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ASSESSMENT OF ENVIRONMENTAL EXPOSURE TO MERCURY IN SELECTED HUMAN POPULATIONS AS STUDIED BY NUCLEAR AND OTHER TECHNIQUES

Report on the Third Research Co-ordination Meeting

Monaco, 6-10 June 1994

INTERNATIONAL ATOMIC ENERGY AGENCY

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NAHRES-27, IAEA, Vienna (1995)

A report prepared by the IAEA's
Section of Nutritional and Health-Related Environmental Studies
Division of Human Health
Department of Research and Isotopes

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ABSTRACT

A Co-ordinated Research Programme (CRP) on assessment of environmental exposure to mercury in selected human populations as studied by nuclear and other techniques was initiated by the IAEA in 1990. The purpose of this CRP is to promote national and regional studies to evaluate the exposure of selected population groups to mercury and methylmercury and to estimate potential risks in these groups. The programme is focused on the analysis of human head hair for the determination of mercury and methylmercury. The CRP has two main components: (i) identifying population groups that are at risk, and ii) studying health effects in the exposed persons, particularly pregnant women and the babies born to them. This document reports the discussions held during the third Research Co-ordination Meeting (RCM) for the CRP which took place at the IAEA, Monaco Laboratory.

I. INTRODUCTION

The toxicity of high levels of methylmercury (MeHg) on the human nervous system has been well-established. More recently, there has been additional concern over MeHg, due to the recognition that prenatal exposure at previously considered "low" levels of this mercury species can affect the developing nervous system. Of special concern are populations with diets rich in fish, since MeHg can be concentrated to high levels in fish. For these reasons, monitoring the levels of forms of mercury in many population groups has become important. The use of hair analysis for such monitoring has proven to be an effective method, due to the bio-concentration of MeHg in hair and the relative ease of sample collection, as compared to blood samples. Hair samples also provide an integrated history of mercury exposure over months, or even years, in contrast to blood samples whose mercury concentration reflects only very recent exposures.

Although methods for the determination of MeHg were previously unavailable in many countries and have not been simple and straightforward, it appears to be of great advantage to distinguish in hair analytically between MeHg, believed to reflect incorporation of mercury by humans through food and other sources, and total Hg, which may also be partly an external contaminant. The utility of such measurements and the establishment and validation of methods for determining MeHg in hair have been the core of an IAEA co-ordinated research programme, "Assessment of Environmental Exposure to Mercury in Selected Human Populations as Studied by Nuclear and Other techniques". The purpose of this CRP is to promote national and regional studies to evaluate the exposure of selected population groups to mercury and methylmercury and to estimate potential risks in these groups. The programme is focused on the analysis of human head hair for the determination of mercury and methylmercury.

Two components of this CRP were foreseen. The main component (the "core programme") is focused on identifying population groups that are at risk. This is being done mainly by hair analysis. In addition, various kinds of "supplementary" studies are also being done. For example, some participants are analyzing fish and other seafood that could be contributing a significant amount of methylmercury to the diet.

The third Research Co-ordination Meeting was held at the Agency's Laboratory in Monaco from 6-10 June 1994. The participants presented summaries of their work since the last RCM, which was held in August of 1992. The meeting in Monaco presented an opportunity to discuss recent results and exchange information on the different analytical methods being utilized. The meeting was attended by all nine research contract or agreement holders, in addition to one observer and four of the Agency's staff members. A complete list of the participants and the agenda of the meeting are given in the pages immediately following this introduction.

There were several important outcomes of the meeting. First, from the individual reports on the status of each project within the CRP, a network of laboratories now exists that can reliably determine MeHg in human hair samples for risk assessment purposes, showing that this technology is feasible and can be transferred worldwide. A further result of the programme is that risks may be confined to a few specific population groups, but that neonatal concentrations from significant numbers of the populations being studied may exceed levels that are currently believed to constitute a risk for fetal neurological development, although the mothers do not fall into the risk groups according to their hair levels, as currently defined.

The individual working papers that were presented at the meeting are given in Part II, and are listed in the table of contents. A number of seminar presentations were also made at the meeting, either by invitation or were proposed by the participants. In general, most of the workshop presentations dealt with specific problems that the participants have encountered in their research, experience obtained in the applications of analytical techniques or other subjects related to the CRP objectives. A list of these presentations is given at the end of the agenda.

Included in the seminar presentations was a report by the technical officer on the preparation of the IAEA human hair intercomparison materials, IAEA-085 and IAEA-086. In addition, a summary of the preliminary analytical results on Hg and MeHg by the CRP participants was given. It was agreed that the analysis of the intercomparison materials would be finalized and submitted before the intercomparison deadline, at the end of November 1994.

After the presentations of the working papers and seminars, there was a time for extensive discussions among the participants, as well as some practical demonstrations in the laboratory. The discussions were guided by, but not limited to, a list of the discussion topics that was distributed at the meeting. The list of discussion topics, as well as the summary of the discussions, are given as the final sections of Part I of this report.

All the participants at the meeting would like to express their warmest appreciation to the staff at IAEA's Monaco Marine Environmental Laboratory, especially Dr. Milena Horvat, for the excellent arrangements for the meeting. The help and hospitality that were given enabled the meeting to be successful.

RESEARCH CO-ORDINATION MEETING (RCM) ON
ASSESSMENT OF ENVIRONMENTAL EXPOSURE TO MERCURY
IN SELECTED HUMAN POPULATIONS AS STUDIED
BY NUCLEAR AND OTHER TECHNIQUES
MONACO, 6-10 JUNE 1994

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RESEARCH CO-ORDINATION MEETING (RCM) ON
ASSESSMENT OF ENVIRONMENTAL EXPOSURE TO MERCURY
IN SELECTED HUMAN POPULATIONS AS STUDIED
BY NUCLEAR AND OTHER TECHNIQUES

AGENDA

MONDAY, 6 JUNE 1994

08:45 - 09:00 **Registration**

09:00 - 09:15 **OPENING**

Welcome *Dr. M. Baxter*
Director
Marine Environmental Laboratory, Monaco

09:30 - 12:00 **SESSION 1:** Chair: *M. Horvat, S. Stone*

Adoption of the agenda

Status report on the Co-ordinated Research Programme and administrative arrangements for the meeting
(S. Stone)

PROGRESS REPORTS (presentation of working papers)

Brazil	M. Vasconcellos
Chile	C. Bruhn
China	Chai Chifang
Czech Republic	K. Kratzer/P. Benes

13:30 - 17:30 **SESSION 2:** Chair: *Chai Chifang, K. Kratzer*

PROGRESS REPORTS (presentation of working papers)
(continuation)

India	S. Gangadharan
Italy	G. Ingrao
Malaysia	S. Sarmani
Slovenia	A. Byrne
Vietnam	Tac Anh Nguyen

17:30 **Reception**

TUESDAY, 7 June 1994

09:00 - 12:00 **SESSION 3:** Chair: *G. Ingrao, S. Sarmani*

SEMINARS (See separate list)

13:30 - 17:30 **SESSION 4:** Chair: *A. Byrne, C. Bruhn*

GENERAL DISCUSSION
(See separate list of discussion topics)

WEDNESDAY, 8 June 1994 Laboratory demonstrations/discussion

THURSDAY, 9 June 1994 Laboratory demonstrations/discussion

FRIDAY, 10 June 1994

SESSION 9: Chair: *M. Vasconcellos, Tac Anh Nguyen*

08:30 - 10:30 Continuation of Discussions

10:00 - 11:00 Special Seminar: Nuclear Research and Development Programme of India - *Dr. R. Chidambaram, Chairman, Atomic Energy Commission, India*

11:00 - 12:30 Final discussions
Report of the meeting

CLOSING OF THE MEETING

14:00 - open end Personal discusions

LIST OF SEMINAR TOPICS

1.	M. Horvat	IAEA-MEL's reference materials for organomercury compound analysis
2.	R. Zeisler	Operating procedures and quality management in an analytical laboratory
3.	S. Stone	Preparation of a human hair intercomparison material for total mercury and methylmercury/progress report on results submitted by CRP participants.
4.	S. Gangadharan	The role of hair as an indicator of environmental exposure
5.	A. Byrne	Contribution to the intake of inorganic mercury from dental amalgams

CO-ORDINATED RESEARCH PROGRAMME ON ASSESSMENT OF ENVIRONMENTAL EXPOSURE TO MERCURY IN SELECTED HUMAN POPULATIONS BY NUCLEAR AND OTHER TECHNIQUES

REPORT ON THE THIRD CO-ORDINATED RESEARCH MEETING MONACO, 10-14 JUNE 1994

HIGHLIGHTS FROM INDIVIDUAL PROJECTS

Brazil (CSI Vasconcellos)

Project: Determination of Total Mercury and Methylmercury in Human Head Hair by Radiochemical Methods of Analysis

The most important achievement of the project was to identify population groups (several Indian tribes) living at a reservation in the Amazon region (Xingu park) with very high amounts of mercury in hair as compared to a control group. These tribes could be at risk as regards mercury contamination, and the region deserves further study with regard to possible sources of contamination, such as fish, water and air. Also important to the success of the project was the participation in intercomparison exercises, in order to validate the analytical methodology.

Chile (CSI Bruhn)

Project: Total Mercury and Methylmercury in Pregnant Women, Nursing Women, and Preschool Children Residents of Fisheries in the Eighth Region of Chile

Important achievements of the project have included:

1. The establishment and validation of analytical methodology for total mercury and methylmercury determination human head hair.
2. The site selection and conduct of two surveys, which consisted of the collection of scalp head hair from pregnant and nursing women, and accompanying information in the form of questionnaires. The hair samples in the study group were collected from 153 women from 11 fishing villages located within the coastal zone of the eighth region of Chile, and from 26 women in a control group. The mean total Hg concentration in the hair of the study group was 1.81 ± 1.52 mg/kg (range 0.14 - 9.72 mg/kg), which was significantly higher than the level obtained in the control group, 0.42 ± 0.15 mg/kg (range: 0.20 - 0.79). These results were characterized according to geographical location of the fishing villages, frequency of fish and seafood consumption, residence period in the same fishing village, and age of the women. By using statistical tests, (ANOVA, Tuckey), significant differences ($p<0.05$) were confirmed in at least six of the fishing villages in 1991, seven in 1992-1993, and nine in the combined 1991-1993 period. All of these fishing villages are located within the more polluted coastal zone of the Eighth Region of Chile. In particular, the relatively high total mercury found in five villages apparently indicate high risk populations with regards to mercury exposure through diet. In the defined zones, there was a significant difference between the total mercury in the hair of women who consumed seafood more than once a week compared with those who consumed less seafood.
3. Scalp hair and blood samples of the pregnant women in the identified high risk group are being collected as well as from a corresponding control group. These samples will also be analysed for methylmercury. The results for total and methylmercury will be examined for

possible correlation between concentration in hair and blood. Also planned is to obtain hair and blood (or umbilical cord blood) of newborns to the women in the study.

China (CSI, Chai Chifang)

Project: Study on total and methylmercury levels in human scalp hair of lying-in women and newborns by NAA and other techniques

Scalp hair samples have been collected from 1179 fishermen living at a typical polluted region in Northeast China, and from 27 lying-in women and their newborns in Beijing. Total and methylmercury have been determined by INAA, GCEC and other techniques. The longitudinal mercury patterns of the lying-in women hair samples obtained by INAA and synchronous radiation-based x-ray fluorescence spectrometry show a gradually decrease of Hg during pregnancy. Further, the mercury contents of the hair samples from newborns are generally above or close to those of their mothers, which confirms the mechanism that the methylmercury is readily able to penetrate the placental barrier and is accumulated in the fetus.

Czech Republic (CSI, Kratzer)

Project: The study of chemical forms of mercury in human hair and other bio-environmental samples

During this project two separation procedures were developed, employing radiotracers, that were suitable for the determination of methylmercury and inorganic mercury in human hair. One procedure is based on the extraction of methylmercury iodide from dissolved hair using benzene. The second procedure uses selective leaching of the methylmercury with 2M HCl at the volume/mass ratio of 40 mL/g for 4 h. The separation procedures were coupled with the determination of mercury in hair or extracts after separation of the organic and/or inorganic mercury, using a single purpose atomic absorption spectrometer AMA-254. Good reproducibility of the determination of mercury was found in several samples of hair and in fish (IAEA-350 and IAEA MA-B-3). There was also good agreement between the results of the different procedures, and of the reference materials with the recommended values. The good agreement has provided evidence that the basic assumptions of the radiotracer method applied in the development of the analytical procedures were correct.

India (CSI, Gangadharan)

Project: Preparation of standard hair material and development of analytical methodology

A batch of 65 kg of human scalp hair have been procured, from a place of worship. This material has been physically cleaned and washed by standard procedures to obtain 10.5 kg of clean hair. Ten kg of the hair in two cartons was radiation sterilized to 50 kGy and shipped to IAEA, Vienna. This material was used to prepare two intercomparison materials to be used for quality assurance in hair analyses for total and methylmercury. In addition, an analytical methodology was developed for the determination of total Hg (CVAAS, INAA) MeHg (GC-ECD, following alkaline digestion), along with additional elements (Se, Zn, Cd, and Co) by spectrochemical and electrochemical methods. A study of mercury in hair samples from different population groups did not show higher levels of Hg than those of control groups. Finally, the stability of Hg content in hair has been assessed over a two year period, with no statistically significant changes being shown.

Italy (CSI, Ingrao)
Project: Mercury levels in defined population groups

Four population groups having a fish consumption above the national average were selected from coastal towns in Southern, Central, and Northern Italy. The range of Hg concentrations in hair was 0.23 - 28.5 mg/kg and the median was 3.7 mg/kg, significantly higher than the values found for the general population. The highest values were observed in the subjects living near the lagoon of Merano and Grado, with a median value of 5.1 mg/kg. This location has high natural levels of Hg, due to the discharge of the Isonzo river, a tributary of which crosses the mercury-rich area of Idria in Slovenia. The results obtained for a group of pregnant women and their newborns show that mercury is readily transferred from mother to fetus. The Hg levels in the hair of newborns are equal or exceed those found in the hair of the mothers. Statistically significant correlation coefficients were found for Hg concentrations in hair, pubic hair, placenta and newborn hair. The Se levels in newborns hair was about a factor of 2 higher than in the hair of the mothers. Because of the higher susceptibility of fetuses to adverse health effects caused by Hg exposure during pregnancy, it is suggested to carry out an epidemiological study on babies in critical groups. In fact, some recent studies indicate that developmental effects could appear in babies when the mothers are exposed to mercury levels that are below the current recommended FAS/WHO Provisional Tolerable Weekly Intake (PTWI) of 3.3 μg/kg/week.

Malaysia (CSI, Sarmani)
Project: Intake of mercury through fish consumption

Achievements in this projects include:
1. The establishment and validation of the methodology for total mercury and methylmercury analysis in hair samples, as well as in fish and other environmental samples.
2. To identify population groups with elevated mercury content in hair, as compared to control groups.
3. To create an interest among other scientists to carry out methylmercury analysis.
4. To generate data that can be used by epidemiologists to investigate the possible effect of methylmercury.

Slovenia (CSI, Byrne)
Project: Analytical quality control in the environmental exposure to mercury
Summary of achievements: Advice and expertise in analytical methods appropriate for the determination of total and methylmercury in hair and other samples were provided to participants throughout the project, and particularly in discussions and practical sessions during the research co-ordination meetings. Analytical results were provided to assist in the establishment of the homogeneity of the two hair intercomparison materials IAEA-085 and IAEA-086, and their methyl and total mercury contents, during and after their preparation. In addition, results were provided to participants in batches of samples submitted to the reference laboratory for quality control purposes, though this possibility was not fully taken advantage of by many participants. The laboratory also worked on improving existing methods and the development of new techniques for analysis of total and methylmercury was also an important feature of the work of the reference laboratory, including a modified Westöö method, the isothermal distillation (Convey cell) technique, distillation, and the new

approach of ethylation, allowing simultaneous determination of inorganic and methylmercury.

Viet Nam (CSI Tac Anh Nguyen)

Project: Determination of total mercury and methylmercury in the head hair of pregnant Vietnamese women

The results of the studies undertaken show that the potential health risks of mercury pollution in the Ho Chi Minh city is higher than controls in the south (Dalat, Nha Trang). In Ho Chi Minh city, almost 20% of the samples taken from pregnant women showed mercury concentrations higher than 6 mg/kg, which is above the recommended limit given by FAO/WHO. This is in comparison to the coastal area, where only 5% of the samples were greater than 6 mg/kg, and only 1% in a highland area (Dalat)

RESEARCH CO-ORDINATION MEEETING (RCM) ON ASSESSMENT OF ENVIRONMENTAL EXPOSURE TO MERCURY IN SELECTED HUMAN POPULATIONS AS STUDIED BY NUCLEAR AND OTHER TECHNIQUES

LIST OF DISCUSSION TOPICS

PURPOSE AND SCOPE OF THE CRP

1. The Core Programme

   1.1 Does the Group agree with the present definition of the core programe?

   1.2 If not, how should the statement of the core programme be modified?

2. The Supplementatry Programme

   2.1 Does the Group agree with the present definition of the supplementary programme?

   2.2 What are the priorities among the various topics?

   2.3 Are there any other topics that should be included?

3. What are the expected benefits of this research?

   3.1 For the participating countries?

   3.2 For science in general?

TECHNICAL ASPECTS

(The following questions relate mainly to the core programme. However, their relevance to the supplementary programe should also be discussed if there are any issues of general interest.)

4. Selection of study populations

   4.1 What are the criteria for the selection of sampling sites?

   4.2 How many samples should be collected and over what sampling period?

5. Questionnaire

   5.1 Has the use of the "approved" questionnaire been successful?

   5.2 Are there any changes that should be made?

6. Analysis:

   6.1 What advice is needed on the analytical techniques used in this CRP:

6.1.1 For the determination of total mercury?
6.1.2 For the determination of methylmercury?
6.1.3 For the determination of selenium?

7. Data Processing

7.1 Database management
7.1.1 How are the data being kept, *i.e.* computer database, files, etc.?
7.1.2 Are the data being managed satisfactorily?

7.2 Data evaluation and presentation
7.2.1 What statistical evaluations are being done on:
a) the collected analytical data?
b) the questionnaire data (how is this being summarized?)?
c) the analytical data and the questionnaire data together?
7.2.2 Is further advice needed on any of these points?

8. Quality Assurance

8.1 Quality assurance of sampling - are there any points that need to be discussed?

8.2 Quality assurance of analysis:what procedure are recommended:
8.2.1 For "in-house" use?
8.2.2 As an external quality control scheme?

8.3 Quality assurance of data reporting and evaluation: what procedures are recommended?

8.4 Are the "reference" laboratories (Josef Stefan Institute and Monaco Marine Environment Laboratory) being utilized sufficiently, and for the purposes intended:
8.4.1 As a source of specialized advice?
8.4.2 To assist the collection centers that otherwise do not have sufficient analytical capacity (*e.g.* determination of MeHg)?
8.4.3 For cross-checking selected samples?

9. Intercomparison exercise

9.1 What is the Group's opinion of the prepared materials?

9.2 What are some of the difficulties encountered in the analysis?

10. What should be the final goals for this CRP?

ORGANIZATIONAL ASPECTS

11. Co-operation with others;what suggestions are there for making (or improving) co-operation with others:
11.1 Nationally?

11.2 Internationally *(e.g.* WHO)?

12. Technical co-operation projects and training: are there any suggestions for future activities?

13. Information exchange within the CRP - how can this be best promoted?

14. Publications policy for work done within the framework of the CRP

15. The final RCM: where and when should this be?

RESEARCH CO-ORDINATION MEETING (RCM) ON ASSESSMENT OF ENVIRONMENTAL EXPOSURE TO MERCURY IN SELECTED HUMAN POPULATIONS AS STUDIED BY NUCLEAR AND OTHER TECHNIQUES

SUMMARY OF DISCUSSION POINTS

1. The Core Programme

The Group was reminded of the goals that had been originally defined goals for the CRP, and these were discussed in terms of the results that have been obtained so far. The original goals were to 1) identify groups "at risk" from elevated mercury exposure and 2) study health effects in the exposed persons, particularly pregnant women and babies born to them. For the first goal listed, it is still not clear at what level a group is "at risk" since an actual "at risk" level is still being considered and revised by a World Health Organization (WHO) task group. Currently revised guidelines have stated levels as low as 6 ppm Hg in the hair of pregnant women may constitute a risk for their developing babies.

The Group agreed that the second goal cannot be met without the help of WHO, who for various organizational reasons has not been able to become significantly involved in the programme. None of the participants has any experience in carrying out epidemiological studies, nor has any links to their respective national health organizations.

A revised goal for the CRP was suggested: to define the status of MeHg in selected population areas, from environmental exposure through the food chain. These results should be summarized in a report, even before the final reports are due. The comparable groups at this time are samples from the fishing villages (Brazil, Chile, Malaysia, Italy, Vietnam). A database would also be needed to summarize the results, and participants should submit their results in a suitable format.

2. Supplementary Programme

For the supplementary programme, no universal topic has been specified, since this depends on the research interests of the individual participants. Individual research topics in this area include various dietary analyses, placenta analysis, and the determination of Se in hair and food samples. It was also encouraged, where possible, to collect pubic hair in addition to scalp hair, and compare the results. It was pointed out that this was not possible in the Indian population from Brazil; they have no pubic hair.

3. Expected Benefits of the Programme

The importance of how to promote the results of the programme and how to extend the work once the official CRP is at an end were discussed, and several strategies were suggested. A decision is needed on how to present the collected data, and also how to present the quality of the data. The strategies should be on two levels: 1) at the national level (by the participants) and 2) at the international level (by the Agency). At the international level, several contact possibilities were suggested:1) the Joint UNDP/RCA/IAEA Project on the Use of Isotopes and Radiation to Strengthen Technology and Support Environmentally Sustainable Development: Sub-Project on Nuclear Analytical Techniques; 2) environmental advocates, who would need us to tell them the facts; and 3) WHO. It was pointed out that there were political and economic interests to be considered when starting these contacts. For example, in Brazil, the process of Au extraction is dumping large amounts of Hg into the general environment. Does the economic benefit of the Au extraction to the government outweigh the problems caused by the Hg? In these cases, the

information must be carefully presented, with proper documentation.

On the national level, it was recommended that health organizations should be contacted by participants. Epidemiological studies could then be started on the basis of the results of the studies from the CRP.

Technical Aspects of the CRP

5. Sampling

There were no significant changes to be discussed over the selection of the study populations or study sites; the Group felt that this had been well-defined. The questionnaire was briefly discussed. There was a consensus that one may not be able to put great confidence on the answers given as to current diet, and that the answers depend on the persistence and thoroughness of the health team taking the hair samples.

6. Analysis

For advice on analysis, it was felt that it was important to plan a training course, possibly a regional training course or courses, for the analysis of MeHg, with the emphasis on samples other than for hair, *e.g.* diet samples. The duration of the training course was recommended to be about 3 weeks. It was felt that it would be better to have such a course on a regional basis, so as to concentrate on the practical aspects of solving problems specific to certain areas. It was also stressed that, when planning a regional training course of this sort, it is important to ensure that all materials are available at the selected site, chemicals, instruments, *etc.* It was recommended to make up a checklist of all pertinent items that would be available at the proposed site. Conception, Chile was one possible site that was discussed.

The use of the Convey cells for the separation of MeHg by volatilization was discussed. To provide a supply of these cells, it would be necessary to have a drawing with the proper dimensions in order to obtain quotations. Also, it was indicated that a thick wall is essential for proper construction. A. Byrne (Slovenia) wants to try a modified type of weighing bottle; this would need to be tested.

For total Hg analysis with CV-AAS using a gold trap, it was stressed that self-made gold traps would be much cheaper. M. Horvat (IAEA-MEL) provided a summary describing how such traps can be made from dissolving gold in aqua regia and coating quartz sand with the resulting solution.

In some of the hair samples from Brazil, there is a significantly lower ratio of MeHg to total Hg than expected. The normal ratio is about 80%, but in some of the Indian samples, the ratio was as low as 40-50%. The Monaco laboratory has also found these curious results in a few samples, and it was suggested that there might be some other origin than biological incorporation. Several of the participants have read publications which indicate that inorganic mercury can also penetrate the biological barrier, *i.e.* from blood to hair, probably as neutral forms (HgOH, HgOHCl). In one New Zealand study, there were problems when assuming the constant 0.8 ratio of MeHg to total Hg, when determining only total mercury, because the ratio did vary significantly. The varying Hg/MeHg ratio was also found in the WHO study in the Mediterranean.

In response to discussion on the difficulties of taking blood from newborns in some of the participants' studies, the feasibility of taking placental blood (also known as "cord blood") as the newborn blood samples was discussed. The group from Chile has taken some of these samples, but at the time of the meeting, the samples were not yet analyzed.

7. Data Processing

On the subject of data processing, it was stressed that, for interpretation, one could not assume normal distributions; therefore, distribution-free statistical methods should be employed. Most of the participants are keeping the data in some sort of database programme; the recommended software to be used for collecting the data for the programme is Dbase, QuatroPro, or Excel. It would then be possible to link the data to the data from the questionnaire, which would be in a separate table. It was decided to try to combine only a few of the participant's data at first; G. Ingrao (Italy) and M. Horvat (MEL) will send their data to the technical officer (S. Stone). On the basis of the experience in combining these two datasets, a recommended format will be sent out to the rest of the participants.

For data evaluation and presentation, it was mentioned that the PC program StatGraphics produces very acceptable box plots, as well as performing statistical analyses. Also, StatView for Macintosh computers has been used for graphical presentations and statistical analyses.

8. Quality assurance

For those participants who are exchanging hair samples, or sending samples to the reference laboratory, a minimum sample size of 0.5 g was requested. It was also agreed that it was not necessary to dry the hair prior to sending the samples; the moisture determinations should be made directly before analysis. For the intercomparison samples from Chile, it was agreed to report on a "wet" basis (the samples were packaged in silica gel). If participants are using "in-house" reference materials, it was recommended to send these to the reference laboratory for their analysis. Currently, the participants from China, India, and Slovenia are employing "in-house" reference materials.

The reference laboratory (Ljubljana) encouraged the other participants to send hair samples for check analyses. It was agreed that they could handle about 20 samples per laboratory. It would also be possible to analyze samples other than hair (*e.g.* fish), but this would be done on a bilateral basis, such as authoring a joint paper on the subject, since this was not part of the official role of the reference laboratory.

On the subject of reference materials, the problem of the previous unavailability of hair reference materials was presented as a major stumbling block. This situation however, has now greatly improved. The participant from China gave the information that there were two hair reference materials available from China; one at 2 ppm Hg, and the other at 0.6 ppm Hg. There are also the new IAEA intercomparison materials, and the new NIES material that will soon be issued. These new materials are planned to provide recommended values for MeHg, as well as total Hg. Because of the availability of a variety of reference materials, it was advised to run <u>one</u> particular reference material over a long period of time, interspersed occasionally with supplementary reference materials. In this way, the analyst can obtain a better idea of the stability of their measurement systems, as well as precision and accuracy.

A document containing an interpretation of the ISO guidelines was discussed to see if this would be of interest to the participants. It was stressed that this was only to be used for guidance. For interested participants, this document will be mailed to them following the meeting.

9. Intercomparison exercise

A summary of the preliminary analytical results on Hg and MeHg by the CRP participants was given. There appeared to be no major problems with any of the results; however, there were some apparent discreprencies in the results for MeHg from the reference laboratories in the elevated level hair material that seemed to be method-dependent, and need further investigation. The CRP participants agreed that they would finalize their analyses of the intercomparison materials and would submit them before the intercomparison deadline, at the end of November 1994.

10. Final Goals of the CRP

It was decided that it was of great importance to establish a laboratory system of quality assurance. This is necessary to show that the proper methods are in place to be able to validate the data that have been collected so far in the CRP. One possible way to accomplish this is participation in the proposed UN Project for the Development of Regional Centers for Analytical Quality Assurance, which is being proposed by R. Zeisler, IAEA-Seibersdorf. Participation in such a programme, if it is approved, would be a way to extend the associations with this CRP after it officially ends. This could be the first set of candidate laboratories, and a way to start a system of training. R. Zeisler distributed the draft proposal to the CRP participants, and asked for comments.

For additional information on Quality Assurance, the Chemistry Unit at Seibersdorf has a Quality Assurance Manual that will soon be in print. When it is finalized and printed, it can be distributed to CRP participants as long as it is realized that it is only an example document; such a manual has to be personalized for each laboratory. M. Horvat has another example QA document, "Reference Methods from the Regional Seas Programme, on Contaminant Monitoring", which will be sent to participants.

It was stressed that quality assurance depends on individual laboratory motivation. The quality assurance "stamp of approval" from the ISO 9000 and similar ISO documents require only that the proper checks and balances of management, *etc.* be in place.

Organizational Aspects

Co-operative efforts have already been discussed, both nationally and internationally, as have suggestions for possible training courses and co-operative projects. It was proposed that the final report of the CRP be issued as an IAEA Technical Document. The participant from Chile also suggested that a publication on the summary of analytical methods for hair analysis be produced.

The site for the final RCM was not definitively decided upon, as there were no volunteers to hold the meeting. The approximate time for the meeting will be at the end of 1995. There were suggestions to again have the RCM with a training component incorporated, or that the meeting could be connected with the next EPRI meeting on mercury. M. Horvat will try to obtain more information on this meeting, although this alternative is not favored due to increased organizational difficulties, and that the RCM would be delayed until 1996.

DETERMINATION OF TOTAL MERCURY AND METHYLMERCURY IN HUMAN HEAD HAIR BY RADIOCHEMICAL METHODS OF ANALYSIS

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Abstract

Total mercury has been determined by instrumental neutron activation analysis in the hair of several Indian tribes living in the Xingu Park, located in the Amazonic region of Brazil. Methylmercury and total mercury have been determined in selected samples using cold vapour atomic absorption spectroscopy, at the Nuclear Chemistry Department, Jozef Stefan Institute, Ljubljana, Slovenia. Mercury levels were found to be much higher in the Indian hair samples as compared to the samples from the control population. The arithmetic and geometric means for total mercury in the Indian hair samples ranged from 10 to 20 ppm, compared to values of about 1 ppm for the means of the control group. The results obtained for methylmercury have shown that the majority of the mercury is present in the hair of the Indians as the organic form. The Indian study populations living in the Xingu Park can thus be considered as being at risk with regards to contamination by mercury. With the aim of applying neutron activation analysis for the determination of methylmercury in hair, experiments were done at the IEA-R1 nuclear research reactor irradiating cysteine- and also thioacetamide-impregnated filter papers, on which a methylmercury solution was pipetted. The results obtained have shown that all the mercury was lost from the cysteine-impregnated paper and about 90 % of the mercury remained on the paper impregnated with thioacetamide.

1. INTRODUCTION

As described in the last progress report, three main Brazilian population groups were the object of our study:

(1) A control group of 38 subjects with no suspicion of contamination by mercury (friends, colleagues and students from the University of São Paulo).

(2) A group of 28 people living near the Billings Dam, located in one of the most heavily industrialized parts of the country where there is possibility of pollution by chloralkali and other industries. This group consumes fish caught at the Dam without much control from public health authorities.

(3) Indian tribes living at the Xingu Park, located in the Amazonic region, where the gold extraction activities have caused much concern due to the extensive use of mercury in the extraction process.

The results obtained for analysis of mercury in hair of these groups by instrumental neutron activation analysis have shown that the hair samples from the Indians from three tribes (Suiá, Uaurá and Panará) contained very high amounts of mercury, as compared to the control group. The averages obtained for these tribes were about 9 to 18 times higher than the controls.

Samples of hair from the Suiá tribe were also sent to Jozef Stefan Institute in Ljubljana, Slovenia, for analysis of methylmercury. The results obtained have shown that most of the mercury in the hair of the Indians is present as methylmercury (70 to 100% MeHg, with an average of 89%). The population group living near the Billings Dam, on the contrary, showed an average amount of mercury in hair lower than the controls.

Considering the overall results obtained, it was concluded that the Indian tribes living in the Xingu Park could constitute a group at risk with regards to contamination by mercury and methylmercury. This was quite surprising according to the group of physicians from the São Paulo School of Medicine, who are collecting the hair samples, since the region of the park was considered up until this point as being free from mercury contamination because it is far from the sites of intensive gold exploration in the Amazonic region.

Lacerda and Pfeiffer [1] have carried out a study on mercury contamination arising from gold mining in the Amazon environment and have shown that the mercury concentrations in Amazonian fishes are, in various sites, nearly five times the maximum permissible levels for human consumption. This data confirms the importance of carrying out this kind of study in Brazil, because of the possibility of increased mercury ingestion in the population groups consuming high amounts of fish caught in the Amazonic rivers.

2. METHODS

2.1. Collection and preparation of hair samples

In this phase of the study, hair samples were collected from the following groups of Indians living in the Xingu Park:

(1) Coicuro Tribe (4th Group) - 46 samples collected.
(2) Matipu Tribe (5th Group) - 11 samples collected.
(3) Pavuru Tribe (6th Group) - 44 samples collected.
(4) Juruna Tribe (7th Group) - 49 samples collected.

All the samples were collected and washed according to the procedure recommended by the IAEA [2].

The three first groups studied were: Suiá Tribe, Uaurá Tribe and Panará tribe, of which the results for total mercury (for all three groups) and methylmercury (for the Suiá Tribe) were presented in the last report.

2.2. Determination of total mercury in hair samples of the Indians from the Xingu Park by instrumental neutron activation analysis

About 100 to 200 mg of the prepared hair samples and of the reference material Chinese human hair, SHINR-HH, were weighed in polyethylene envelopes previously washed with diluted nitric acid and deionized water. For each set of five samples, one reference material was analyzed. The standards were prepared by pipetting about 1 μg of mercury in the nitrate form onto sheets of Whatman-40 filter paper previously impregnated with a solution of thioacetamide to prevent mercury losses by volatilization before and during irradiation, as recommended by Noguchi *et al.* [3].

Irradiations were carried out for a period of one hour, in a pneumatic station, under a thermal neutron flux of about 10^{12} $n \cdot cm^{-2} \cdot s^{-1}$. After a decay period of about 70 hours, samples, reference materials and mercury standards were measured in a GMX 20195 ORTEC Ge detector, with a resolution of 1.9 keV in the 1332 keV peak of ^{60}Co. The detector is coupled to an ADCAM 918A Multichannel Buffer and associated electronics. Spectrum analysis was performed by means of VISPECT2 software, developed by D. Piccot, from Saclay, France [4]. For calculation of mercury concentrations, the 77 keV peak of ^{197}Hg ($t_{1/2}$ = 64.1 h) was used.

2.3. Determination of methylmercury in hair samples of the Indians from the Xingu Park

From the second group of Indians analyzed (Uaurá Tribe), whose results for total mercury were presented in the last report (n = 18), ten samples were sent to the Nuclear Chemistry Department of the Jozef Stefan Institute (Ljubljana, Slovenia) for analysis of methylmercury by cold vapour atomic absorption spectroscopy. Total mercury was also analyzed in these samples.

From the sixth group of Indians (Pavuru Tribe), of which 44 hair samples were collected, 20 were analyzed at IPEN for total mercury and 24 were sent to Ljubljana for analysis of total mercury and methylmercury.

The hair samples of the seventh group (Juruna Tribe) are currently being analyzed for total mercury by INAA at IPEN.

2.4. Determination of selenium in hair of some of the population groups studied

As stated in the first report of the CRP on analysis of mercury in hair of selected human populations, selenium is, besides mercury, an element of interest to the programme. Apparently, it protects animals against the toxic effects of methylmercury and alters the tissue distribution and excretion of methylmercury and the inorganic to methylmercury ratio in tissues. This fact is due to the high affinity of methylmercury cations to selenides and diselenides.

In view of the fact that high amounts of mercury were found in the hair of the Indians from the Xingu Park although no symptoms of mercury intoxication could be

detected in these populations, it was decided to start in this project some analysis of selenium in hair by instrumental neutron activation analysis. The groups studied up to now were: control group, group of the Billings Dam and the first group of Indians (Suiá Tribe).

Hair samples already analyzed for total mercury, and the hair reference material SHINR-HH were irradiated for 90 seconds at the IEA-R1 nuclear research reactor, under a thermal neutron flux of 4×10^{11} $n \cdot cm^{-2} \cdot s^{-1}$ together with selenium standards. The selenium standards were prepared by pipetting about 120 g of selenium in the nitrate form onto sheets of Whatman-40 filter paper. After a decay time of about 30 seconds, samples, reference material and standard were measured for 90 seconds in the γ-ray spectrometer already described in Section 2.2. The short-lived radioisotope ^{77m}Se, with a half-life of 17.5s was used for the selenium calculations.

2.5. Experiments of determination of methylmercury by neutron activation analysis

Since no equipment, either of atomic absorption or gas chromatography is available at our department for methylmercury analysis, a nuclear method was selected for the analysis. The method would be based on extraction of MeHg-Cl in toluene followed by back extraction in filter paper impregnated with cysteine, as described by Horvat and Byrne [5]. The paper containing MeHg in cysteine could then be irradiated together with mercury standards, in the same way as described for INAA of total mercury in hair (Section 2.2). Since one of the problems in the irradiation of mercury compounds is the loss of the element by the effect of the radiation, it was decided to start the experiments by this last step.

