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

[1] WORLD HEALTH ORGANIZATION, Methylmercury, Environmental Health Criteria No. 101. WHO Geneva (1990).

[2] NATIONAL ACADEMY OF SCIENCES, Recommended Dietary Allowances, 9th edition, Washington, DC (1980).

[3] HORVAT, M., BYRNE, A.R., Analyst **117** (1992) 665-668.

[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

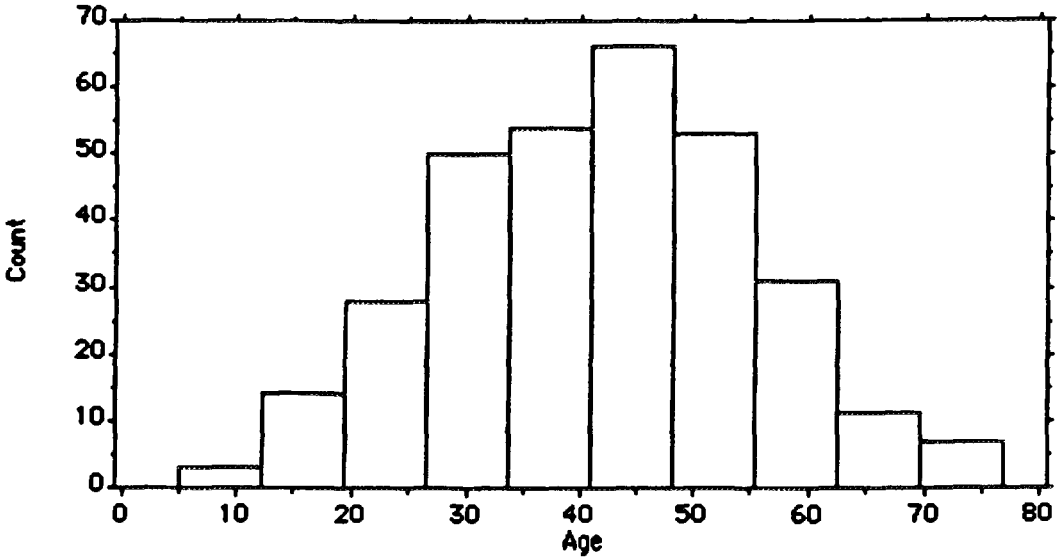

Figure 1. **Age frequency distribution of all subjects.**

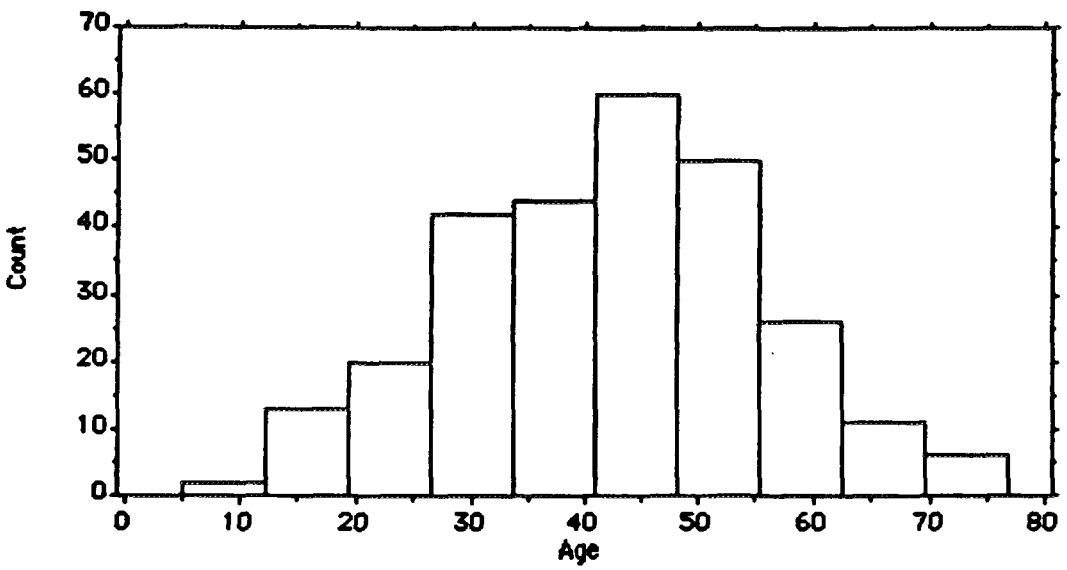

