# 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 $\mu$g) 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 $\gamma$-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 $\gamma$-ray 77.3 keV for correction purposes. We use the QXAS software (distributed by IAEA) for this work. Figure 2 is a typical soft-$\gamma$ 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

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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 |

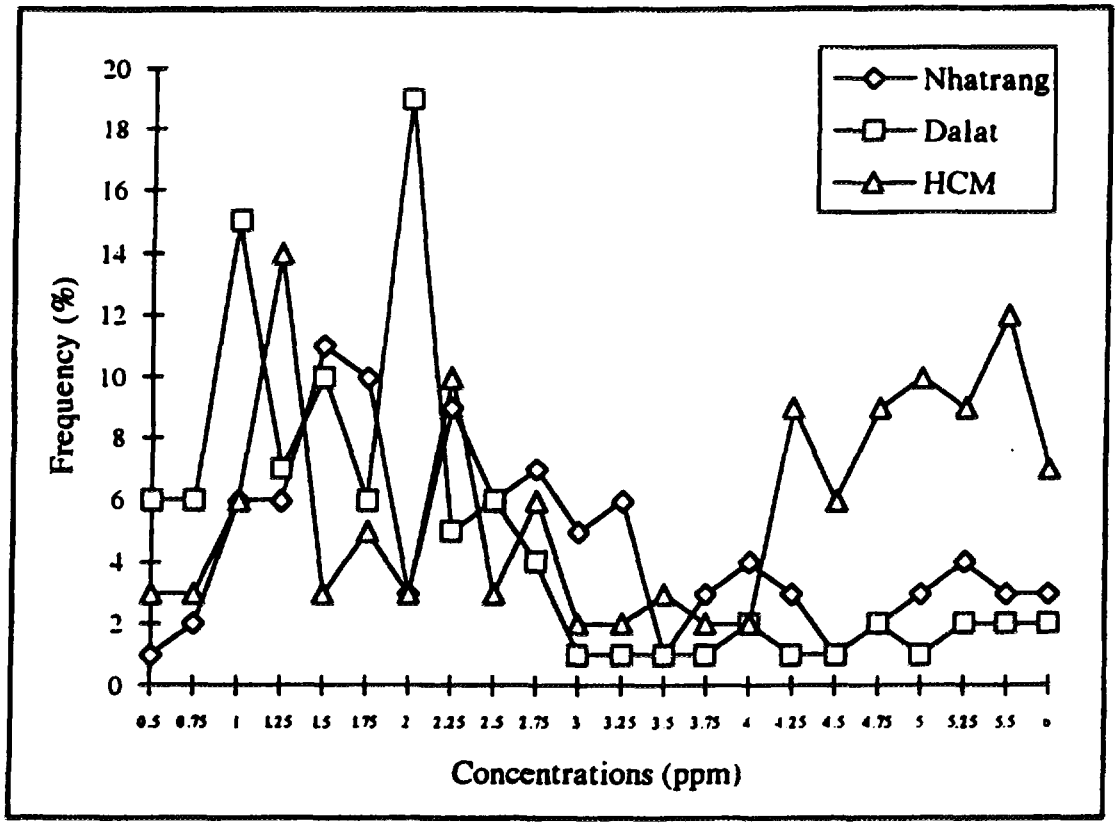


**Figure 1.** **A chart frequency distribution of T-Hg concentration in PW head hair samples from Dalat, Nha Trang and Ho Chi Minh regions.**

