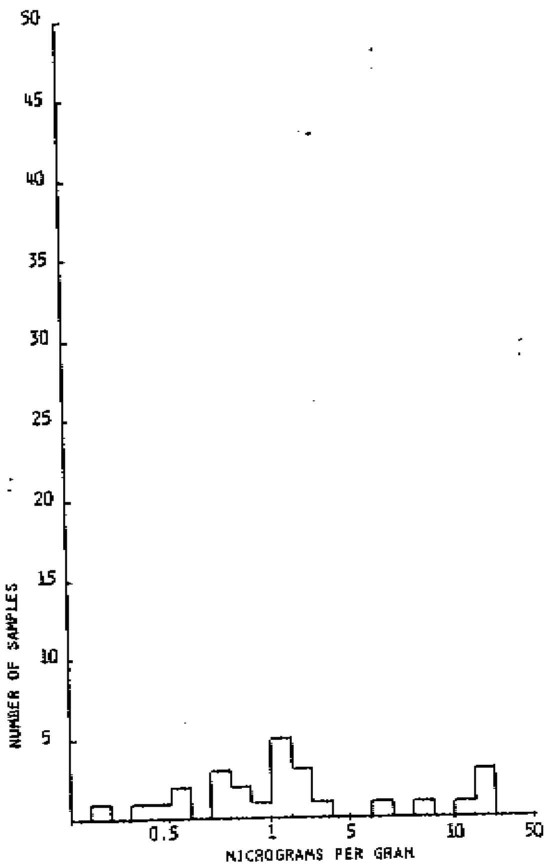

Fig.2(A) Frequency distribution of hair mercury levels in Italian population group (N = 26). Total Mercury.

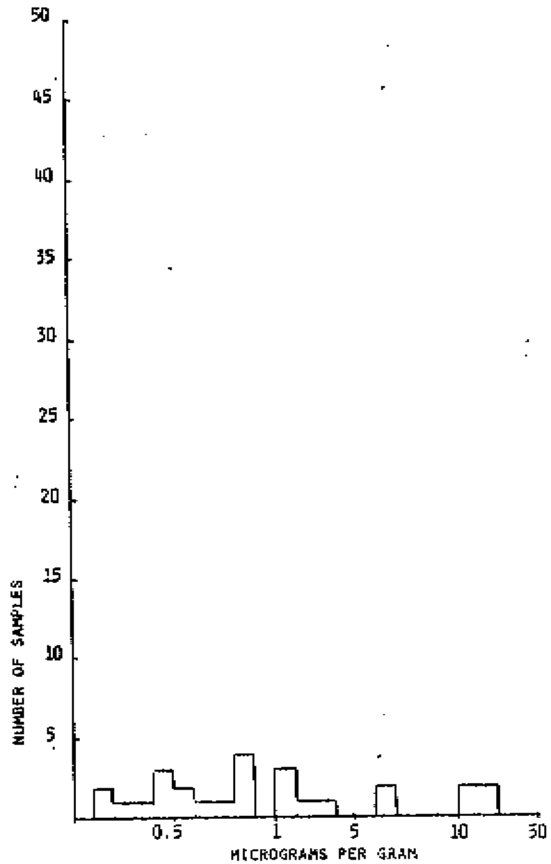

Fig.2(B) Frequency distribution of hair mercury levels in Italian population group (N = 26). Methylmercury.

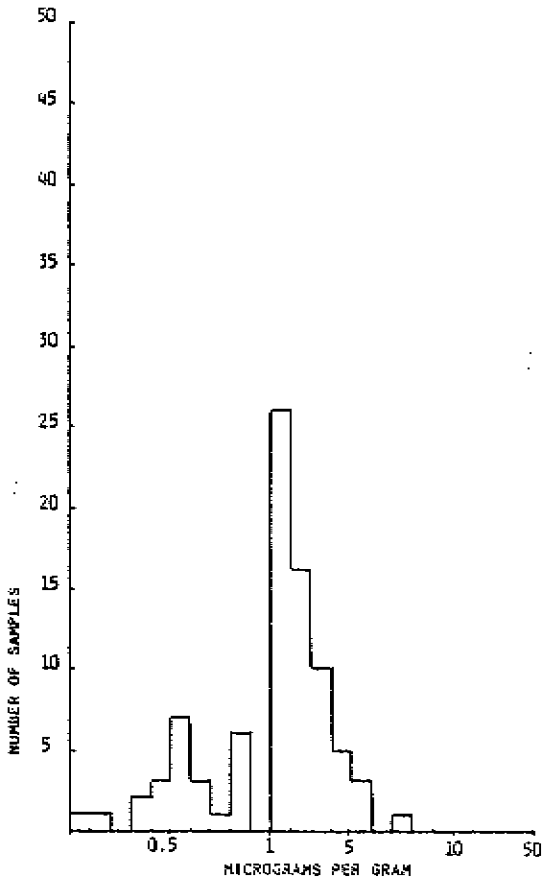

Fig.3(A) Frequency distribution of hair mercury levels in Yugoslav population group (N = 84). <u>Total Mercury.</u>