Figure 2. **Age frequency distribution of male subjects.**

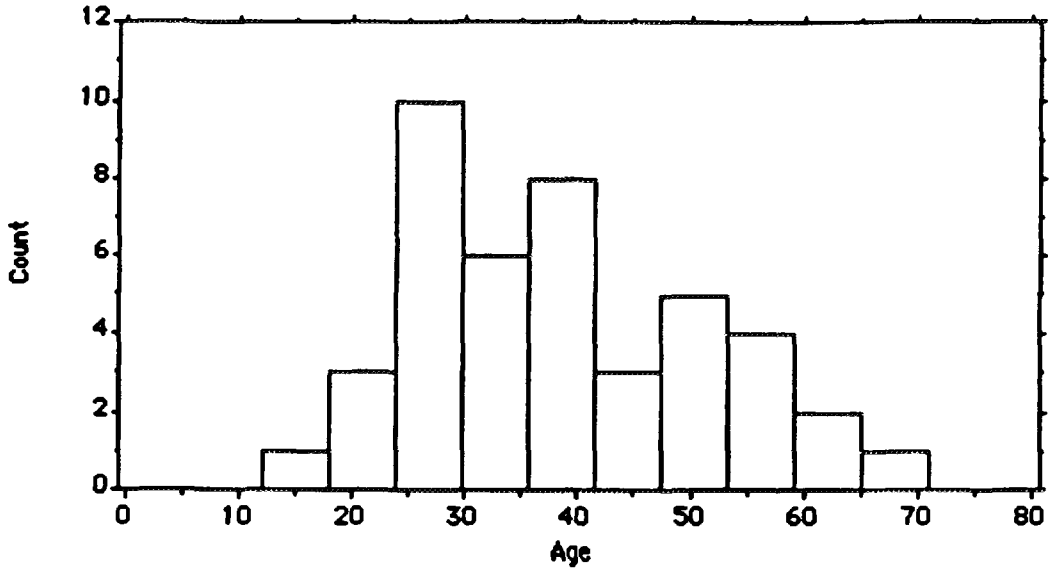

Figure 3. **Age frequency distribution of female subjects.**

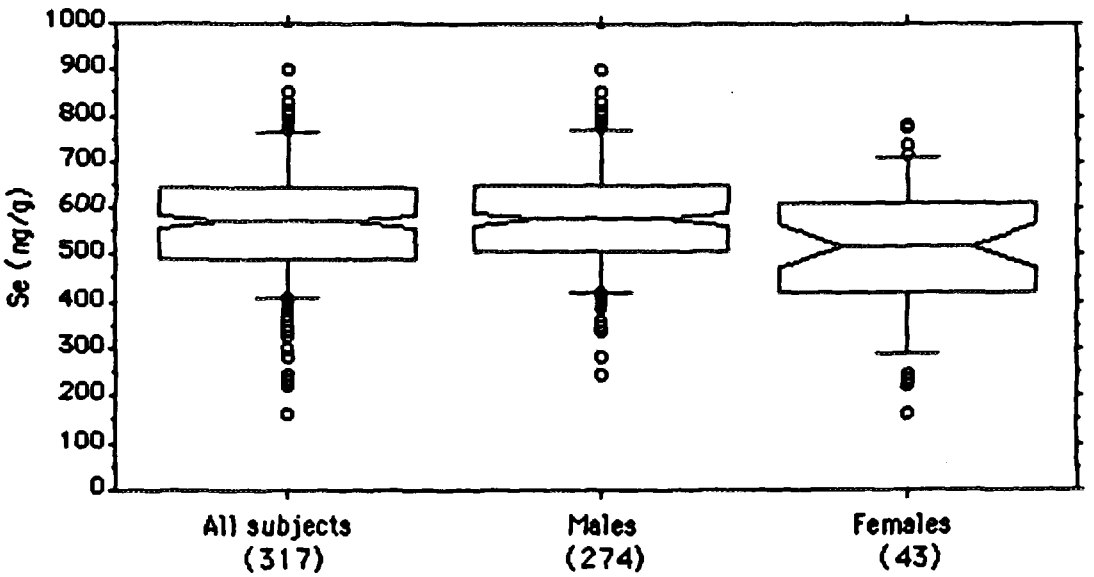