A 200 ppm methylmercury solution was prepared by diluting a standard solution of methylmercury chloride acquired from Johnson Matthey (1000 ppm). As stated in the paper of Horvat and Byrne [5], MeHg is extracted into aqueous cysteine solution only at neutral pH. So, a buffered solution with pH 7.00 was prepared by dissolving 21.20 mg of cysteine in a small amount of HCl (diluted 1:1) and adding a buffer of potassium diphosphate and sodium monophosphate.

An aliquot of 50 μL of the diluted MeHg-Cl solution, corresponding to about 1 g of Hg was pipetted onto a sheet (2 x 2 cm) of Whatman-40 filter paper previously impregnated with 50 μL of either the buffered cysteine solution (described above) or a thioacetamide solution (about 0.1 g thioacetamide dissolved in 50 mL water). The filter papers were irradiated for 1 h under a thermal neutron flux of about $10^{12} n \cdot cm^{-2} \cdot s^{-1}$ together with the Chinese Hair Standard, SHINR-HH-1 (GBW 09101).

3. RESULTS

3.1. Analysis of Reference Materials

The analysis of a group of 10 samples of the Chinese Hair Reference Material, SHINR HH-1, yielded an average of 2.15 ppm of Hg, with a relative standard deviation of 9.1% and a relative error of 0.46% as compared to the certified value of 2.16 - 0.21 ppm.

As to the analysis of selenium in the same reference material, the average obtained for six determinations was 0.60 ppm Se with a relative standard deviation of 21% and a

relative error of 3.4%. This average Se value is in close agreement to the certified value of 0.58 ± 0.05 ppm.

3.2. Analysis of hair samples

Table I presents the results obtained at the Jozef Stefan Institute for analysis of total mercury and methylmercury in Indian hair samples from the second group studied (Uaurá Tribe). These samples have previously been analyzed at IPEN for total mercury, and these results were presented in the last progress report. The results of the first and third groups studied were also presented in the last report.

Table II shows the results for total mercury in hair of the fourth group studied (Coicuro Tribe). The results were obtained at IPEN by INAA.

Table III presents the results for total mercury in hair of the fifth group of Indians (Matipu Tribe), also obtained by INAA.

In Table IV are the results for total Hg obtained by INAA of 20 samples of the sixth group of Indians (Pavuru Tribe). This group comprises of 44 samples in total, of which, 24 were sent to the Jozef Stefan Institute (Ljubljana, Eslovenia) for analysis of total mercury and methylmercury by CVAAS.

Table V presents the results obtained for selenium by INAA in hair samples of 16 individuals from the control group. The very high results obtained for samples IQ3 and TFR9 were not considered for average calculations.

Table VI presents similar results for 15 individuals living near the Billings Dam, a highly industrialized region and whose results for total mercury have been previously presented. The very high result obtained for sample B23 was not considered for average calculations.

Table VII presents the concentrations of selenium obtained by INAA in the sample of the first group of Indians (Suiá Tribe) studied. The results of total mercury obtained by INAA for this group have been previously presented.

In Table VIII, a summary is presented of all the results obtained in this period of the project for total mercury in hair of individuals from three Indian tribes (4th, 5th, 6th group) and for total mercury and methylmercury in the Uaurá Tribe (2nd group).

Table IX presents a summary of the results obtained for the analysis of selenium by INAA, for the control group, the group of the Billings Dam and for the Suiá Tribe (first group) of the Xingu Park.

4. DISCUSSION

The results of the analysis of 10 samples of the Chinese Hair Reference Material, SHINR-HH-1, of 2.15 ppm of total Hg, showed good agreement with the certified value of

2.16 - 0.21 ppm. The relative standard deviation was 9.1%.

The analysis of total mercury in the hair of four Indian tribes (Coicuro, Matipu, Pavuru and Uaurá) showed that the arithmetic means, geometric means and medians obtained were much higher than the corresponding values for the controls (around 1 ppm), which were presented in the last report.

In Table VIII, it can be noted that the mean value of total mercury in the samples from the Coicuro, Matipu and Uaurá Tribes ranged from about 10 to 13 ppm and for the Pavuru Tribe, around 20 ppm.

The results obtained at the Jozef Stefan Institute for MeHg in the Uaurá Tribe showed that most of the mercury contained in the hair of these Indians is present as MeHg (about 80%, on average).

Together with the values for Hg and MeHg presented in the last report, it can be concluded that all the Indian Tribes in Xingu Park studied up until now could be at risk with regards to increased exposure to mercury. It could be concluded that this contamination could arise from consumption of fish caught in the rivers of the Xingu Park, since these populations consume fish almost daily. As stated in the introduction, Lacerda and Pfeiffer [1] have shown that the mercury concentrations in some Amazonian fishes are, in various rivers, nearly five times the maximum permissible ones for human consumption. To further investigate this observation, a more detailed study of several compartments of the Xingu Park, such as water, sediments, aerosols and other foodstuffs consumed by the Indians must be carried out.

As to the analysis of selenium in hair, no significant difference was found among the control Group, the group of the Billings Dam and the Suiá Tribe. The means and medians for this data were very similar; around 0.4 ppm of selenium.

The irradiation experiments carried out for MeHg-Cl solution pipetted on filter paper impregnated with cysteine and thioacetamide showed that in the first case, practically all the mercury was lost. The investigation using paper impregnated with thioacetamide presented better results, as about 89.5% of the Hg pipetted as CH_3HgCl remained on the filter paper after irradiation.

5. PLANS FOR FUTURE WORK

The following scheme is devised for the next period of the Research Contract:

(1) Collection of additional hair samples from Indian tribes living in the Xingu Park.

(2) Preparation of the hair samples collected (cutting, washing, drying) according to the procedure recommended by the IAEA.

(3) Analysis of reference materials.

(4) Analysis of total mercury in the hair samples by instrumental neutron

activation analysis.

(5) Analysis of methylmercury in part of the hair samples by CVAAS, at the Jozef Stefan Institute (Ljubljana, Slovenia).

(6) Experiments for analysis of methylmercury by neutron activation analysis:

- Extraction of MeHg from the samples by hydrochloric acid, as in the method of May *et al.* [6].

- Extraction of MeHg in toluene, twice, as in the method of Horvat and Byrne [5].

- Re-extraction with cysteine solution .

This step can also be substituted by shaking of the toluene phase with cysteine paper. The paper could be irradiated directly for neutron activation analysis.

(7) Analysis of selenium in the hair samples of Indians, via the short lived isotope ^{77m}Se ($t_{1/2}$ = 17.5 s).

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TABLE I. RESULTS OF THE ANALYSIS OF TOTAL MERCURY AND METHYLMERCURY IN HAIR FROM THE UAURÁ TRIBE (2ND GROUP), OBTAINED AT THE JOZEF STEFAN INSTITUTE (LJUBLJANA, SLOVENIA)

Sample Code Number	Total Hg (mg/kg)	Methyl-Hg (mg/kg)	% Methyl-Hg
210	-	7.55	-
418	-	12.9	-
537	11.5	9.22	80
705	13.9	11.5	83
727	10.1	7.70	76
937	21.7	11.6	53
5099	-	10.0	-
5192	12.1	11.0	90
5262	13.1	11.5	88
6029	10.1	9.21	91
	n = 7	n = 10	
	Mean = 13.2	Mean = 10.2	
	std. dev. = 4.0	std. dev. = 1.8	
	$Mean_G$ = 12.8	$Mean_G$ = 10.1	
	std dev_G = 1.3	std dev_G = 1.2	
	median = 12.1	median = 10.5	

TABLE II. RESULTS OF THE ANALYSIS OF TOTAL MERCURY BY INAA IN HAIR OF THE INDIANS FROM THE COICURO TRIBE (4TH GROUP), FROM THE XINGU PARK

Sample Code Number	Total Mercury (mg/kg)
132	12.6
133	16.9
135	14.5
176	25.3
334	16.0
371	17.9
380	13.4
381	17.5
382	13.8
383	10.3
390	16.2
397	20.2
513	10.0
514	12.9
578	12.3
583	11.1
585	15.3
594	12.1
595	13.9
597	11.3
607	11.5
611	16.7
616	6.8
617	10.0
618	11.7
650	12.0
653	15.1

TABLE II. (Cont.)

Sample Code Number	Total mercury (mg/kg)
679	4.8
813	7.2
820	15.8
821	15.8
895	7.3
917	9.9
928	13.7
957	13.0
5018	10.2
5040	17.8
5051	11.1
5055	16.9
5057	13.3
5085	11.8
5127	16.7
5147	13.0
5148	10.0
5185	12.6
5200	8.8

n = 46; Mean = 13.2; std dev = 3.8;
range = 4.8 - 25.3;
$Mean_G$ = 12.7; std dev_G = 1.4;
median = 13.0

TABLE III. RESULTS OF THE ANALYSIS OF TOTAL MERCURY BY INAA IN HAIR OF THE INDIANS FROM THE MATIPU TRIBE (5TH GROUP), FROM THE XINGU PARK

Sample Code Number	Total Mercury (mg/kg)
3	8.3
286	11.5
316	13.0
389	9.2
633	7.3
634	1.7
653	15.1
682	12.2
692	10.4
5156	12.9
5198	14.7

n = 11; Mean = 10.6; std dev = 3.9;
range = 1.7 - 15.1;
$Mean_G$ = 9.4; std dev_G = 1.9;
median = 11.5

TABLE IV. RESULTS OF THE ANALYSIS OF TOTAL MERCURY BY INAA IN HAIR OF THE INDIANS FROM THE PAVURU TRIBE (6TH GROUP), FROM THE XINGU PARK

Sample Code Number	Total Mercury (mg/kg)
305	18.1
425	18.7
435	13.0
438	24.4
440	18.6
444	22.7
447	28.2
448	17.6
449	17.5
452	8.1
458	12.2
463	13.0
466	13.0
480	21.6
524	27.7
883	21.8
5115	20.5
5278	18.9
5280	18.8
5353	57.3

n = 20; Mean = 20.6; std dev = 10.0;
range = 8.1 - 57.3;
$Mean_G$ = 19.0; std dev_G = 1.5;
median = 18.8

TABLE V. RESULTS OF THE ANALYSIS OF SELENIUM BY INAA IN HAIR OF 16 INDIVIDUALS FROM THE CONTROL GROUP

Sample Code Number	Selenium Concentration (mg/kg)
C1	0.42
C3	0.47
C4	0.39
C5	0.34
C6	0.50
IQ1	0.43
IQ3	84.3
TFR1	0.42
TFR2	0.44
TFR3	0.44
TFR4	0.48
TFR5	0.36
TFR6	0.46
TFR7	0.40
TFR8	0.45
TFR9	7.43

n = 16; Mean = 0.43; std dev = 0.04;
range = 0.34 - 84.3;
$Mean_G$ = 0.43; std dev_G = 1.11
median = 0.43

Obs.: For the calculation of averages and of the median the values for samples IQ3 and TFR9 were not considered.

TABLE VI. RESULTS OF THE ANALYSIS OF SELENIUM BY INAA IN HAIR OF 15 INDIVIDUALS FROM THE GROUP OF THE BILLINGS DAM

Sample Code Number	Se Concentration (mg/kg)
B2	0.33
B3	0.26
B4	0.53
B5	0.43
B6	0.33
B7	0.27
B8	0.46
B9	0.39
B10	0.33
B11	0.52
B12	0.30
B13	0.41
B14	0.17
B15	0.64
B23	26.8

n = 15; Mean = 0.38; std dev = 0.12;
range = 0.17 - 26.8;
$Mean_G$ = 0.36; std dev_G = 1.41;
median = 0.36

TABLE VII. RESULTS OF THE ANALYSIS OF SELENIUM BY INAA IN HAIR OF 15 INDIVIDUALS FROM THE FIRST GROUP (SUIÁ TRIBE) FROM THE XINGU PARK

Sample Code Number	Selenium Concentration (mg/kg)
1225	0.33
1226	< 0.28
1228	< 0.28
1230	0.57
1234	0.34
1241	0.37
1242	0.86
1244	< 0.28
1245	0.40
1247	< 0.28
1248	0.64
1250	0.39
1251	0.25
1253	0.84
1255	0.35
1269	0.50
1274	< 0.28
1277	< 0.28
1278	< 0.28
1280	0.53
1281	0.46
1285	0.32
1286	< 0.28
1293	0.41
1324	0.50
1341	0.51
1652	< 0.28

n = 27; Mean = 0.47; std dev = 0.01;
range = < 0.28 - 0.86;
$Mean_G$ = 0.45; std dev_G = 1.39;
median = 0.43

TABLE VIII. SUMMARY OF THE RESULTS OBTAINED FOR TOTAL MERCURY AND METHYLMERCURY CONTENTS IN THE HAIR OF THE POPULATIONAL GROUPS STUDIED (IN mg/kg)

POPULATION GROUP	Mean	std dev	$Mean_g$	std dev_g	Median	Range
Total mercury (COICURO TRIBE - 4th Group)	13.2	3.8	12.7	1.4	13.0	4.8 - 25.3
Total mercury (MATIPU TRIBE - 5th Group)	10.66	3.9	9.4	1.9	11.5	1.7 - 15.1
Total mercury (PAVURU TRIBE - 6th Group)	20.66	10.0	19.0	1.5	18.8	8.1 - 57.3
Total mercury (UAURÁ TRIBE - 2nd Group)	13.2	4.0	12.8	1.3	12.1	11.5 - 21.7
MeHg (UAURÁ TRIBE-2nd Group)	10.2	1.8	10.1	1.2	10.5	7.7 - 12.9

TABLE IX. SUMMARY OF THE RESULTS OBTAINED FOR SELENIUM CONTENTS IN HAIR OF THREE OF THE POPULATIONAL GROUPS STUDIED (In mg/kg)

POPULATIONAL GROUP	Mean	std dev	$Mean_g$	std dev_g	Median	Range
CONTROL GROUP	043	0.04	0.43	1.11	0.43	0.34 - 84.3
BILLINGS DAM GROUP	0.38	0.12	0.36	1.41	0.36	0.17 - 26.8
SUIÁ TRIBE (1st Group)	0.47	0.01	0.45	1.39	0.43	< 0.28 - 0.86

TOTAL MERCURY AND METHYLMERCURY LEVELS IN PREGNANT WOMEN, NURSING WOMEN AND PRESCHOOL CHILDREN - RESIDENTS OF FISHERIES IN THE EIGHTH REGION OF CHILE

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Abstract

In the 1991-1993 period, efforts were concentrated on establishing and validating the analytical methodology for determining total mercury (Hg-T) and methylmercury (MeHg) in human hair, and to identify any high risk populations in the study group. Two surveys were conducted during this period, which involved the collection of scalp hair samples that were prepared and analyzed for Hg-T, and also for MeHg in selected samples. The mean hair Hg-T concentration determined in the study group (1.81 ± 1.52 mg/kg, as dry weight) was significantly higher than the level obtained in the control group (0.42 ± 0.15 mg/kg). These results were characterized according to geographical location of the FVs, frequency of fish and seafood consumption and residence period in the same FV. Multiple comparison tests confirmed significant differences between the arithmetic means obtained in each FV and the control group. Five of the FVs with higher Hg-T concentrations in PW and NW were selected for further and more in-depth studies. A new survey, which is now in progress, is also described, which targets these five FVs and the associated control group.

1. INTRODUCTION

The coastal zone of the eighth region of Chile receives discharges of industrial residues, wastewater and domestic wastes. These discharges have included, until 1993, inorganic Hg from two chloralkali plants that was dumped into river and estuary waters flowing towards the coast. The food chain is the main pathway leading to humans for most trace elements that may be present in these contaminated waters. Hence, the primary aim of this project, carried out in collaboration with the Health Regional Ministry Office, (SEREMI-Salud) is to perform a descriptive study on levels of total mercury (Hg-T) and methylmercury (MeHg). The study employs the analysis of scalp hair specimens of a selected human population at high risk with respect to Hg exposure through the diet (*i.e.*, pregnant women (PW), nursing women (NW) and preschool children (PSCH) residing in fishing villages (FVs) within this coastal zone, who have fish and shellfish as main food

components in their diets. The mercury concentrations in these hair samples are compared to the concentrations hair samples from an appropriate control group. The MeHg/Hg-T ratio in scalp hair will enable interpretation of the results with respect to the degree of contamination by Hg, and the dietary habits of the sample donors of each FV. In addition, Se levels in scalp hair of PW with relatively high Hg content will be investigated for possible correlation with MeHg levels. As the analysis of samples of PSCH are beyond the scope of this CRP, these results are reported elsewhere.

2. METHODS

2.1. Quality assurance

A number of approaches were followed for both internal and external analytical quality control (AQC) for our studies. Internal AQC was performed for Hg-T through the periodic analysis of either NIES RM No. 5 (Human Hair) or a laboratory-prepared human hair sample. The latter is segmented but not powdered, and is available in a few grams, with an established Hg-T value by CVAAS (1.10 ± 0.05 mg/kg, 95% conf. int. in 16 independent determinations). This sample was used as internal control to assess intra-laboratory variability. Its homogeneity was estimated at the 100 mg level by comparing the relative standard deviations (RSDs (%)) obtained in independent determinations of Hg-T in several sub-samples, with the RSD (%) found when independent sub-samples of RM NIES No. 5 were analyzed in a similar way. Also during this period, Hg-T and MeHg were analyzed in human hair RMs, which are certified for Hg-T and have reference values for MeHg. Moreover, Hg-T was analyzed in two blood samples from the CTQ (Québec, Canada).

External AQC was approached two ways; first, by comparing the results obtained for Hg-T by CVAAS and INAA in several biological and environmental SRMs and CRMs, including two RMs of human hair, and second, through intercomparison exercises. Using the second approach, five intercomparison exercises have been performed so far and a sixth one is in progress.

2.2. Study and control population groups

A new survey was performed in 1992-93 that included PW in 11 FVs of the coastal zone who had no occupational exposure to Hg, and who consumed at least one fish meal per week. The subjects (95 women, age 14 - 40) also regularly attended the health posts for medical check-ups. The survey included a control group (19 women, ages 15 - 38, who were in good health, had negligible fish or shellfish consumption, and resided in the rural towns of Pinto and El Carmen). The questionnaire from the previous survey (1991-92) was improved, and now included the frequency of fish and shellfish consumption per week, the quantity per meal, the mode of preparation and origin, the number of conceptions and lastly, the expected delivery date.

2.3. Sampling and pretreatment

The scalp hair samples were collected and analyzed according to the approved protocol [1]. The hair was cut from the proximal ends with stainless steel scissors into segments of 2 - 5 mm, transferred to a Pyrex bottle, washed with acetone for 10 minutes

and dried at room temperature. At this point, we introduced a modification in the sample preparation procedure, and instead of continuing the washing sequence using the small hair segments, the dry sample was powdered in a PTFE homogenizer (*i.e.*, using a microdismembrator) after freezing in liquid nitrogen, and the washing sequence was resumed afterwards with water and acetone, according to the protocol. The powdered sample was dried, protected from dust and draughts and stored in a plastic container at room temperature.

As was agreed in the second RCM, in addition to human hair reference materials (NIES RM No. 5, GBW 09101 and BCR CRM-397), several biological materials certified for Hg-T and with reference MeHg contents were analyzed. These included Trace Metals in Tuna Fish (IAEA-350 in three bottles: No. 220, 346 and 400), Shrimp Tissue Homogenate (MA(S)-MED-86/TM in bottle No. 131), Shrimp Tissue Lyophilized (MA-A-3/TM in bottle No. 171) and Fish Tissue Lyophilized (MA-B-3/OC in bottle No. 194). Also, samples No. 1 and No. 2 from the first analytical quality exercise (AQCE) were reanalysed for MeHg.

2.4. Analytical methods

Hg-T was determined by cold vapour atomic absorption spectrometry (CVAAS) and by instrumental neutron activation analysis (INAA) in 20% of the scalp hair samples for periodic external quality control. MeHg was determined by gas liquid chromatography with electron capture detector (GC-ECD). Se was determined by INAA.

2.4.1. CVAAS

Human hair samples were digested with HNO_3 in sealed Pyrex ampoules [2], and biological materials were digested in acid (HNO_3), either in PTFE bombs or in sealed Pyrex ampoules, as described in the second RCM report [3]. However, for biological materials and blood, the digestion in Pyrex ampoules was performed in a HNO_3 : $HClO_4$ mixture (3:1) [2], rather than in pure nitric acid, to enhance the oxidation of organomercury compounds in such highly organic matrices. The analytical procedure for CVAAS has been described previously in detail [3,4].

Using this CVAAS procedure, Hg-T was determined in several reference materials (RMs), in two blood samples of the Interlaboratory Comparison Programme of the Centre de Toxicologie du Québec (CTQ), in two blind hair samples sent for the second AQCE, in 114 new scalp hair samples of the study and control population groups, and more recently, in scalp hair and blood samples of PW residing in the FVs selected for more in-depth studies.

2.4.2. INAA

The analytical methodology for Hg-T and Se was already described in the previous RCM report [3]. The following RMs were used as solid standards: Citrus Leaves (NIST, SRM 1572), Human Hair (BCR-397), Oyster Tissue (NIST, SRM 1566), and additionally, the IAEA RMs: Trace Metals in Tuna Fish (IAEA-350), Fish Tissue Lyophilized (MA-B-3/OC) and Shrimp Tissue Lyophilized (MA-A-3/TM). These were prepared and irradiated in the same form as the hair samples. In parallel, standard solutions of Hg (5.0 μg/mL) and Se (30.0 μg/mL) were encapsulated in quartz (Vitreosil) and surrounded by an iron ring. Samples and standards were irradiated in the nuclear reactor RECH-1 for 24 hrs periods with a neutron flux oscillating between 0.9 - 2.0 x 10^{13} n/seg x cm^2. After an appropriate decay period (*ca.*

10 days), the vials were cooled with liquid nitrogen, opened and transferred to polyethylene containers for measurement by γ spectrometry. Samples were counted for 1 hr on a γ - spectrometer (FWHM = 2.4 keV at the 1332 keV ^{60}Co photopeak). A pneumatic sample changer, controlled through a IBM/PC compatible computer, was used for the analyses.

No losses were detected as a result of the irradiation process. Hg-T and Se were determined in certified reference materials (human hair and biological) and in scalp hair samples of the study and control population groups. The results from the analyses of the two blind human hair samples of the second AQCE are not yet available, but are in progress. As usual, the interference produced by the ^{75}Se peak in this γ emission was corrected by calculating the relation between this emission and the emission at 264 keV.

2.4.3. GC-ECD

According to the results obtained in the first AQCE in two "blind" RMs provided by the IAEA [5], while our results for Hg-T were quite consistent with the target values, the MeHg results were not satisfactory. In this exercise, sample No. 1 had a very low MeHg content and fairly high amount of ethylmercury (Et-Hg) (Et-Hg > 7x MeHg), and our MeHg result was significantly high, partially due to the poor detection power of MeHg. Moreover, in sample No. 2, the recovery of MeHg was low (63.3%), which we attributed to incomplete isolation and extraction of MeHg under the alkaline disintegration approach. Therefore, we decided to change our sample treatment and adopted a procedure based on the selective isolation of MeHg from human hair by volatilization in a microdiffusion cell (Convey dish). This procedure is described in detail by M. Horvat *et al.* in the second RCM report (Alternative 2 direct method) [6] and has three advantages compared to the alkaline digestion approach. First, the volatilization procedure provides a much cleaner extract resulting in simpler chromatograms with few interferences from other peaks (*e.g.*, fatty acids); second, it requires much less sample and leads to more concentrated toluene extracts, which are more amenable for determinations in large sample numbers; and third, it requires less reagents, and is, therefore, less expensive.

In the microdiffusion approach, the cysteine paper containing the MeHgCN (trapped on it following volatilization) was acidified with 4M KBr and 2M H_2SO_4 (saturated with $CuSO_4$). The MeHg (now as aqueous MeHg-Br) was extracted into toluene (1 mL x 2) under mechanical shaking for 10 min. This was followed by a 10 min centrifugation for phase separation. After transferring the toluene phase into a separate glass vial, the toluene was dehydrated with a few anhydrous Na_2SO_4 crystals. Prior to sample analysis, the glass silanized chromatographic column was pre-conditioned [3]; first, for 24 hours with a saturated solution of $HgCl_2$ in benzene. Following this, a 1 μL injection of a 10 μg/mL MeHg solution was applied into the column and was left for another 12 hrs before use. For actual sample analysis, the separation conditions used were: Column temperature, 170 °C; Injector temperature, 230 °C; Detector temperature, 230 °C; gas flow, 60 ml/min (ultrapure N_2); injection volume, 1-2 μL.

Preliminary recovery of MeHg in pure solutions was 89.5 ± 2.5%. As this result was slightly lower than expected, we cross-checked our MeHgCl working stock solution with a MeHg-Cl standard solution provided by the reference laboratory. The MeHg and Et-Hg chromatographic peaks were also confirmed with standard solutions provided by the reference laboratory. No significant difference was found between the concentration of our MeHg-Cl stock solution cross-checked with the MeHg-Cl standard, compared with the

nominal value (1012.5 vs 1000 μg/mL expressed as Hg). New recovery results obtained for MeHg in aqueous solutions prepared by dilution of the standard supplied by the reference laboratory (at 0.50, 0.10, 0.05, and 0.025 μg/mL) by the microdiffusion technique yielded between 85.8 ± 3.2% (n=3) (at 0.025 μg/mL final conc.) and 91.8 ± 2.6%(n=3) (at 0.5 μg/ml). These recoveries were considered fair and quite reproducible for pure MeHg-Cl solutions. However, recoveries were better when MeHg was analyzed in real samples, as is shown in the RMs that were analyzed (see below).

3. RESULTS AND DISCUSSION

3.1. Quality assurance

Figures 1 and 2 present, as control charts, the variations of Hg-T concentration measured in both of the human hair materials. Observing these charts, it is possible to realize the importance of analysing a very homogeneous powdered human hair samples (*e.g.* NIES No. 5) as a control samples, rather than a segmented hair sample (our internal control sample). Based on these results, the Hg-T determination in powdered hair is under statistical control, but the results obtained in our control sample showed that only 6 of 13 independent determinations are within ± 1 SD of the target value.

The results of the analyses of the human hair RMs and of the CTQ blood samples are given in Table I. The values for Hg-T were found to be satisfactory, with the exception of the low recovery in one blood sample, which had a relatively high Hg value. The results obtained for MeHg show improvements in recoveries with respect to our last results reported at the second RCM [3]. In particular, excellent agreement was obtained with the reference MeHg content in NIES RM No. 5.

In comparing the results of the analysis of the various RMs by CVAAS and INAA using the t-test, there were no significant differences ($p < 0.05$) in the results of both methods, which showed a correlation coefficient of 0.993 [3,4].

Of the intercomparison exercises we performed, the first exercise was a periodic external AQC using INAA as reference method for Hg-T, which consisted in the analysis of 20% of the scalp hair samples collected in this study and analyzed by CVAAS. A comparison made by regression analysis in 21 samples of the study and control groups collected in 1991 and 1992 showed a significant correlation between results obtained by CVAAS and INAA for all the women studied ($r^2 = 0.88$, $p < 0.0001$), and no significant differences were established by paired t-test (mean difference = 0.178, std. error = 0.155, $t_{calc.} = 1.152$, $p = 0.2628$) [4].

The second intercomparison exercise was the intercalibration procedure based on the analysis of Hg-T (by CVAAS and INAA) and MeHg (by GC-ECD) in the two"blind" RMs that were provided by the IAEA. These results have been already presented and discussed at the second RCM [3].

The third exercise was the delivery at the second RCM of human hair samples collected and homogenized in our laboratory to other participant laboratories for analysis of Hg-T. Table II shows a comparison between our results and the results received from three of these laboratories. These samples corresponded to hair that was cut into 2-5 mm

segments but was not powdered because at that time, there was no microdismembrator available yet in our laboratory. According to these data, and considering the different analytical techniques used in it, the results are quite consistent, particularly at low Hg-T concentrations. However, at very high Hg concentration (> 10 mg/kg), which is very unusual in our study population group, significant difference was observed in the result of one sample between our data (by CVAAS) and data obtained by NAA in another participating laboratory.

The fourth exercise is the intercomparison of Hg-T and MeHg in three biological reference materials provided by the Marine Environmental Studies Laboratory (MESL) of the IAEA-MEL: Trace Metals in Tuna Fish IAEA-350 (in bottles 220, 346 and 400), Shrimp Tissue Homogenate (MA(S)-MED-86/TM in bottle No. 131), Shrimp Tissue Lyophilized (MA-A-3/TM in bottle No. 171) and Fish Tissue Lyophilized (MA-B-3/OC in bottle No. 194). The results are shown in Table III. In this table, the results on IAEA Samples No. 1 and 2, that were reanalysed for MeHg, are also reported. In general, reasonably good agreement was obtained with the certified and reference values for Hg-T and MeHg in RMs IAEA-350 (in bottles Nos. 220 and 346) and MA-B-3/OC. Relatively high Hg-T and MeHg results were obtained in RM MA(S)-MED-86/TM and MA-A-3/TM, and also for Me-Hg in IAEA Samples No. 1 and No. 2. Three of these samples have in common relatively low MeHg and high Et-Hg contents and apparently, the chromatographic peaks were not well resolved under these conditions. Further studies are in progress to solve this problem.

3.2. Study and control groups

3.2.1. Hg-T

Hg-T was determined (in duplicate) by CVAAS, in 95 and 19 scalp hair samples collected in 1992 (and early in 1993) of new PW from 11 FVs (study group) and two rural towns (control group), respectively. In this period, all samples corresponded to PW that have not been surveyed or sampled previously. The pooled standard deviation (SD_p) obtained in duplicate determinations of 104 samples of powdered hair with Hg-T content between 0.14 and 9.72 mg/kg was 0.198 mg/kg, similar to the SD_p obtained in 1991 (SDp = 0.173 mg/kg in 66 specimens of segmented hair), showing also consistent results between subsamples. In this case, the uncertainty of Hg-T determination was not significantly different for either segmented or powdered hair.

Table IV shows a comparison of results for Hg-T in scalp hair obtained in this period (1992) and in 1991, grouped per FV (study group), including the control group, the number of PW, the arithmetic mean, the standard deviation and the range. Also, a summary of mean results for both sampling periods is given for the study group in Table V, in comparison with the control group. Upon application of t-test to the Hg-T concentration means of the study group in 1991 (2.09 ± 1.46 mg/kg), 1992 (1.63 ± 1.53 mg/kg) and combined data 1991-1992 (1.81 ± 1.52 mg/kg) with respect to the means of the control group in the same periods (1991, 0.43 ± 0.19 mg/kg; 1992, 0.42 ± 0.14 mg/kg; and 1991-1992, 0.42 ± 0.15 mg/kg), significant differences ($p < 0.01$) were established between the two populations. Although no dietary survey has been made in Chile to our knowledge regarding fish consumption by the general population, the national gross average is estimated between 3.5 and 4.0 kg per year [10], varying with the proximity to the coast.

Three main features deserve comment regarding these results. First, the mean concentration of Hg-T in the study group was at least 4 to 5 times higher than the mean of the control group confirming the validity of our hypothesis in both study periods. Second, the Hg-T mean concentration in 1992 was lower by 22% with respect to the 1991 period, probably as a result of two events: first, as of 1991, the dietary habits regarding fish and seafood consumption were temporarily altered (due to sanitary recommendations from the Chilean health authorities because of an outbreak of cholera morbus in Perú, limitrophe in Chile); and second, decreased amounts of Hg were discharged from chloralkali plants into the river waters in 1992 (due to partial replacement of the Hg electrolytic cells by diffusion membranes). Third, it is important to notice the consistency and low dispersion of the results in the control group. The mean Hg-T concentration found in the control group was the same, despite the different study periods, PW involved, and number of women considered in both periods. As expected, the result obtained in this group was low, denoting the rather scarce consumption of fish and fish products due to their location inland far from the coast.

Important conclusions were obtained when these results were examined concerning the effects of the geographical location of the FVs, the approximate frequency of fish and seafood consumption of the study women, and the age and residence period in the same FV, and may be summarized as follows:

(1) By application of multiple comparison tests (Anova and Tukey) to the Hg-T concentration means obtained in each FV with respect to the control group, significant differences ($p < 0.05$) were confirmed in at least six FVs in 1991, seven FVs in 1992 and nine in the 1991-1992 period, all located within the more polluted coastal zone. By multiple comparison tests (DSL and DSH) in 1991, it was confirmed that Punta Lavapié and Tumbes presented the highest risk concerning mercury exposure through the diet. In particular, the relatively high Hg-T results obtained in PW and NW of Chome, Tumbes, Lenga, Isla Sta. María and Punta Lavapié after two study periods apparently indicate high risk population groups with regards to mercury exposure through the diet. Therefore, these FVs were selected for further studies in the 1993-1994 contract period. The results obtained in two FVs (Dichato and Quidico), at the northern and southern limits, respectively, of this zone, were consistently low as expected because they are out of the critical zone.

(2) Among PW and NW resident in FVs of the polluted zone, a statistically significant difference was established between the Hg-T results of women who ate fish and seafood one or more times weekly and those consuming a lower amount. However, no significant differences in Hg-T content were evident between women with different consumption frequencies above the level of once per week.

(3) Within PW and NW who ate fish and seafood at least one time per week, a statistically significant difference was found in Hg-T content in two subgroups; the first one, with less than 20 years of residence (A), and the second one beyond 20 years of residence (B) in the same FV. Subgroup B showed higher levels in the two study periods: 1.76 ± 1.15 mg/kg(A) vs. 2.57 ± 1.67 mg/kg(B) in 1991, and 1.24 ± 1.00 mg/kg(A) vs. 2.27 ± 1.97 mg/kg (B) in 1992. However, there was no evidence to find significant

difference with age, at least between two subgroups of women less and beyond 25 years old having similar fish and seafood dietary habits.

Also, as already mentioned, a new survey is in progress in 1994 to collect scalp hair and blood samples of PW from the five FVs selected as high risk group due to exposure to Hg through the diet. Results on some of these samples were presented at the current RCM. This study is linked to the health plans of the Health Regional Ministry Office, and the samples are being collected according to the facilities provided by the health services. Recently, 22 samples were collected and are being analysed.

3.2.2. MeHg and MeHg/Hg-T ratio

Results obtained for MeHg in duplicate by GC-ECD in 19 samples of the study and control groups collected in 1992 (and early in 1993) are shown in Table VI, including the ratio of MeHg/Hg-T. The ranges of MeHg (as Hg) were 0.523 - 2.015 mg/kg and 0.169 - 0.372 mg/kg in the study group and control groups, respectively, and the MeHg/Hg-T ratio varied between 0.232 and 0.875 with a mean of 0.628 ± 0.168. These ratios are significantly higher than the MeHg/Hg-T ratios obtained by the former MeHg methodology that was abandoned. The MeHg/Hg-T ratio was consistently high (ca. 0.76) for PW of the Isla Sta. María, which is an island located about 20 miles from the coast. This island was considered as one FV, although there are two FVs settled there. PW living on the island depend on fish and seafood products for their diet, due to the difficulties to travel to the mainland to get other food products. The information obtained through the questionnaire about the frequency of fish and seafood consumption and the number of meals per week of these PW showed a mean of 6.5 meals/week and an estimated amount of 45.5 kg/person/year of fish consumption. Therefore, the relatively high MeHg/Hg-T ratio of PW residing in this island can be directly connected to their dietary habits.

4. PLANS FOR FUTURE WORK

In the third year, we have validated the new methodology for MeHg determination in scalp hair and plan to finish the analysis of the hair samples collected in the 1992 study period in order to have a complete map of the distribution of Hg-T and MeHg in the FVs of the study group. In 1994, a new survey is in progress to identify and collect hair and blood samples of PW living in FVs selected as high risk population due to mercury exposure through the diet. These samples will be analyzed for Hg-T and MeHg to examine and correlate the MeHg/Hg-T ratio with the dietary habits (fish consumption) of the PW, to establish the source of Hg.

During the fourth year, we plan to finish the analysis of these samples and based on their results, eventually, hair and blood samples of some newborns of these PW (and/or umbilical cord blood) will be collected at the time of birth and analyzed too, for Hg-T and MeHg to study possible correlations between these species levels in hair and blood, as well as in the mother and newborn, and to assess the degree and extent of Hg contamination. Based on these results, seafood and water consumed by these PW will be sampled and analyzed to establish the main sources of Hg in the group at risk.

In those samples with relatively high Hg-T and MeHg levels, selenium will be determined by hydride generation atomic absorption spectrometry to establish a possible correlation between MeHg intake and Se levels, particularly in blood.