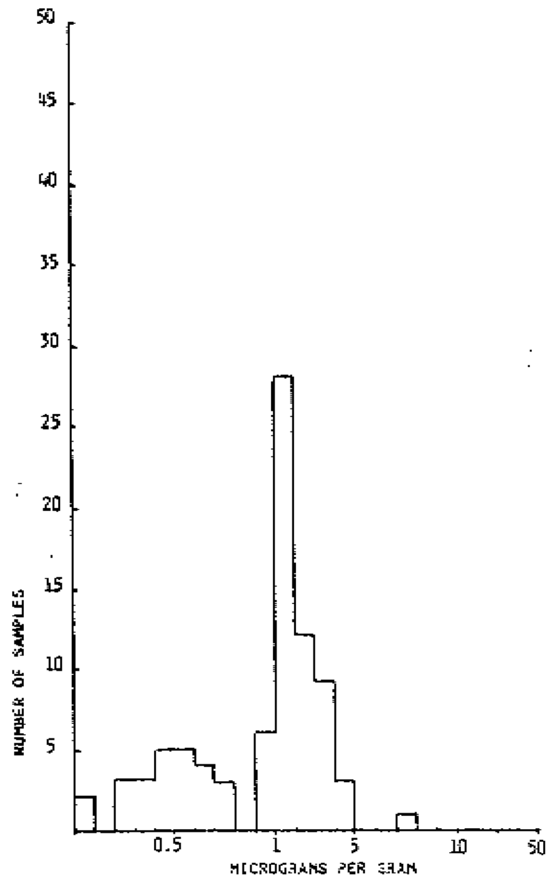

Fig.3(B) Frequency distribution of hair mercury levels in Yugoslav population group (N = 84). <u>Methylmercury.</u>