Figure 4. **Se concentration in hair of subjects from all selected areas.**

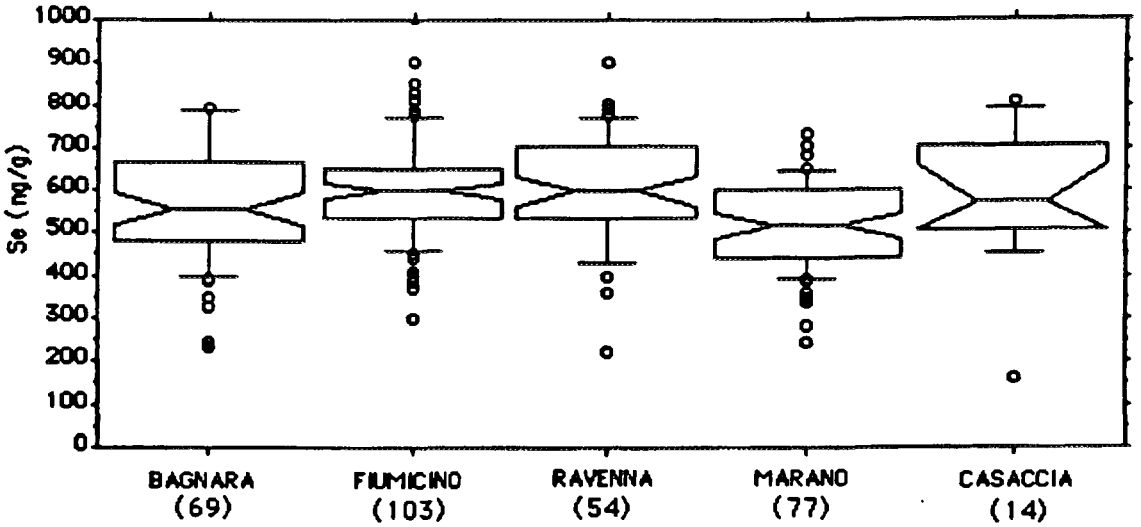

Figure 5. **Se concentration in hair of subjects by area.**

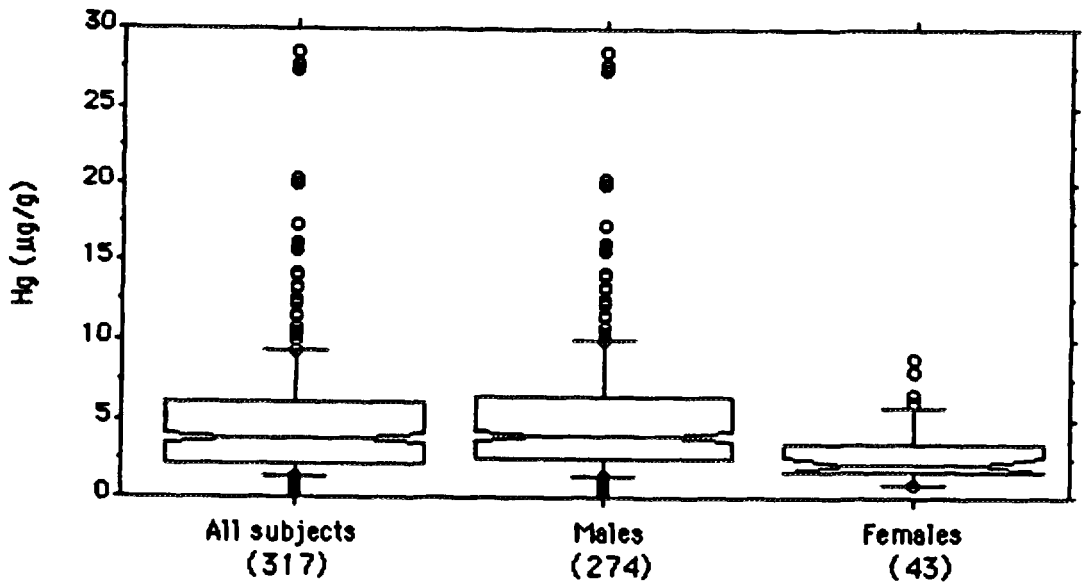

Figure 6. **Hg concentration in hair of subjects from all selected areas.**