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TABLE I. ANALYTICAL RESULTS OF Hg-T AND MeHg IN HUMAN HAIR RMs AND OF Hg-T IN TWO BLOOD SAMPLES OF THE INTERLABORATORY COMPARISON PROGRAMME OF THE CTQ[a]

SAMPLE	Hg-T (mg/kg)		MeHg (mg/kg as Hg)	
	CVAAS	CERTIFIED OR TARGET	GC-ECD	REFERENCE
NIES No. 5 Human Hair	4.25 ± 0.18 (13)	4.40 ± 0.40[b] 4.50 ± 0.5[c]	3.37 ± 0.164	3.3 - 3.4 [7]
BCR-CRM-397 Human Hair	11.63 ± 0.22 (5)	12.3 ± 0.60		(0.646) (± 0.11) [7]
GBW 09101 HH Human Hair	2.12 ± 0.10 (5)[d] 2.72 ± 0.11 (2)[e]	2.16 ± 0.21	1.89 ± 0.252 (3)	0.90 ± 0.04 [6] 1.54 ± 0.04 [6]

[a]Centre de Toxicologie du Québec (Québec, Canada)
[b]Certificate value
[c]Ref. [6]
[d]Result obtained in May 1992
[e]Result obtained in May 1994

BLOOD SAMPLE	Hg-T (nmol/l)		ACCEPTABLE RANGE
	CVAAS	TARGET [8]	
M-9314	45.07 ± 8.86 (3) (9.1 ± 1.8 ng/ml) 35.4 - 52.8	48	33 - 63
M-9315	130.1 ± 10.8 (3) (26.7 ± 2.6 ng/ml) 118.3 - 139.2	180	140 - 220

TABLE II. COMPARISON OF RESULTS FOR Hg-T AND Se IN SEVEN HUMAN HAIR SAMPLES OBTAINED IN CHILE AND IN OTHER PARTICIPATING LABORATORIES AS PART OF THE EXTERNAL QUALITY CONTROL

Sample	Hg-T (mg/kg)		Se (mg/kg)	
	Our Results	Others*	Our Results	Others
2 - 91	3.40 ± 0.25 (2) I 4.00 ± 0.09 (2) II	2.50 ± 0.25 (2) [CH]	0.78 ± 0.11 (2)	–
14 - 92	223.3 ± 21.6 (4) I 294.3 ± 16.9 (2) II	160.0 ± 3.9 (4) [CH]	< 0.19 (2)	–
31 - 92	0.60 ± 0.06 (2) I	0.71 ± 0.07 (2) [BR]	–	–
62 - 92	1.38 ± 0.14 (2) I	1.4 ± 0.1 [BR]	–	–
99 - 91	4.37 ± 0.21 (2) I 4.75 ± 0.09 (2) II	4.0 ± 0.4 [IT]	< 0.24 (2)	0.08 ± 0.03 [IT]
101 - 92	0.56 ± 0.01 (3) I	0.42 ± 0.06 [IT]	–	0.68 ± 0.07 [IT]
257 - 91	0.44 ± 0.04 (2) I	0.485 ± 0.007 (2) [CH]	–	–

The number in parenthesis is the number of independent determinations; quoted variation is one standard deviation.

*[BR] = Brazil; [CH] = China; [IT] = Italy

TABLE III. RESULTS FOR TOTAL MERCURY, METHYLMERCURY AND SELENIUM OBTAINED IN IAEA RMs CERTIFIED FOR TOTAL MERCURY AND SELENIUM IN SAMPLES DELIVERED AT THE SECOND RCM IN KUALA LUMPUR (AUGUST 1992)

		Hg-T (mg/kg)			MeHg (mg/kg as Hg)		Se (mg/kg)	
REF. MATERIAL	BOTTLE NO.	CVAAS	INAA	CERTIFIED	GC-ECD	REFERENCE	INAA	CERTIFIED
IAEA-350 (Trace metals in tuna fish)	220	4.89 ± 0.213(12) (4.48 - 5.53) 4.84 ± 0.267(6)A (4.55 - 5.23) 4.95 ± 0.438(6)B (4.48 - 5.75)		4.99 ± 0.26	3.38 ± 0.180(6) (3.14 - 3.54)	3.65 ± 0.31[a]		
IAEA-350 (Trace metals in tuna fish)	346	5.13 ± 1.323(4)B (4.33 - 6.02)	5.20 ± 0.229(2)	4.99 ± 0.26	3.83 ± 0.189(6) (3.58 - 4.12)	3.65 ± 0.31[a]	5.92 ± 0.77(3)	5.57 4.42 - 5.97
IAEA-350 (Trace metals in tuna fish)	400	5.27 ± 0.973(4)B (4.93 - 5.70)		4.99 ± 0.26	N.D.	3.65 ± 0.31[a]	6.02 ± 0.15(2)	5.57 4.42 - 5.97
MA(S)-MED-86/TM (Shrimp tissue homog.)	131	2.50 ± 0.372(4) (2.37 - 2.76)	0.976 ± 0.077(2)	1.79 ± 0.24 (Prov.val.)	0.110 ± 0.015(6) (0.093 - 0.129)	0.08 - 0.09[b]	3.17 ± 0.04(2)	2.02 ± 0.41
MA-A-3/TM (Shrimp tissue lyophilized)	171	2.06 ± 0.177(7) (1.80 - 2.36)		1.79 ± 0.24 (Prov.val)		0.08 - 0.09[b]		
MA-B-3/OC (Fish tissue lyophylized)	194	0.513 ± 0.048(7) (0.447 - 0.063)	0.560 ± 0.098(2)	0.510 ± 0.070	0.398 ± 0.052(3) (0.378 - 0.419)	0.411 - 0.503[b]	1.61 ± 0.07(4)	1.46 1.35 - 1.70
IAEA Sample #1 (IAEA-MA-A-1/TM) (Dried Copepods)		0.280 ± 0.028(6)[3]	0.264 ± 0.019(2)	0.28 ± 0.01	0.205 ± 0.050(3)[c] (0.184 - 0.224)	0.018 ± 0.002[a]	3.42 ± 0.11(2)	3.03 2.43 - 3.39
IAEA Sample #1 (IAEA-MA-A-2/TM) (Fish Homogenate)		0.479 ± 0.053(8)[3]	0.477 ± 0.011(2)	0.47 ± 0.02	0.397 ± 0.048(3)[c] (0.380 - 0.418)	0.300 - 0.318[a]	1.44 ± 0.18(2)	N.A.

A = Acid digestion in PTFE bomb; B = Acid digestion in sealed Pyrex ampoules.
[a]Result of intercalibration IAEA/RUN TUNA 350 [9]; [b]Result provided by M. Horvat *et al.* [6].
[c]Sample of the first analytical AQCE reanalyzed for MeHg; N.A. = Reference value not available.

TABLE IV. CONCENTRATION OF Hg-T (mg/kg) IN SCALP HAIR SAMPLES OF PREGNANT WOMEN IN THE STUDY AND CONTROL GROUPS. RESULTS PER FISHING VILLAGES IN THE PERIOD 1991 - 1992

FISHING VILLAGE	1991			1992			1991 - 1992		
	No.	x ± SD*	RANGE	No.	x ± SD	RANGE	No.	x ± SD	RANGE
DICHATO	13	0.82 ± 0.39	0.16 - 1.80	14	0.69 ± 0.36	0.14 - 1.51	27	0.75 ± 0.38	0.14 - 1.80
COLIUMO	4	2.13 ± 1.59	0.43 - 3.81	5	0.94 ± 0.56	0.45 - 1.75	9	1.47 ± 1.22	0.43 - 3.81
COCHOLGUE	7	2.63 ± 0.31	2.14 - 2.97	7	1.51 ± 0.65	0.77 - 2.50	14	2.07 ± 0.76	0.77 - 2.97
TUMBES	5	3.53 ± 1.19	1.64 - 4.62	10	2.68 ± 2.54	1.19 - 9.72	15	2.97 ± 2.17	1.19 - 9.72
CHOME	3	3.25 ± 0.49	2.71 - 3.65	1	4.40 ± 0.0	--	4	3.54 ± 0.70	--
LENGA	3	2.56 ± 0.56	2.04 - 3.15	3	2.69 ± 0.38	2.31 - 3.06	6	2.63 ± 0.43	2.04 - 3.15
ISLA STA. MARIA	--	--	--	10	3.23 ± 2.27**	1.27 - 9.12	10	3.23 ± 2.27	1.27 - 9.12
LARAQUETE	12	1.61 ± 0.52	0.97 - 2.63	18	1.40 ± 0.89	0.48 - 3.09	30	1.48 ± 0.76	0.48 - 3.09
TUBUL	4	1.21 ± 0.61	0.84 - 2.13	8	1.68 ± 1.36	0.76 - 4.93	12	1.53 ± 1.16	0.76 - 4.93
PTA. LAVAPIE	4	4.99 ± 2.48	1.70 - 7.11	7	2.01 ± 0.89	0.661 - 3.07	11	3.07 ± 2.09	0.61 - 7.11
QUIDICO	3	0.90 ± 0.39	0.58 - 1.33	12	0.52 ± 0.35	0.18 - 1.33	15	0.60 ± 0.38	0.18 - 1.30
CONTROL	7	0.43 ± 0.19	0.20 - 0.79	19	0.42 ± 0.14	0.25 - 0.73	26	0.42 ± 0.15	0.20 - 0.79

*SD = Standard deviation.
**Samples collected in 1992

TABLE V. CONCENTRATION OF Hg-T (mg/kg) IN SCALP HAIR SAMPLES OF PREGNANT IN THE STUDY AND CONTROL GROUPS. PERIOD 1991 - 1992

GROUP	1991			1992			1991 - 1992		
	No.	x ± SD	RANGE	No.	x ± SD	RANGE	No.	x ± SD	RANGE
STUDY	58	2.09 ± 1.46	0.16 -7.12	95	1.63 ± 1.54	0.14 - 9.72	153	1.81 ± 1.52	0.14 - 9.72
CONTROL	7	0.43 ± 0.19	0.20 - 0.79	19	0.42 ± 0.14	0.25 - 0.73	26	0.42 ± 0.15	0.20 - 0.79

SD = Standard deviation.

TABLE VI. RESULTS OF Hg-T, MeHg AND MeHg/Hg-T RATIO IN SCALP HAIR SAMPLES OF PREGNANT WOMEN FROM THE STUDY AND CONTROL GROUPS COLLECTED IN 1992 - 1993

FISHING VILLAGE	SAMPLE	Hg-T (mg/kg)	SD (mg/kg)	MeHg[a] mg/kg (as Hg)	SD (mg/kg)	MeHg/Hg-T[b]
LENGA	3 - 92	2.710	0.260	1.419	0.139	0.524
TUMBES	5 - 92	2.870	0.120	1.789	0.029	0.623
	9 - 92	1.190	0.014	0.523	0.023	0.439
	12 - 92	2.940	0.060	1.632	0.300	0.555
	14 - 92	223.3	21.600	1.801	N.A.	0.008*
PUNTA LAVAPIE	44 - 92	3.070	0.212	1.113	0.128	0.363
	47 - 92	2.670	0.297	1.944	0.132	0.728
	62 - 91	4.480	0.010	2.015	0.130	0.450
ISLA STA. MARIA	106 - 93	1.570	0.056	1.138	0.117	0.725
	109 - 93	1.650	0.122	1.284	0.016	0.778
	110 - 93	1.270	0.014	0.986	0.081	0.776
	111 - 93	2.160	0.028	1.652	0.089	0.765
CONTROL	85 - 92	0.360	0.007	0.315	0.024	0.875
	86 - 93	0.510	0.042	0.352	0.011	0.690
	87 - 92	0.520	0.014	0.345	0.000	0.663
	88 - 92	0.500	0.001	0.372	0.040	0.744
	95 - 93	0.450	0.064	0.303	0.013	0.673
	96 - 93	0.280	0.050	0.196	0.020	0.700
	97 - 93	0.730	0.014	0.169	0.013	0.232

* Hair sample contaminated with inorganic Hg from hair conditioner.

[a]Range: 0.523 - 2.015 (mg/kg) (FVs); 0.169 - 0.372 (mg/kg) (control).
[b]Mean : 0.628 ± 0.168 (0.232 - 0.875); 0.611 ± 0.153 (FVs); 0.654 ± 0.200 (control)

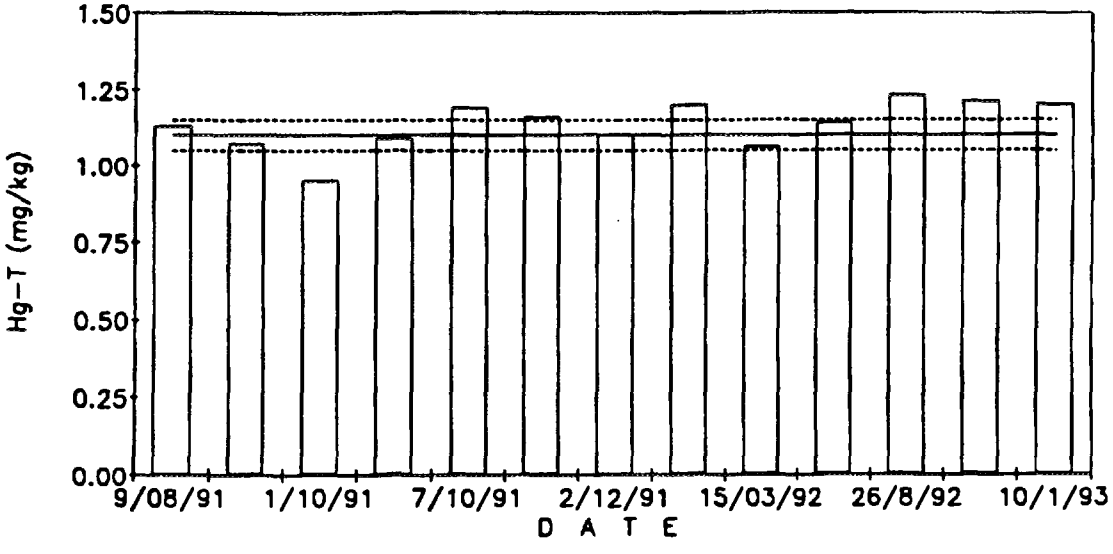

Figure 1. **Control chart on Hg-T determination in internal control sample.**

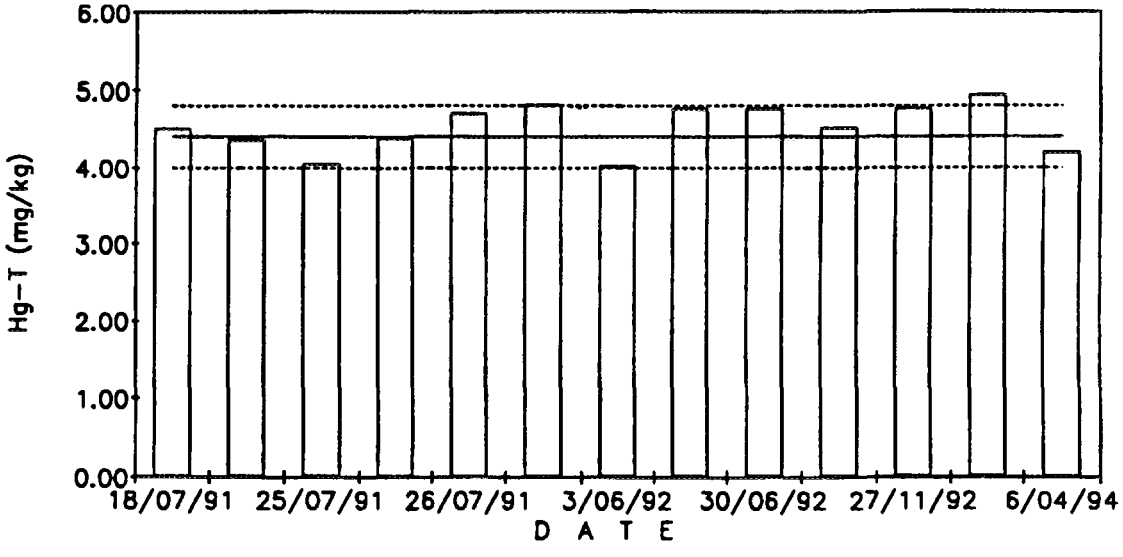

Figure 2. **Control chart on Hg-T determination in NIES RM NO. 5 human hair.**

STUDY ON TOTAL AND METHYL MERCURY LEVELS IN HUMAN SCALP HAIRS OF LYING-IN WOMEN AND NEWBORNS BY NAA AND OTHER TECHNIQUES

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Abstract

Since the Second Research Co-ordinating Meeting in Malaysia, 24-28 August 1992, our research group has completed the analysis of total and methylmercury in scalp hair samples of 1179 fishermen living at a typical Hg-polluted region in Northeast China and of 27 lying-in women and their newborns in a Beijing hospital by INAA, GC(EC) and other techniques. The longitudinal Hg patterns of the lying-in women show a gradually decreasing tendency during the pregnancy period. Further, the hair Hg contents of the newborn babies are generally above or close to those of their mothers, confirming the mechanism that the methylmercury, an organic species of Hg with high toxicity, is readily able to penetrate the placental barrier and accumulate in the fetus. Thus, the mercury exposure has occurred at the early stage of pregnancy.

1. INTRODUCTION

Mercury is a toxic element to humans. Its specific toxicity lies in the methylated species, *i.e.* methylmercury (MeHg), which is readily able to enter human body via the food chain [1,2] and accumulate through various biological membranes. Recent clinical observations have indicated that the MeHg affects the early stages of fetal development and the later mental ability of children whose mothers were exposed during pregnancy to 3-4 times the tolerable weekly intake set by WHO and FAO [3].

Mercury pollution is still an environmental problem to be solved in some regions of China [4]. In order to study the level of Hg pollution in the Second Songhuajiang River System, a typical Hg-polluted area, we collected hair samples of 1179 fishermen there and determined the total mercury and methylmercury in them by NAA, GC(EC) and other techniques in the framework of the IAEA Co-ordinated Research Project on "Assessment of environmental exposure to mercury in selected human populations as studied by nuclear and other techniques".

It is known that during the prenatal process, the methylated species of Hg is easily transferred from mothers to fetus through placental tissue. In order to study the hereditary toxicity of Hg, the correlation between Hg contents in scalp hairs of lying-in women and

their newborn babies has been investigated in this work. Also, the variation of Hg contents in the hair of pregnant women during their pregnancy was studied by NAA and by synchronous radiation-based XRF.

2. EXPERIMENTAL METHOD

2.1. Sampling

We followed the sampling procedure outlined in the United Nations Environment Programme (UNEP) on "The determination of methylmercury, total mercury and total selenium in human hair", Reference Methods for Marine Pollution Studies No. 46 (draft), October 1987, prepared in co-operation with WHO and IAEA [5]. Head hair samples from 1179 people from the population living at the Second Songhuajiang River System were collected. In addition, 27 sets of hair samples from mothers and their newborn babies were taken, either immediately at or within a few days after delivery in the Beijing Zhong-Guan-Chen Hospital. At the time of hair collection, information, such as, name, age, sex, occupation and nutritional habits was also obtained. All hair samples were taken from the occipital area and as close as possible to the scalp. The length of the samples was at least 10 cm long and about 10 g of hair were obtained from the lying-in women whose hair was without waving or dying in the latest 10 months. Due to limited amount of hair available, the weights of the hair samples from the babies ranged from 0.5 to 1.0 g. The hair washing procedure recommended by IAEA was followed to remove dirty materials from the hair samples, *i.e.* washing by acetone - 3 times bi-distilled water-acetone. The hair was then left to dry in a clean desiccator for later analysis.

2.2. Analysis of total mercury

Neutron activation analysis (NAA), mercurymetry (MM) and atomic fluorescence spectrometry (AFS) were used to determine the total mercury in the hair samples for this work. Here, only the methodology of NAA will be briefly described; detailed descriptions for the determination of total mercury using MM and AFS are given in Refs. 6 and 7.

Hg has two stable nuclides, ^{196}Hg and ^{203}Hg. Both of them are able to be readily determined by NAA. We count the two peaks of 68.8 keV and 77.3 keV from ^{197}Hg produced by ^{196}Hg (n,γ) reaction in a heavy water reactor with neutron flux of 4 - 5 x 10^{13} n·cm^{-2}·sec^{-1} from a 30 min irradiation. The counting was done after 3 d decay using a planar high-pure Ge detector with a better energy resolution in low energy region than normal HPGe detectors. For additional details, please refer to our previous working paper [8].

2.3. Analysis of methylmercury

Our procedure for MeHg analysis of hairs is shown in Figure 1. The detailed experimental conditions are given in our previous report [9]. Two sets of gas chromatography devices are available. One is a Varian Model Vista 6000; the other is Shimadzu Model GC-9A with a C-92A data processor. The chromatographic column is a 10% PEG-20M (ϕ=3.2 mm x 1.1 m). For carrier, 60-80 mesh Chromosorb W is used. The electron capture detector is a 10 mCi source of ^{63}Ni.

2.4. Hg profile analysis of hair

Two independent methods were used to investigate the longitudinal variation of Hg contents from the bottom to the top of hair samples, mainly for pregnant women. One technique was "sectional neutron activation analysis", which means that the long hair sample was cut into 1 cm long portions. The other technique that was used was synchronous radiation-based X-ray fluorescence spectrometry (SRXRF). A Beijing Electron-Positron Collider (BEPC) is available with 2.8 GeV energy and 150 maximum currency intensity. A two-dimensional scanning unit with a size-adjustable slit from 10 x 10 μm to 1 x 1 mm was used. The 9.987 keV L_α line of Hg was employed to calculate the Hg content. One typical SRXRF spectrum of hair is shown in Figure 2.

3. RESULTS AND DISCUSSION

3.1. Total and MeHg contents of 1179 hair samples of fishermen

Our results indicate that most of the hair samples of 1179 fishermen contain low mercury, below 5 mg/kg. Only 18 samples have high Hg contents (see Table I). The No. 5 sample contains the highest Hg content, up to 113 mg/kg. The MeHg contents are also included in Table I. It can be seen that the methylation rates vary from 10% to almost 100%, but the MeHg percentages of most hair samples exceed 50 %. As a comparison, we also determined total and MeHg in hair samples from several tens of workers at a Hg mineral district located in southwest China, which show low MeHg compositions [10]. This fact indicates a different pathway of mercury absorption between fishermen and workers.

3.2. Correlation of hair Hg contents of lying-in women with those of their newborns

The mercury concentrations in hair samples from 27 lying-in women and their newborns determined by INAA are listed in Table II. Figure 3 shows the correlation between them. It can be seen that the hair Hg contents of 22 babies are close to or above those of their respective mothers. Only 5 babies have a slightly lower Hg content. The statistical treatment states that the average Hg value for all babies is 0.66 ± 0.31 mg/kg, while 0.59 ± 0.25 mg/kg for mothers. In addition, there is no significant difference between them ($p>0.05$). Lauwerys, *et al.* [11] reported higher Hg contents of newborns' blood (umbilical cork) than those of mothers' blood (intravenous) by 10-15 % in a non-Hg-polluted area in Belgium. Similar results were also given by Pitkin, *et al.* [12]. Thus, our results agree with those of blood samples. At Hg-polluted districts, the hair Hg contents of babies are likely to be even higher.

3.3. Longitudinal variations of Hg contents in hairs of pregnant women

It is known that the growth rate of human head hair is about 300-400 μm/d, *i.e.* 1 cm per month. Thus, a strand of a 10 cm long hair can cover the time of the whole pregnancy period. INAA was used to determine the Hg contents of hair taken from lying-in women, in portions of 1 cm each. Table III lists the longitudinal variations of hair Hg contents of 3 representative lying-in women obtained by INAA. Our results show that the hair Hg contents of the lying-in women decrease with the increase of pregnancy time. The regression analysis of 3 specimen indicates a significant correlation between them (r_1 =

-0.966, τ_2 = -0.900 and τ_3 = - 0.871, $P<0.001$) (see Fig. 4). Similarly, MeHg also exhibits the same variation tendency (Fig. 5). The SRXRF results are shown in Figure 6.

Our results confirm the mechanism that Hg is gradually transferred from mothers to their fetus during pregnancy period. The animal test claims that the Hg species play a great role in its transfer. The inorganic Hg is basically unlikely to go through placental tissue, while the MeHg, an ester-loving material, readily penetrates it to enter the fetus. It is likely that the fetus is easily able to absorb ester-like materials from the placenta [13]. As the early stage of pregnancy is an important period in which infants develop their cerebrum and nervous system, Hg pollution is more dangerous to fetus than to mothers.

4. PLANS FOR FUTURE WORK

In the final period of this research programme, we plan to:

(1) Improve the available analytical methods by interlaboratory comparison exercise and establish two new methods based only on INAA for MeHg analysis of hair.

(2) Collect additional hair samples from populations living in Hg-polluted regions, especially mother-baby paired hair samples.

(3) Complete the analysis of total and methylmercury contents in all the samples collected during this CRP activity.

(4) Submit a final report and prepare publications of the summarized results of this work.

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TABLE I. TOTAL AND METHYMERCURY LEVELS IN HAIR SAMPLES TKAEN FROM SELECTED FISHERMEN LIVING AT THE SECOND SONG-HUA-JIANG RIVER SYSTEM (mg/kg)

Sample No.	T-Hg (NAA)	T-Hg (MM)	T-Hg (AFS)	MeHg (as Hg) (GC-EC)
1	9.90 ± 0.30	11.9	--	7.92
2	9.82 ± 0.30	10.7	--	8.68
3	11.3 ± 0.3	12.0	--	6.96
4	9.70 ± 0.36	12.0	--	7.22
5	113 ± 10	121	101	60.9
6	10.2 ± 0.2	11.3	10.9	5.35
7	7.86 ± 0.34	10.0	--	6.84
8	9.11 ± 0.27	10.4	--	6.10
9	10.5 ± 0.3	11.6	--	1.50
10	16.7 ± 0.2	15.6	14.9	13.7
11	14.5 ± 0.2	16.8	--	1.90
12	8.45 ± 0.34	13.0	--	4.64
13	16.2 ± 0.1	15.0	--	7.64
14	8.64 ± 0.34	10.1	9.9	3.96
15	13.2 ± 0.1	13.3	--	13.2
16	9.87 ± 0.20	12.7	--	4.57
17	34.6 ± 0.1	54.1	35.5	--
18	13.0 ± 0.1	20.4	15.5	--

TABLE II. Hg CONTENTS OF 27 LYING-IN WOMEN AND THEIR NEWBORNS DETERMINED BY INAA (mg/kg)

Sample No.	Mother	Baby
1	0.83	1.16
2	0.52	0.76
3	0.55	0.66
4	0.42	0.59
5	0.36	0.40
6	0.30	0.39
7	0.92	1.35
8	0.67	0.65
9	0.46	0.45
10	1.01	1.10
11	0.79	0.66
12	0.61	0.82
13	0.76	1.23
14	1.12	0.98
15	0.60	0.46
16	0.40	0.48
17	0.47	0.47
18	0.29	0.42
19	0.95	1.07
20	0.22	0.33
21	0.44	0.43
22	0.41	0.30
23	0.71	0.82
24	0.23	0.29
25	0.80	0.64
26	0.72	0.73
27	0.28	0.30
MEAN ± SD	0.59 ± 0.25	0.66 ± 0.31

TABLE III. VARIATION OF Hg CONTENTS OF LYING-IN WOMEN DURING PREGNANCY (mg/kg)

pregnancy time (month)	Sample No.		
	1	2	3
1	1.17	0.75	1.48
2	1.16	0.64	1.45
3	1.03	0.62	1.03
4	0.90	0.54	0.92
5	0.84	0.49	0.88
6	0.86	0.40	0.75
7	0.61	0.47	0.61
8	0.59	0.47	0.63
9	0.63	0.42	0.69
10(at delivery)	0.55	0.38	0.74

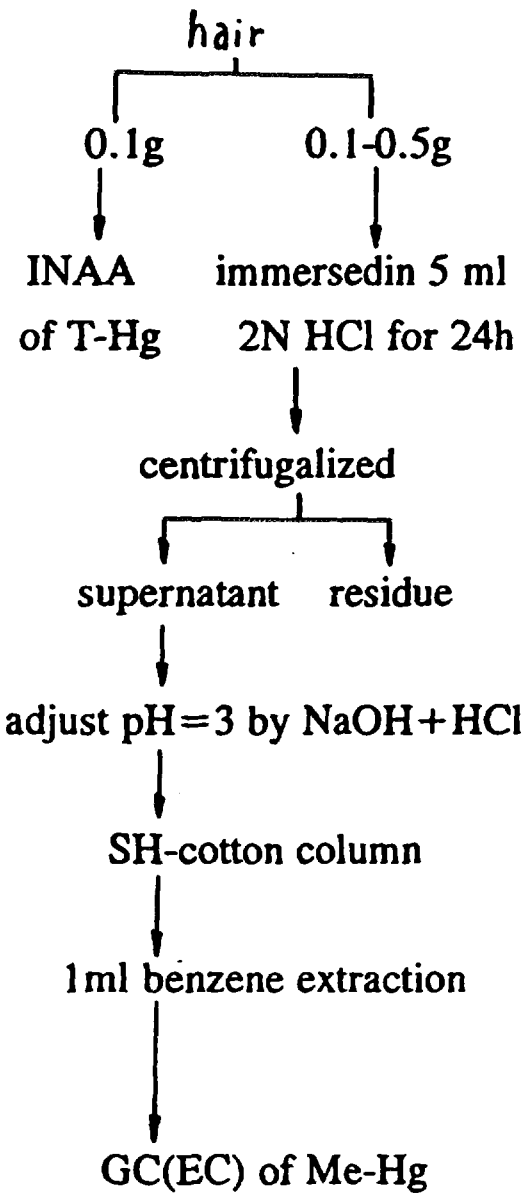

Figure 1. **Experimental procedure for MeHg analysis of hair samples.**

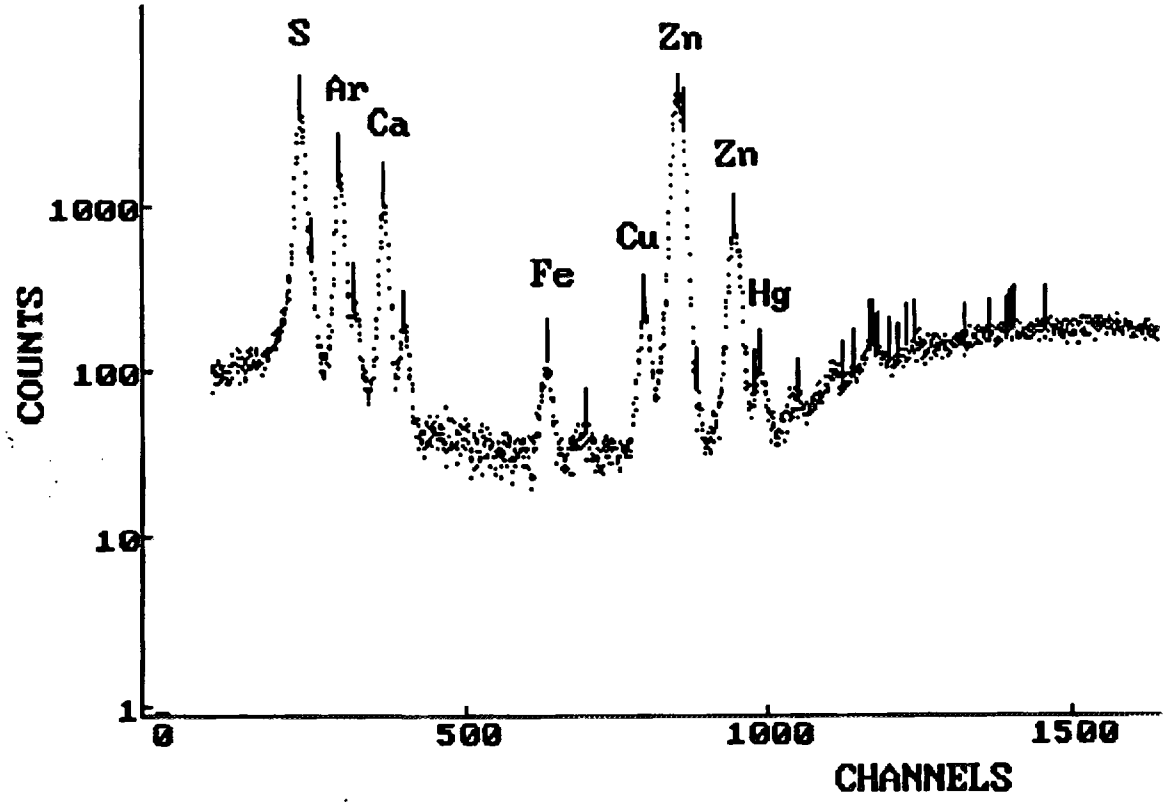

Figure 2. The SRXRF spectrum of a lying-in woman's hair.