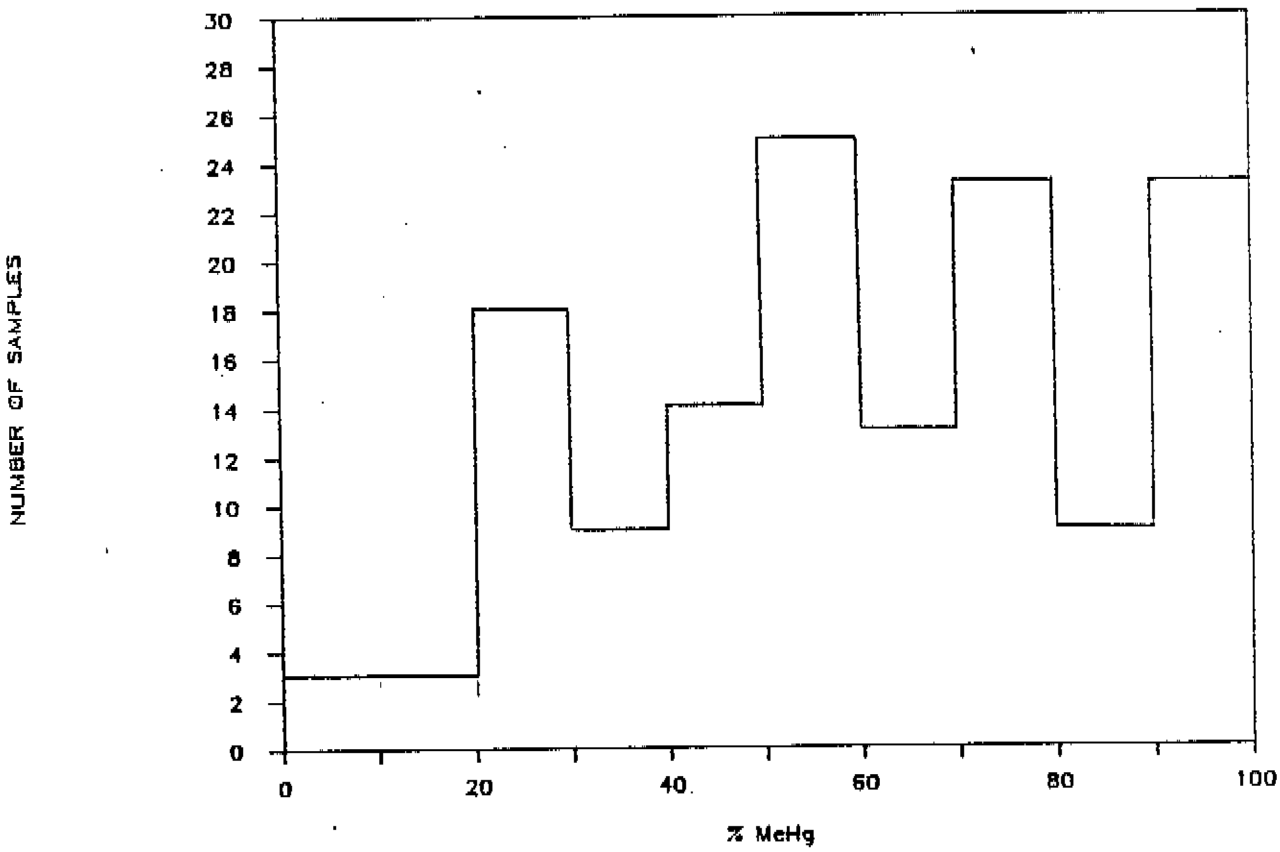

Fig.4 Frequency distribution of methylmercury percentage of total mercury hair content in Greek population group (N = 140).

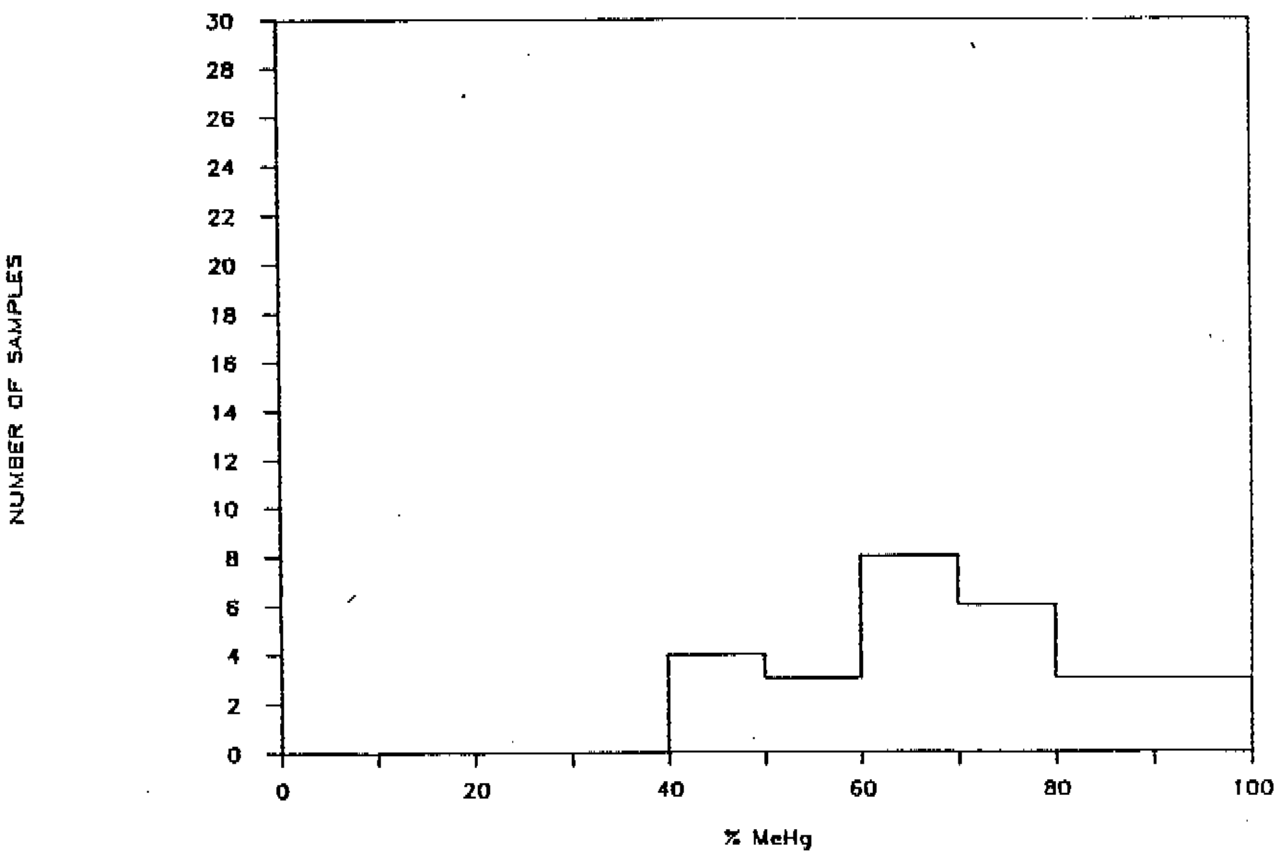

Fig.5 Frequency distribution of methylmercury percentage of total mercury hair content in Italian population group (N = 26).