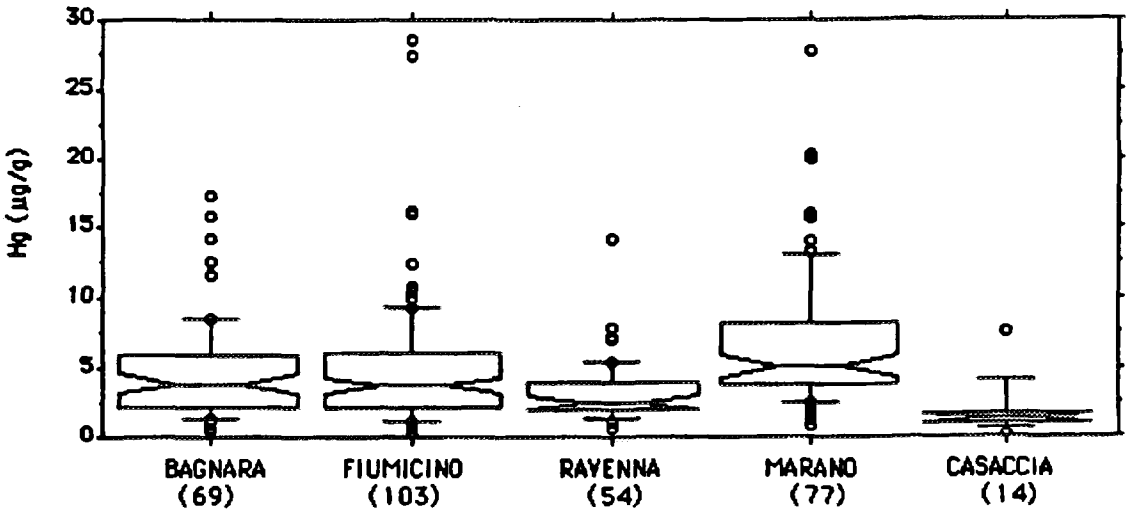

Figure 7. **Hg concentration in hair of subjects by area.**

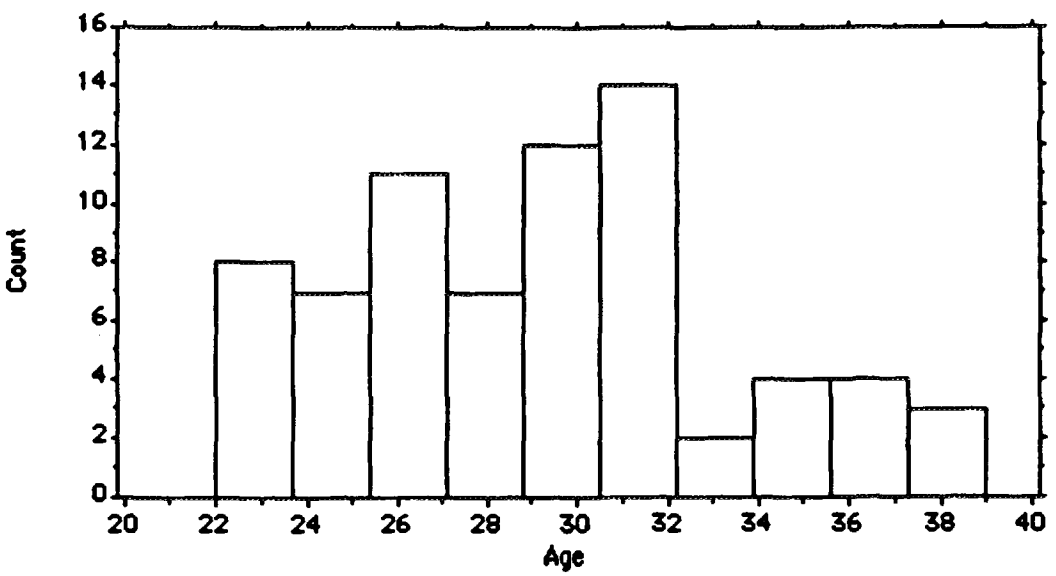

Figure 8. **Age frequency distribution of pregnant women.**

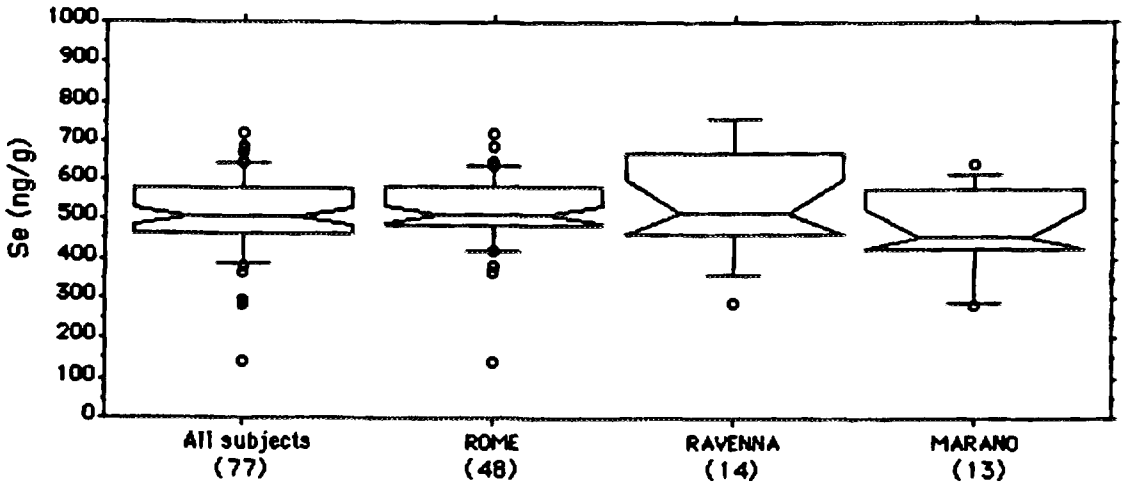