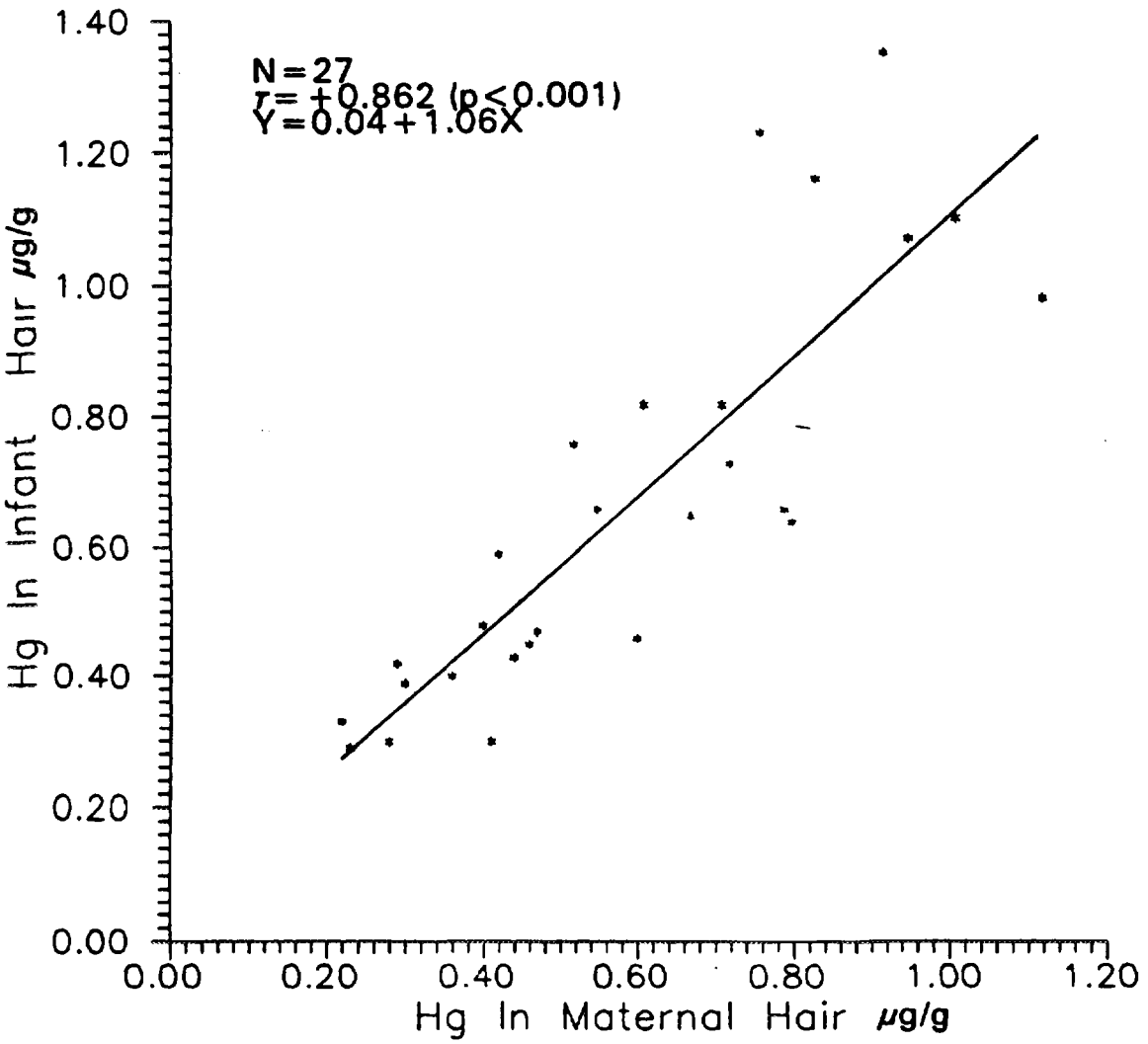

Figure 3. **Correlation between hair Hg contents of lying-in women and their newborns.**

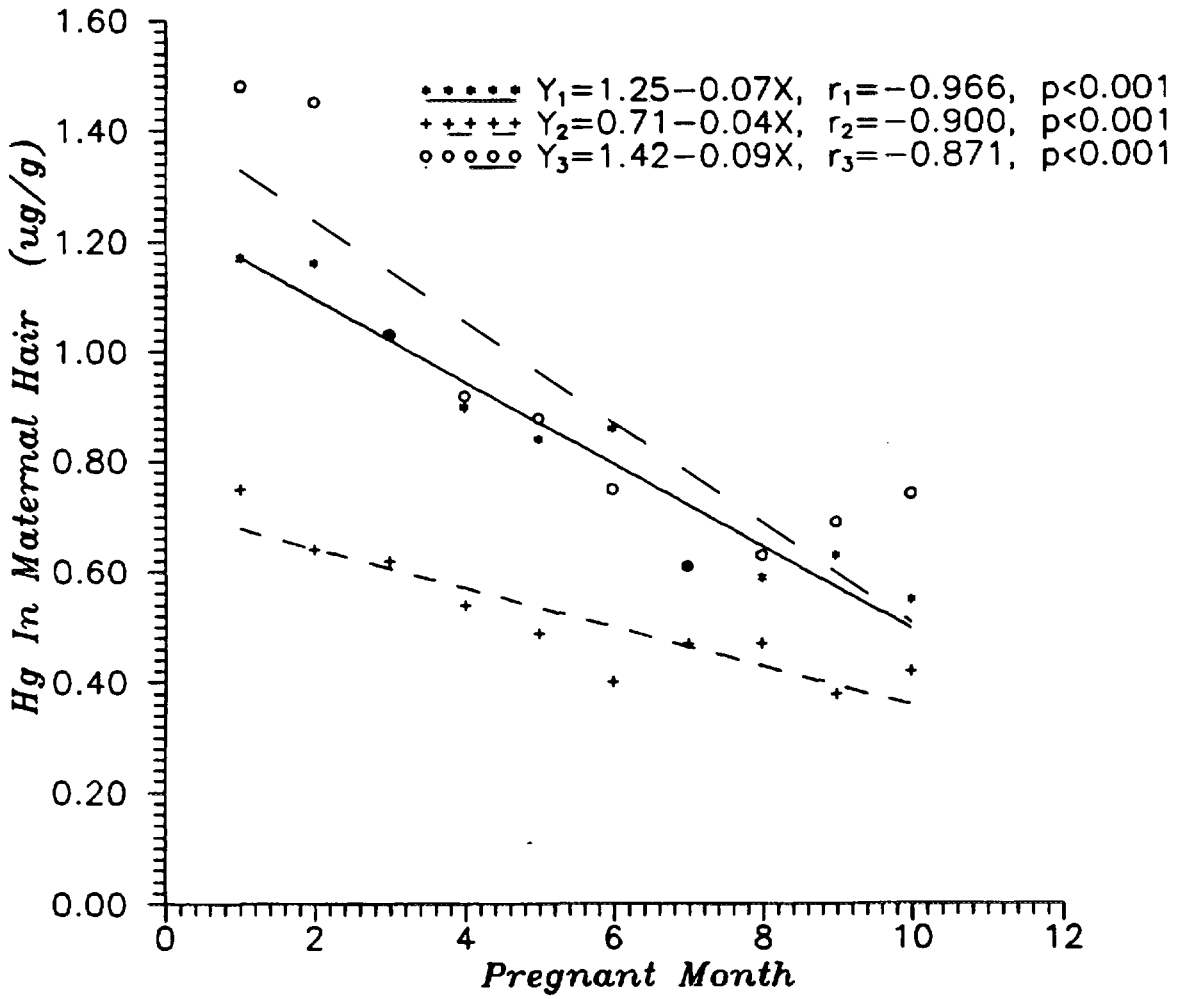

Figure 4. **Variations of hair Hg contents of three pregnant women with time.**

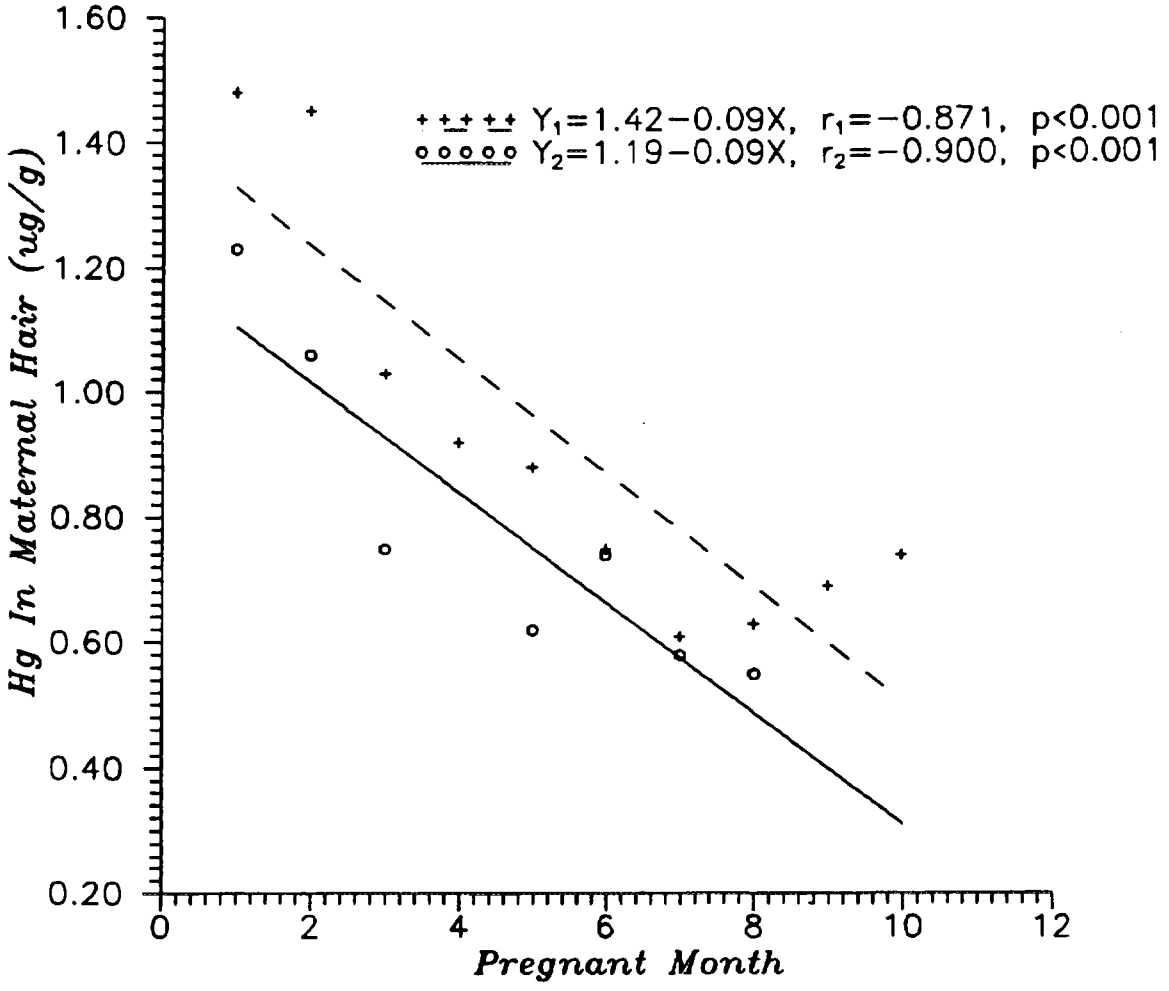

Figure 5. **The changes of Hg and MeHg contents in one pregnant women's hair during her pregnancy determined by sectional INAA and GC(EC).**

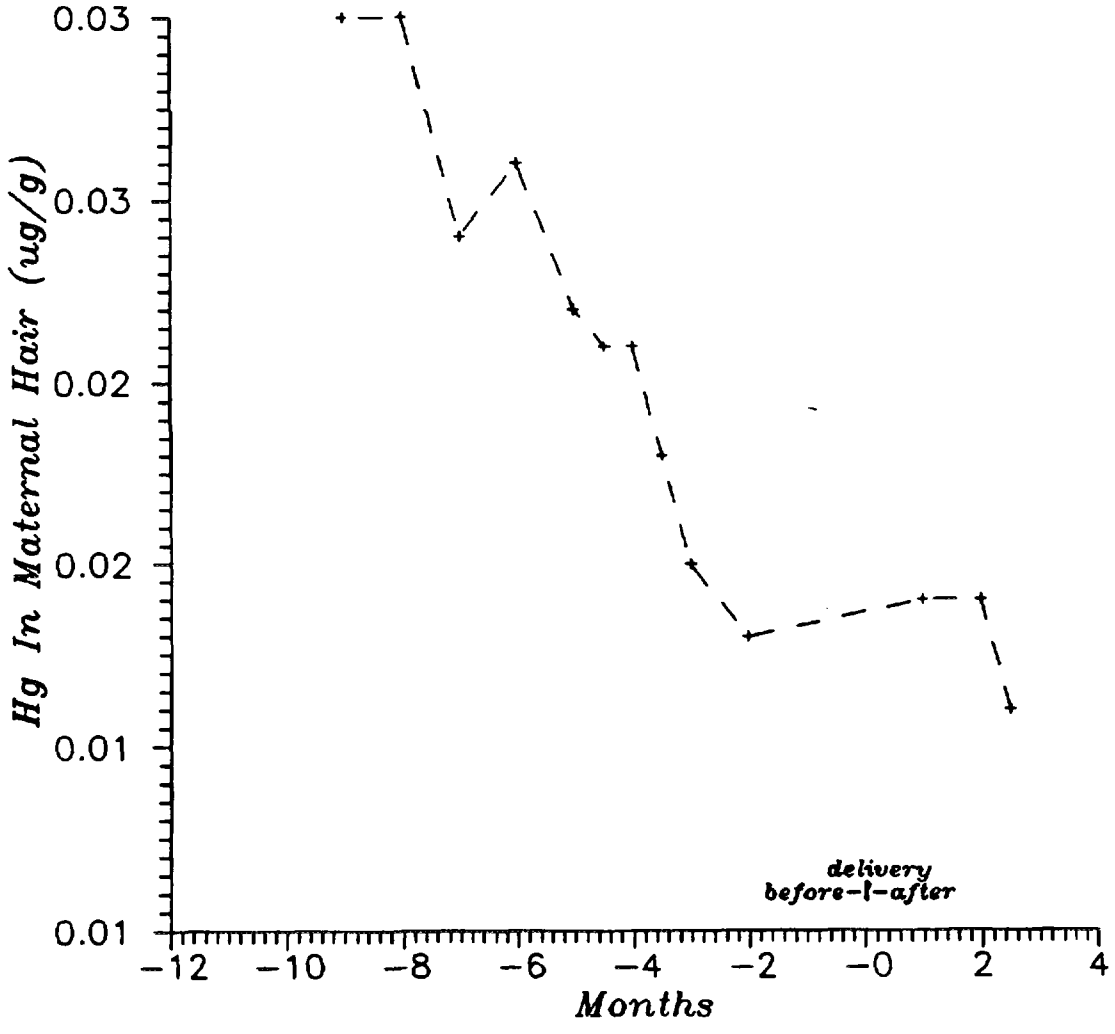

Figure 6. **The variation of Hg contents in one woman's hair during and immediately following pregnancy as determined by SRXRF.**

THE STUDY OF CHEMICAL FORMS OF MERCURY IN HUMAN HAIR AND OTHER BIO-ENVIRONMENTAL SAMPLES

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Abstract

The results of the continued studies on methods of analysis for forms of mercury in hair, and of the distribution of mercury among inorganic and organic forms in human hair are described. A new method for determining methylmercury has been developed, based on the selective leaching of methylmercury from hair using 2M hydrochloric acid. This method was used, in combination with the determination of mercury by atomic absorption spectrometry, for the analysis of mercury forms in three samples of human hair and two samples of fish homogenate. Good reproducibility of parallel determinations was obtained. The results were compared with literature data for the samples, or with the results of the analysis of the same samples by extraction method described earlier. Good agreement was also found between these methods. Further experiments were concerned with the study of the effect of radiation sterilization on the forms of mercury in hair, of the speciation of ^{203}Hg formed by irradiation of hair in nuclear reactor and with the labelling of a large batch of human hair with methylmercury.

1. INTRODUCTION

Since the last RCM held in Kuala Lumpur, our working group continued to elaborate analytical techniques suitable for determination of methylmercury (MeHg) and inorganic mercury (Hg_{in}) species in human hair. The work done within the framework of the CRP can be divided into several topics:

(1) The study of the speciation in hair of ^{203}Hg formed by irradiation in a nuclear reactor.

(2) The study of the effect of radiation sterilization on mercury species in hair.

(3) Finding suitable conditions for selective leaching of methylmercury from hair.

(4) Comparison of solvent extraction and acid leaching separation procedures.

(5) Preparation of a large batch of human hair labelled with methylmercury.

(6) The analysis of the intercomparison materials IAEA-085 and IAEA-086.

The results achieved are described below.

2. METHODS

2.1. The Study of The Speciation in Hair of ^{203}Hg Formed by Irradiation in a Nuclear Reactor

Neutron activation analysis using a nuclear reactor is one of the methods frequently employed for the analysis of mercury in environmental samples. It has also been used for determination of mercury in hair. It appeared interesting to examine whether the activation product of mercury formed during the irradiation of hair samples in a reactor remained in the original form of stable mercury. If this were the case, separation of the forms could be made after the irradiation of samples and, in this way, the forms could be determined. Therefore, we irradiated cut hair samples spiked with either inorganic mercury (Hg_{in}), or methylmercury (MeHg). The method described in the our last report [1] was used for the spiking.

100-150 mg hair aliquots were heat-sealed into 25 mm diameter disk capsules made of polyethylene (PE). A mercury standard was prepared in the same way using chromatographic paper impregnated with a 10 % solution of thiourea to prevent mercury losses from PE capsules during irradiation.

The samples and standards were irradiated in a LWR-15 nuclear reactor at the Nuclear Research Institute, Řež for 10 hours, in a thermal neutron flux density of $3 \times 10^{13} \cdot cm^{-2}s^{-1}$. The PE discs (wrapped in Al foil to ensure adequate heat transfer) were stacked to form a column in an Al irradiation container in which up to 30 samples and/or standards could be accommodated. Neutron flux monitors (discs of about 50 mg Fe) were inserted between each six samples and/or standards to be able to correct for the axial neutron flux gradient; this gradient amounts to 10 - 30 % along the height of the column in various irradiation positions of the LWR-15 reactor.

After a cooling period of about 1 month, the PE disc capsules were counted for 120 min each on a 21 % relative efficiency HPGe detector coupled to a Nuclear Data computer controlled γ-spectrometric system. Prior to counting, the Al foil was removed from the PE capsules and the capsule surfaces were cleaned by washing with water and ethanol. Reproducible positioning of the samples and standards towards the detector (± 0.1 mm) was ensured by using special perspex holders (source-to-detector cap distance was 2 cm). Measurements were done on the ^{203}Hg peak at 279.1 keV. Data treatment and calculation of final results were carried out using adapted Nuclear Data software for neutron activation analysis on a PDP 11/73 computer.

Following the measurement for total mercury, the samples were unpacked and treated with the procedure [1] recommended for the separation of methylmercury: Irradiated hair samples of 100 - 150 mg were dissolved in centrifugation tube in 0.2mL 10M NaOH at 90-95°C. Then, 1.8 mL of distilled water was added and pH was adjusted to 0.5 - 1 using concentrated H_2SO_4. The solution was cooled down to room temperature and 150 mg of solid KI was added. After 10 min shaking with 2 mL of benzene the aqueous and organic phases were separated by centrifugation and the activities of ^{203}Hg in both phases were measured.

2.2. The Study of the Effect of Radiation Sterilization on Mercury Species in Hair.

Hair samples used for standardization purposes in the analysis of hair should be sterilised by irradiation to prevent biological changes of the samples and infection. It is therefore important to know whether radiation sterilization can change the speciation of mercury in hair. To invetigate this, we irradiated about 1 g of cut hair labelled with $Me^{203}Hg$ (3 μgHg/g using the standard procedure [1]) in GAMMACELL 220 (AECL Canada) with the dose 25kGy. An activity of 50 - 150 mg of irradiated hair was measured (A_{tot}), and methylmercury was then separated using the recommended extraction procedure [1]. The activity of the organic phase (A_{org}) was then determined.

2.3. Selective Leaching of Methylmercury from Hair

Earlier, we described the separation of methylmercury from inorganic mercury in hair by solvent extraction [1]. Our next step consisted of developing an alternative separation method based on selective leaching of methylmercury directly from hair. To release mercury species bound in solid samples, acid leaching is often used [2-8], occasionally in presence of cupric salt. However, only limited knowledge still exists on the behaviour of individual mercury species in the leaching process, and on possible changes in the speciation of mercury during leaching. This also applies for the isolation of mercury from hair. Therefore, we studied the leaching of methylmercury and inorganic mercury from hair using hydrochloric acid of various concentrations, and the effect of the presence of cupric ions on the extraction.

A radiotracer method was used throughout this work. The application of radioactively-labelled mercury species greatly facilitates the study of the behaviour of the species during the separation. The labeled species can be easily traced during the separation by measurement of the activity of samples, if isotope exchange between the labelled species and other mercury species is negligible or slow. The rate and extent of the isotope exchange between methylmercury and inorganic mercury in aqueous solution is known [9]. Therefore, conditions could be selected to maintain a negligible isotopic exchange. The methylmercury used in these studies was labelled with ^{203}Hg, and was prepared by the isotope exchange method [1,9].

About 0.5 kg of human hair (a mixture obtained from different persons) was cut to less than 5 mm long pieces by stainless-steel scissors, washed according to the procedure recommended by IAEA and WHO [7] and homogenized by mixing. A part of hair was ground down in an agate mill. Radioactively-labelled hair was then prepared by two alternative procedures:

Procedure A: 20 mL of aqueous phase containing 0.01 M acetate buffer (pH 4.7), 0.001M NaCl and 0.05 - 0.1 μg/mL of Hg as radioactively labelled Hg_{in} or MeHg is stirred with 1 g hair for 1 hour. The hair is separated by centrifugation, washed twice with 40 mL of distilled water, twice with 40 mL of acetone and air dried.

Procedure B: 20 mL of 2 M HCl containing 0.05 - 0.1 μg/mL of $^{203}Hg_{in}$ is shaken on a mechanical shaker with 1 g hair for 100 hours. The hair is separated by centrifugation, washed twice with 40 mL of distilled water, twice with 40 mL of acetone and air dried.

For the study of leaching of mercury species from hair using HCl in the concentration range 0.1 - 5 M , the following experiments were carried out: After determining its initial activity(A_0), 10 - 150 mg of radioactively labelled (with either $Me^{203}Hg$ or $^{203}Hg_{in}$) hair were shaken in centrifugation tube for appropriate time with 1 - 2 mL of leaching solution which contained hydrochloric acid of the required concentration and in certain cases also 1M $CuCl_2$ solution acidified by HCl. After centrifugation the activity of the separated aqueous phase (A) was measured and the distribution coefficient (K_D) was calculated:

$$K_D = \frac{A_0 - A}{A} \cdot \frac{V}{M} \qquad (1)$$

where V is the volume of the aqueous phase and m is mass of the hair sample.

2.4. Comparison of Solvent Extraction and Acid Leaching Procedures

Both the solvent extraction [1] and the acid leaching procedures for separation of MeHg from hair were developed using radiotracer method. The basic problem in the use of the radiotracer method for this purpose was to achieve equal behaviour between the labelled mercury species added to hair samples and mercury species naturally present in the hair. The equal behaviour cannot be easily proved by model experiments and must be checked in practical analyses. Comparison of results obtained by two different methods may be very helpful in this connection. Therefore we carried out analyses of mercury species in an unlabelled hair sample using two methods developed by us.

The extraction method [1] was slightly modified for this purpose (Figure 1): 1.4 mL of 10 M NaOH was added to 1 g sample of hair in a centrifuge tube. The tube was kept in a thermostat at 90 - 95°C for 30 min. Then 5 mL of distilled water was added to the dissolved sample and its pH value was adjusted to 0.5 - 1.0 using concentrated H_2SO_4. The sample was cooled to room temperature, 1 g of solid KI was added and MeHg was extracted by 30 min shaking with 4 mL of benzene. After separation of the phases by centrifugation the content of MeHg was determined directly in the organic phase and/or after re-extraction into 2 M NaOH.

A single purpose atomic absorption spectrometer AMA-254 was used for the determination of nanogram amounts of mercury. Its principle and use has been described in the literature [10,11]. 10 - 50 mg of solid hair or 50 - 100 μL of liquid sample is placed on a boat, then automatically transferred to a combustion furnace where it is initially dried and than burned in a stream of oxygen. The combustion products pass through a catalytic furnace. Here the oxidation is completed. The combustion products are then passed by a stream of oxygen through an amalgamator where mercury is entrapped. By a short-time heating of the amalgamator to a high temperature the entrapped mercury is released and driven to tandem measuring cells where absorbance is measured. The whole analytical run including all the parameters affecting the sensitivity and reproducibility of the determination are checked and controlled by a computer.

2.5. Analysis of Fish Homogenate

The proposed acid leaching method is based on the difference in the distribution of mercury species between a solid sample and the leaching solution. It can be expected that the values of distribution coefficients will strongly depend on the nature of analyzed material. The applicability of the leaching method must therefore be checked for each type of matrix.

For verification of the applicability of this method for determining methylmercury in fish samples, two IAEA certified samples (IAEA-350 and IAEA-MA-B-3) were analyzed using the recommended procedure (Figure 2) for the determination of MeHg. The content of total mercury was determined directly with the AMA-254.

2.6. Preparation of a Large Batch of Human Hair Labelled with Methylmercury

A large batch of human hair labelled with methylmercury was needed for an analytical intercomparison exercise and for the preparation of a standard reference hair. We modified the method developed earlier [1] in order to prepare 5 kg of human hair containing an elevated amount of methylmercury (MeHg). The method is based on the spiking of hair by MeHg from a solution which contains acetate buffer and sodium chloride. Unless otherwise stated, all reagents were of analytical reagent grade purity. The MeHg stock solution contained 172.5 mg of methylmercury chloride (Riedel de Haen, analytical standard) dissolved in 100 mL of 0.01 M NaOH. The solution was kept in a refrigerator. The acetate buffer stock solution (pH = 4.7) contained 143 mL of glacial acetic acid and 50 g of NaOH in 1 L. The NaCl stock solution was 1 M. Finally, the loading solution was prepared from 25 L of distilled water mixed with 100 mL of acetate buffer, 25 mL of NaCl and 20 mL MeHg stock solutions in a polyethylene bottle. This solution was prepared immediately before use. Hair cut to less than 10 mm pieces was supplied by IAEA. The prcedure used 1.25 kg of hair which was thoroughly soaked in 25 L of distilled water. The wet hair was taken out of water with plastic sieve, placed into a 40 L plastic vessel and mixed with loading solution in about 10 s. After 1 hour of stirring with a plastic agitator, the hair was taken out again, washed three times batchwise with 25 L of distilled water for 15 min and dried on air in a thin layer for 3 days.

The spiking was done in 4 batches (A,B,C,D). The portions A,B and C were prepared as described above. In batch D only 900 g of hair and 14.4 mL of MeHg stock solution were used.

2.7. IAEA-085 and IAEA-086 Intercomparison Study

The intercomparison material IAEA-085 (human hair with an elevated level of methylmercury) and IAEA-086 (human hair with a low level of mercury) were analyzed to check the content of mercury and methylmercury. IAEA 350, Tuna Homogenate (sample No: 346, distributed at 2nd RCM) was used as quality assurance material. The labeling described in the previous section (2.6) resulted in the base material for intercomparison material IAEA-085.

The total content of mercury was determined directly by AMA-254. The method of acid leaching was used for the determination of the content of methylmercury (organic bound mercury) and inorganic mercury. For the determination of water content in samples, 250 mg portions of each material were taken at the time of analysis and dried at 80°C for 24 hours. Six independent determinations were made for both total mercury and methylmercury in each material.

3. RESULTS AND DISCUSSION

The results of the study of stability of mercury forms during reactor irradiation were as follows: In the non-spiked hair, 0.32 ± 0.02 μg/gtotal mercury was determined; in hair spiked with MeHg, 7.3 ± 0.3μg/g was found; in hair spiked with Hg_{in}, 8.3 ± 0.3 μg/g was determined. The samples and mercury levels were selected to represent hair with natural mercury content and hair with different raios of MeHg/Hg_{in}, respectively. Following the separation procedure on these samples, in all cases, less than 0.5 % total activity was found in the organic phase. These results indicate that the radioactive ^{203}Hg originated during irradiation is present in the irradiated hair in inorganic form.

A summary of the results obtained from the studies on the effects of radiation sterilization is given in Table I. From these results, it is seen that methylmercury present in hair does not decompose during radiation sterilization.

The distribution of labelled mercury species between hair and HCl solution of various concentrations after 4 h of leaching is shown in Figure 3 (cut hair) and Figure 4 (ground hair). No differences have been found between leaching of mercury species from ground hair labelled by Procedures A and B. However, in the case of cut hair, the distribution coefficient of Hg_{in} depends on the method of spiking used (Fig. 3, curves 3 and 5). The presence of Cu^{2+} in the leaching solution causes a decrease in K_D of both MeHg and Hg_{in} and makes the separation of these forms less efficient.

The dependence of K_D on time of leaching using 2 M HCl (Fig. 5) indicates that the equilibrium is reached within approximately 4 hours. Only for cut hair labelled with Hg_{in} by Procedure A does the quick transfer of the label into the aqueous phase followed by the slow re-uptake by hair occur (Fig.5, curve 3). Similar uptake is observed when non-spiked hair is treated with 2 M HCl containing labelled Hg_{in} (curve 4). The differences in the behaviour of Hg_{in} spiked on cut hair by Procedure A and B suggest that in Procedure A Hg_{in} is adsorbed on the surface of hair whereas in Procedure B it is absorbed into hair.

On the basis of the above results, while respecting the natural level of mercury species in hair and the sensitivity of the analytical method used, the leaching with 2 M HCl for 4 hours at the ratio V/m = 40 mL/g was chosen for separation of mercury species in hair. Based on these results, the recommended procedure is now (Fig. 2): 90 - 125 mg of cut hair is shaken with 3.6 - 5 mL of 2 M HCl (the ratio V/m is kept at 40 $mL \cdot g^{-1}$) on a mechanical shaker for 4 hours. The aqueous phase is separated by centrifugation, its mercury content is determined and from it the concentration of methylmercury in the original hair sample is calculated. The separated hair is washed twice with 5 mL of distilled water, air dried and the content of inorganic mercury in hair is determined.

The results obtained by analysis of the same hair sample using the solvent extraction method (Figure 1) and the acid leaching method (Figure 2) are given in Tables II and III. The results show that consistent values were obtained for the content of methylmercury using both methods. The differences are not statistically significant. Results obtained by the direct determination of total mercury in the hair are presented in Table IV. A good agreement was reached between these results and the sum of contents of mercury species found by the acid leaching method (Table II). It confirms that the value obtained by analysis of hair after the acid leaching represents the content of Hg_{in}.

The good agreement of the data obtained on the speciation of natural mercury in hair using two very different separation methods also suggests that natural mercury species from hair behave in the separation process similarly to radioactively-labelled species, used in the development of both separation methods. This confirms the validity of basic assumptions of radiotracer methods applied in the development of the procedure. The proposed leaching method enables a simple and rapid determination of both methylmercury and inorganic mercury in a 100 mg sample of human hair. The method is suitable for serial analysis.

Table V shows the results obtained for the analysis of the IAEA Fish Homogenate samples are compared with literature values [6,12]. The good agreement between the data proves that the acid leaching method is suitable also for determination of methylmercury in fish samples.

Following the preparation of the labeled human hair material, the four batches of spiked hair were handed over to Agency personnel for further preparation. The batches were combined and provided the basis for the intercomparison material IAEA-085 [13], following homogenization and further preparation. The analytical results of the intercomparison materials IAEA-085, as well as of the natural level material IAEA-086, and of the quality assurance materials are presented in Table VI. These results are expressed on dry weight basis.

4. PLANS FOR FUTURE WORK

For the final year of this CRP, we plan to select a polluted (heavily industrialized) area and a relatively clean area in Czech republic. Hair samples will be collected from the population groups in chosen areas and analyzed for methylmercury and total mercury. The acid leaching method will be used for separation of mercury species and AMA-254 will be used for mercury determination.

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TABLE I. STABILITY OF MeHg IN HAIR DURING RADIATION STERILIZATION

Hair weight [mg]	A_{tot} [count/100s]	A_{org} [count/100s]	$100A_{org}/A_{tot}$ [%]
61.0	27106	27554	101.6
107.5	48788	49408	101.3
119.0	59786	58543	97.9
121.1	59781	58486	97.8
mean			99.7 ± 1.8 %

TABLE II. DETERMINATION OF Hg SPECIES IN HAIR USING THE ACID LEACHING METHOD

Hair weight [mg]	MeHg found [ng $Hg \cdot g^{-1}$]	Hg_{in} found [ng $Hg \cdot g^{-1}$]	Total Hg [ng $Hg \cdot g^{-1}$]
125	135	283	418
107	124	291	415
117	136	287	423
115	128	298	426
111	128	285	413
103	132	283	415
110	125	309	434
112	120	293	413
mean ± CI	129 ± 5	291 ± 8	420 ± 12

CI - 95% confidence interval

TABLE III. DETERMINATION OF MeHg IN HAIR BY USING THE SOLVENT EXTRACTION METHOD

Hair weight [g]	C_6H_6 phase MeHg found [ng $Hg \cdot g^{-1}$]	NaOH phase MeHg found [ng $Hg \cdot g^{-1}$]
0.888	144	103
1.032	114	121
1.088	113	99
1.076	120	99
1.024	127	118
1.000	123	130
1.000	122	135
mean ± CI	123 ± 10	115 ± 12

CI - 95% confidence interval

TABLE IV. DIRECT DETERMINATION OF TOTAL Hg IN HAIR BY AMA-254

Hair weight [mg]	Hg found [ng]	Hg total [ng $Hg \cdot g^{-1}$]
11.52	5.09	441
15.90	6.96	437
16.12	6.82	423
16.31	6.93	424
15.42	6.67	432
23.46	9.75	415
11.38	4.98	437
17.24	7.42	430
18.03	7.82	433
20.03	8.47	422
mean ± CI		429 ± 6

CI - 95% confidence interval

TABLE V. RESULTS FOR METHYLMERCURY AND TOTAL MERCURY IN IAEA REFERENCE MATERIALS

Reference Material	Methyl mercury		Total mercury	
	found	published*	found	published*
IAEA-350 Tuna fish homogenate	3.77 ± 0.07	3.32 - 4.01	4.87 ± 0.04	4.99 ± 0.26
IAEA MA-B-3 Fish tissue lyophilized	0.470 ± 0.006	0.411 - 0.503	0.525 ± 0.005	0.510 ± 0.07

* literature values published in [6,12]

Results are given in μg Hg/g, dry weight, quoted variation is 95% confidence interval

TABLE VI. ANALYTICAL RESULTS FOR METHYLMERCURY, INORGANIC MERCURY AND TOTAL MERCURY COMPOUNDS IN THE INTERCOMPARISON MATERIAL

Sample code	Dry/wet ratio	MeHg	Hg_{in}	Total Hg
IAEA - 085	0.90	20.9 ± 0.1	1.8 ± 0.1	23.4 ± 0.4
IAEA - 086	0.88	0.28 ± 0.01	0.37 ± 0.03	0.60 ± 0.01

Results are given in μg Hg/g, dry weight, quoted variation is one standard deviation.