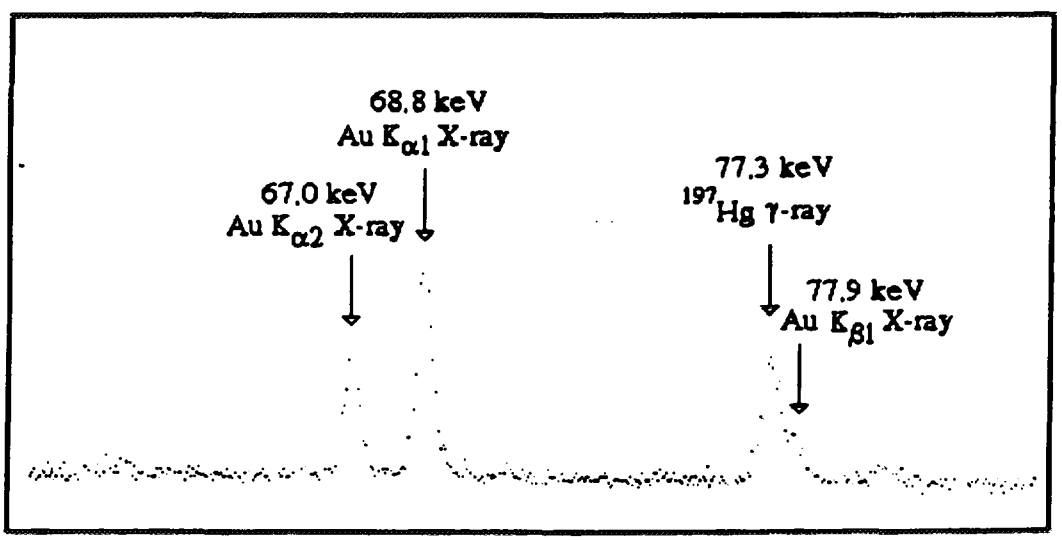


**Figure 2.** **A Typical soft-γ spectrum of human head hair sample (measured on planar HPGe detector; Ti = 1 h, Td = 3 d and Tc = 3600s)**

