



## 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  $^{203}\text{Hg}$  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 ( $\text{Hg}_i$ ) 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  $^{203}\text{Hg}$  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 $^{203}\text{Hg}$ 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 ( $\text{Hg}_m$ ), 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  $3 \times 10^{13} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ . 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  $\gamma$ -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 ( $\pm 0.1$  mm) was ensured by using special perspex holders (source-to-detector cap distance was 2 cm). Measurements were done on the  $^{203}\text{Hg}$  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  $\text{H}_2\text{SO}_4$ . 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  $^{203}\text{Hg}$  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 investigate this, we irradiated about 1 g of cut hair labelled with  $\text{Me}^{203}\text{Hg}$  ( $3 \mu\text{gHg/g}$  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 ( $A_{\text{tot}}$ ), and methylmercury was then separated using the recommended extraction procedure [1]. The activity of the organic phase ( $A_{\text{org}}$ ) 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  $^{203}\text{Hg}$ , 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  $\mu\text{g/mL}$  of Hg as radioactively labelled  $\text{Hg}_\text{n}$  or  $\text{MeHg}$  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  $\mu\text{g/mL}$  of  $^{203}\text{Hg}_\text{n}$  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:

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

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  $\mu L$  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 procedure 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,  $0.32 \pm 0.02 \mu\text{g/g}$  total mercury was determined; in hair spiked with MeHg,  $7.3 \pm 0.3 \mu\text{g/g}$  was found; in hair spiked with  $\text{Hg}_{\text{in}}$ ,  $8.3 \pm 0.3 \mu\text{g/g}$  was determined. The samples and mercury levels were selected to represent hair with natural mercury content and hair with different ratios of MeHg/ $\text{Hg}_{\text{in}}$ , 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  $^{203}\text{Hg}$  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  $\text{Hg}_{\text{in}}$  depends on the method of spiking used (Fig. 3, curves 3 and 5). The presence of  $\text{Cu}^{2+}$  in the leaching solution causes a decrease in  $K_{\text{D}}$  of both MeHg and  $\text{Hg}_{\text{in}}$  and makes the separation of these forms less efficient.

The dependence of  $K_{\text{D}}$  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  $\text{Hg}_{\text{in}}$  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  $\text{Hg}_{\text{in}}$  (curve 4). The differences in the behaviour of  $\text{Hg}_{\text{in}}$  spiked on cut hair by Procedure A and B suggest that in Procedure A  $\text{Hg}_{\text{in}}$  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  $V/m = 40 \text{ mL/g}$  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  $40 \text{ mL}\cdot\text{g}^{-1}$ ) 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  $Hg_m$ .

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			$99.7 \pm 1.8 \%$

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

Hair weight [mg]	MeHg found [ng Hg·g <sup>-1</sup> ]	Hg <sub>in</sub> found [ng Hg·g <sup>-1</sup> ]	Total Hg [ng Hg·g <sup>-1</sup> ]
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	$129 \pm 5$	$291 \pm 8$	$420 \pm 12$

CI - 95% confidence interval

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

Hair weight [g]	C <sub>6</sub> H <sub>6</sub> phase MeHg found [ng Hg·g <sup>-1</sup> ]	NaOH phase MeHg found [ng Hg·g <sup>-1</sup> ]
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·g <sup>-1</sup> ]
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  $\mu\text{g Hg/g}$ , 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 <sub>in</sub>	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  $\mu\text{g Hg/g}$ , dry weight, quoted variation is one standard deviation.