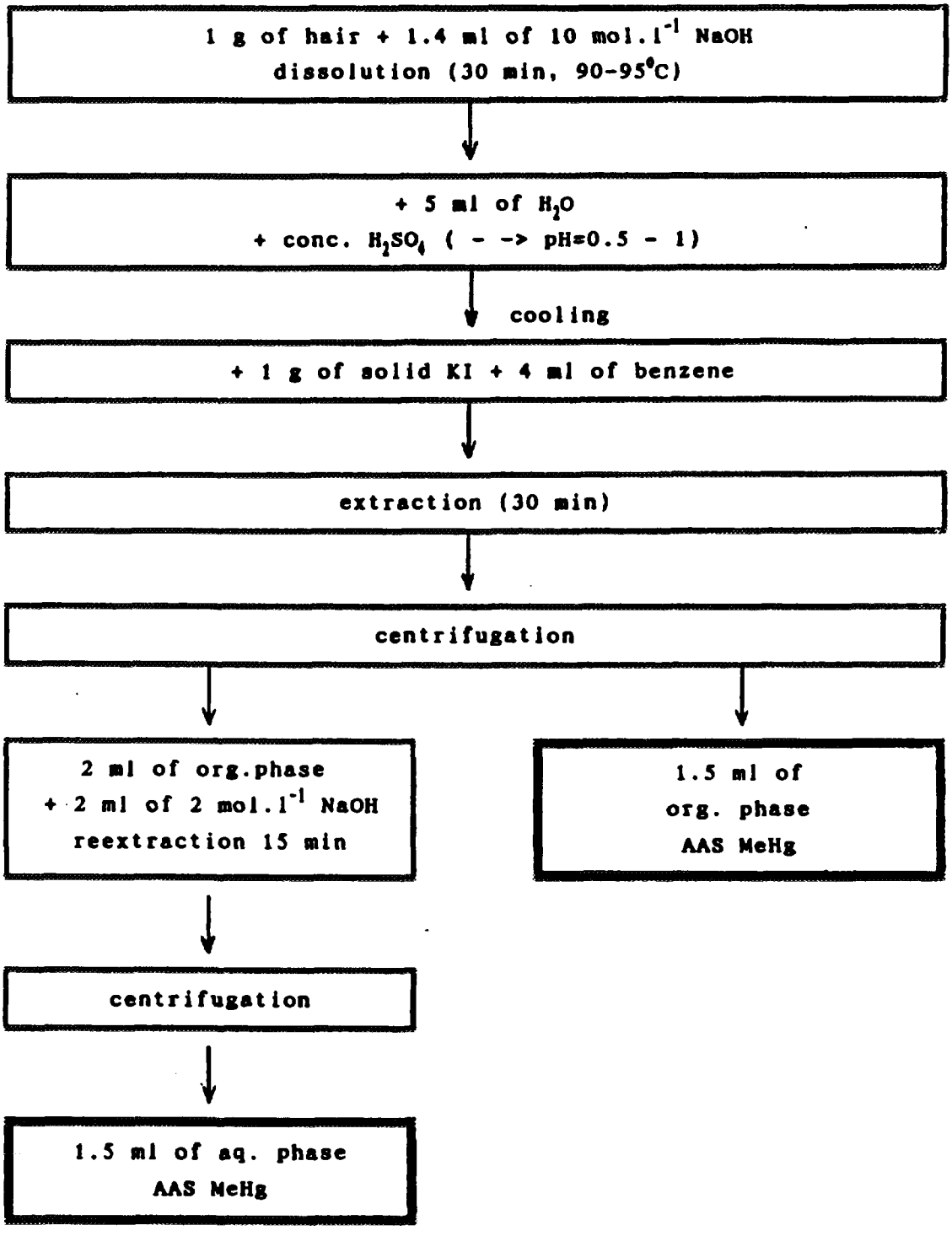


Figure 1. The solvent extraction method

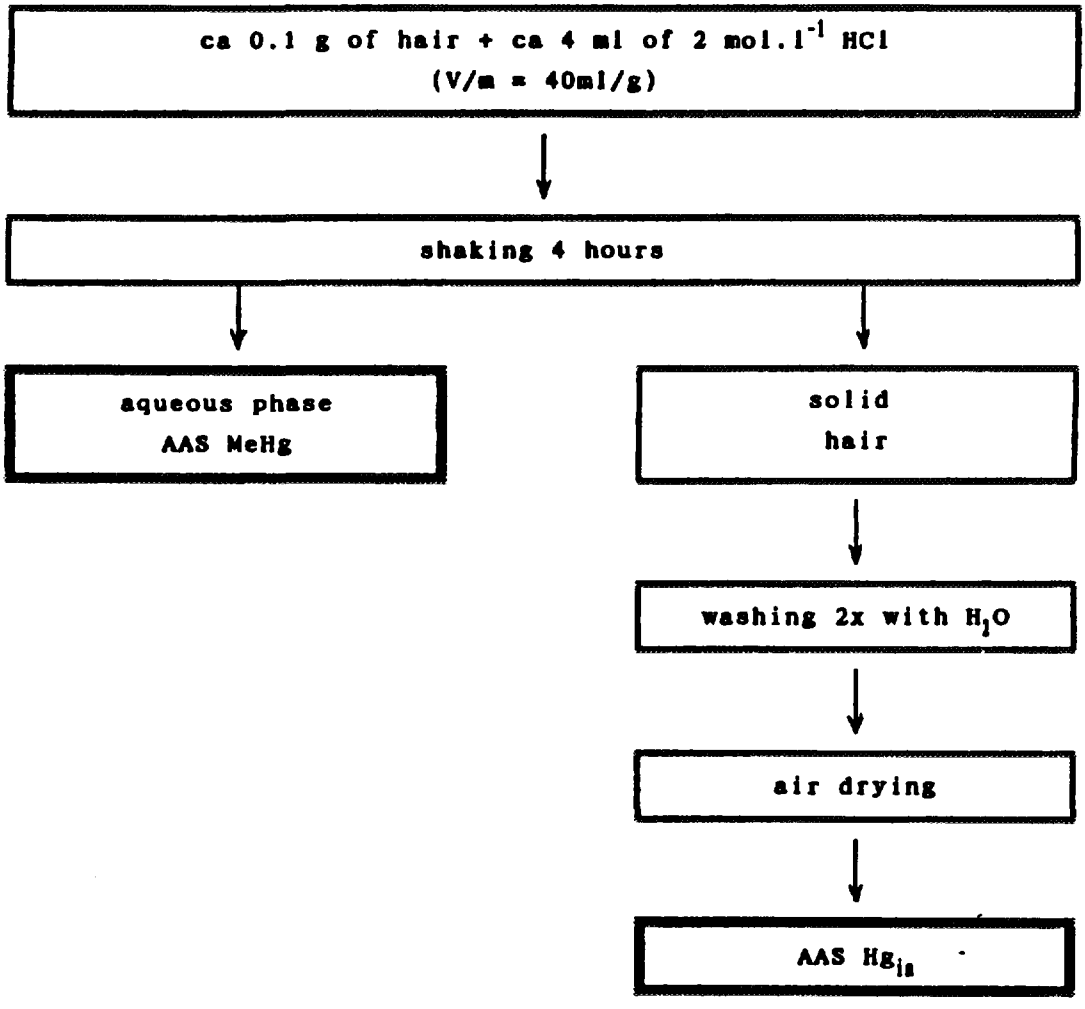
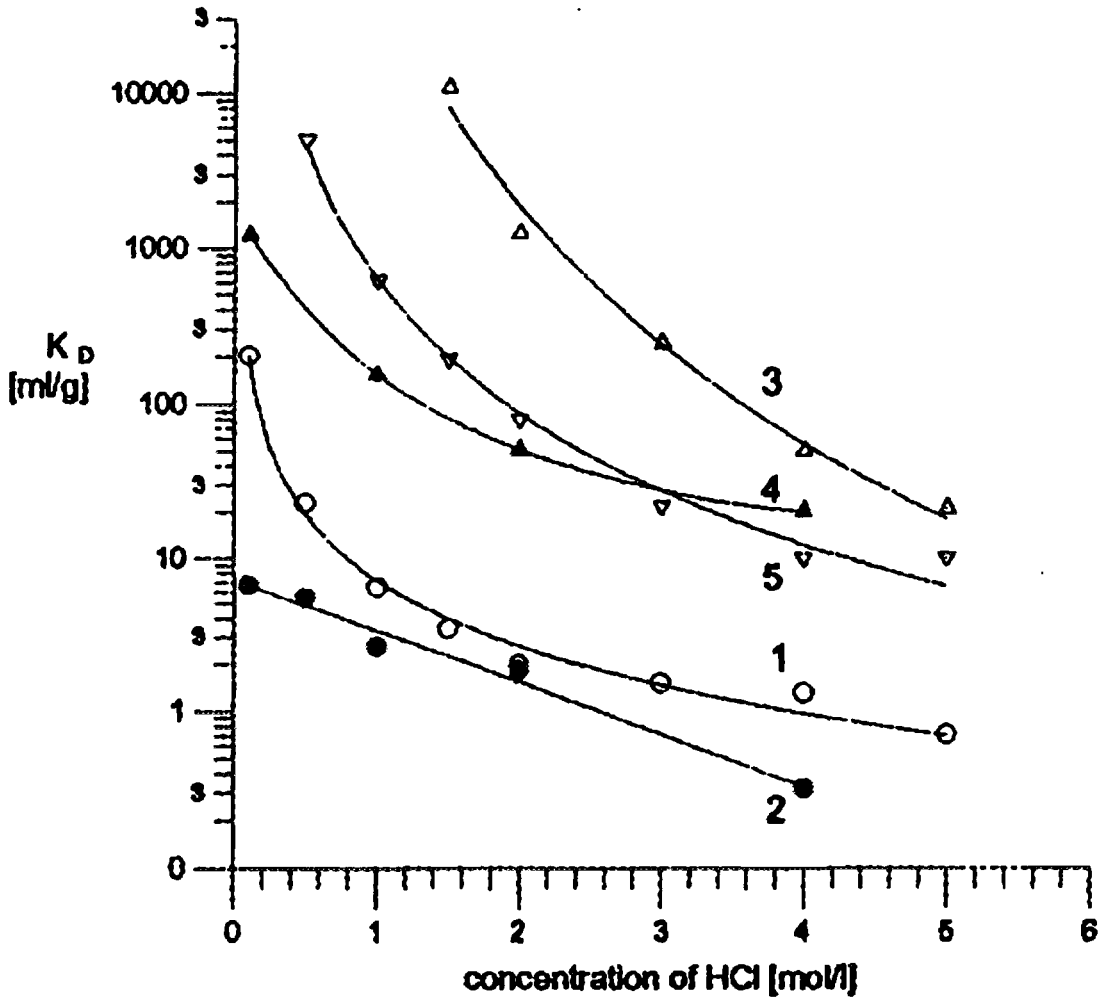
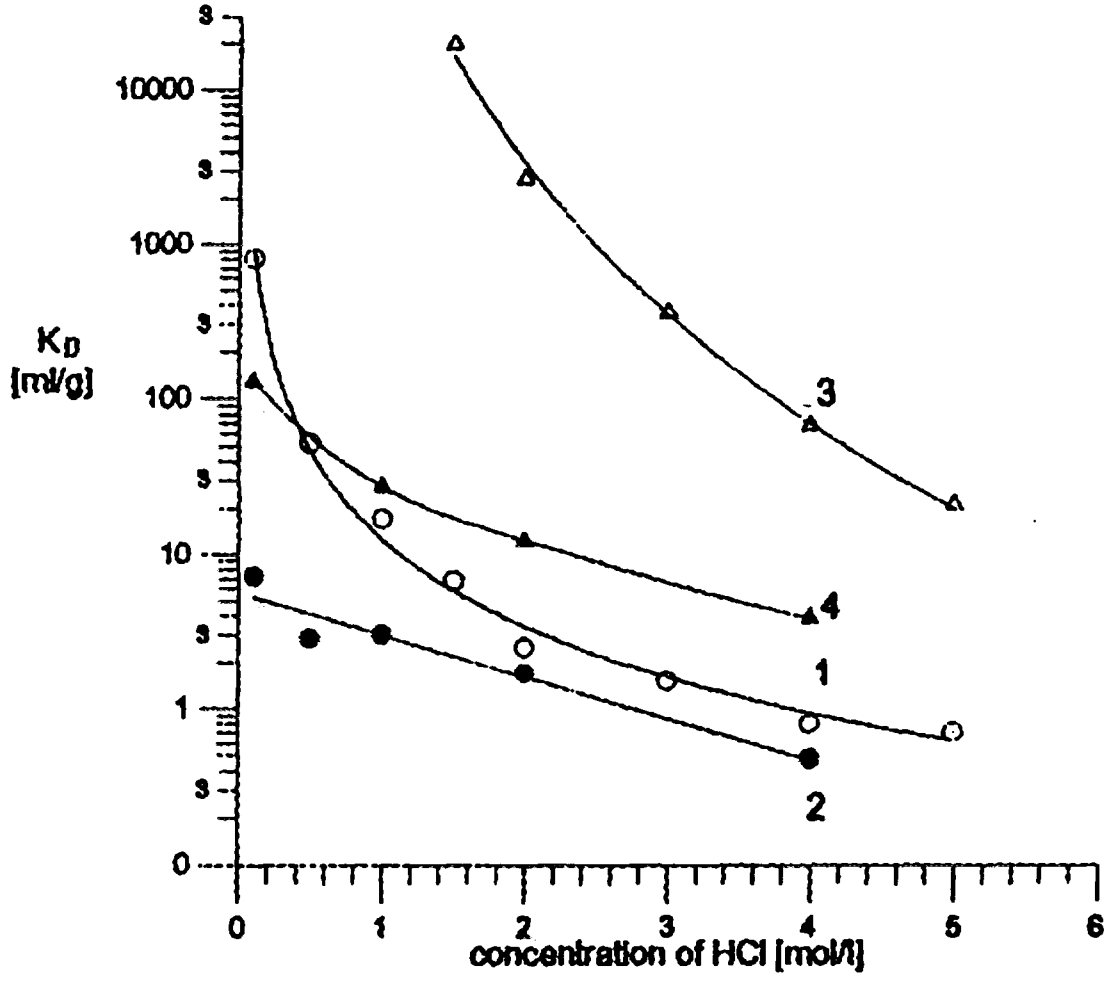


Figure 2. The acid leaching method



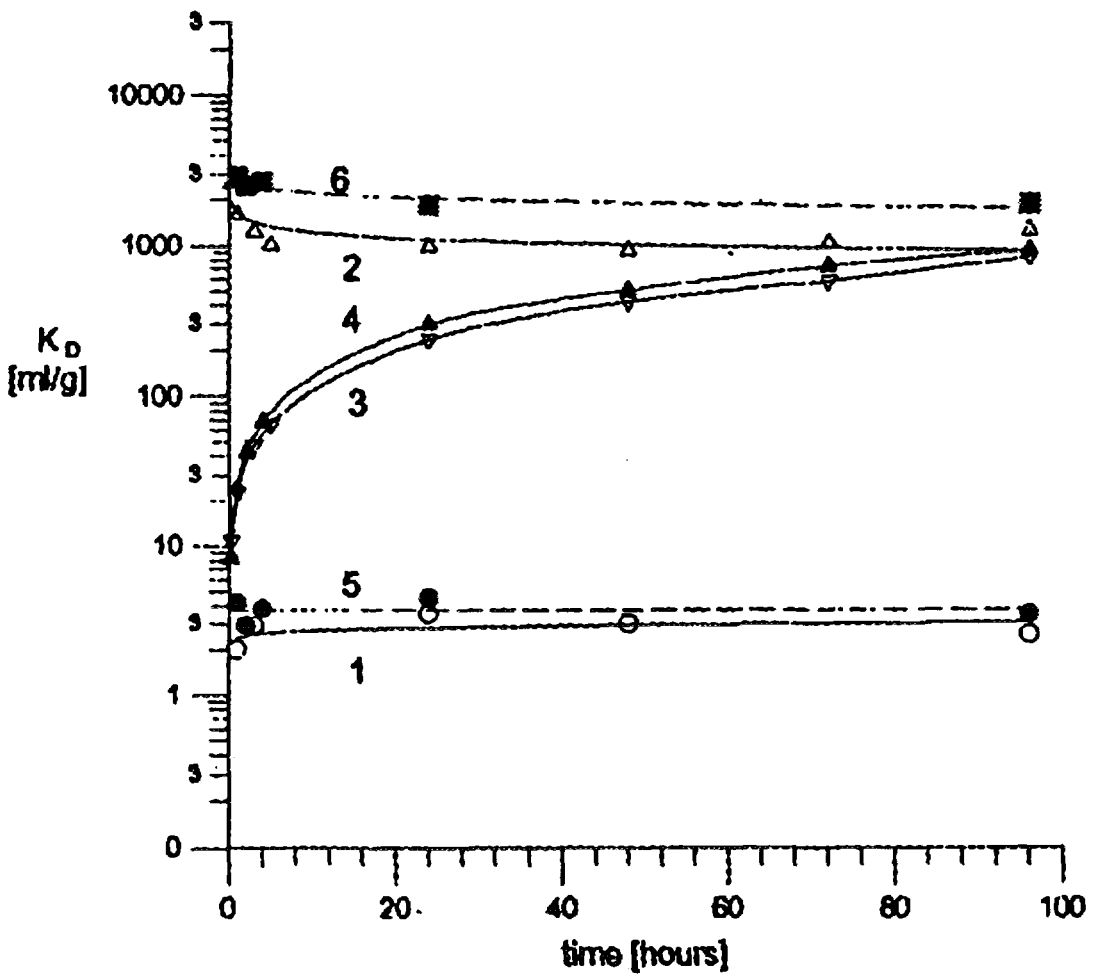
1 - MeHg; 2 - MeHg, in the presence of 1 mol·l^{-1} Cu^{2+}; 3 - Hg_{in}, prepared by procedure B; 4 - Hg_{in}, prepared by procedure B, in the presence of 1 mol·l^{-1} Cu^{2+}; 5 - Hg_{in}, prepared by procedure A

Figure 3. Leaching of mercury species from cut hair with HCl.



1 - MeHg; 2 - MeHg, in the presence of 1 mol·l^{-1} Cu^{2+}; 3 - Hg_{in}; 4 - Hg_{in}, in the presence of 1 mol·l^{-1} Cu^{2+}

Figure 4. Leaching of mercury species from ground hair with HCl.



1 - MeHg, cut hair; 2 - Hg_{in}, cut hair prepared by procedure B; 3 - Hg_{in}, cut hair prepared by procedure A; 4 - uptake of Hg_{in} from 2M HCl by cut hair; 5 - MeHg, ground hair; 6 - Hg_{in}, ground hair

Figure 5. Kinetics of leaching of mercury species from hair with 2 $mol \cdot l^{-1}$ HCl.

PREPARATION OF STANDARD HAIR MATERIAL AND DEVELOPMENT OF ANALYTICAL METHODOLOGY

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Abstract

The concept of the use of human scalp hair as a first level indicator of exposure to inorganic pollutants has been established by us earlier. Efforts towards the preparation of a hair reference material are described. The analytical approaches for the determination of total mercury by cold vapour AAS and INAA and of methylmercury by extraction combined with gas chromatography coupled to an ECD are summarized with results on some of the samples analyzed, including the stability of values over a period of time of storage.

1. INTRODUCTION

The potential of hair as an indicator of environmental exposure was discussed at the first Advisory Group Meeting in 1976, when we had suggested a possible approach [1]. This approach was substantiated by our study of countrywide student population and general population of metropolitan city of Bombay [2] , which clearly established the role of human scalp hair as an effective first level monitor in a multi-level scheme of monitoring environmental exposure to inorganic pollutants. It is in this context that we offered to provide an adequate material of hair, subjected to minimal chemical treatment as dictated by the social customs and the ready availability of relatively large quantities. The characterization of the hair sample with respect to trace elements in general, and mercury in particular, requires the cross validation for which different analytical techniques need to be developed and applied. The programme of work carried out since the second meeting of CRP had the following objectives:

(a) To prepare a large quantity of hair material for despatch to Vienna towards a reference material;

(b) to measure total and methylmercury in a variety of hair samples; and

(c) to measure mercury levels in a restricted population, whose staple diet happens to be fish.

2. STANDARD MATERIAL OF HAIR

The first lot of 35 kgs of hair purchased from a temple at Tirupati did not give a sufficient amount of clean hair (< 10 kg) after processing at the Bhabha Atomic Research

Centre, which followed the preliminary processing at Tirupati. Accordingly, additional 25 kg hair was procured and, after the preliminary processing of physical cleaning, washing with acetone-water and finally with acetone at Tirupati, this was mixed with the other major portion. The total sample was then cut into small pieces, between 5 to 10 mm lengths, and then subjected to the standard washing procedure (acetone, followed by three times with deionized distilled water, and finally again with analytical grade acetone), followed by air drying). All these operations were carried out in a "clean" room of the laboratory using non-metallic containers, which were initially cleaned in 1% nitric acid. It is to be noted that a more careful examination of the thoroughly cleaned sample gave a ratio of weight of uncleaned (as procured) to final processed hair at nearly 6:1 instead of 2.5:1 as reported earlier.

Several aliquots of these samples were analyzed for both total mercury and methylmercury, the total mercury determination using both cold vapour atomic absorption and instrumental neutron activation analysis. The other elements determined were selenium, zinc, cadmium and cobalt using Cold Vapour Atomic Absorption Spectrometry (CVAAS), Instrumental Neutron Activation Analysis (INAA), Graphite Furnace Atomic Absorption Spectrometry (GFAAS) and Square Wave Cathodic Stipping Voltammetry (SWCSV). Ten kg of this final material was packaged in two containers, each containing about 5 kg, sealed in polyethylene bags, radiation sterilized to 50 KGy at ISOMED of Board of Radiation & Isotope Technology, and was shipped by air to Vienna in May 1993.

We are glad to note that the homogenization process has been successfully completed as also the spiking and that the samples are already out for intercomparison experiments.

3. SAMPLES ANALYZED

The samples that have been analyzed during this period are: 1) aliquots of hair samples drawn from the bulk of the material; 2) hair samples collected from other rural population groups whose staple diet is fish; 3)the old fish homogenate of the IAEA; and 4) the new homogenized hair samples 085 and 086. One vial of sample containing 085 which was received in February 1994 for possible use at the Agency's Workshop on Environmental and Industrial Applications of Nuclear Analytical Techniques was analyzed for both total mercury and methylmercury. The two samples, 085 and 086 sent for intercomparison exercises were received only in May 1994 and as such, these could not be analyzed for the required constituents. These results will be reported at a later time.

The basic principles of the experimental approaches followed for the determination of total mercury by CVAAS and INAA and of methylmercury by gas chromatography are briefly summarized below.

3.1. Total mercury: CVAAS

The hair sample is digested repeatedly with a mixture of nitric and sulphuric acid in a modified Bethge's apparatus. The last traces of organic matter are destroyed by fuming with perchloric acid. Mercury vapour is generated by stirring a sample aliquot with $SnCl_2$ solution which is then flushed with purified air into the absorption cell. A mercury vapour

lamp is used as the source of the incident beam. The concentration of mercury in the sample is worked out from a calibration graph.

3.2. Total mercury : INAA

The samples and standard solutions are sealed independently in quartz ampoules and irradiated at a flux of $10^{12} cm^{-2} \cdot s^{-1}$ in a pool reactor in which the temperature of water does not rise beyond 40°C; blank quartz ampoules are also included in the irradiation. Quantitation of radionuclides is based on two time-dependant measurements and, wherever possible, on more than one γ-ray energy. These measurements are carried out using a high purity germanium detector coupled to multi-channel pulse height analyzer.

3.3. Methylmercury

The hair sample is disintegrated in a solution of sodium hydroxide in presence of L-cysteine hydrochloride. MeHg is extracted from an aliquot of the solution into benzene and after the removal of emulsion, a small volume is injected into a gas chromatograph, which is equipped with a column of diethylene glycol succinate on Supelcoport, 100-200 mesh. MeHg is detected with an electron capture detector and its amount is determined by comparing the peak heights/areas with those of appropriate standards.

4. RESULTS

Some of the results are summarized in Tables I through IV. The analysis of samples from the West Coast of India from small towns and villages, who are basically fishermen, showed a large variation in the concentration of mercury. However, the maximum value was only marginally higher than that for normal population. The stability of values for total mercury and also methylmercury has been demonstrated by analysis of the same sample over a period of 1-2 years, as indicated in Tables I and II.

5. PLANS FOR FUTURE WORK

In future work we plan to look at other approaches to the determination of forms mercury, particularly methylmercury. More importantly, we also plan to analyse for selenium in some of the samples that we have already collected from specific population groups for determining mercury.

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[2] GANGADHARAN, S., ARUNACHALAM, J., BHAT, K.R., YEGNASUBRAMANYAN, S., Neutron Activation Analysis in the Monitoring of Health-related Trace Element Pollutants, IAEA/TECDOC-330 (1985) 147-154.

TABLE I. TOTAL MERCURY (CVAAS) μg/gm

Hair Sample	30/07/92	17/09/93	04/0294
TPT 4	0.44	0.40	0.47
TPT 5	0.39	0.41	0.38
TPT 9	0.39	0.37	0.34
TPT 11	0.37	0.37	0.40

(Values reported on 04/02/94 are from the recent IAEA Workshop)

TABLE II. STABILITY OF METHYMERCURY IN HAIR SAMPLE

Sample	MeHg (μg/g ± SD)	
TPT 4	18/01/93	25/01/94
	0.40 ± 0.02	0.40 ± 0.02

TABLE III. MERCURY AND METHYLMERCURY

Sample	Concentration μg/g	
	Total Hg (INAA, CVAAS)	MeHg (GC - ECD)
Fish Homogenate (MA-A-2)	0.5 ± 0.03	0.35 ± 0.04
Fish Homogenate (305)	0.41 ± 0.03	0.31 ± 0.01
Hair (IAEA-085) (Received Feb. 1994)	19.5 ± 0.5	12.2 ± 0.2
Indian Population Hair	0.4 ± 0.03	0.35 ± 0.02

TABLE IV. TOTAL MERCURY IN HAIR

Sample	CVAAS	INAA	
		^{197}mHg	^{203}Hg
IAEA-085 (Bottle No. 3)	22.0 ± 0.4	26 ± 0.1	32
IAEA-086 (Bottle NO. 4)	3.3 ± 0.1	0.5	0.6

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MERCURY LEVELS IN DEFINED POPULATION GROUPS

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Abstract

Hair samples from subjects living in the areas of Bagnara Calabra, Fiumicino and Ravenna, having a fish consumption above the national average, have been analyzed. A new location close to the Lagoon of Grado and Marano, located near the border with Slovenia, has been selected because of the high natural levels of mercury in this lagoon due to the discharge of the Isonzo river, a tributary of which crosses the mercury rich area of Idria in Slovenia. During the last year, a group of pregnant women were selected in Rome, Bagnara Calabra, Ravenna and the area of the Lagoon of Grado and Marano. Samples of hair, pubic hair and placenta were collected from each of the subjects. A sample of the newborn hair was also collected whenever possible. The preliminary results indicate higher mercury levels in the subjects living in the area around the Lagoon of Grado and Marano.

1. INTRODUCTION

The purpose of this study is to assess the levels of exposure to mercury in selected groups of the general Italian population, using hair as a bio-monitor of the mercury contamination of the human body to verify that the tolerable recommended weekly intake of mercury set by FAO and WHO [1] are not exceeded, and to evaluate the effects of elevated intakes of mercury. It is well known that excluding occupational exposure, the consumption of fish and other seafood is the major pathway through which methylmercury enters the human body. The average consumption of fish at a national level is rather small in Italy, 12.5 kg per year. Therefore, there is only a negligible risk of exposure to elevated levels of mercury through the diet for members of the Italian general population. However, some population groups consume a diet rich in seafood. These groups are usually found in coastal towns and include subjects working as fishermen, fish dealers, restaurant workers and their families. Because of the higher susceptibility of fetuses to adverse health effects caused by mercury exposure during pregnancy, a group of pregnant women has also been taken into consideration to evaluate the transfer of mercury from the mother to the fetus. Because of the protective action of selenium against the adverse health effects of mercury, both these elements are determined in all the samples. This project is carried out with the collaboration of the National Institute of Nutrition and local health institutions.

2. METHODS

Hair samples were collected from subjects living in the areas of Bagnara Calabra, Fiumicino and Ravenna, having a fish consumption above the national average. These towns, in Southern, Central and Northern Italy, respectively, are located along the coast and have active fishing ports. A new location near the Lagoon of Grado and Marano that

is located near the border with Slovenia was selected because of the high natural levels of mercury in this lagoon due to the discharge of the Isonzo river, a tributary of which crosses the mercury rich area of Idria in Slovenia. A group of pregnant women was also examined in Rome, Bagnara Calabra, Ravenna and the area of the Lagoon of Grado and Marano. Samples of hair, pubic hair and placenta were collected from each subject. A sample of the newborn hair was also collected whenever possible soon after delivery. The hair samples were collected from the back of the head, cutting roughly the first 2 cm of the hair closer to the scalp. The weight of the samples was usually in the range 0.1 - 0.3 grams, but sometimes, particularly in the case of newborn hair, it was only a few milligrams.

2.1. Sample treatment

Hair samples are washed according to the IAEA protocol, once in acetone, three times in distilled water and once more in acetone. During each wash, the samples stand at room temperature for 10 minutes with the solvent while being stirred constantly. Placenta samples and total diets are first freeze dried and then homogenized with a blender having titanium knives.

2.2. Analytical methods

2.2.1. Determination of total mercury and selenium

The instrumental neutron activation analysis is the analytical method used for the determination of total mercury and selenium. The samples are enclosed in pure quartz vials and irradiated in the 1MW Triga reactor at the Casaccia research centre for about 14 hours in a thermal flux of approximately $2.6 \cdot 10^{12} n \cdot cm^{-2} \cdot sec^{-1}$. Standard reference materials (NIST, BCR and IAEA) are also irradiated at each run. The continuous rotation of the irradiation facility ensures a uniform neutron flux for all the samples. After an appropriate cooling time, the samples are transferred to polyethylene containers and measured by γ spectrometry using high-purity germanium detectors with relative efficiency of about 25% and resolution (FWHM) of 1.9 KeV at the 1332 KeV peak. The EG&G Ortec Computer program Omnigam is used for the analysis of the γ spectra.

2.2.2. Determination of methylmercury

Capillary gas chromatography with electron capture detection is used for the determination of methylmercury. Samples are digested in alkaline solution/toluene in ultrasonic bath at about 50°C. After cooling and treatment with HCl 6 N and $CuSO_4$ saturated solution, the organic phase is extracted with a cystein solution. Methylmercury is back extracted in toluene by adding $CuSO_4$ and KBr and analyzed by GC/ECD using a DB17 capillary column. The practical detection limit of the method for methylmercury is 50 ng/g using 100 mg of hair sample.

3. RESULTS AND DISCUSSION

To date, 394 hair samples have been analyzed including 14 subjects from the Casaccia research centre living in the Rome area and 77 samples collected from pregnant women. The age frequency distribution for the 317 subjects from the selected areas is shown in Figure 1. The median is 41 years and the range is 5 ÷ 76 years. Figures 2 and

3 show the age frequency distribution for the 274 male subjects and the 43 female subjects, respectively. The median age of the selected women is lower than that for men, 36 and 42 years, respectively.

For a more effective presentation, the Se and Hg concentration distributions are shown in the figures in the form of box plots. The Se concentration in hair of subjects from all selected areas is shown in Figure 4. Also shown are the data for male and female subjects. The range of Se concentration is 0.24 ÷ 327.8 μg/g with a median of 0.58 μg/g for male subjects. For female subjects, the median is 0.52 μg/g, slightly lower than that for males, and the range is 0.17 ÷ 0.78 μg/g. The Se concentration values above 1.5 μg/g, including the very high value of 327.8 μg/g observed in 7 male subjects were caused by the use of a particular shampoo very rich in selenium. As can be seen from Figure 5, the distribution of Se concentration in hair among the various areas is very similar. In fact, the range of the median concentrations for all the selected areas is 0.52 ÷ 0.60 μg/g. Figure 6 shows the Hg concentration for all the subjects from the selected areas and for male and female subjects separately. The range of Hg concentration for all the subjects is 0.23 ÷ 28.5 μg/g and the median value is 3.7 μg/g. Male subjects generally have higher Hg levels than females. The observed range of mercury concentration in hair of female subjects is in fact 0.76 ÷ 8.8 μg/g and the median value is 2.1 μg/g compared to 3.9 μg/g for male subjects. These values are correlated to the amount of fish consumed, the median fish consumption for male subjects is in fact 1000 grams, while it is 600 grams for female subjects. The data have been analyzed separately for each of the selected locations, the concentrations are presented in Figure 7. The highest values were observed in the area of Marano with a range of 0.86 ÷ 27.8 μg/g and a median of 5.1 μg/g. Slightly smaller Hg concentrations are present in the subjects from Fiumicino and Bagnara Calabra, the median value for both locations is 3.9 μg/g and the ranges are 0.26 ÷ 28.5 μg/g and 0.50 ÷ 17.4 μg/g, respectively. The range of Hg concentration is 0.58 ÷ 14.3 μg/g and the median value 2.4 μg/g for the subjects from Ravenna. Significantly lower values were observed in the small group of subjects from the Casaccia research with a range of 0.23 ÷ 7.6 μg/g and a median value of 1.3 μg/g.

The BCR Human Hair Reference Material 397 and 12 hair samples were analyzed for the determination of total mercury and methylmercury concentration and the results are reported in Table I. These hair samples were collected from adult subjects having a high fish consumption. It can be observed that the percentage of methylmercury is higher than 75% of total mercury. In some cases, the methylmercury concentration is higher than the total mercury concentration. This is probably due to an insufficient homogeneity of the samples and to the relatively high standard deviation of the methods used (> 10%).
To estimate the dietary intakes of Hg and Se in the area of Marano, four total diets were analyzed. The selection of the diets was based on a dietary survey carried out by the National Institute of Nutrition for the population group living in this area. All the food items necessary for the preparation of the diets were purchased in the town of Marano. The preparation of all diets was carried out in our laboratory following the local recipes. The diets were then homogenized and freeze dried. The concentration and daily ingestion of Hg and Se are reported in Table II. The variability of Hg concentration for the 4 diets is rather wide, 0.025 ÷ 0.090 μg/g. The results indicate that if diets having Hg concentrations in the upper end of the range are consumed daily, the weekly Hg intake would be higher than 200 μg, that is, the provisional tolerable weekly intake of methylmercury established by a FAO/WHO Expert Committee on Food Additives [1]. The Se daily intake appears to be rather satisfactory being within the recommended range of 50 ÷ 200 μg [2].

The age frequency distribution of the pregnant women examined to date is shown in Figure 8. The Se concentration in hair of subjects from all the selected area is shown in Figure 9. All the three groups present very similar Se concentrations, the median value for all the subjects is 0.51 μg, while it is 0.51 μg, 0.52 μg and 0.46 μg for the groups from Rome, Ravenna and Marano, respectively. The Hg concentration is very similar in hair of subjects from Rome and Ravenna with a median value of 1.13 μg/g and 1.17 μg/g, respectively, while it is significantly higher in hair of subjects from Marano where the median value is 2.16 μg/g and the range 0.27 ÷ 9.4 μg/g. A similar pattern is observed for the Se and Hg concentrations in pubic hair, shown in Figures 11 and 12, respectively, and in newborn hair, shown in Figures 13 and 14, respectively. The Se levels in the hair of the newborn are higher than those found in the hair and pubic hair of the mother. In fact, while the median Se concentration in the hair of newborn is 1.05 μg/g, it is 0.51 μg/g and 0.62 μg/g in the hair and pubic hair of the mother. The levels of Hg in hair and pubic hair of the mother and in the hair of the newborn are instead very similar, the Hg median values are in fact 1.17 μg/g, 1.32 μg/g and 1.27, respectively. The data for the Se concentration in the freeze dried placenta are shown in Figure 15, the values are similar for the three locations, the median value for all the subjects is 0.99 μg/g, while it is 1.04 μg/g, 1.0 μg/g and 0.91 μg/g for the groups from Rome, Ravenna and Marano, respectively. The Hg concentration is higher in the placenta of the subjects from Marano with a median value of 0.10 compared to the values of 0.08 and 0.06 found for the subjects from Ravenna and Rome respectively. The Hg data regarding the pregnant women have been analyzed to check if correlations were present among the concentrations in hair, pubic hair, newborn hair and placenta. Statistically significant correlations were found for all cases. In particular, the following correlation coefficients were found: $R = 0.88$ ($p = 1.0E-4$) between hair and pubic hair, $R = 0.89$ ($p = 1.0E-4$) between hair and placenta, $R = 0.78$ ($p = 1.0E-4$) between hair of mother and newborn hair, $R = 0.80$ ($p = 1.0E-4$) between pubic hair and placenta, $R = 0.82$ ($p = 1.0E-4$) between pubic hair of mother and newborn hair, $R = 0.68$ ($p = 3.0E-4$) between placenta and newborn hair. The strong correlation between mercury concentration in hair and pubic hair suggests that both types of hair can be used as an indicator tissue. The results on mercury concentration in newborn hair indicate that the placenta is not a barrier for mercury and therefore hair or pubic hair of pregnant women can be used to monitor the mercury levels in the fetus.

4. PLANS FOR FUTURE WORK

During the next year, we plan to complete the collection of the samples and carry out the determination of mercury and methylmercury giving priority to the samples from pregnant women.

5. REFERENCES

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[4] GRIEPINK, B., QUEUVAUVILLER, P., MAIER, E.A., VERCOUTERE, K., MUNTAU, H., The Certification of the Contents (Mass Fractions) of Cd, Hg, Pb, Se, and Zn in Human Hair (CRM 397). Rep. EUR-13433 EN-Luxembourg (1991).

TABLE I. TOTAL MERCURY AND METHYLMERCURY (AS μg/g OF Hg) CONCENTRATION IN HAIR SAMPLES

Sample	Total Hg	MeHg
BCR 397*	12.1	0.95
F46	27.5	25.5
F25	9.9	8.2
B2	12.6	16.8
B16	6.7	5.0
M6	19.9	15.5
M7	14.1	10.9
M1	5.7	5.8
M8	7.0	6.5
F11	12.4	17.2
R6	7.2	9.5
B3	12.5	18.9
F4	8.8	7.9

*BCR 397: Certified value for total Hg 12.3 ± 0.6 μg/g, literature values for MeHg 0.646 ± 0.011 [3], 0.840 ± 0.040 [4].