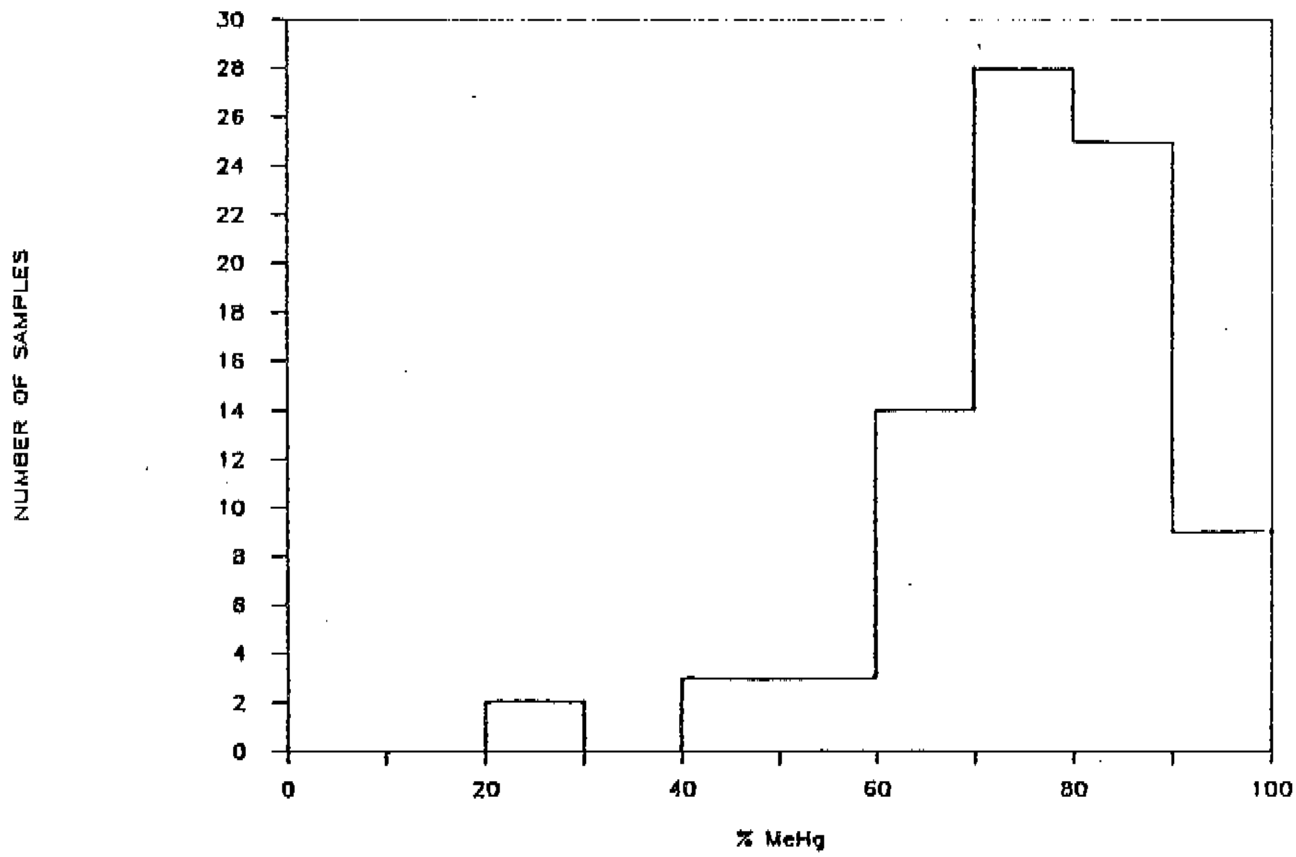

Fig.6 Frequency distribution of methylmercury percentage of total mercury hair content in Yugoslav population group (N = 140).