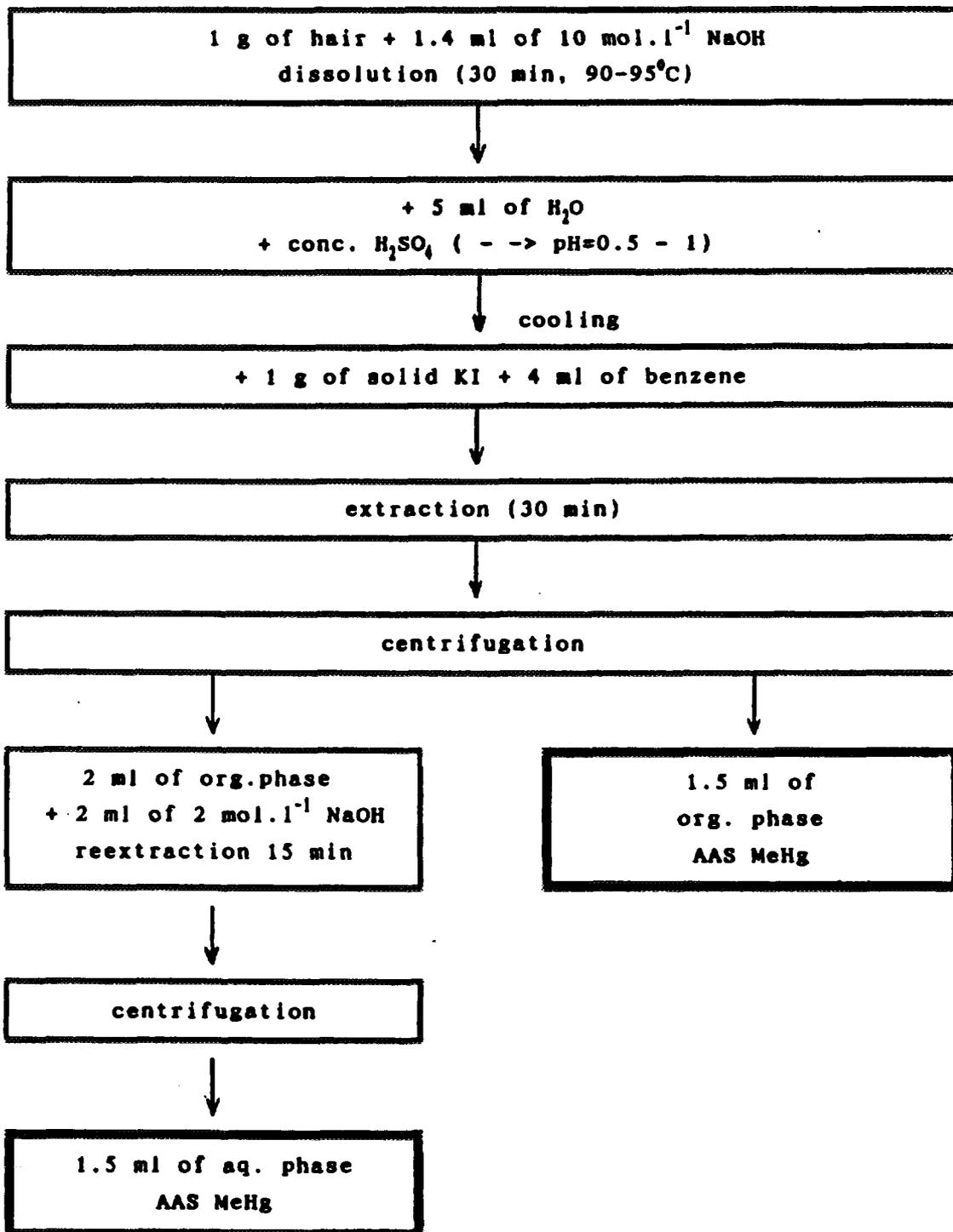


Figure 1. The solvent