TABLE II. CONCENTRATION LEVELS IN TOTAL DIETS AND DAILY INGESTION OF Se AND Hg

Diet	Amount of fish (g)	Concentration in freeze dried diet (μg/g)		Daily ingestion (μg)	
		Se	Hg	Se	Hg
A	75	0.131	0.030	46.5	10.6
B	122	0.234	0.029	122.0	15.1
C	107	0.219	0.090	92.6	38.1
D	174	0.183	0.025	85.1	11.6

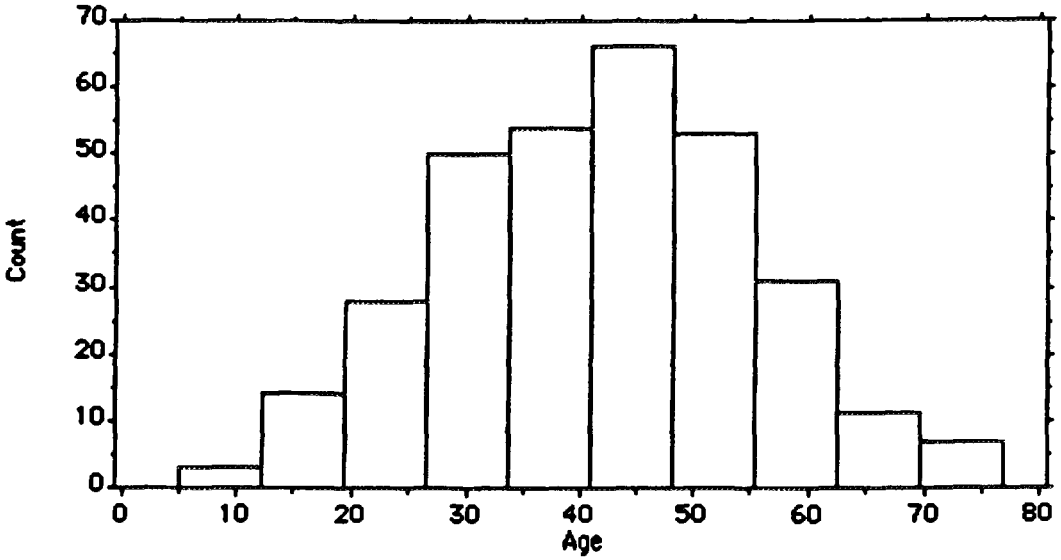

Figure 1. **Age frequency distribution of all subjects.**

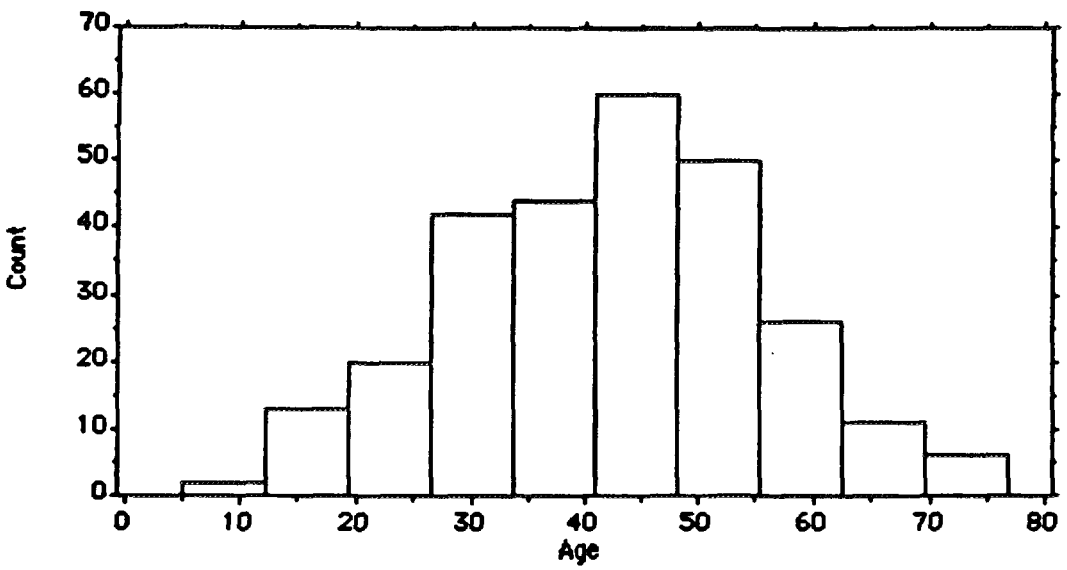

Figure 2. **Age frequency distribution of male subjects.**

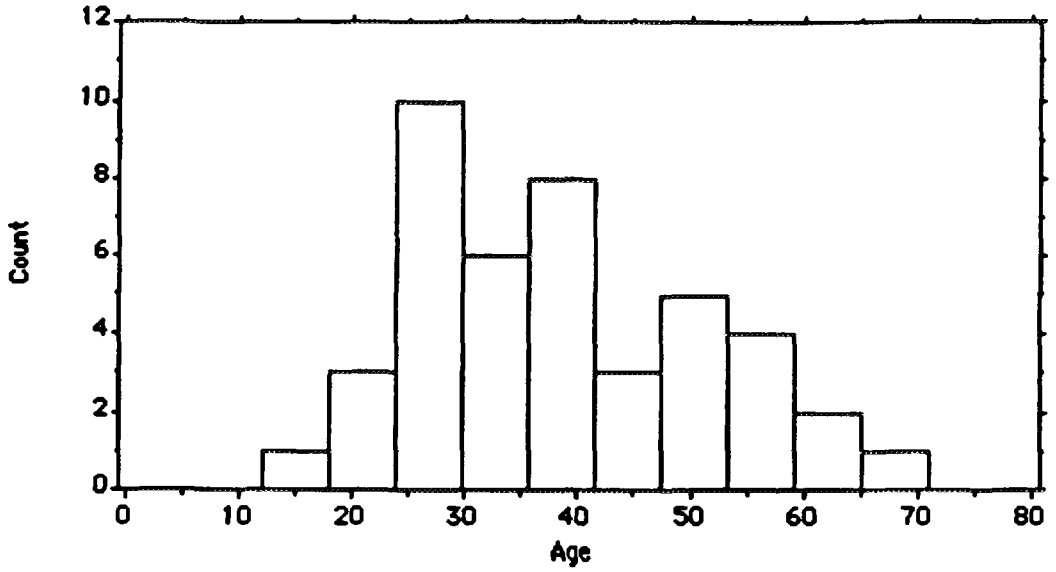

Figure 3. **Age frequency distribution of female subjects.**

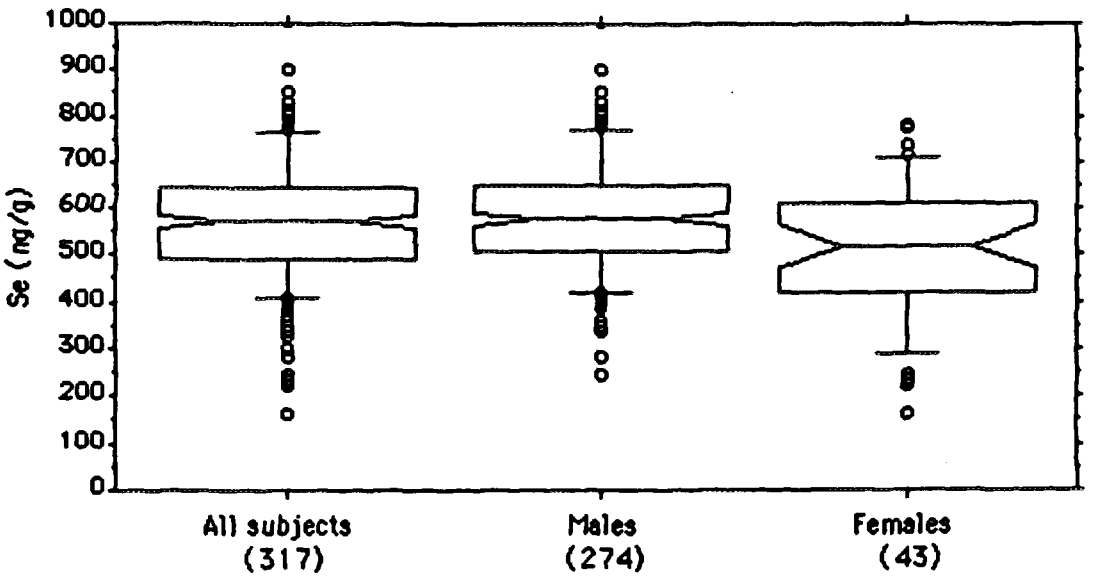

Figure 4. **Se concentration in hair of subjects from all selected areas.**

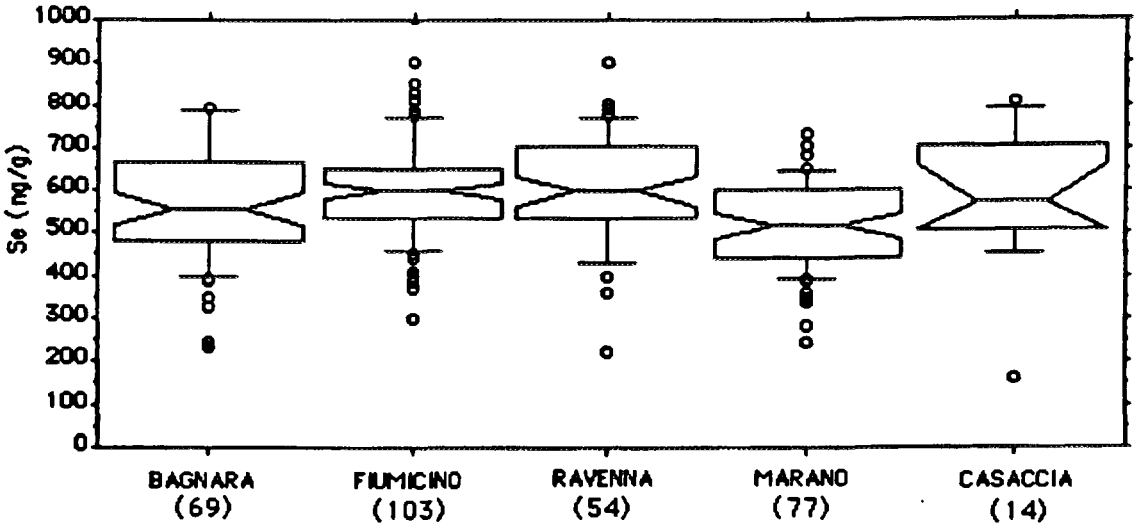

Figure 5. **Se concentration in hair of subjects by area.**

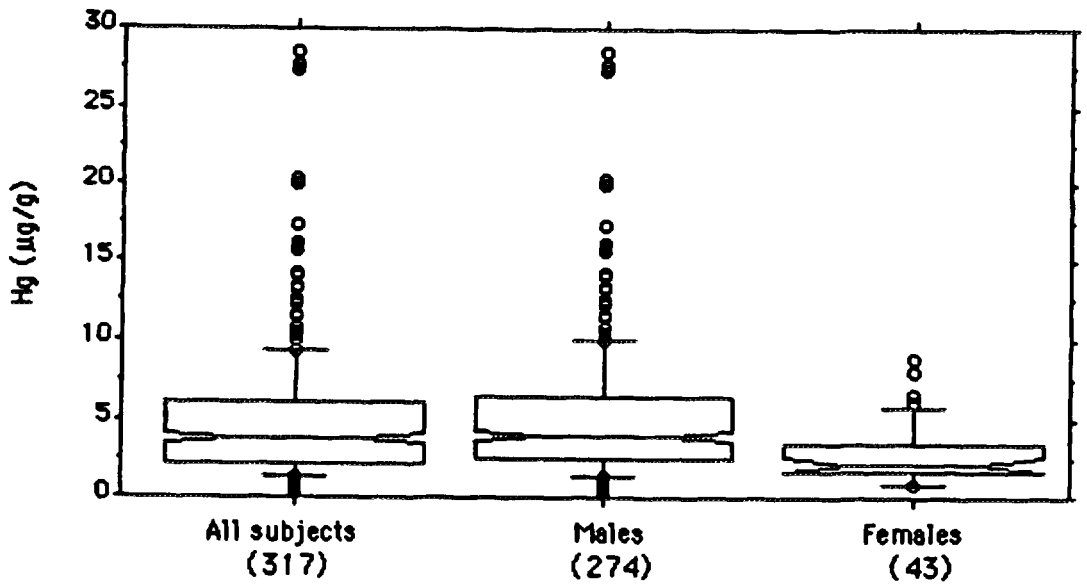

Figure 6. **Hg concentration in hair of subjects from all selected areas.**

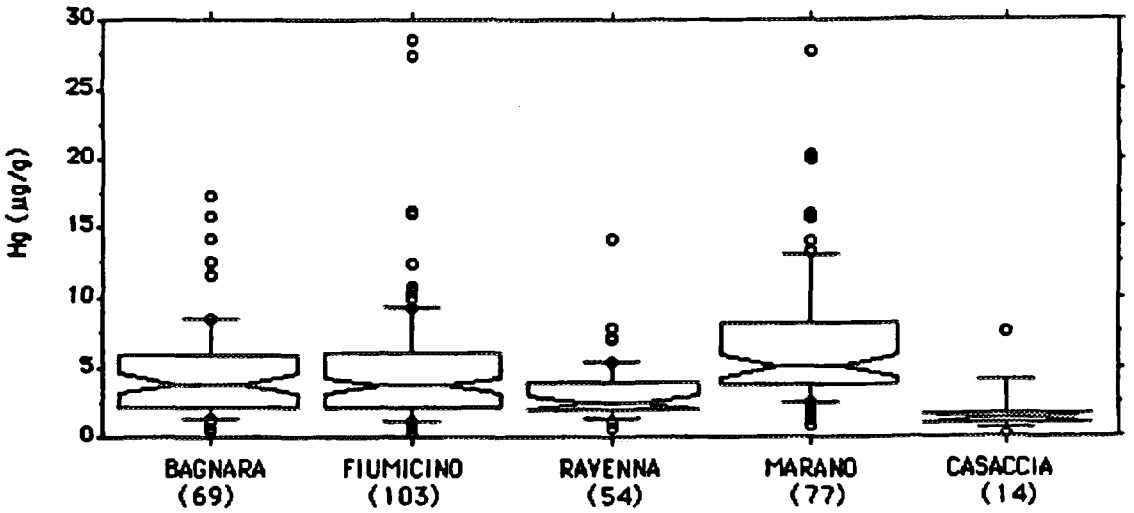

Figure 7. **Hg concentration in hair of subjects by area.**

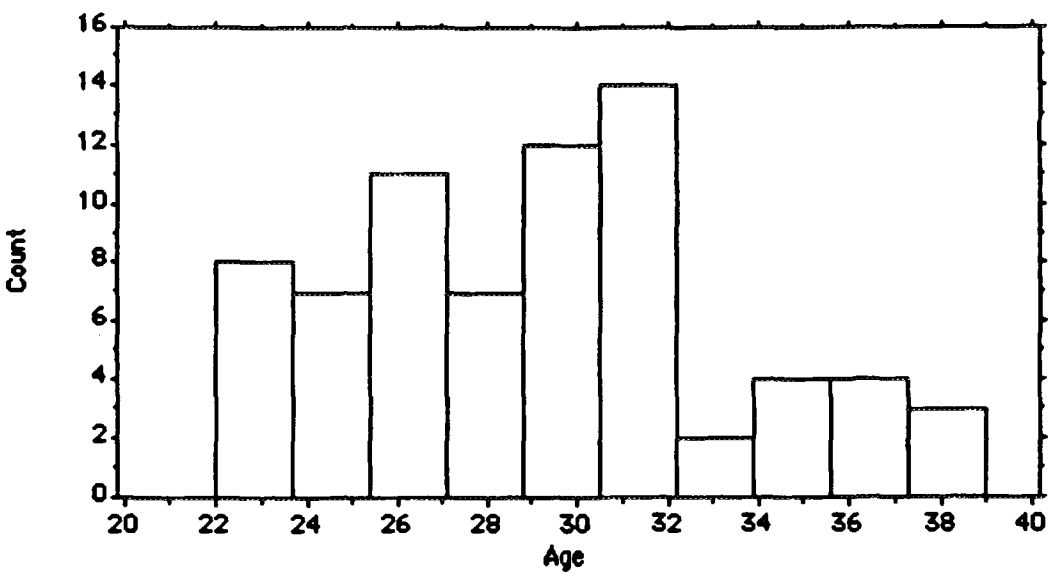

Figure 8. **Age frequency distribution of pregnant women.**

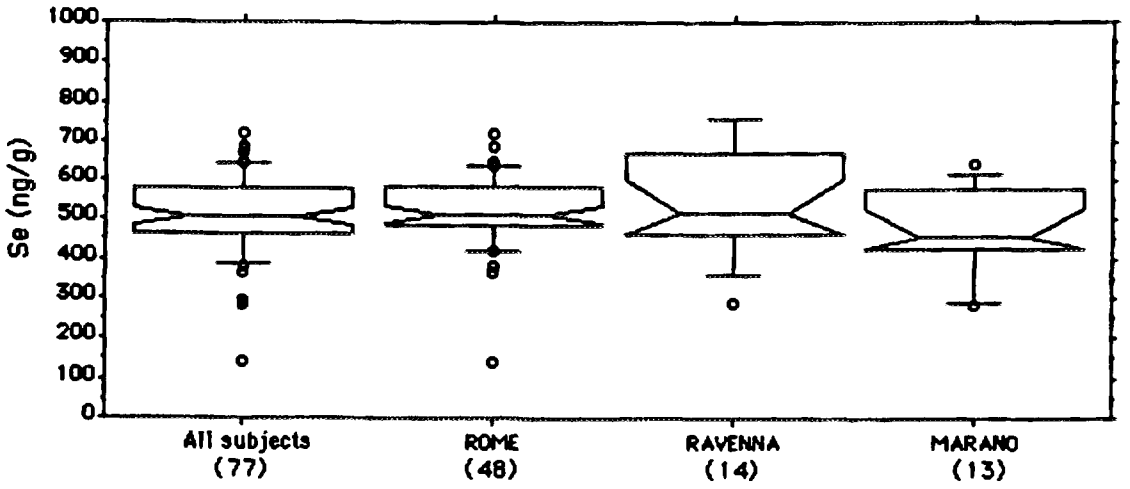

Figure 9. **Se concentration in hair of pregnant women.**

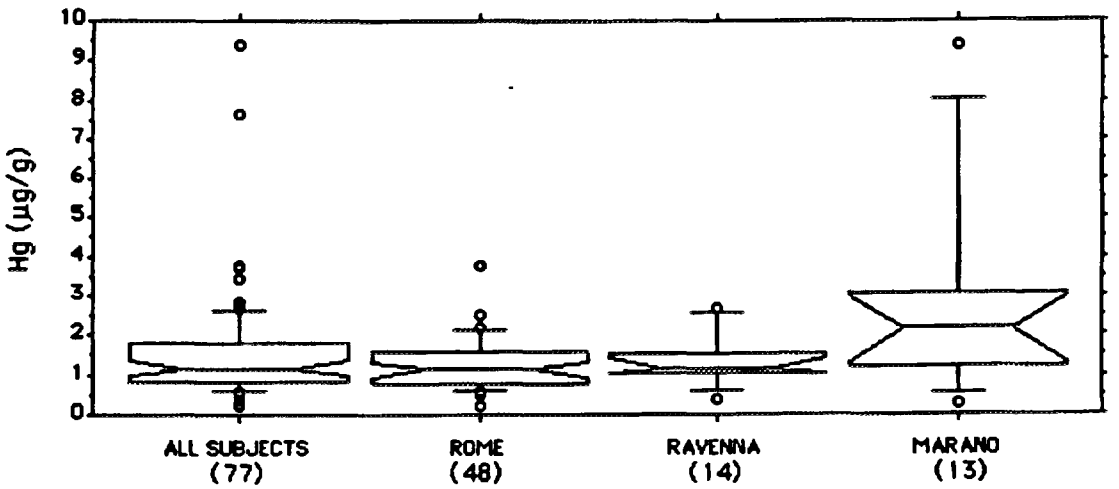

Figure 10. **Hg concentration in hair of pregnant women.**

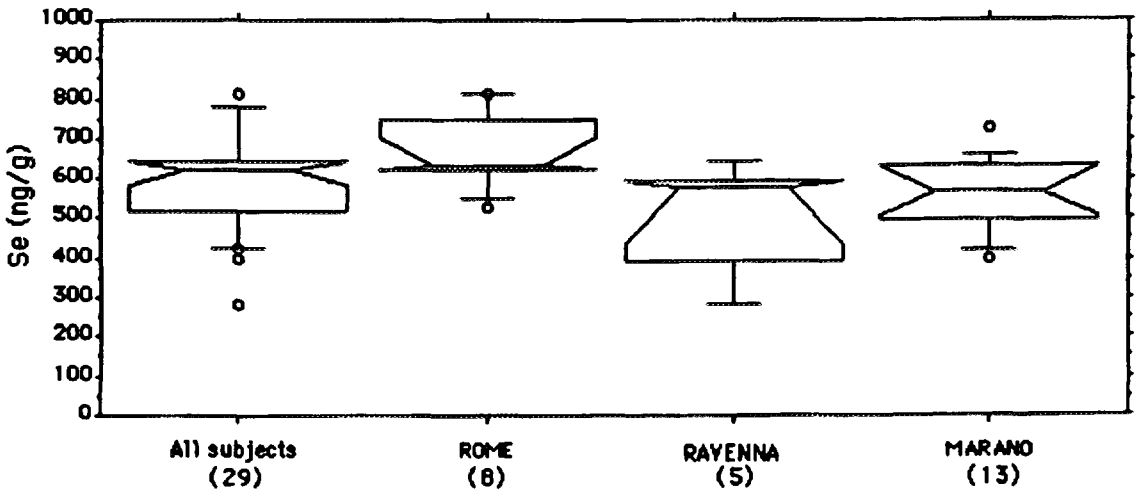

Figure 11. **Se concentration in pubic hair of pregnant women.**

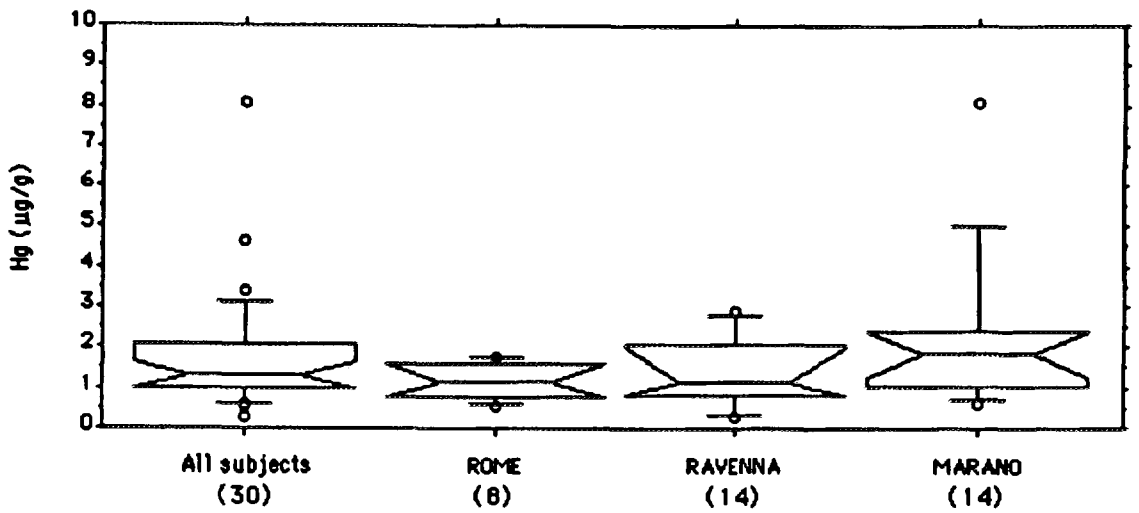

Figure 12. **Hg concentration in pubic hair of pregnant women.**

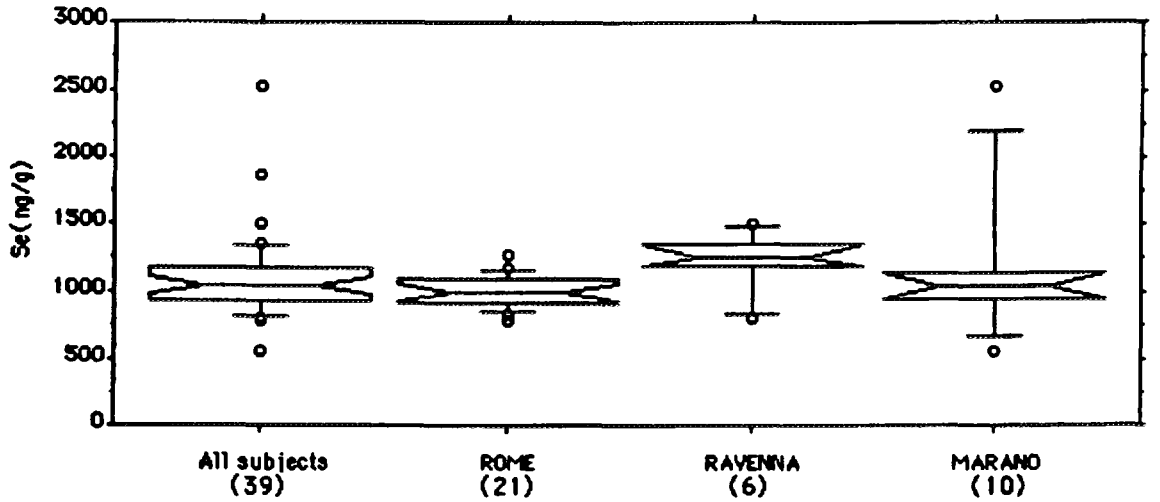

Figure 13. **Se concentration in hair of newborn.**

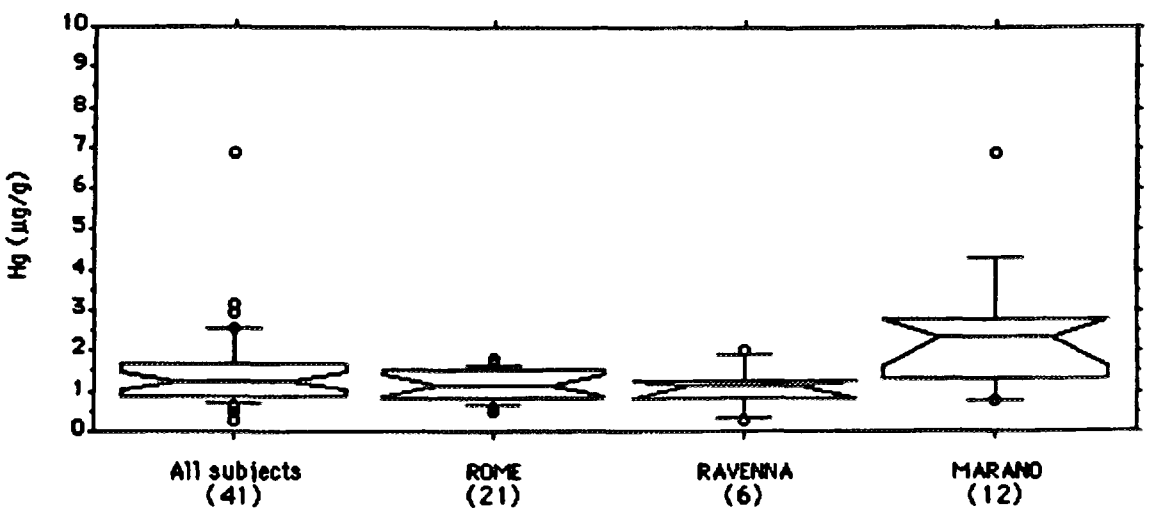

Figure 14. **Hg concentration in hair of newborn.**

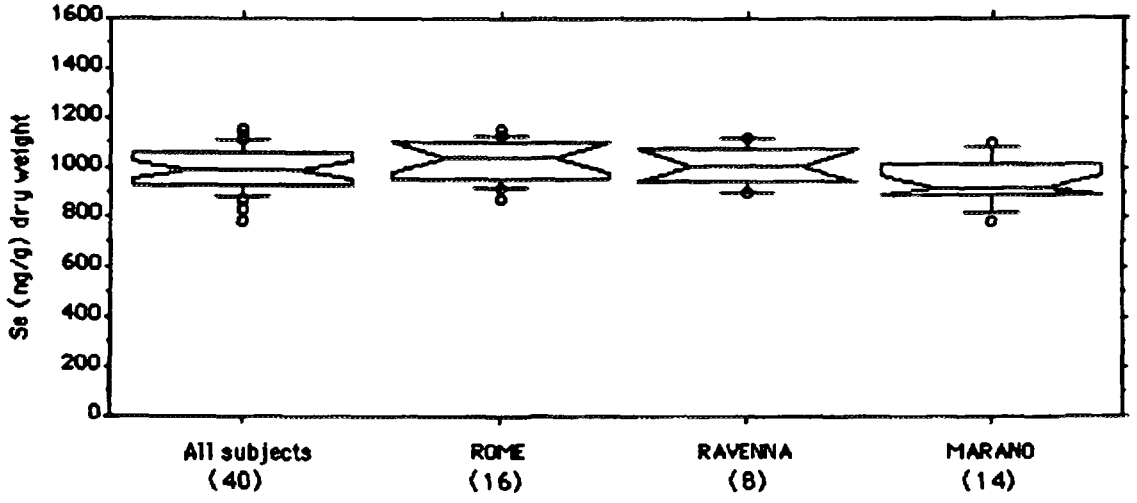

Figure 15. **Se concentration in freeze dried placenta.**

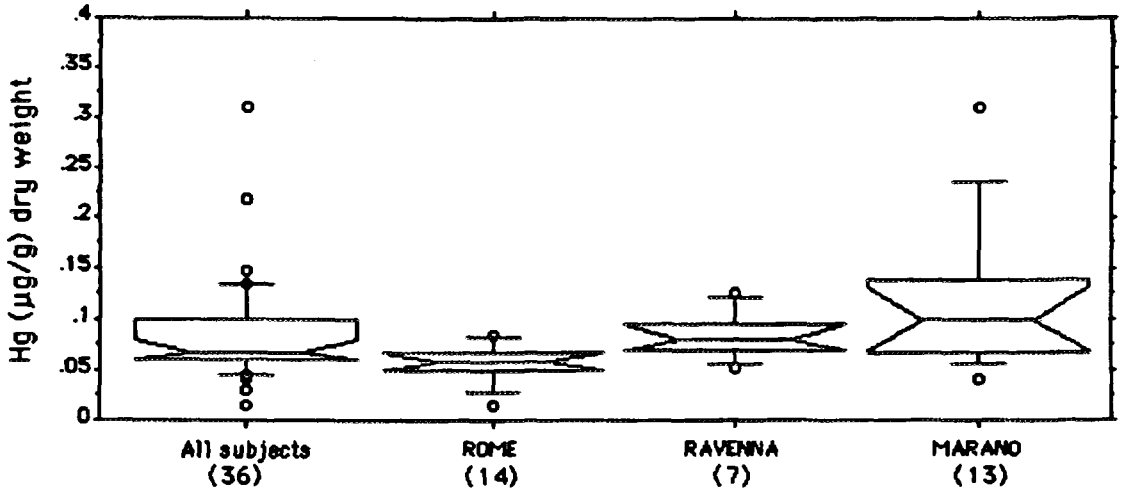

Figure 16. **Hg concentration in freeze dried placenta.**

INTAKE OF MERCURY THROUGH FISH CONSUMPTION

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Abstract

Fish has been known as a source of non-occupational mercury exposure to fish consuming population groups, and this is shown by the high hair mercury levels. In this study, hair samples collected from fishermen and their families, and commercial marine fishes were analyzed for mercury and methylmercury by neutron activation and gas chromatography. The results showed a correlation between hair mercury levels and fish consumption patterns. The levels of mercury found in this study were similar to those reported by other workers for fish consuming population groups worldwide.

1. INTRODUCTION

Being a peninsular state, fishing is an important industry in Malaysia, providing people with employment as well as supplying them with protein from the fish. Fish comprises about 70 % of the protein intake of the population, with consumption at about 100 g/d/person. The average mercury levels in commercial marine fishes marketed in Malaysia as reported in our study range from 0.06 - 0.42 mg/kg fresh weight. With such a relatively high intake of fish, it is apparent that fish is a possible source of non-occupational mercury exposure to the general population. The Provisional Tolerance Weekly Intake (PTWI) as set by the WHO and FAO at 0.2 mg Hg may have been achieved by some population groups and this is reflected in the hair mercury concentrations of 5 - 6 mg/kg [1].

In recent years, industrialization and urbanization have encroached onto many coastal fishing villages, which in many cases, cause water pollution. As a result, the livelihood of small inshore fishermen have been threatened or destroyed. At the same time, coastal fishes are contaminated by pollutants from industrial wastes, especially the toxic heavy metals. It is expected that the coastal fishermen and their families are exposed to a higher intake of mercury in the form of methylmercury. In this study, hair samples were collected from fishermen and their families residing in two fishing villages. The aim of this study was to identify certain population groups that may have ingested mercury higher than the PTWI as set by the WHO. We are collaborating with researchers from the Nuclear Energy Unit, the Department of Chemistry and also the Department of Public Health for these studies.

2. METHODS

2.1. Study area

The project is focused on two fishing villages: Kuala Juru in the State of Pulau Pinang and Chendering in Trengganu. Pulau Pinang is one of the most industrialized states in Malaysia. Rapid industrialization of this state has caused many environmental pollution problems and one of the affected areas is Kuala Juru. Chendering in Trengganu serves as a good model for a non-industrialized region in Malaysia.

2.2. Sample preparation

Hair samples were collected from residents of the study areas according to the IAEA protocols. The hair samples are successively washed in acetone, thrice in water, and once more in acetone, as is described in the IAEA protocol. Sufficient amounts of the solvents are added to cover the sample entirely. At each wash, the sample is allowed to stand at room temperature for 10 minutes in contact with constant stirring. After each wash, the liquid is decanted and fresh solvent added. The washing is carried out in a dust-free enclosure.

Fish samples were bought from coastal fishermen at landing jetties. The samples were kept refrigerated and brought to the laboratory. The samples were then cleaned with distilled water. Only body muscle was used in the analysis. The cleaned samples were kept at -50°C and then dried in a freeze dryer. The dried samples were blended and homogenized in a clean blender with stainless steel blades. The homogenized samples were kept in plastics bottles.

2.3. Extraction of methylmercury

The extraction of methylmercury from fish tissue samples was carried according to the methods described in [2]. Two cm^3 of 2M H_2SO_4 saturated with $CuSO_4$, 2 cm^3 of KBr solution and 3 cm^3 toluene were added to 1 g of dried fish tissue sample. The mixture was shaken by vortex for 10 minutes and was centrifuged. The extraction process was repeated with another 3 cm^3 toluene. The toluene layer was then separated quantitatively. Cysteine paper was added to the toluene and shaken for 15 minutes to extract the organic mercury. The paper was then rinsed once with toluene and dried in an acid-free box. The cysteine paper was packed in a polythene vial for neutron activation analysis.

The extraction of methylmercury from hair samples was carried out as in [3]. Each hair sample (approx. 0.1 g) was disintegrated with 2 ml of 7.5M NaOH in a water bath at 90°C for 30 min. Cysteine hydrochloride solution was added to enhance the extraction of methylmercury. The mixture was diluted with 1% NaCl solution to 10 ml. One ml of the solution was then acidified with $CuSO_4$-H_2SO_4, 2 ml 4M KBr was added and was then extracted into 3 ml toluene. Two ml of the toluene was transferred into a test tube and the MeHg was back extracted into thiosulphate solution. The aqueous solution was then acidified and MeHg was extracted into benzene or toluene. The organic solution was then ready for GC analysis.

2.4. Analysis by gas chromatography

A Perkin Elmer Autosystem GC with an Electron Capture Detector (^{63}Ni) and an HP 3390A intergrator was used for the analysis of methylmercury. A Supelco 2-4044 capillary column, 0.32 mm x 30 m with 0.25 micron film packed with SPB-1 (100% dimethyl polysiloxane), was used. The operational temperature was set at 200°C for the injector, 300°C for the detector, and 60 - 300°C for the column. The nitrogen gas flow rates were 30 ml min^{-1} for the detector and 2 ml min^{-1} for the column. The injection volume was 5 μl for standards and samples.

2.5. Neutron activation analysis

2.5.1. Sample irradiation

About 150 mg of each sample (hair or fish tissue) was packed in a pre-cleaned polyethylene vial. Triplicate analysis was carried for each sample. The cysteine paper was also packed in a separate polyethylene vial for activation. Standards were prepared from standard chemical solutions pipetted on ashless filter paper, which were packed in a polyethylene vial after drying. About 50 μL of 10% solution of thioacetamide was pipetted on the filter paper beforehand to preserve mercury. Samples and standards were irradiated in the PUSPATI TRIGA Reactor for 6 hours at a neutron flux of 2.3 x 10^{12} $n \cdot cm^{-2} \cdot s^{-1}$.

2.5.2. Measurement of activities

Mercury was determined by both the ^{197}Hg and^{203} Hg radionuclides. The determination via the 77 keV of ^{197}Hg was carried out by using a low energy photon detector (LEPD). The irradiated samples were cooled for 3 days and counted for 30 minutes. The determination of mercury via the 279.1 keV photopeak of ^{203}Hg is interfered by the 279.6 keV of ^{75} Se, and correction has to be made for the photopeak contribution. The samples were cooled at least for 2 weeks prior to counting. The γ-ray activities of the samples and standards were also measured by a high resolution γ-spectroscopy system comprising of a high purity coaxial N-type Germanium detector with thin Be window (Tennelec), microcomputer based MCA system with 80386 SX data processor and 8192 channel ADC, and TC-244 spectroscopy amplifier (Tennelec).

2.6. Quality control

Accuracy and precision of the analytical technique was evaluated by analyzing IAEA standard reference materials MAA-1 (Copepoda), MAA-2 (Fish Homogenate) and MAB-3 (Fish Tissue) and IAEA-350 (Tuna).

3. RESULTS AND DISCUSSION

Accuracy and precision for the analysis of mercury and organic mercury in the standard reference materials by neutron activation are shown in Table I. The results obtained were in good agreement.

Mercury in fish and hair samples can be analyzed quite easily and conveniently by instrumental neutron activation. Thus, tedious radiochemical separation steps can be

avoided. By employing a LEPD, mercury can be analyzed more quickly through the 69 and 77 keV photopeaks of ^{197}Hg. However, better accuracy and precision can be achieved by analyzing the 279 keV photopeak. The detection limit of the technique used was calculated at 0.01 mg/kg. However, this method is not capable of differentiating the organic mercury into its various species.

The hair mercury levels in the two population groups studied are shown in Table II. The results for the two groups differ significantly. The population in Chendering has lower mercury levels as it is located in a non-industrialized area. About 30% of the Kuala Juru samples contained mercury levels higher than 5 mg/kg. This may be an indication about the percentage of the study population that might have exceeded the PTWI intake of mercury. From the information gathered, some of the donors, especially the adult male population, consumed more than 100 g of fish daily. This is also reflected in the distribution of mercury concentrations in relation to the age of the donors as shown in Table III. Based on the concentrations of mercury in fishes marketed in Malaysia, the PTWI limit may be achieved by consuming about 500 g of fish weekly.

Preliminary results of methylmercury in hair samples are shown in Table IV. The results indicated that the percentage of methylmercury in hair ranged from 14 - 88%. The source of hair methylmercury is the consumption of fish. The concentrations of mercury in the hair samples analyzed showed a positive correlation with the amount of fish consumed. The correlation is within the statistical relationship established by Airey [4] *i.e.*,

$$\text{Hair mercury concentration} = 1.67 + 0.13(\text{Fish consumption}), \quad (1)$$

where the hair mercury concentration is expressed in mg/kg , and annual fish consumption expressed in kg/person/year.

Under this project, we are also trying to establish the levels of mercury and methylmercury in sea fishes marketed in Malaysia. Tuna has been known to contain high levels of mercury, higher than most other fish analyzed. Since tuna is quite popular among certain population groups, tuna samples obtained from local markets were analyzed for mercury. There are three species of tuna available in Malaysia. They are *Euthynnus affinis, Auxis thazarg* and *Thunnus tonggol*. The results of total mercury are shown in Table V. The highest mercury levels were found in the liver.

The total mercury and organic mercury levels of some other popular fish types as analyzed by neutron activation are shown in Table VI. The organic mercury levels were 45 - 95% of the total mercury depending on the species, as well as the location of the samples. Generally, the samples collected along the West Coast contained higher mercury levels, probably due to increased industrialization.

4. PLANS FOR FUTURE WORK

Methylmercury was not analyzed during the previous contract period as we faced difficulty in obtaining a methylmercury standard. Recently, we managed to procure methylmercury from Johnson Matthey in England. However, sharing a GC with other users is not practical and it became important to obtain our own GC. We have now bought a new Perkin Elmer Autosystem GC, which will be dedicated for the analysis of methylmercury.

Currently, we are developing our capability in analyzing methylmercury by both neutron activation method and by gas chromatography. The detection limit for GC analysis is better than the NAA method that we used. In addition, smaller sample sizes can be used in the determination of methylmercury by the GC method. For NAA, we normally used >1 g samples for the extraction of organic mercury, as compared to the 0.1 g samples used for the GC analysis. Another important factor is the analysis time. The determination of methylmercury by NAA requires about 3 weeks of analysis time. However, we are still keen to develop and apply the NAA method.