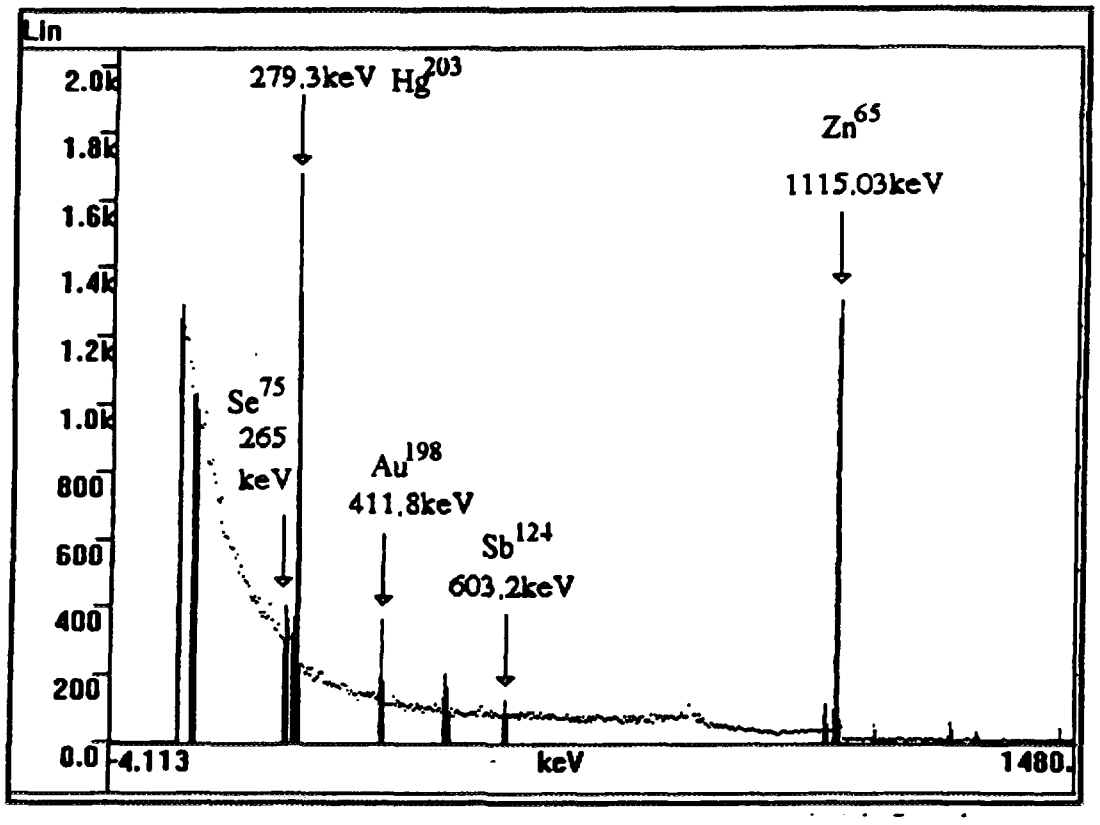


Figure 3. A typical γ-ray spectrum of a human head hair sample (measured on coaxial HPGE detector; Ti = 20 h, Td = 15 d, Tc = 3600s).

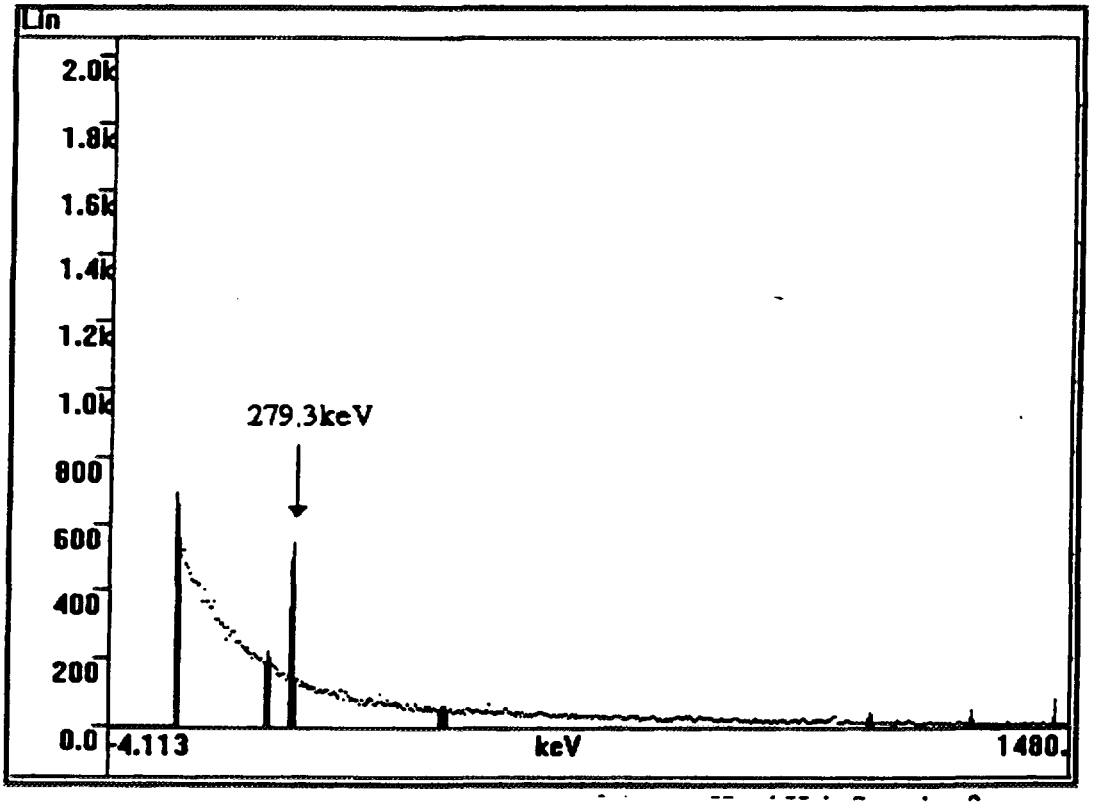


**Figure 4.** A typical γ-ray spectrum of a human head hair sample after separating MeHg (Measured on coaxial HPGe detector; Ti = 20 h, Td = 15 d, Tc = 3600s).