extraction method

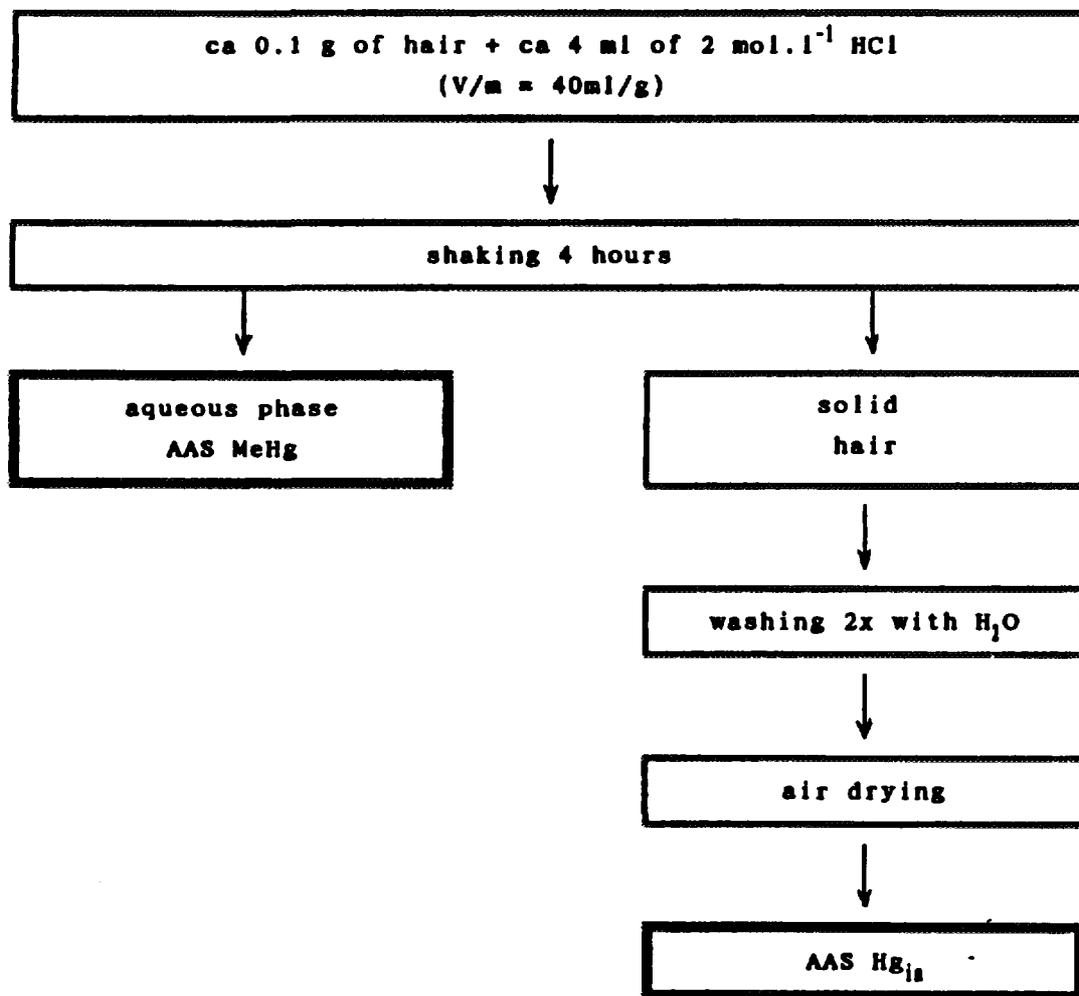