When we have developed the capability in the routine analysis of methylmercury, our major work in the future is in the application of the methods. We will take an active part in the interlaboratory analysis, as well as larger scale screening work for identifying critical population groups. In this regard, we have included in our group researchers from the Department of Public Health, Faculty of Medicine who are interested in the heavy metal poisoning. There has been some isolated cases of arsenic poisoning reported by the department in Malaysia. We have plan to screen the hair mercury levels of pregnant women among the general population groups.

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TABLE I. MERCURY IN IAEA STANDARD REFERENCE MATERIALS (mg/kg)

	This work	Certified value
MAA - 1	0.33 ± 0.05	0.28 ± 0.01
MAA - 2	0.49 ± 0.14	0.47 ± 0.02
MAB - 3	0.54 ± 0.19	0.510 ± 0.070
IAEA - 350	3.88 ± 0.41	4.99 ± 0.26
IAEA - 350	3.31 ± 0.09[a]	3.96 ± 0.27[b]

[a] - total organic mercury
[b] - Analysis by M. Horvat in Ref. [1]

TABLE II. TOTAL MERCURY IN HAIR (mg/kg)

	Kuala Juru	Chendering
Sample	106	98
Range	0.45 - 16.87	0.34 - 9.07
Arith. mean	3.61	2.20
Median	2.96	1.77
Geom. mean	3.49	1.87

TABLE III. DISTRIBUTION OF MERCURY CONCENTRATIONS

Age (y)	Range (mg/kg)	Mean (mg/kg)
1 - 9	0.45 - 8.47	3.27
10 - 19	0.58 - 10.43	3.16
20 - 29	0.87 - 16.87	3.83
30 - 39	1.04 - 6.75	3.09
40 - 49	1.91 - 12.55	5.22
50 - 59	1.94 - 8.33	4.05
60 above	1.29 - 16.65	6.25

TABLE IV. TOTAL MERCURY AND METHYLMERCURY IN HAIR (mg/kg)

Sample	Age	Sex	Hg-T	MeHg	%MeHg
T05	69	M	2.16	1.34	62
T07	12	F	1.68	0.24	14
T22	8	F	1.84	0.73	40
T23	6	M	1.69	1.05	62
T29	46	F	1.56	0.68	43
T49	34	F	1.09	0.96	88
T64	29	F	0.52	0.44	83
T71	63	F	2.13	1.24	58
T77	12	M	2.89	2.54	88
T81	9	M	1.76	1.04	59

TABLE V. MERCURY IN MALAYSIAN TUNA (mg/kg) FRESH WEIGHT

Organ	Hg	Se	Zn
White muscle	0.81 ± 0.18	1.64 ± 0.51	11.47 ± 1.04
Red muscle	1.20 ± 0.08	1.98 ± 0.07	9.78 ± 0.58
Liver	3.35 ± 0.27	8.02 ± 0.64	50.79 ± 1.60
Roe	1.29 ± 0.79	4.55 ± 0.21	151.72 ± 2.96
Spleen	1.96 ± 0.62	7.83 ± 0.55	115.59 ± 7.22
Intestine	1.39 ± 0.21	4.37 ± 0.19	13.51 ± 0.92

TABLE VI. TOTAL MERCURY AND ORGANIC MERCURY IN FISH (mg/kg) FRESH WEIGHT

Sample	Location	Hg-T	Hg-O	Hg-O/Hg-T (%)
Indian mackerel	Mersing	0.126	0.084	67
	Bachok	0.059	0.048	81
	K. Trengganu	0.059	0.053	90
	Paka	0.092	0.062	67
	Marang	0.064	0.048	74
Spanish mackerel	Mersing	0.076	0.064	85
	K. Perlis	0.076	0.071	94
	Batang Tiga	0.074	0.044	59
	Benut	0.069	0.044	63
	Kuala Kedah	0.140	0.113	80
Squid	Kuala Perlis	0.163	0.144	84
	Mersing	0.086	0.058	67
	Kuantan	0.138	0.096	70
	Pekan	0.067	0.038	57
	Kuala Selangor	0.093	0.053	79
Prawn	Kuantan	0.273	0.145	53
	Kuala Kedah	0.255	0.115	45
	Rompin	0.158	0.123	78
	Mersing	0.255	0.140	55
	Sg. Buloh	0.215	0.175	81
Cockle	Morib	0.056	0.040	71
	Juru	0.110	0.088	80

ANALYTICAL QUALITY CONTROL IN STUDIES OF ENVIRONMENTAL EXPOSURE TO MERCURY

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Abstract

The work of the laboratory for quality control in this co-ordinated project for the period from November 1993 to June 1994 is presented. The major effort was devoted to assisting in establishing the homogeneity and total methylmercury levels in two new hair reference materials prepared as control materials for the project, numbered 085 (spiked) and 086 (natural level). Results for some hair materials from participants are also given.

1. INTRODUCTION

The general aims of the programme and the role of the Nuclear Chemistry Department, J. Stefan Institute, as a reference laboratory for purposes of ensuring the achievement of the quality assurance programme of this CRP were discussed in our first and second annual reports, and at the previous research co-ordination meetings (RCMs) [1,2].

Most of our activities have been performed in collaboration with the IAEA in Vienna (Dr. S. Stone) or the IAEA Marine Environmental Laboratory in Monaco (Dr. M. Horvat). The topics discussed in this report include co-operative analysis of the new mercury in hair standards, 086 natural level and 085 high level (spiked), prepared by the IAEA, during and after its preparation, analysis of human hair samples received from participants in the programme, and improvements and developments in analytical procedures for mercury analysis.

2. METHODS

In the report period, the method used for hair analysis was basically the technique described by May *et al.* [3], which uses an anion exchange separation of extracted inorganic from organic mercury species, followed by destruction of organic species by UV irradiation, with the usual CV-AAS finish.

About 100 mg of hair was shaken with 10 ml 6 M HCl for 24 h in the dark and centrifuged. Protected from the light, the sediment was washed twice, re-centrifuged and the washings combined with the centrifugate, which was then passed down a Cl^- form Dowex-1 anion exchange column to absorb inorganic Hg^{++}. The presence of Hg^{++} in the eluate was tested for by reduction with $SnCl_2$ and CV-AAS; none was found. The eluate was then subjected to 24 h irradiation from a UV lamp to decompose MeHg to Hg(II), and Hg(II) determined by CV-AAS.

Total mercury in hair was determined by destruction of up to 100 mg of hair with 2 ml conc. HNO_3 in a sealed tube by heating in a block for several hours (or preferably overnight) at 90°C, followed by CV-AAS determination [4].

3. RESULTS AND DISCUSSION

3.1. Analysis of IAEA Hair reference materials 086 and 085

The analytical control function of our laboratory was heavily engaged in the report period in assisting in the establishment of total mercury and methylmercury levels during the preparation of two new human hair reference materials for the CRP, prepared at IAEA Seibersdorf by spiking a natural, low-level mercury content human hair batch from India (Sample 086) with methylmercury to form a high level standard (Sample 085).

The preliminary results for methylmercury after spiking and initial homogenization are shown in Table I and revealed satisfactory homogeneity, with a MeHg level of about 22 μg/g.

Results for total and MeHg in three bottles each of two subsamples (*i.e.* 6 bottles in all) of the homogenized non-spiked hair 086 are shown in Table II. Agreement for both analytes between the bottles and between the two batches was excellent. The total mercury level is about 0.60 $\mu g \cdot g^{-1}$, of which, about 47% (0.28 $\mu g \cdot g^{-1}$) is present as MeHg. Quality control analyses using BCR-A human hair and the Canadian CRC certified reference material TORT-1 (Lobster haematopancreas) gave excellent results.

The spiked hair sample 085 was further homogenized and three sub-batches H-46, H-47 and H-48 bottled. Three bottles of each sub-batch were then analyzed in our laboratory for total Hg and MeHg as shown in Table III. The results indicate that the homogeneity both within and between the batches is excellent, and the results were also in very good agreement with the preliminary results after spiking (Table I). The quality control analyses were also in excellent agreement with the certified values.

After the final stage of preparation of the two new hair reference materials 085 and 086, which involved radiation sterilization and remixing, followed by bottling, a final control of the as-bottled samples was performed as shown in Table IV. As for the spiked material (085), the results are virtually identical to those obtained in Table III, while for the unspiked base material 086, the results are in very close agreement with those obtained before, as shown in Table II.

Thus, on the basis of these extensive analyses, both materials appear to be very homogeneous and stable samples, which should enable participants to test their methods and carry out routine control analyses, even if unfortunately towards the end of the programme, rather than nearer the beginning.

Further analyses of total mercury by radiochemical neutron activation using the volatilization technique [5] are in progress to check the results reported here by CV-AAS.

3.1.1. Note on moisture content of 085 and 086

Some evidence was obtained from periodic determination of moisture that there is a tendency for moisture to increase and some variation between samples 085 and 086. Therefore, we suggest as a precaution, that moisture content be determined on a separate sub-aliquot on every occasion that the hair materials are used for quality control, or at least if the interval between the last determination of moisture is more than one month.

3.2. Results for hair samples from participating laboratories

Two batches of hair were sent from Brazil (Dr. M. Vasconcellos) for analysis, being collected from Indians in the Amazon basin (for a fuller description of the samples and an interpretation of the results, see M. Vasconcellos *et al.* these Proceedings). As shown in Table V and VI, both groups are high and the first group in particular has a very high and constant percentage of MeHg (89 ± 6 %).

We regret that these samples were the only hair material we have received in the report period, and that participating laboratories still seem reluctant to make use of the opportunity to have their results checked by independent analysis. Perhaps, this is mainly due to the absence so far in the programme, of a readily available hair reference material for laboratories to use to establish their own quality assurance. Nevertheless, just because of the absence of such materials, quality control by comparative analysis with the reference laboratory is more desirable.

3.3. Analytical developments

In co-operation with the group of N. Bloom, two pulications on the speciation of merucry using the ethylation-GC-CVAFS techniques have appeared [6,7], allowing sensitive and simultaneous determination of inorganic and organic species.

3.4. Supplementary programme

Due to the continuing unrest and war situation in Croatia, our programme on pregnant women has been unable to progress further. Recently, in co-operation with Swedish scientists, we showed that there was no detectable in-vivo methylation of mercury in chloralkali workers exposed to merucry vapour [8]. Previous work on this topic had produced conflicting or insufficient evidence.

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TABLE I. PRELIMINARY RESULTS FOR MeHg IN FOUR BOTTLES OF IAEA SPIKED HAIR AS $\mu gHg \cdot g^{-1}$ (dry weight)[a], DETERMINED NOV. 1993

Sample	Results	Mean ± σ	n
A	(13.5)[b], 21.8, 22.6, 22.0	22.1 ± 0.4	(3)
B	18.6, 21.6, 22.1, 21.5	21.0 ± 1.6	(4)
C	22.5, 21.6, 22.0, 21.7	22.0 ± 0.4	(4)
D	24.1, 24.6, 21.6, 22.1	23.1 ± 1.5	(4)
	overall mean	22.0 ± 1.3	(15)

[a]corrected for moisture factor f_{H2O} (freshweight/dry weight) of 1.1086
[b]rejected on statistical grounds since outside overall mean of all 16 results minus 3σ (21.5 ± 2.48 (16): 3σ = 7.44)

TABLE II RESULTS FOR TOTAL Hg AND MeHg (as ng $Hg \cdot g^{-1}$, dry weight) IN THREE BOTTLES EACH OF TWO SUBSAMPLES OF UNSPIKED HAIR, CODE 086, DETERMINED IN MARCH 1994

Sample	Total Hg results	MeHg results	f H_2O
IAEA 086/1			
1/1	619.5 , 585.2	254.9 , 292.2	1.1215
1/2	591.3 , 583.1	273.6 , 251.8	
2/1	563.8 , 620.2	317.9 , 260.5	1.1201
2/2	605.3 , 622.9	295.7 , 283.6	
3/1	571.6 , 632.9	257.1 , 292.2	1.1203
3/2	608.0 , 588.4	290.7 , 303.4	
mean	599.4 ± 22.0	281.1 ± 21.3	
IAEA 086/2			
1/1	619.2 , 597.1	281.0 , 287.3	1.1271
1/2	623.7 , 639.2	271.3 , 277.5	
2/1	634.7 , 622.3	274.9 , 249.4	1.1324
2/2	579.0 , 589.9	291.5 , 262.4	
3/1	610.8 , 619.8	288.0 , 276.0	1.1324
3/2	590.1 , 608.8	266.7 , 272.5	
mean	605.0 ± 21.4	278.0 ± 17.2	
BCR-397 Human Hair	12.1, 11.6, 12.1, 13.3* mean: 12.3 ± 0.7 (4)* certified: 12.3 ± 0.5*		
CRC TORT-1		121.9, 122.2, 127.1 mean: 124 ± 2.9 (3) certified: 128 ± 14	

*units here are $\mu g\ Hg \cdot kg^{-1}$

TABLE III. RESULTS FOR TOTAL Hg AND MeHg IN IAEA HAIR 085, THREE BOTTLES EACH OF THREE BATCHES AFTER HOMOGENIZATION, IN $\mu g \cdot g^{-1}$ (dry weight), DETERMINED IN FEBRUARY 1994

Sample	total Hg, $\mu g \cdot g^{-1}$ (dry weight)		MeHg, $\mu g \cdot g^{-1}$ (dry weight)	
H-46				
1/1	24.6 , 24.6	25.6	21.1 , 21.1	21.4
1/2	26.8 , 26.3		21.0 , 22.2	
2/1	26.3 , 25.9	26.4	23.3 , 23.3	21.7
2/2	26.9 , 26.6		19.8 , 20.4	
3/1	25.7 , 26.4	26.2	21.7 , 22.9	21.1
3/2	27.0 , 25.7		19.7 , 20.1	
H-47				
1/1	26.2 , 25.9	25.5	21.8 , 22.0	22.0
1/2	24.3 , 25.5		21.6 , 22.4	
2/1	25.6 , 26.8	25.9	23.8 , 24.2	22.9
2/2	25.5 , 25.6		22.1 , 21.6	
3/1	26.1 , 26.3	26.1	22.6 , 22.0	22.5
3/2	26.1 , 25.8		21.7 , 23.5	
H-48				
1/1	25.7 , 23.5	25.3	21.2 , 21.4	21.7
1/2	27.2 , 24.6		21.6 , 22.6	
2/1	25.3 , 25.1	25.5	22.0 , 22.2	22.0
2/2	27.3 , 24.4		21.8 , 21.8	
3/1	25.3 , 25.1	26.0	21.8 , 21.0	22.0
3/2	25.7 , 28.0		23.7 , 21.6	
	Grand mean:	25.8 ± 0.4 (9)	Grand mean:	21.9 ± 0.5 (9)
Quality control				
BCR-397 Human Hair	12.5, 11.3, 12.3, 12.7 12.2 ± 0.6 (4) certified: 12.3 ± 0.5		0.631, 0.756, 0.742 0.71 ± 0.07 (3) not certified	
CRC TORT-1	0.338 ± 0.004 (3) certified: 0.330		0.126, 0.130, 0.128, 0.120 0.126 ± 0.004 (4) certified: 0.128 ± 0.014	

TABLE IV. FINAL RESULTS FOR TOTAL AND MeHg IN IAEA 085 SPIKED and 086 UNSPIKED HAIR SAMPLES, IN $\mu g \cdot g^{-1}$ (dry weight) AND $ng \cdot g^{-1}$ (dry weight), RESPECTIVELY, DETERMINED IN MAY 1994, AFTER RADIATION STERILIZATION AND FINAL BOTTLING

Sample	total Hg	MeHg
085 (No. 10)[a]		
1	24.9 , 25.6	22.3 , 21.7
2	25.0 , 25.9	23.3 , 23.1
3	26.1 , 26.4	21.8 , 22.2
4	24.5 , 26.7	22.1 , 23.6
5	26.0 , 25.6	21.4 , 21.7
	mean: 25.7 ± 0.7	22.3 ± 0.8
086 (No. 105)[b]		
1	564 , 636	290 , 282
2	628 , 608	272 , 282
3	596 , 589	277 , 290
4	637 , 593	276 , 289
5	597 , 597	262 , 293
	mean: 607 ± 20	mean: 282 ± 10
Quality control:		
BCR-397	12.4, 11.2, 12.0,	TORT-1 131.3, 124.6,
Human Hair	12.5, 12.4	126.3, 130.3
	mean: 12.1 ± 0.5 (5)	mean: 128 ± 14
	Certified: 12.3 ± 0.5	Certified: 128 ± 14

[a]moisture factor : f_{H_2O} = 1.1143
[b]moisture factor : f_{H_2O} = 1.1349

TABLE V. TOTAL Hg AND MeHg IN HAIR SAMPLES FROM BRAZIL IN $\mu g \cdot g^{-1}$ (dry weight). SAMPLE IDENTIFICATION CODES REFER TO THOSE OF BRAZILIAN PROGRAMME.

Sample	Total Hg	MeHg	% Me / Hg
482	21.5	10.0	47
940	12.1	7.97	66
944	10.9	6.67	61
972	11.8	8.41	71
2092	18.9	8.28	44
5028	13.9	6.98	50
5098	22.4	16.4	73
5121	10.4	8.29	80
5219	11.1	6.89	62
5225	10.1	7.83	78
5226	16.6	11.0	66
5279	11.3	7.56	67
5317	17.9	15.5	87
5354	19.1	12.4	65
5355	11.7	8.89	76
5361	29.5	14.2	48
5428	13.2	10.0	76
6031	15.4	12.7	82
6032	10.1	8.48	84
6033	15.8	10.1	64
6097	11.7	8.99	77
6099	7.70	5.94	77
6106	16.9	11.1	66
cocoyea	23.5	16.8	71

TABLE VI TOTAL Hg AND MeHg IN BRAZILIAN HAIR SAMPLES IN $\mu g \cdot g^{-1}$ (dry weight). SAMPLE IDENTIFICATION CODES REFER TO THOSE OF BRAZILIAN PROGRAMME.

Sample No.	Total Hg	MeHg	% Methyl
1225	18.3	16.3	89
1226	15.3	13.1	86
1228	19.2	16.7	87
1230	13.1	12.4	95
1234	21.3	18.4	86
1241	17.7	14.9	84
1242	16.7	13.7	82
1244	18.1	15.0	83
1245	15.9	14.4	91
1247	14.2	14.2	100
1250	14.7	13.4	91
1251	11.5	9.47	82
1253	20.1	18.5	92
1255	26.5	23.3	88
1269	11.3	10.1	89
1274	20.6	18.0	87
1277	5.36	4.79	90
1278	19.1	16.3	85
1280	15.5	14.2	92
1281	17.7	15.4	87
1285	19.9	18.4	92
1286	20.6	20.6	100
1293	14.7	10.2	70
1324	26.4	25.7	97
1341	16.4	15.4	94
1652	24.1	22.7	95

DETERMINATION OF TOTAL AND METHYLMERCURY COMPOUNDS IN THE IAEA HUMAN HAIR INTERCOMPARISON SAMPLES - EXPERIENCE OF THE IAEA-MEL

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1. INTRODUCTION

The IAEA-MEL, Marine Environmental Studies Laboratory (MESL) in Monaco has developed considerable expertise in Quality Assurance/Quality Control for non-radioactive contaminants (trace elements and trace organics). It provides AQCS services to the Member States through the number of activities, such as, organization of intercomparison exercises, production and distribution of reference materials and provision of training courses for trace elements, and trace organic contaminant determinations. Most of its activities are funded through the UNEP's Regional Seas Programme. In recent years, MESL has also performed a lot of research on organometals in the marine environment (organomercury and organotin compounds), particularly on method development and testing. As a result, two reference materials were given a recommended value for methylmercury: IAEA-350, Tuna Fish Homogenate [1] and IAEA-356, Polluted Marine Sediment [2]. Future intercomparison samples for trace elements will also include methylmercury as one of the important parameter to be measured. In 1994, two such intercomparison samples will be prepared and distributed: a mussel tissue and a sea plant homogenate.

The programme of this CRP is focused on the analyses of human hair samples. There are only two human hair samples certified for total mercury, and no RMs for methylmercury compounds is available. One of the main objectives of the this CRP is to produce, through the IAEA AQCS Programme, a human hair intercomparison material for quality assurance requirements in population monitoring programmes for total and methylmercury exposure.

Through the reporting period, MESL has introduced a new method for simultaneous determination of total and methylmercury in biological samples. As the laboratory has close collaboration with the CRP's Reference Laboratory in Ljubljana, Slovenia, it has also been actively involved in the quality assurance component of this CRP. This report represents a summary of the results for total and methylmercury in two intercomparison samples, IAEA-085 and IAEA-086 using newly developed method.

2. EXPERIMENTAL

2.1. Determination of methylmercury compounds

The method consists of alkaline digestion (KOH/methanol) followed by aqueous phase ethylation, room temperature precollection on Tenax (or Carbotrap) separation of ethylated mercury species on the GC column (a wide bore capillary column Supelco SPB ™, 30 m long, operated isothermally at 58°C). Separated mercury species were then transformed into elemental Hg by heating at 900°C and swept into atomic fluorescence detector (CV

AFS). The method is schematically presented in Figure 1 and described in details in other publications [3,4].

A comparative method based on the modified Westoo extraction procedure and GC-ECD detection was also used. Precise description is given in the publication [5]. Briefly after acidification of the alkaline digested sample, methylmercury bromide was extracted into toluene. In the clean-up step, methylmercury was adsorbed onto the paper impregnated with cysteine. After the cysteine paper was washed with fresh portions of toluene and dried, methylmercury bromide was back extracted into toluene after acidification. Finally, methylmercury was then measured by gas chromatography equipped with electron capture detector (ECD).

2.2. Determination of total mercury

Approximately 0.1 to 0.25 g of the sample was digested with 4 mL HNO_3 (Merck, Selectipur, 70%) and 0.5 mL of HCl (Merck, Suprapur, 36%) in closed Teflon vials (25 ml, Savillex Corp., USA) at 90°C for 6 to 12 hours. Before samples were diluted to the final volume (50 mL), 1 mL of BrCl oxidation solution was added. Samples were left for few hours before 0.1 mL of 10% hydroxyl amine hydrochloride solution was added to remove the excess of BrCl oxidizing solution. Samples were then diluted to the final volume with deionized water (MilliQ). An aliquot of the sample was introduced to the $SnCl_2$ reduction, aeration, amalgamation process. Finally, mercury was detected by CV AAS (Perkin-Elmer 1100, MHS-20) and/or CV AFS (double amalgamation technique coupled with a Brooks Rand, Ltd. AFS detector).

2.3. Data quality control

To control the accuracy of the measurements, a CRM, NIES No. 5, Human Hair sample was initially used. It was analyzed in duplicates in each batch of the analyses. Results obtained for total mercury compared well with certified value. Methylmercury has not been certified in this material. However, data obtained compared well with the results of previously methodological intercomparison study [6,7]. Unfortunately, during the reporting period, this sample was no longer unavailable. Therefore, a new intercomparison Human Hair sample from NIES No. 13 was used. Results for total and methylmercury in this sample are very similar to NIES No. 5. Results compared well with the preliminary results obtained from NIES (personal communication).

The accuracy of the results for both total and methylmercury determinations was also checked by analyzing a RM IAEA-350, Tuna Fish Homogenate. Results are given together with Tables of the results.

3. RESULTS AND DISCUSSION

3.1. Determination of methylmercury in IAEA-086 (spiked) human hair sample before grounding

Spiked human hair samples, cut in 2 - 4 mm segments, were received in four batches, marked as A, B, C, and D. Two types of experiments were performed.

(a) Methylmercury was determined in each batch of the sample marked as A, B, C, and D. Analyses were performed in duplicates (sample weight varied from 20 to 50 mg) using an alkaline digestion, followed by aqueous phase ethylation, room temperature precollection on Carbotrap, isothermal GC and CV AFS detection. Comparative analyses by a modified Westoo procedure (extraction, GC-ECD) was also done.

(b) The samples (without any pretreatment) were leached with toluene in order to check any possible release of methylmercury from the surface. Measurements were performed by GC-ECD.

Results are given in Table I. The overall methylmercury concentration was 26.34 ± 2.00 mg/kg (RSD = 7.6 %) (expressed as Hg). Relatively high RSD is probably related to the small sample weights. No significant difference of the results between the batches A, B, C, and D could be observed.

To control the accuracy of the measurements, a CRM NIES No. 13, Human Hair sample was analyzed together with the rest of the samples. The result obtained for methylmercury was 3.80 ± 0.18 mg/kg based on the analysis of four independent aliquots. Aliquots of the alkaline digested samples A(b) and C(c) were also processed using a modified Westoo procedure [6]. Two results presented in Table I compared well with other results.

3.1.1. Leaching with toluene

In order to check whether spiked methylmercury is well bound to the matrix, a leaching into toluene has been performed. Approximately 200 mg of each batch of the sample was put in a centrifuge Teflon vials and 5 mL of toluene was added. Each batch was done in duplicate. After two hours of shaking, an aliquot of 2 μL was injected onto the GC column. No detectable amounts of methylmercury were measured (the absolute detection limit of measurements was 10 pg of methylmercury). Measurements were repeated after 24 hours of leaching. Even after this long period, there was no methylmercury detected. This indicated that methylmercury is mainly bound to the human hair structure.

3.2. Determination of methyl and total mercury after grounding and homogenization

3.2.1. Methylmercury

IAEA-085 (spiked) was received in nine different bottles. Methylmercury was isolated from the sample using three preseparation procedures. Sample weights varied from 0.03 to 0.05 g.

(1) Alkaline digestion (25% KOH in methanol) at 90°C for 12 hours

(2) Distillation (aqueous phase, NaCl/sulphuric acid)

(3) HCl leaching at room temperature (6M HCl, 12 hours)

For each preseparation technique, the following detection system was used. An aliquot of the sample (alkaline digested, or distillate, or HCl leachate) was transferred into a bubbler for aqueous phase ethylation, room temperature precollection on Carbotrap (or Tenax), separation of ethylated mercury species on a GC column and determination by CV AFS.

The IAEA-086 was received in six bottles and the analyses were performed only with alkaline digestion method, followed by ethylation, GC, and CV AFS detection.

Each result in Tables II and III (IAEA-086, IAEA-085) is given as an average of two measurements. Alkaline digestion was done in duplicates. Evidently, results obtained by alkaline digestion and distillation are in very good agreement (Table II), while HCl leaching resulted in smaller results. Summary of the results is given in Table IV. It was assumed that not all of the methylmercury could be released by HCl leaching. In order to verify this conclusion, the residue after the leaching was thoroughly washed with water and submitted to distillation. Some of the sample was lost during this procedure. The methylmercury found in residues was around 0.5 mg/kg, which only partly covered the difference mentioned above. The question is, where is the missing methymercury? Some of it has most probably been lost during washing of the sample and/or it was decomposed during leaching.

It is interesting to note that earlier method intercomparison study performed on human hair samples [5,6,7] have shown that HCl leaching coupled with ion-exchange separation compared well with other methods.

3.2.2. Total mercury

Spiked IAEA-085 sample was analyzed by both CV AAS and CV AFS. Results obtained by two different final detection systems are presented in Table II and summarized in Table IV. IAEA-086 sample was only analyzed by CV AAS. Evidently, a good agreement of the results have been obtained. Initially, when only HNO_3 acid was used for digestion, lower results (for 20 to 30 %) were obtained. This was mainly due to uncompleted digestion of the sample. Most probably, methylmercury was not completely decomposed to inorganic mercury and consequently could not be reduced with $SnCl_2$. It was found that addition of BrCl improved the results. Similar difficulties could also be expected in other laboratories. It is suggested to compare these results with another independent method, such as neutron activation analysis (preferably with a radiochemical separation).

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TABLE I. METHYLMERCURY IN HUMAN HAIR INTERCOMPARISON SAMPLE BEFORE GROUNDING

Sample	Weight (mg)	Result (mg/kg) as Hg	
		Ethylation CV AFS*	Extraction GC-ECD
A (a)	0.019	25.46	
A (b)	0.50	27.56	26.9
A (c)	0.030	23.05	
B (a)	0.020	25.13	
B (b)	0.028	28.49	
B (c)	0.025	29.38	
C (a)	0.019	25.41	
C (b)	0.028	29.31	
C (c)	0.038	25.57	26.1
D (a)	0.019	26.52	
D (b)	0.025	24.01	
D (c)	0.022	26.23	
X ± SD		26.3 ± 2.0	

*Each result is expressed as the mean of two determinations.

TABLE II. RESULT FOR TOTAL AND METHYLMERCURY IN IAEA-085 (SPIKED)

Bottle No.	Moisture content (%)	Total - Hg mg/kg, dry weight		Methyl-Hg (as Hg) mg/kg, dry weight		
		CV AAS	CV AFS	Alkaline dig.	Distillation	HCl leaching
46A	8.9	25.44	26.61	24.46	24.87	23.78
		24.77	25.88	24.51		
46B	8.9	25.45	27.02	23.96	25.59	22.48
		27.48		27.02	24.67	
					26.94	
46C	9.1	26.65	26.11	25.42		23.13
		25.26		24.83		
47A	8.5	27.02	26.17	24.87	26.80	24.17
		26.32	28.62	24.19		
47B	8.3	23.92	27.23	24.56		
		26.15		22.96		
47C	7.9	26.71	24.24	26.39		
		29.08		27.26		
48A	8.0	26.07	27.07	24.57		
		26.60		21.88		
48B	8.5	24.47	25.19	26.48		
		27.42		27.80		
48C	8.4	24.44	26.83	26.20		
		26.90		23.79		
Mean ± SD	**8.5 ± 0.4**	**26.11 ± 1.29**	**26.45 ± 1.15**	**25.06 ± 1.55**	**25.77 ± 1.06**	**23.39 ± 0.74**
NIES No.13	8.8	4.72	4.32	3.84	3.75	
		4.27	4.53	3.64	3.74	
		4.63	4.54			
		4.27			3.80	
Mean ± SD		**4.51 ± 0.22**	**4.46 ± 0.12**	**3.74 ± 0.14**	**3.76 ± 0.03**	
Recomm. value*		4.10 - 4.30		3.90		
IAEA 350 Tuna Hom.	6.2		4.79			
			4.70			
Certified value			4.68			
			95% Conf. Int. (4.36 - 4.91)			

*NIES No. 13 is not as yet certified. Values recommended from NIES are given for comparison.

TABLE III. RESULTS FOR TOTAL AND METHYLMERCURY IN IAEA-086

Bottle No.	Moisture content (%)	Total-Hg mg/kg, dry weight	Methyl-Hg mg/kg, dry weight
		CV AAS	Alkaline digestion
086/1A	9.6	0.38	0.28
		0.39	0.28
086/1B	11.0	0.41	0.30
		0.40	0.27
086/1C	10.3	0.37	0.29
		0.36	0.31
086/2A	11.3	0.37	0.29
		0.37	0.31
086/2B	10.9	0.38	0.25
		0.41	0.28
086/2C	11.1	0.36	0.32
		0.38	0.30
Mean ± SD	**10.3 ± 0.6**	**0.38 ± 0.02**	**0.29 ± 0.02**
NIES No. 13	8.8	4.42	4.00
		4.51	4.12
		4.38	3.72
		4.53	
		4.36	
Mean ± SD		**4.44 ± 0.080**	**3.95 ± 0.21**
Recomm. value*		4.10 - 4.30	3.90

*NIES No. 13 is not as yet certified.
Values recommended from NIES are given for comparison.

TABLE IV. SUMMARY - TOTAL AND METHYLMERCURY IN IAEA-085 AND IAEA-086 HUMAN HAIR SAMPLES (CONCENTRATIONS ARE GIVEN IN mg Hg/kg, DRY WEIGHT

	IAEA-085 (spiked)		IAEA - 086 (non-spiked)	
	Hg-total	MeHg	Hg-total	MeHg
After spiking Before grounding (2 - 4 mm segments)		26.3 ± 2.0 (A) n = 12 26.9, 26.1 (B) n = 12 25.0 ± 1.5 (A)** n = 4	- - -	- - -
After grounding and homogenization	26.1 ± 1.3 (CV AAS) n = 18 26.5 ± 1.2 (CV AFS) n = 11	25.1 ± 1.6 (A) n = 12 25.8 ± 1.1 (C) n = 5 23.4 ± 0.7 (D) n = 4 26.6 ± 1.8 (A)** n = 6	0.38 ± 0.02 (CV AAS)* n = 12 0.61 ± 0.41 (CV AFS) n = 6	0.29 ± 0.02 (A) n = 12
Intercomparison sample	24.41 ± 1.21 (CV AAS) n = 6 26.11 ± 1.60 (CV AFS)** n = 3	23.33 ± 0.91 (A) n = 6 22.17 ± 1.16 (F) n = 4 23.35 ± 1.33 (A)** n = 5	0. 57 ± 0.02 (CV AFS) n = 6	0.29 ± 0.02 (A) n = 6

(A) alkaline digestion, ethylation, GC, CV AFS;(B) akaline digestion, extraction, GC-EC; (C) distillation, ethylation, GC, CV AFS;
(D) HCl leaching, ethylation, GC, CV AAS; (F) microvolatilization, extraction, GC-ECD

* Low results due to matrix interference during reduction/amalgamation step.

** Samples re-analyzed in December 1994 by Dr. Lian Liang

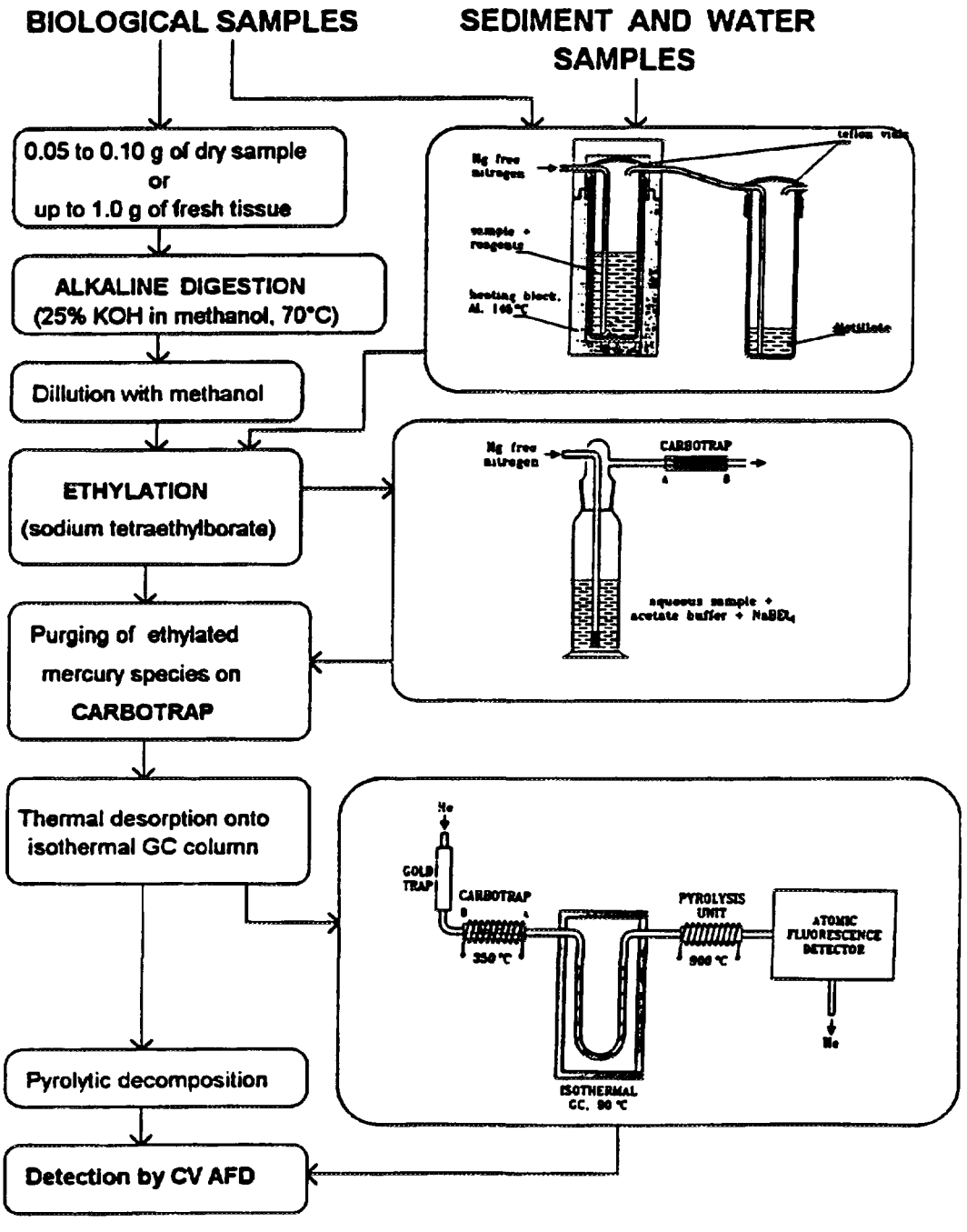

Figure 1. **A schematic flow-chart for methylmercury determination by ethylation, room temperature preconcentration, GC separation and CV AFS detection.**

DETERMINATION OF TOTAL MERCURY AND METHYLMERCURY IN THE HEAD HAIR OF PREGNANT VIETNAMESE WOMEN

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Abstract

Neutron Activation Analysis (NAA), including both the non-destructive (Instrumental NAA) and destructive (Radiochemical NAA) forms, is used as the principal technique to determine total mercury (T-Hg) and methylmercury (MeHg) in samples of human head hair. Head hair samples taken from pregnant women has been the focus for sample collection in this project. In addition, a special population group (Buddhist monks) and a normal population (control) group have also been selected for study. The defined population groups are residing in distinct regions which represent highland, coastal and industrial areas in Vietnam. Preliminary results from the determination of T-Hg and MeHg in hair samples from the defined groups indicate that the consumption of fish and seafood products is the main source of intake of MeHg; this agrees with the results from other investigators. The mean T-Hg in human hair samples from Ho Chi Minh City (an industrial region) was statistically higher than the corresponding mean values from samples taken in Dalat and Nha Trang cities, which suggest that industrial activities may be discharging an appreciable amount of mercury into the environment around Ho Chi Minh City.