Figure 9. **Se concentration in hair of pregnant women.**

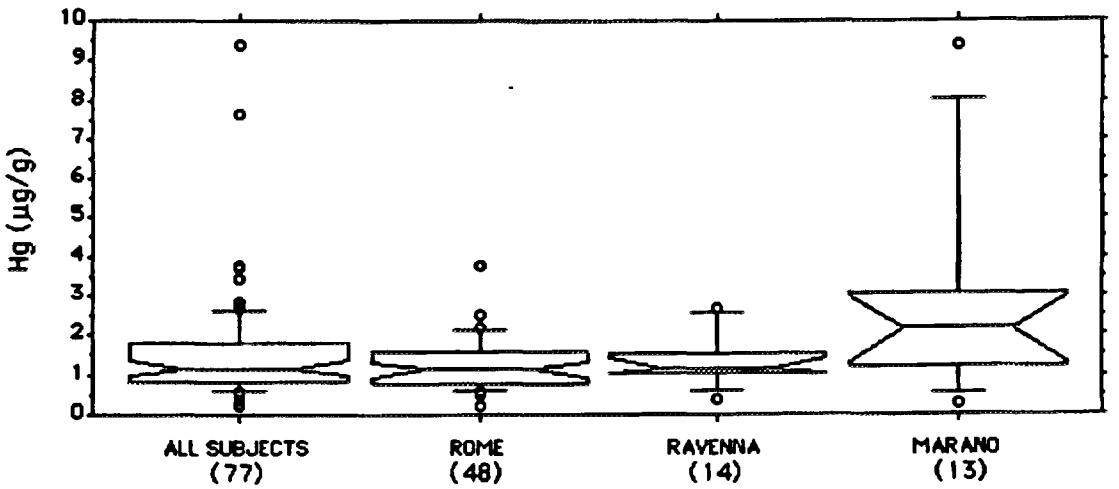

Figure 10. **Hg concentration in hair of pregnant women.**

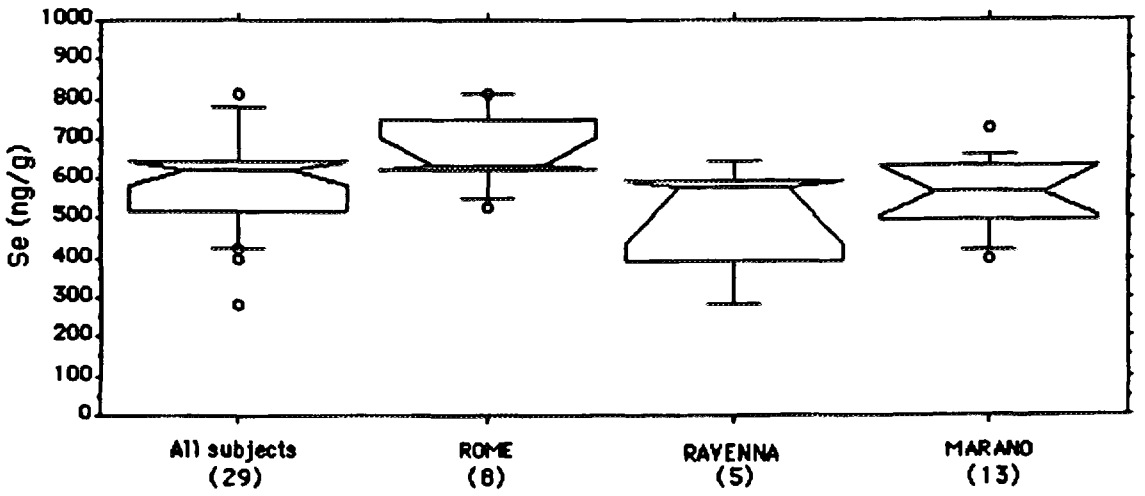

Figure 11. **Se concentration in pubic hair of pregnant women.**