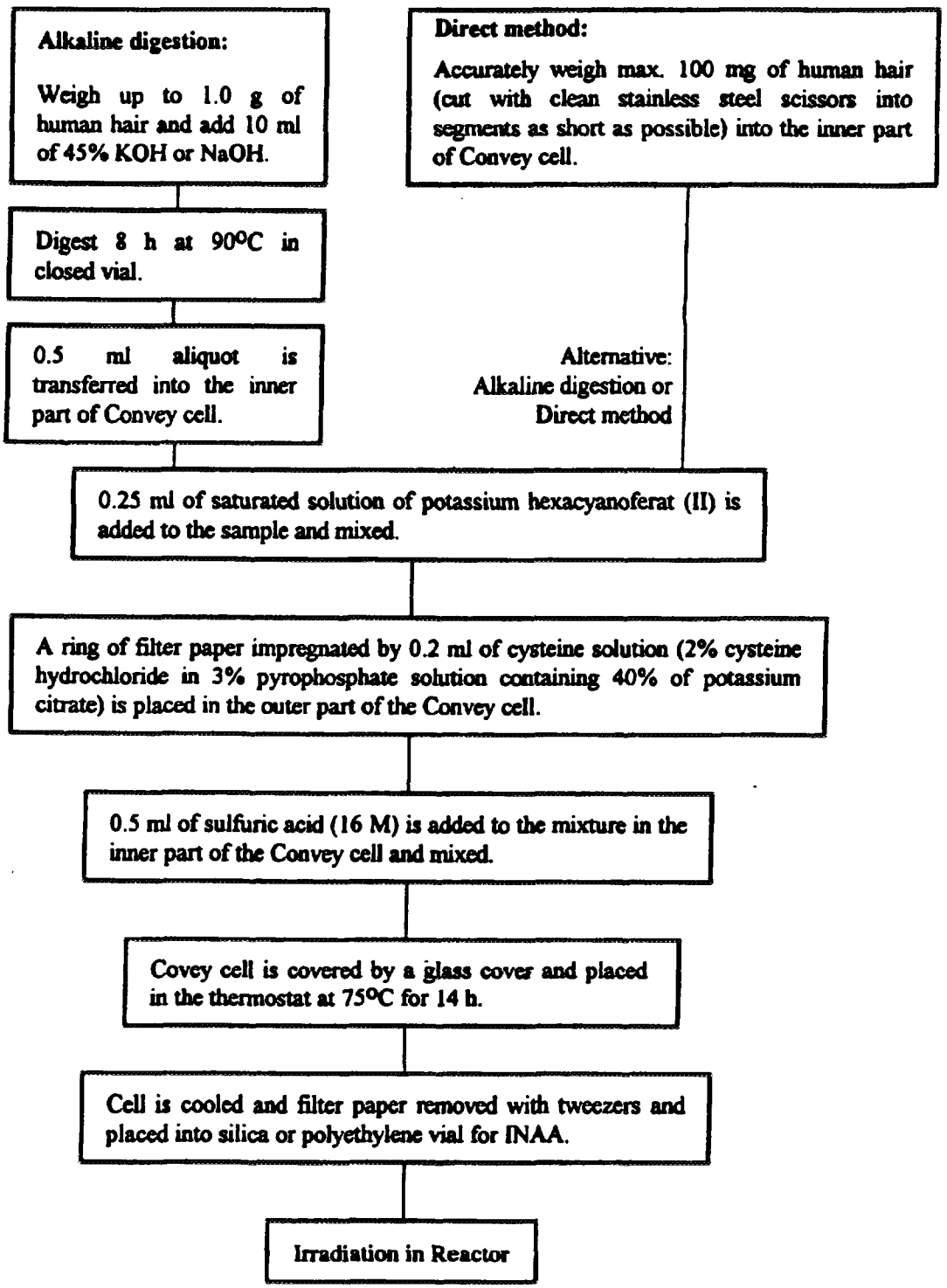


**Figure 5.** **A flow chart showing steps for isolation of MeHg by microdiffusion and volatilization technique in convey cell.**