1. INTRODUCTION

The study on environmental pollution to mercury in Vietnam has never been of great concern until recently, when the interest in the determination of total mercury (T-Hg) and methylmercury (MeHg) in human head hairs at the Nuclear Research Institute (NRI) in Dalat was raised. Prenatal life is more sensitive to the toxic effects of mercury than are adults, and prenatal exposure to MeHg can result in inhibition of early childhood development and overall mental ability of children [1]. Therefore, it is of primary importance to carry out investigations on this subject. Since mercury levels in the body are faithfully reflected in mercury content of the human hair [2], samples of the head hair from pregnant women were selected to be studied. As comparisons, a special population group (Buddhist monks), who very rarely consume fish or fishery products, and a normal (control) population group have also been selected for sample collection.

These investigation has been sponsored by the IAEA (via the Co-ordinated Research programme (CRP) on Assessment of Environmental Exposure to Mercury in Selected Human Populations) and by the NRI, Dalat (via the National Environmental Programme). The collection of samples and evaluation of data have also been performed in collaboration with health institutions in Dalat, Nha Trang and Ho Chi Minh cities. The purpose of these

investigations was to make a concrete survey of mercury levels and trace elements in head hair from defined population groups in Vietnam.

2. METHODS

2.1. Sample collection and preparation

Dalat, Nha Trang and Ho Chi Minh City have been selected as three typical places representing common characteristics related to mercury contamination problem. The first area is a highland region, where the population consumes fish and seafood products in relatively small amount. The second area is a coastal city where fish and seafood products are nearly the main foodstuff for the population. The third area (Ho Chi Minh City) is an industrial region with many chemical plants that were suspected of discharging an appreciable amount of mercury into the environment.

The procedure for collection and preparation of human head hair samples from the selected subjects followed the guidelines outlined in the UNEP on the determination of MeHg, T-Hg and Se in human hair [3]. The samples have been collected in collaboration with local health institutions in terms of occupation, age, weight, height, state of pregnancy and type of diet. Hair was sampled, using stainless steel scissors, from the occipital region (neck area), and as close as possible to the scalp, in an amount that corresponded to about 2 - 3 grams. Clean, sealed polyethylene bags were used for sample storage. The scalp hair samples were first washed with distilled water, dried at 50°C and then dipped into an acetone/alcohol solution with volume ratio 50 : 50 for 3 hours. Final drying was at 50°C for 5 hours. The hair sections taken for analysis were cut with clean stainless steel scissors into segments as short as possible, and then washed with acetone. Tuna fish samples have also been collected from the market. They are divided into two parts: muscle and liver. These tuna fish samples were freeze-dried and then pulverized.

Several reference materials were analyzed with the hair samples, for quality assurance purposes. These included Human Hair (NIES-RM-5), Horse Kidney (IAEA-H-8), and Fish Flesh (IAEA-M-A-2). About 150 - 200 mg of each material was used for these analyses. For the MeHg standard, CH_3HgCl (approximately 1 μg) was used.

2.2. Instrumental neutron activation analysis (INAA)

The samples and standards were sealed in quartz ampoules and irradiated either for 1 or 20 hours (depending on the selected measurement method that will be described below) at a thermal neutron flux of about 2.5 - 5.0 x 10^{12} $n \cdot cm^{-2} \cdot s^{-1}$, either at the peripheral irradiation rack or in the core of Dalat Nuclear Reactor. The samples, reference materials and standard chemicals were counted in fixed geometry condition with a HPGe detector coupled to a computer-based Multi-Channel Analyzer. Using a planar HPGe detector with FWHM of about 500 eV at 122 keV of ^{57}Co, the Au X-rays 67.0 and 68.8 keV are resolved from one another, while the Au X-ray at 77.9 keV and the ^{197}Hg soft γ-ray 77.3 keV appear as a doublet. So, a computer program is used in order to fit the overlapping peaks, using the Au X-ray at 77.9 keV and the ^{197}Hg soft γ-ray 77.3 keV for correction purposes. We use the QXAS software (distributed by IAEA) for this work. Figure 2 is a typical soft-γ spectrum of hair sample that was measured on a planar HPGe detector.

Using a coaxial HPGe detector with FWHM of about 2.0 keV at 1332 keV of ^{60}Co coupled to AT-386 computer-based APTEC PCMCA/WIN, the 279 keV γ-ray of ^{203}Hg (46.9 days) and ^{75}Se (120 days) was measured and the interference produced by ^{75}Se peak in this γ emission was corrected for by calculating the relation between this emission and the emission at 265 keV.

The measurements done on planar HPGe detector are useful in relatively short T_i (irradiation time) and T_d (decay time). By measuring ^{203}Hg (279 keV) on a coaxial detector, the required T_i and T_d are 20 h and 15 d, respectively, while those required for measurements of ^{197}Hg (77.3 keV) on a planar detector are only 1 h and 3 d, respectively. Table I shows good agreement between the obtained results based on two measurements (planar and coaxial HPGe detector).

2.3. Radiochemical neutron activation analysis (RNAA)

The T-Hg content of hair sample was determined by INAA because the method is without background interference from other activation products. Figure 3 shows a typical γ-ray spectrum of human head hair sample. In contrast, the fish sample measurements are often hindered by activation products with radiations overlapping that of the mercury nuclides. Therefore, the destructive type of activation analysis (RNAA) is often necessary for fish samples. The procedure applied in our laboratory is as follows: After a decay period of 3 - 5 days, the separation of mercury in fish samples was performed. Irradiated samples are mineralized in a Teflon bomb with a solution of 6 mL concentrated nitric acid and 2 mL of concentrated sulphuric acid. A carrier of 20 mg non-radioactive mercury is also added and the solution is heated for 6 hours in a dry furnace at 120°C. The samples are then left to cool down. The sample solution is then neutralized with sodium carbonate and ammonium hydroxide to pH = 6, and the reagents KSCN and $ZnCl_2$ are added and stirred to form $HgZn(SCN)_4$. The resulting precipitate is at last dried under an infrared lamp. The reference and standard samples were also treated in the same way. By using a gravimetric method, the chemical yield for this procedure was determined to be 96.4%.

2.4. Methylmercury (MeHg) determination using the volatilization method

The study of chemical forms of mercury in human hair and other bio-environmental samples by radiochemical methods [4] indicated that it would also be possible to apply methodologies in which MeHg is selectively separated from inorganic mercury by volatilization in a microdiffusion cell. By this technique, MeHg cyanide formed in the inner part of the cell is volatilized at an elevated temperature and trapped on a cysteine paper that is placed in the outer part of the cell [5]. The cysteine paper is then acidified and mercury is determined using INAA. A flow chart describing this technique is presented in Figure 5.

2.5. Analytical quality assessment

The scientific emphasis of these studies depends on analytical quality assurance. Therefore, considerable attention has been given to the analytical procedures to ensure that the obtained results are of good quality as regards accuracy and precision. We have carried out six determinations of T-Hg in various reference materials, *e.g.*, Horse Kidney (IAEA-H-8), Fish Flesh (IAEA-MA-A-2) and Human Hair (NIES-CRM-5) under practical conditions in order to control internal analytical quality. Table II shows T-Hg content in the reference materials analysis and their corresponding certified values.

On the occasion of the second RCM in Kuala Lumpur, August 1992, two IAEA biological specimens (samples #1 and #2) were sent as blind samples for analytical quality control exercises. Recently, we have analyzed these samples for T-Hg and MeHg repeatedly as shown in Table III. The results for the new hair intercomparison materials, IAEA-085 and IAEA-086, arrived recently; the results for these materials will be reported at a later date.

The comparison analysis of mercury contents in hair samples has been carried out by the combination of dithizone extraction and ECD-gas chromatography method at National Institute for Minamata Disease in Japan (A) and by microdiffusion and volatilization method in convey cell and NAA in reactor (B) as shown in Table IV.

3. RESULTS AND DISCUSSION

The study and successful application of the microdiffusion and volatilization method for the isolation of MeHg in convey cells has permitted the determination of MeHg in hair samples as planned. However, the analysis of many samples has proved to be difficult because of the lack of proper convey cell sets. We hope that the problem is going to be overcome. To date, we have determined T-Hg in almost all the collected hair samples and MeHg in about 30% of the samples.

Tables V, VI and VII showed that the arithmetic mean (Mean) of T-Hg content of Pregnant Woman (PW) head hair in Nha Trang region is about 1.8 times higher than the corresponding values from Dalat. The samples from Ho Chi Minh City are about 3.0 times the Dalat values. However, the mean of MeHg content from Nha Trang and Ho Chi Minh City is respectively about 2.8 times and about 3.3 higher than that of Dalat. In addition, the ratio of MeHg/T-Hg in Nha Trang region is also about 1.6 times higher than the corresponding values in Dalat region. The results will be examined for possible correlation with the dietary habits (fish and sea food products) of this population group as shown in column "Fish consumed (grams/week)" including sea and freshwater fish kinds of Tables V, VI and VII.

Figure 1 is a chart of frequency distribution of T-Hg concentration in PW head hair from Dalat, Nha Trang and Ho Chi Minh regions. This figure shows that the frequency line of merucry concentrations from Ho Chi Minh City (in the range 0.5 - 6.0 mg/kg) was wider than the line from Nha Trang and much higher than Dalat (in the ranges 0.5 - 4.0 and 0.5 - 3.0 mg/kg, respectively).

Table VIII shows some preliminary results on the determination of T-Hg and MeHg contents in tuna fish samples. In two parts of tuna fish, the ratio of MeHg/T-Hg in liver is higher than that in muscle.

Table IX shows T-Hg and MeHg contents in head hair samples of Buddhist monks (BM) and normal population in terms of sex (male and female) and age (20-50) from Dalat and Ho Chi Minh City regions. These results have indicated that the consumption of fish and fish products may be the main source of intake of mercury for the population. The comparison the mean of mercury values between the samples from BM and the normal population has also contributed information for understanding the source of mercury intake in the different populations.

4. PLANS FOR FUTURE WORK

We expect to expand the determination of mercury in head hair samples of the defined population groups. Furthermore, the study on chemical yield for MeHg by the volatilization technique will be carried out to bring the method into analytical routine. Finally, the sample collection of head hair samples from mental patients and the determination of mercury and trace elements in these samples, begun in early 1994, will be continued.

ACKNOWLEDGMENT

We would like to thank the financial support of the Agency (via Research Contract No. VIE-6931) so that we were able to carry out the collection of head hair samples from pregnant women and to organize the in- depth work. We would also like to thank the health institutions in Dalat, Nha Trang and Ho Chi Minh cities for their co-operation in this project.

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TABLE I. T-Hg CONTENT BASED ON TWO MEASUREMENTS, IN mg/kg ± STANDARD DEVIATION (SD)

Samples	77.3 keV on planar detector	279 keV on coaxial detector	Ratio of Values
Hair	3.25 ± 0.07	3.16 ± 0.15	1.03
Fish	0.23 ± 0.02	0.21 ± 0.12	1.09

TABLE II. T-Hg IN REFERENCE MATERIALS OF EXPERIMENTS AND THEIR CERTIFIED VALUES (MEAN, mg/kg ± SD; NUMBER OF INDEPENDENT DETERMINATIONS = 6)

Reference Materials	T-Hg	
	Experiments	Certified Values
IAEA-H-8	1.10 ± 0.05	0.91
IAEA-MA-A-2	0.53 ± 0.05	0.47
NIES-CRM-5	4.70 ± 0.10	4.40

TABLE III. T-Hg AND MeHg IN IAEA BIOLOGICAL SAMPLES OF EXPERIMENTS AND CERTIFIED VALUES (MEAN, mg/kg ± SD; NUMBER OF INDEPENDENT DETERMINATIONS = 6)

Samples	Experiments		Certified Values	
	T-Hg	MeHg	T-Hg	MeHg
#1	0.31 ± 0.02	< 0.15	0.28	0.018
#2	0.53 ± 0.05	0.27 ± 0.02	0.47	0.300

TABLE IV. THE COMPARISON ANALYSIS OF MERCURY CONTENTS IN HAIR SAMPLES

Samples	T-Hg (mg/kg)		MeHg (mg/kg)		Ratio MeHg/ T-Hg	
	A	B	A	B	A	B
NT#1	2.55	2.515	1.70	1.935	0.667	0.7696
NT#2	5.05	4.915	3.15	3.405	0.624	0.6929

A = by the microdiffusion and volatilization in convey cell and NAA method.
B = by the combination of dithizone extraction and ECD-gas chromatography method.

TABLE V. T-Hg AND MeHg CONTENTS IN PW HEAD HAIR FROM DALAT REGION (MEAN, mg/kg ± SD)

Age	Fish consumed (grams/week)		T-Hg	MeHg	Ratio MeHg/ T-Hg
	Sea	Fresh			
20 + 35	150 + 300	150 + 400	1.70 ± 0.61	0.65 ± 0.21	0.38

TABLE VI. T-Hg AND MeHg CONTENTS IN PW HEAD HAIR FROM NHA TRANG REGION (MEAN, mg/kg ± SD)

Age	Fish consumed (range in grams/week)		T-Hg	MeHg	Ratio of MeHg/T-Hg
	Sea	Fresh			
20 - 35	150 - 700	150 - 300	3.03 ± 1.09	1.82 ± 1.19	0.60

TABLE VII. T-Hg AND MeHg CONTENTS IN PW HEAD HAIR FROM HO CHI MINH CITY (MEAN, mg/kg ± SD)

Age	Fish consumed (range in grams/week)		T-Hg	MeHg	Ratio of MeHg/T-Hg
	Sea	Fresh			
20 - 35	150 - 1050	150 - 350	5.05 ± 1.29	2.12 ± 1.19	0.42

TABLE VIII. T-Hg AND MeHg CONTENTS IN TUNA FISH PARTS FROM NHA TRANG REGION (MEAN, mg/kg ± SD)

Muscle		Liver	
T-Hg	MeHg	T-Hg	MeHg
0.28 ± 0.03	0.14 ± 0.02	0.43 ± 0.05	0.39 ± 0.03

TABLE IX. T-Hg AND MeHg CONTENTS IN HEAD HAIR SAMPLES OF NORMAL AND BM POPULATIONS FROM HO CHI MINH CITY AND DALAT REGIONS (MEAN, mg/kg ± SD; AGE: 20 - 50)

Regions	Sex	Normal		Buddist monk	
		T-Hg	MeHg	T-Hg	MeHg
HCM City	Female	2.72 ± 0.91	-	-	-
HCM City	Male	2.22 ± 0.74	-	-	-
Dalat	Male	1.05 ± 0.15	0.35 ± 0.15	0.55 ± 0.05	< 0.15

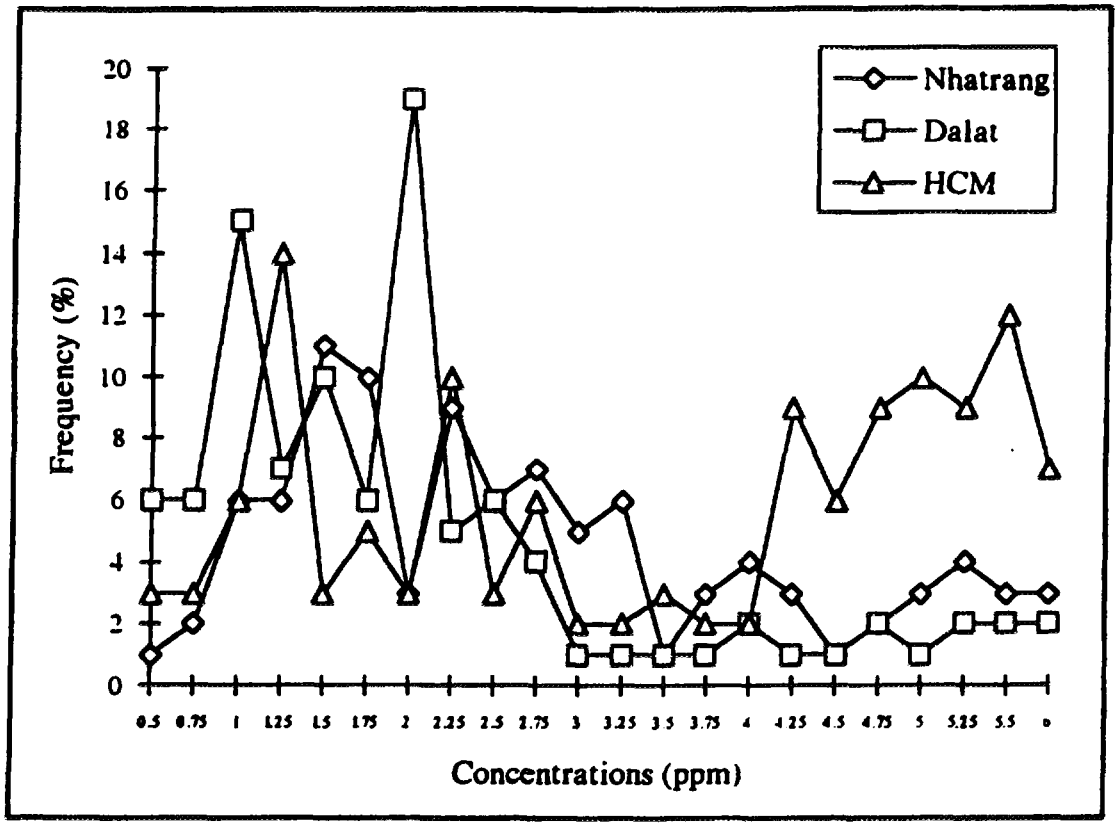

Figure 1. **A chart frequency distribution of T-Hg concentration in PW head hair samples from Dalat, Nha Trang and Ho Chi Minh regions.**

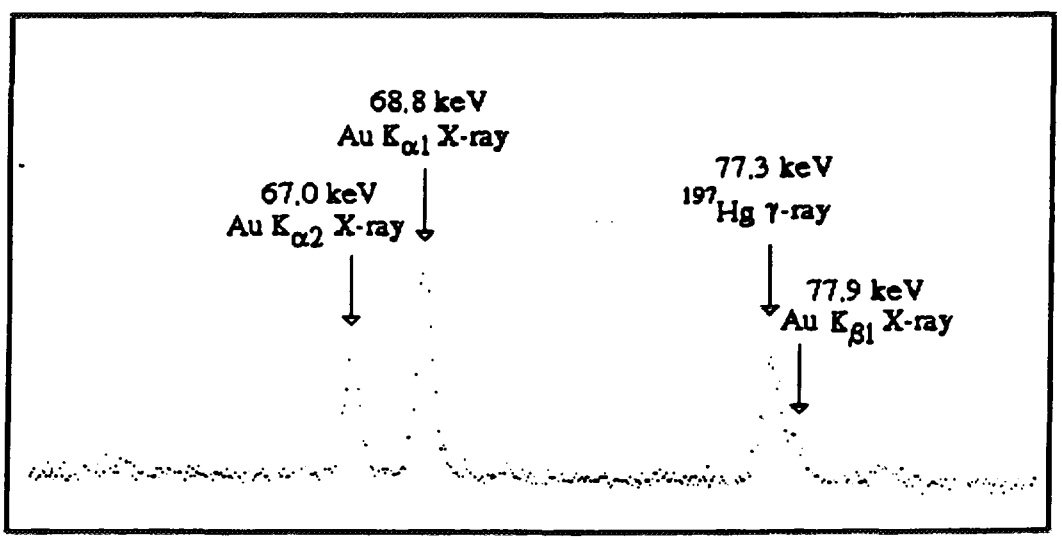

Figure 2. **A Typical soft-γ spectrum of human head hair sample (measured on planar HPGe detector; Ti = 1 h, Td = 3 d and Tc = 3600s)**

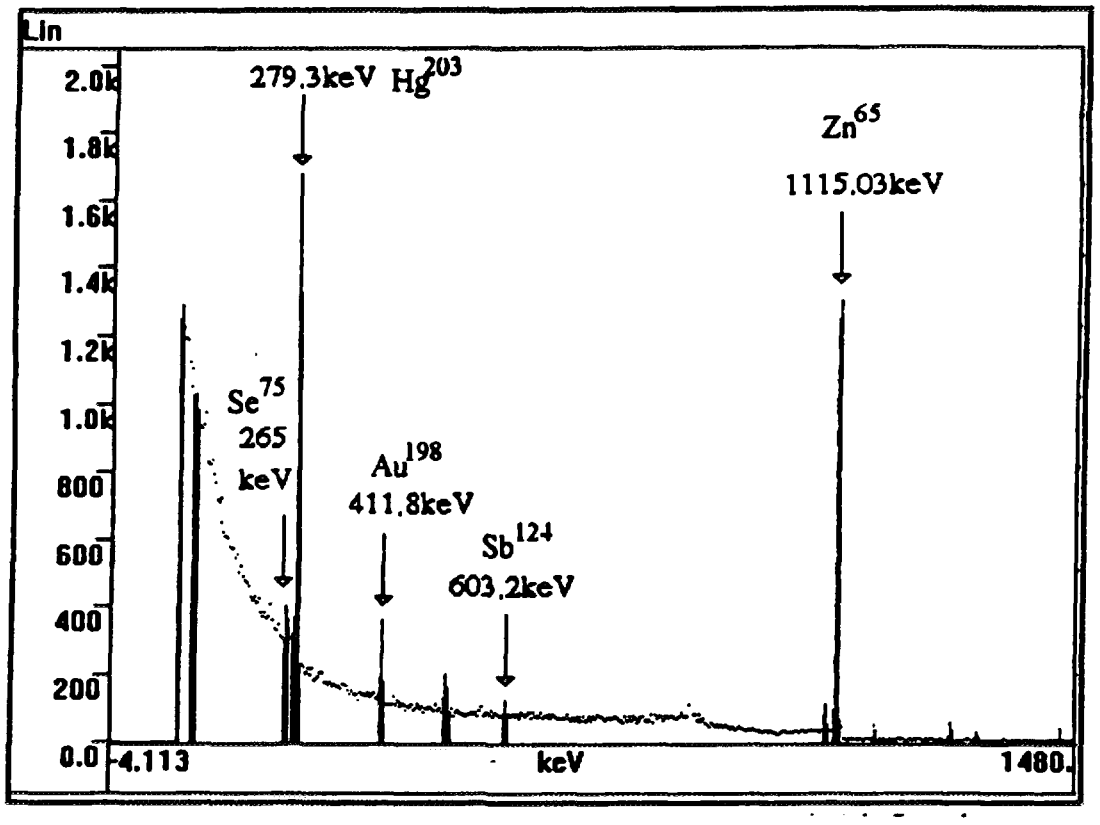

Figure 3. A typical γ-ray spectrum of a human head hair sample (measured on coaxial HPGE detector; Ti = 20 h, Td = 15 d, Tc = 3600s).

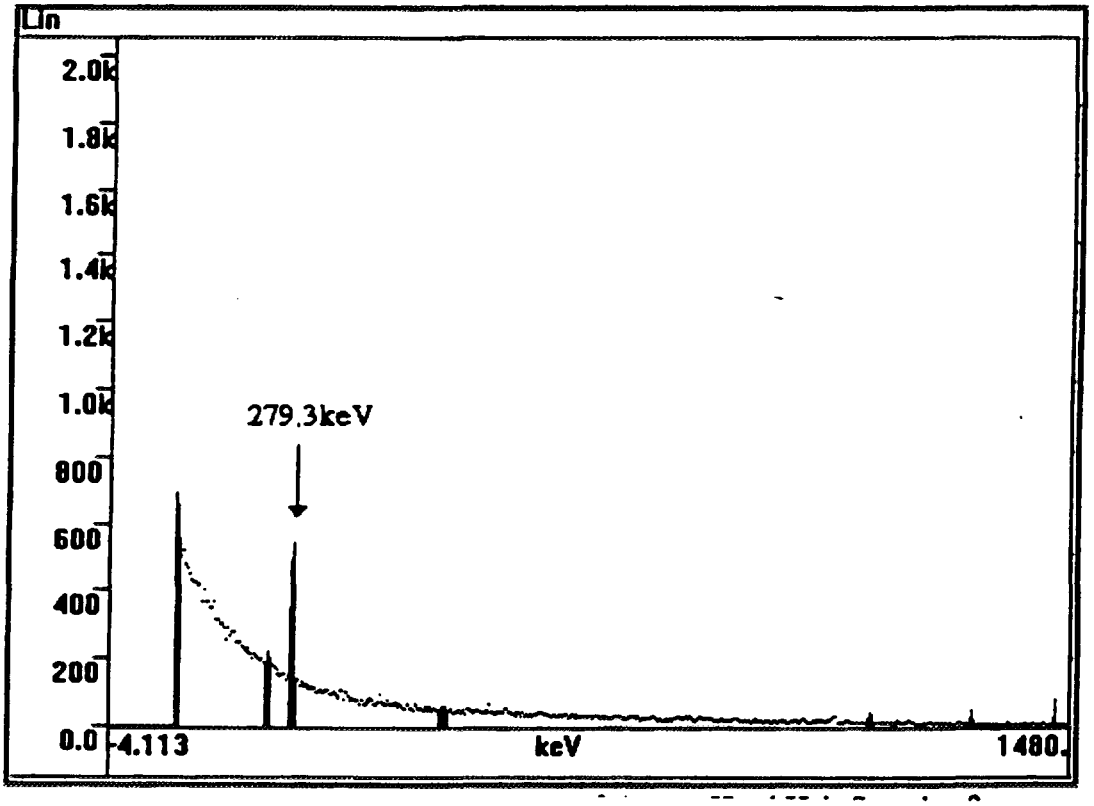

Figure 4. A typical γ-ray spectrum of a human head hair sample after separating MeHg (Measured on coaxial HPGe detector; Ti = 20 h, Td = 15 d, Tc = 3600s).

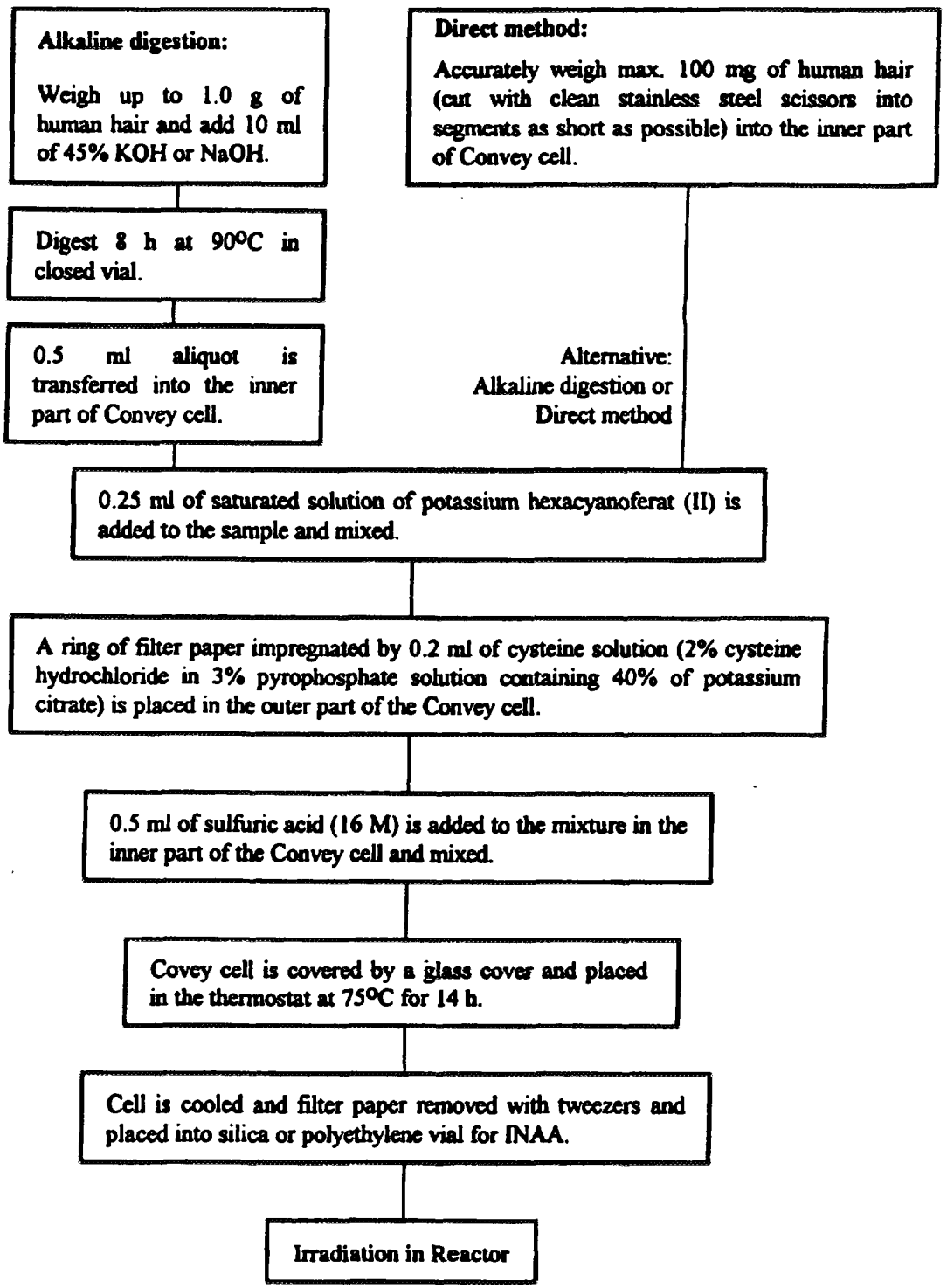

Figure 5. **A flow chart showing steps for isolation of MeHg by microdiffusion and volatilization technique in convey cell.**

Appendix I

IAEA-085 AND IAEA-086 INTERCOMPARISON STUDY ON THE DETERMINATION OF METHYLMERCURY, TOTAL MERCURY AND OTHER TRACE ELEMENTS IN HUMAN HAIR

Information Sheet

Description of the Material

The intercomparison materials IAEA-085 and IAEA-086 have been prepared from human hair; IAEA-085 represents hair with an elevated level of methylmercury and IAEA-086 contains a low level of mercury. Ten kg of human hair were collected and donated to the Agency for preparation of these materials. The material had been previously cut into uniform (1 cm) lengths, and cleaned with acetone and deionized water following the procedure developed by IAEA [1]. The material was split into two portions, each approximately 5 kg, and was radiation sterilized at 50 kGy. One portion (5 kg) was labeled with methylmercury using an established procedure [2] to achieve an elevated level of methylmercury. Both the labeled and unlabeled portions of the hair were cryogenically homogenized using the stainless steel "CryoPalla" mill at the KFA-Jülich Specimen Bank facility [3]. The hair was subjected to consecutive millings, until approximately 70% of each material was below 0.071 μm grain size. We refrained from removing the larger particles to avoid excessive contamination during sieving (it is also assumed that the larger particles prevent agglomeration of the powder).

The materials were then bottled, with 750 units of 5 g each, for IAEA-085, and IAEA-086, respectively. The materials were sterilized in the bottles at 12 kGy using a ^{60}Co Source. One bottle of each material is being distributed to each participant in the intercomparison.

Scope of the Study

The aim of the study is to evaluate the accuracy with which the participating laboratories are determining mercury and methylmercury in hair. For this purpose the results on the reported concentrations will be statistically evaluated and most probable values will be determined. Potential bias in procedures or laboratory results will be reported to the participants. Of course, all results will be treated anonymously, and only laboratory codes will be used throughout the study. Participants will be informed only of their own laboratory code.

The participants are requested to determine total mercury and methylmercury as primary goals; however, additional trace element analyses would also be welcome. All participants are requested to make at least three, but preferably six independent determinations for each element or species in each material. Homogeneity for mercury has been established down to the 10 mg level, however, samples sizes of 50-100 mg are recommended.

Following statistical evaluation of the intercomparison data, a report containing these results will be issued and sent to the participants. After satisfactory evaluations have been completed, these materials will be made available for use by any laboratory that is interested in quality assurance in population monitoring for total mercury and methylmercury.

Analytical Quality Control

Procedures of good laboratory practice (GLP) and laboratory quality assurance should be strictly applied to these analyses. The practice of quality control with certified reference materials is highly recommended. AQCS is unable to provide free of charge an additional quality assurance material for use in this intercomparison; however, BCR-CRM-397 human hair is certified for total mercury, and the IAEA-350, Tuna Homogenate, is certified for methylmercury, as well as for total mercury. Both of these CRMs are certified for additional selected trace elements. The analysis of one of these materials, or another suitable QA material, should be appropriately interfaced with the determinations on the intercomparison materials and the same procedures must be applied. The number of QA determinations should be similar to the number of actual determinations in the intercomparison materials. The results of the analyses of the QA material(s) should be reported along with the intercomparison materials on the forms and diskette provided (see Reporting of Results, below).

AQCS recommends for quantitation the use of physical principles (through fundamental constants and parameters) or primary comparator standards such as quantitative solutions made form pure metals or compounds. Reference materials are in most instances not suitable for standardization and should only be used to overcome the lack of standards or other means for quantitation for some of the elements. All uncertainties of such secondary means must be propagated to the results. Unfortunately, AQCS does not have the resources to provide primary standards.

Moisture Determination

All results are to be reported on a dry weight basis. For the determination of water content, a 250 mg portion of each material should be taken at the time of analysis and

lyophilized in a freeze dryer for 48 hours. Alternately, if a freeze-drier is not available, the portion may be dried at 80 °C for 24 hours. The analytical results are to be corrected for the determined moisture loss. For reference, please include the results of the moisture determination in the reporting form.

Reporting of Results

The results, based on dry weight, (for IAEA-085, IAEA-086, and an appropriate Quality Assurance material) should be reported to AQCS both on the Reporting Forms and on the computer diskette provided with the intercomparison materials. Please list the results for methylmercury and total mercury first, followed by any additional elements that may be determined. The results for methylmercury should be reported in mg/kg, expressed as mercury. Please also indicate the appropriate unit (μg/kg, mg/kg, etc.) for each determinant, and continue the listing with the results for the control material(s). When reporting results for the quality assurance materials, give the reference material - number in the first field of entry; *e.g.* BCR-CRM-397:Hg.

Results should include an estimate of a combined uncertainty (in the same unit, not % relative) for each determination. Estimated uncertainties in an analytical measurement consist of components which can be grouped into categories according to the way in which their value is estimated:

A: those which are evaluated by applying statistical methods to a series of repeated determinations,

B: those which are evaluated by other means.

The components are expressed in terms of estimates of variance (*e.g.* s^2 or σ^2). The combined uncertainty is characterized by the numerical value obtained by applying the usual method for the combination of variances. The combined uncertainty and its components are expressed in the form of standard deviations.

Various components which make up the total uncertainty can typically include:

a) reproducibility of measurement;
b) uncertainty in calibration;
c) bias or drift of measurement;
d) uncertainties in sample preparation (mass, dilution, *etc.*);
e) uncertainty of blank;
f) uncertainty in instrument readings (*e.g.* peak integration).

This is not an exhaustive list. It should be noted that the uncertainties listed above as examples may consist of uncertainties of both category A and B. The limit of detection should also be included for each determinant for the determined concentrations and values below the limit of detection.

A classification of the analytical procedures used (*e.g.*: acid digestion (HNO_3/HF)-liquid/liquid extraction (aqu./CCl_4/aqu.) - ETAAS, or: none-none- INAA) should be listed for each determinant on page 5. A summary description of the applied procedure including relevant reference should be given on page 6. Please use copies of the forms if more space is required.

On the DOS-formatted computer diskette, spreadsheet files have been provided both in EXCEL (XLS) and in Lotus WK1 format. If you do not have access to a spreadsheet programme, please provide the data in a TAB-DELIMITED text format (.TXT) following the column format of the example reporting form. By providing your data both in hard copy and on diskette, you will greatly facilitate our evaluation and avoid possible transcription errors.

The deadline for reporting of results is 31 November 1994. Any results received after that date will still be of interest to us, but it may not be possible to include them in the first report.

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