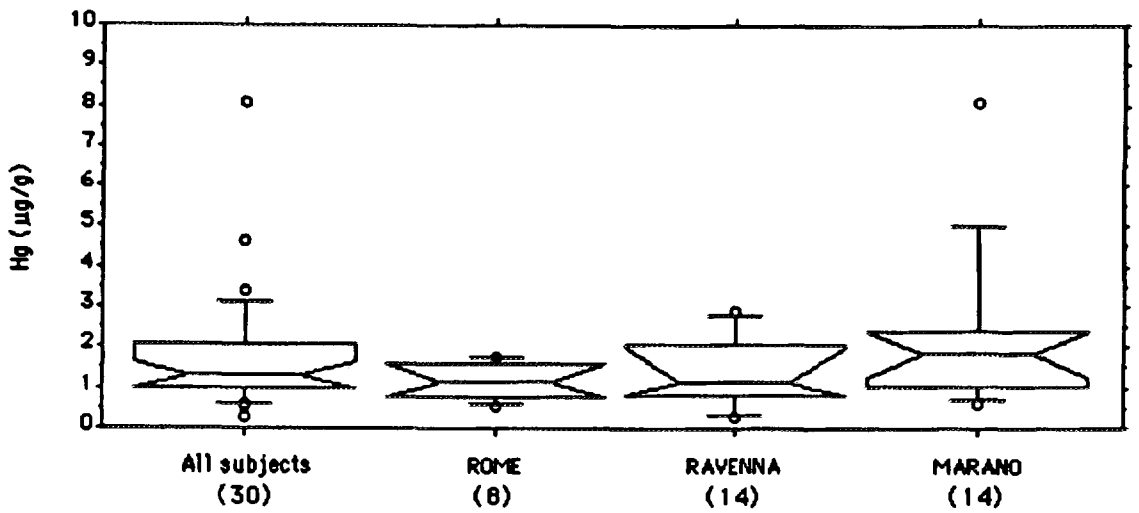

Figure 12. **Hg concentration in pubic hair of pregnant women.**

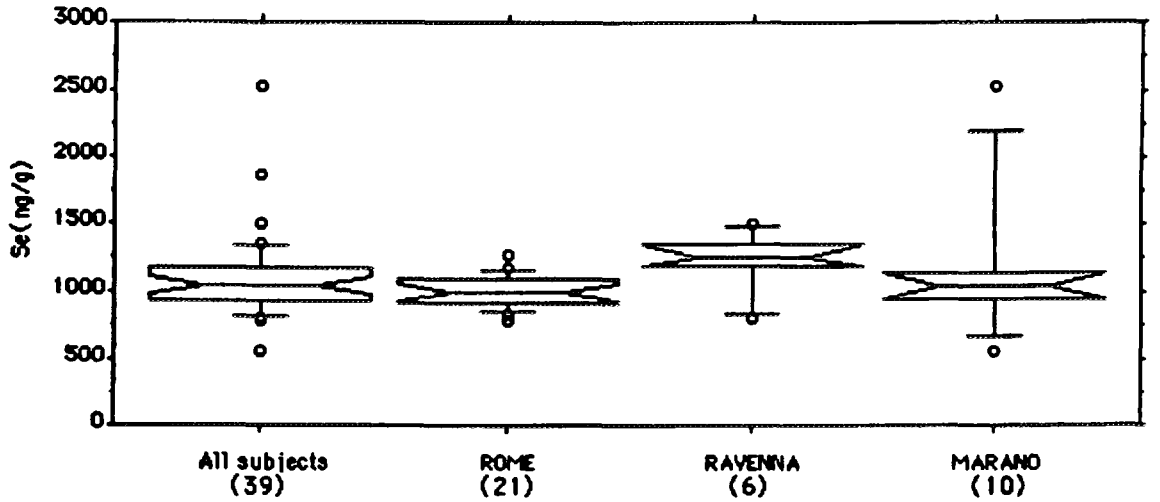

Figure 13. **Se concentration in hair of newborn.**

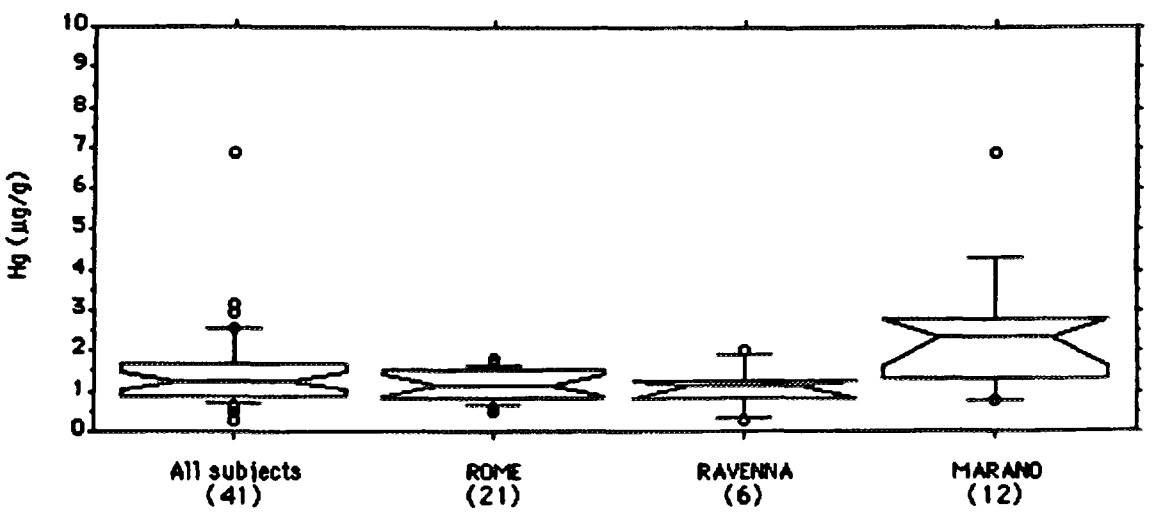