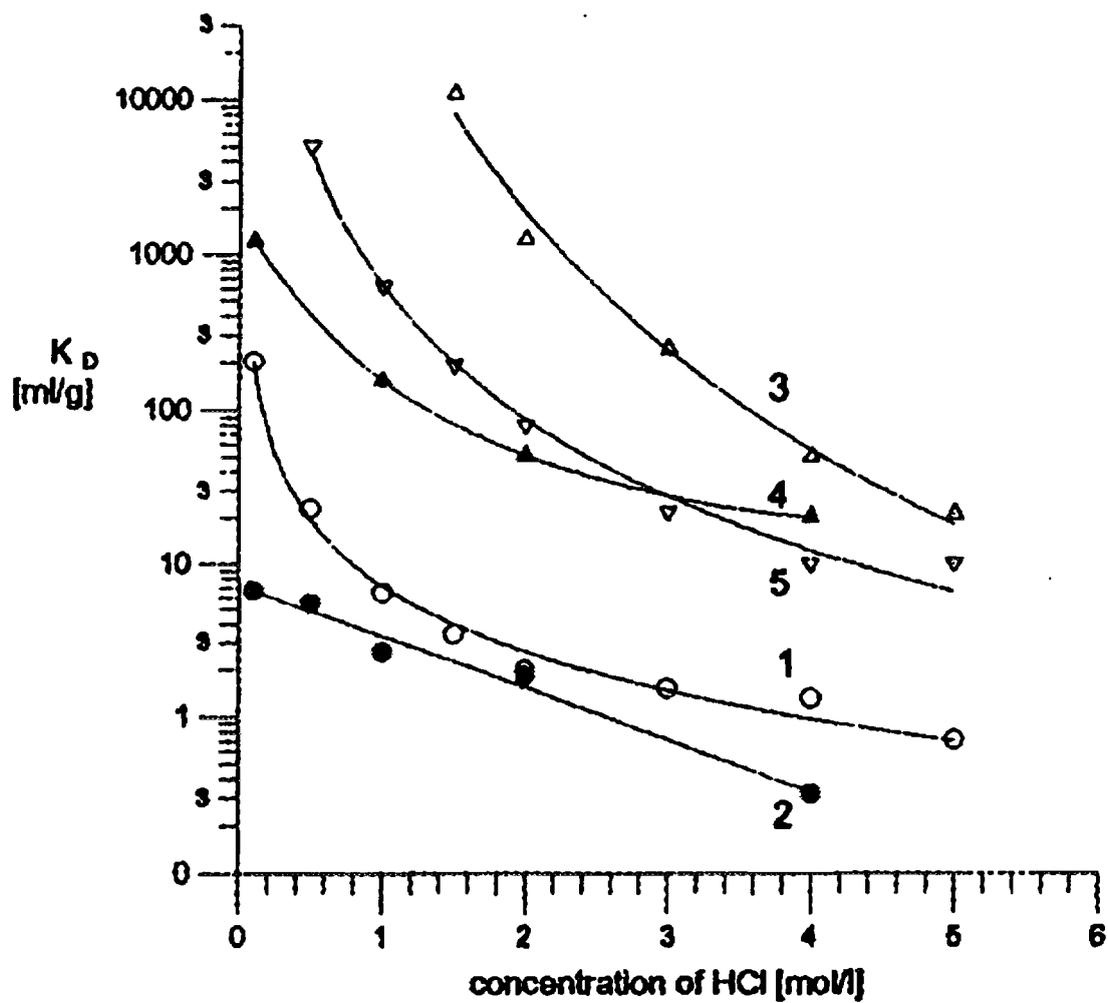
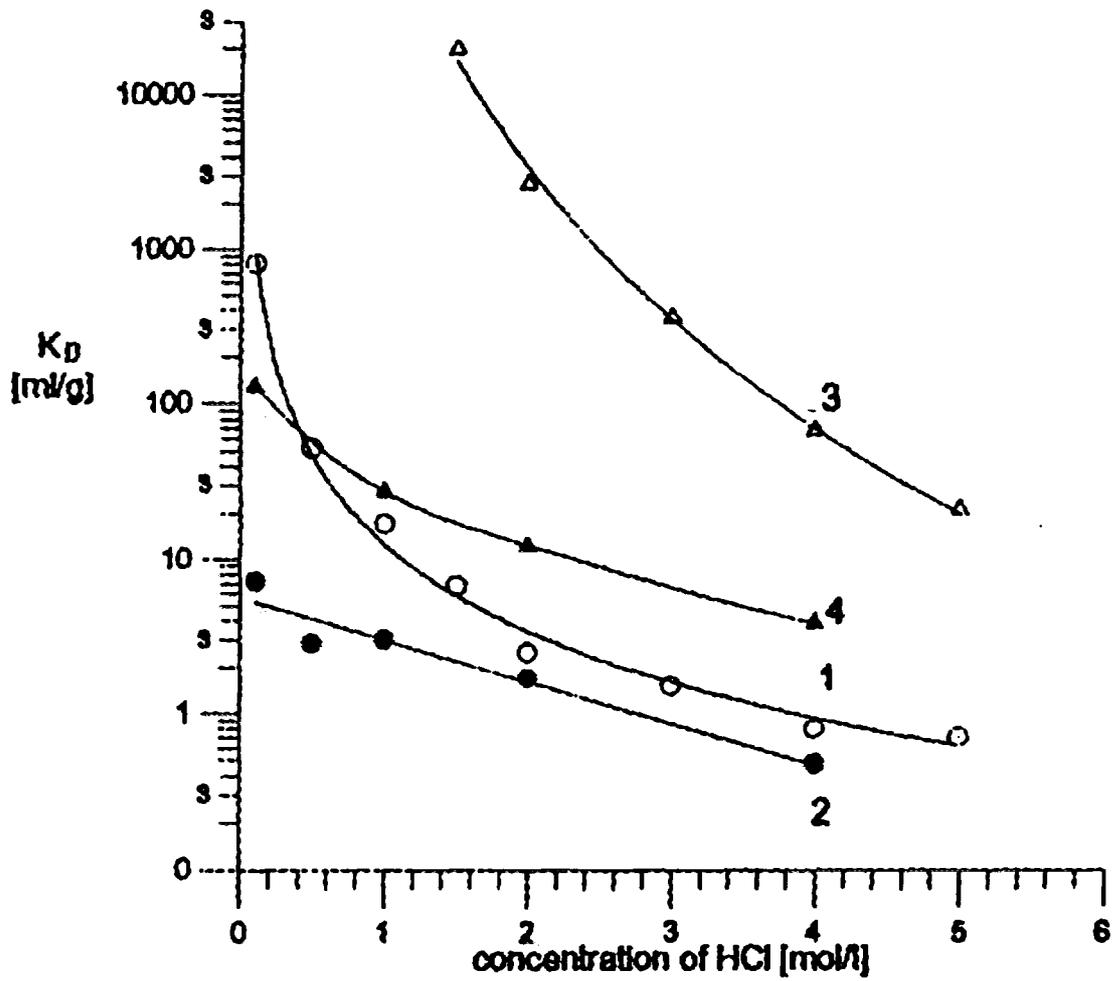