Annex 5

LIST OF PARTICIPANTS

TEMPORARY ADVISERS

Dr Abdel Aziz el Shafei
Toxicology Department, Imbaba Centre, Ministry of Health, Cairo, Egypt.

Professor Dr R. Buzina
Institute for Endocrinology, Metabolic Diseases and Diabetes "V. Vrhovac", Medical Faculty, University of Zagreb, Zagreb, Yugoslavia.

Dr Kornelia Suboticanec-Buzina
Insitute of Public Health of SR Croatia, Zagreb, Yugoslavia.

Professor R. Capelli
Istituto di Chimica Generale, Università di Genova, Genoa, Italy.

Dr A. Chalkias
Athens School of Hygiene, Department of Nutrition and Biochemistry, Athens, Greece.

Professor R. Gigioli
Istituto di Medicina del Lavoro, Clinica del Lavoro L. Devoto, Milan, Italy.

Dr E. del Guidice
Cattedra di Clinica Pediatrica, 2° Facoltà di Medicina e Chirurgia, Università degli Studi di Napoli, Naples, Italy.

Ms Milena Horvat
Univerza E. Kardelja, Institut "Josef Stefan", Ljubljana, Yugoslavia.

Mr G. Kamizoulis
Sanitary Environment Protection Division, Ministry of Health and Welfare, Athens, Greece.

Dr Mohamed Najib Loubaris
Chief, Toxicology Department, National Institute of Hygiene, Rabat, Morocco.

Dr F. Martinez Saura
Environmental Health Service, National Institute of Health, Madrid, Spain.

Professor J. Micheloyiannis
Department of Neurology, University of Crete, Iraklion, Crete.

Dr Vincenzo Minganti
Università di Genova, Facoltà di Farmacia, Istituto di Chimica Generale, Genoa, Italy.

Professor B. Momcilovic
Institute for Medical Research and Occupational Medicine, Zagreb, Yugoslavia.

Dr Sheena Nakou
Institute of Child Health, Aghia Sophia Children's Hospital, Athens, Greece.

Professor J. Piotrowski
Department of Toxicological Chemistry, Institute of Environmental Research and Bioanalysis, Lodz, Poland.

Professor Antonia Polychronopoulou-Trichopoulou
Athens School of Public Health, Department of Nutrition and Biochemistry, Athens, Greece.

Dr E. Polychronopoulos
Athens School of Public Health, Department of Nutrition and Biochemistry, Athens, Greece.

Professor A. Renzoni
Environmental Biology Department, Siena University, Siena, Italy.

Dr V. Silano
Director, Laboratory of Comparative Toxicology and Ecotoxicology, Istituto Superiore di Sanità, Rome, Italy.

Dr Peter Stegnar
Head, Nuclear Chemistry Section, E. Kardelja Institute, "Josef Stefan" Institute, Ljubljana, Yugoslavia.

Mr Y. Thibaud
IFREMER - Centre de Nantes, Département Environnement et Recherches Océanoques, Nantes, France.

Professor G. Tomassi
Istituto Nazionale della Nutrizione, Rome, Italy.

Dr D. Yeroukalis
Consultant to the Minister on Mental Health, Ministry of Health and Welfare, Athens, Greece.

REPRESENTATIVES OF OTHER ORGANIZATIONS

Food and Agriculture Organization (FAO)

Dr G.P. Gabrielides
Senior Fishery Officer (Marine Pollution), Coordinating Unit for the Mediterranean Action Plan, Athens, Greece.

Intergovernmental Oceanographic Commission (IOC)

Dr A. Jernelov
Director of Research, Swedish Institute for Environmental Research, Stockholm, Sweden.

International Atomic Energy Agency (IAEA)

Mr E. Cortez Toro
Department of Research and Isotopes, Division of Life Science, Vienna, Austria.

United Nations Environment Programme

Dr F.S. Civili
Marine Scientist, Coordinating Unit for the Mediterranean Action Plan, Athens, Greece.

Dr L. Jeftic
Senior Marine Scientist, Coordinating Unit for the Mediterranean Action Plan, Athens, Greece.

WORLD HEALTH ORGANIZATION

Regional Office for Europe

Dr D. Kello
Consultant, Toxicology, Environmental Health Service.

Dr L.J. Saliba
Senior Scientist, WHO/EURO Project Office, Coordinating Unit for the Mediterranean Action Plan, Athens, Greece.

Headquarters

Professor M. Berlin
Monitoring and Assessment Research Centre (MARC), London, United Kingdom.

Dr T. Kjellström
Epidemiologist, Prevention of Environmental Pollution, Division of Environmental Health.