Figure 14. **Hg concentration in hair of newborn.**

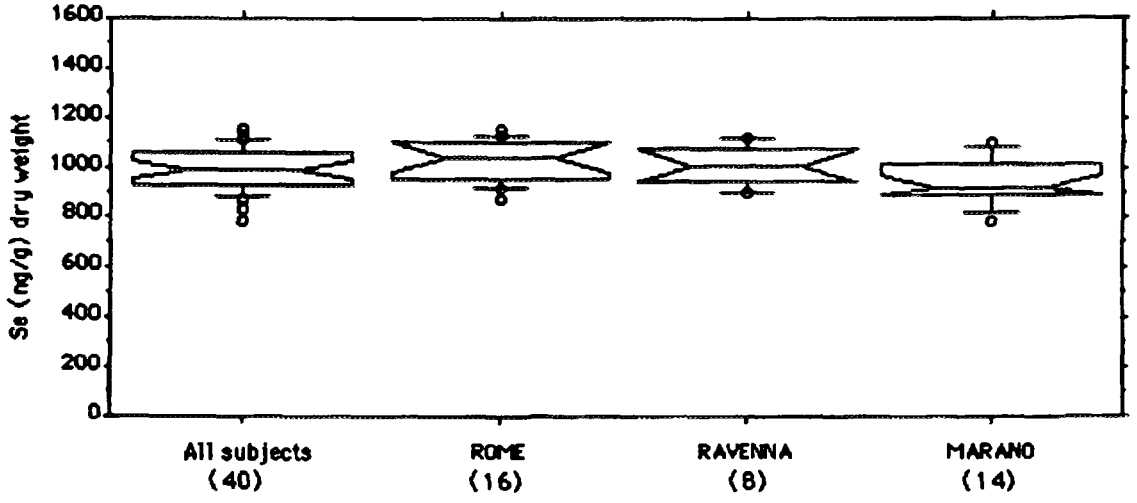

Figure 15. **Se concentration in freeze dried placenta.**

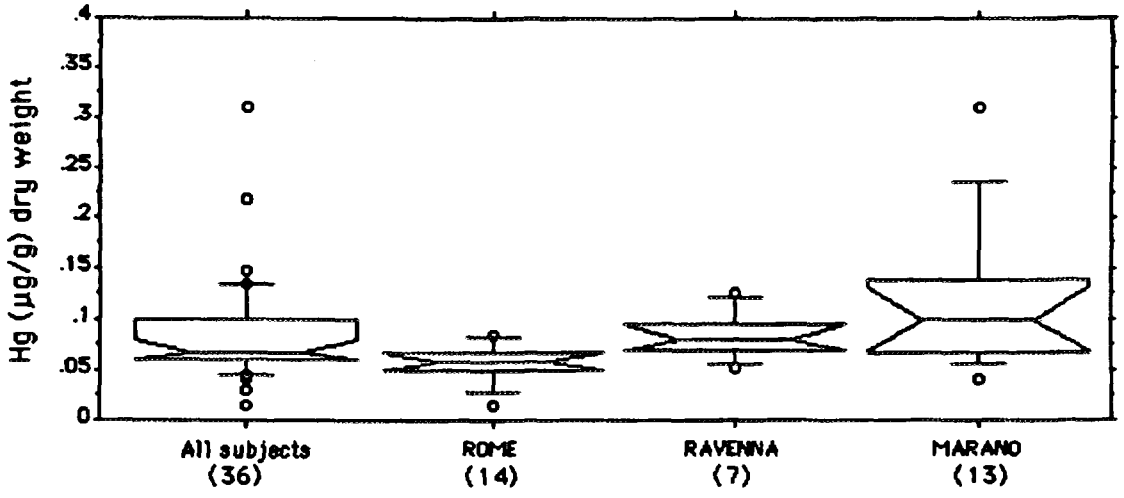

Figure 16. **Hg concentration in freeze dried placenta.**