Figure 2. The acid leaching method



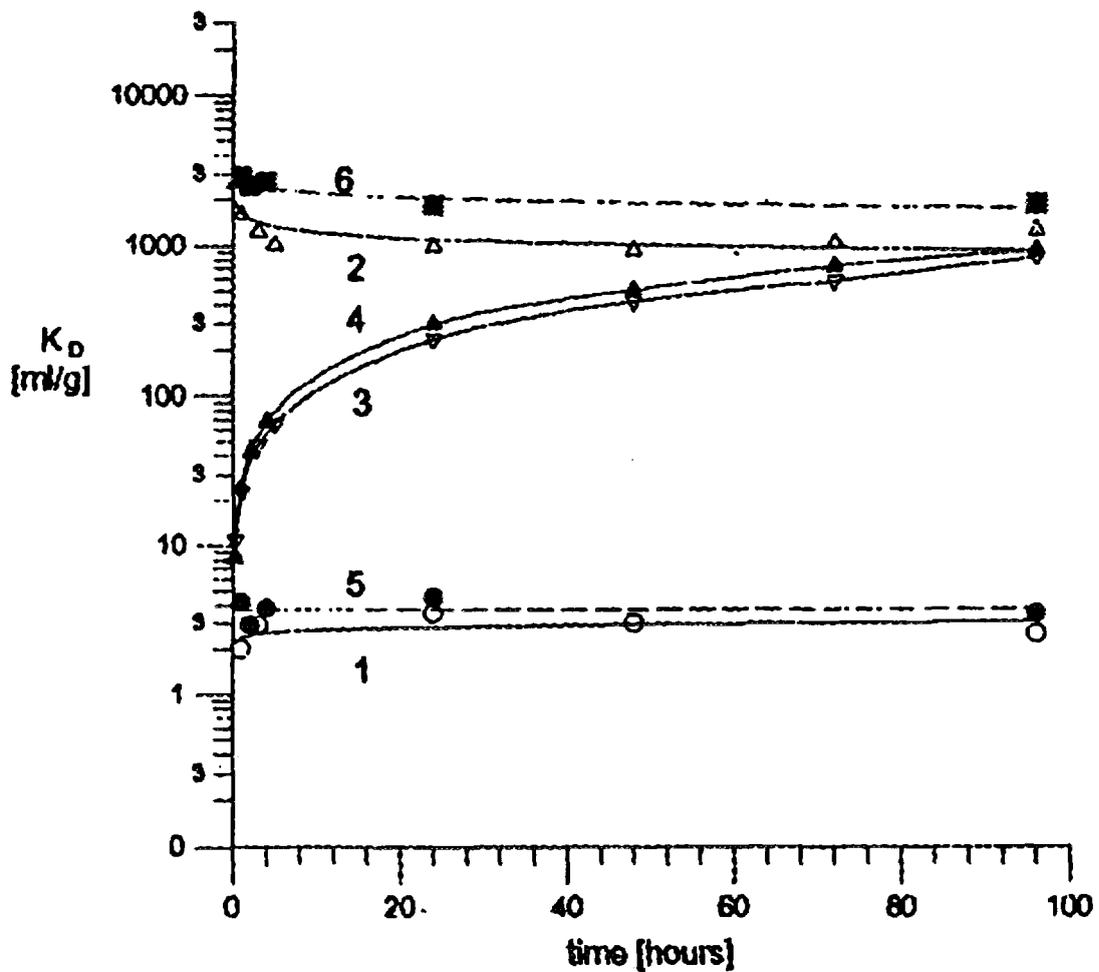
1 - MeHg; 2 - MeHg, in the presence of  $1 \text{ mol}\cdot\text{l}^{-1} \text{ Cu}^{2+}$ ; 3 -  $\text{Hg}_{\text{in}}$ , prepared by procedure B; 4 -  $\text{Hg}_{\text{in}}$ , prepared by procedure B, in the presence of  $1 \text{ mol}\cdot\text{l}^{-1} \text{ Cu}^{2+}$ ; 5 -  $\text{Hg}_{\text{in}}$ , prepared by procedure A

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



1 - MeHg; 2 - MeHg, in the presence of  $1 \text{ mol}\cdot\text{l}^{-1} \text{ Cu}^{2+}$ ; 3 -  $\text{Hg}_{in}$ ; 4 -  $\text{Hg}_{in}$ , in the presence of  $1 \text{ mol}\cdot\text{l}^{-1} \text{ Cu}^{2+}$

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



1 - MeHg, cut hair; 2 - Hg<sub>m</sub>, cut hair prepared by procedure B; 3 - Hg<sub>m</sub>, cut hair prepared by procedure A; 4 - uptake of Hg<sub>m</sub> from 2M HCl by cut hair; 5 - MeHg, ground hair; 6 - Hg<sub>m</sub>, ground hair

Figure 5. Kinetics of leaching of mercury species from hair with 2 mol·l<sup>-1</sup> HCl.