MERCURY LEVELS IN DEFINED ITALIAN POPULATION GROUPS

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1. INTRODUCTION

The consumption of fish and seafood usually is the main source of intake of methylmercury for members of the general population. Therefore subjects having a diet rich in these food items present a high risk of exceeding the tolerable recommended weekly intake of mercury set by FAO and WHO. The average consumption of fish at a national level is rather small in Italy, 12.5 kg per year consequently the risk of exposure to elevated levels of mercury through the diet for members of the Italian general population is rather negligible. However, fish is one of the main components of the diet of some population groups. These groups are usually found in coastal towns close to fishing ports and include subjects working as fishermen, fish dealers, restaurant workers and their families. The purpose of this research programme, carried out with the collaboration of the National Institute of Nutrition is to determine the levels of mercury and methylmercury in hair samples of subjects having a higher than average fish consumption and to evaluate the effects of elevated intakes of mercury.

2. MATERIALS AND METHODS

The towns selected to carry out this study are Bagnara Calabra, Fiumicino and Ravenna, in Southern, Central and Northern Italy respectively. All these towns are located along the coast and have active fishing ports. To facilitate the selection of subjects willing to participate in this study it was decided to request the collaboration of local health institutions in each town. This is particularly important in getting the participation of pregnant women to the study. A collaboration with the health institutions assisting pregnant women will allow the selection of subjects that are followed and controlled until the delivery. The first few months of the programme were devoted to the implementation of collaborations with local health institutions. The response was generally very good,

allowing the organization of a few field trips to the three towns in the second semester of last year and the first semester of 1992 to collect hair samples from the selected subjects. A form containing all the relevant information was filled out for each subject. The hair samples were collected in a standardized way from the back of the head, cutting roughly the first 2 cm of hair closer to the scalp. The amount of the sample collected was usually in the range 0.2 - 0.3 g but in some cases, when the hair was very short or the subject was very concerned about the size of the sample to be cut, the amount taken was less than 0.1 g. Up to now, about 70 hair samples have been collected in Bagnara Calabra, 100 in Fiumicino and 60 in Ravenna. Also 25 hair samples have been collected from pregnant women in an obstetrical clinic. Placenta, pubic hair and newborn hair is also collected whenever it is possible at delivery. The measurements that will be carried out include the determination of total mercury and selenium in all samples and of methylmercury in the samples presenting higher levels of total mercury.

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 min in contact with the solvent while being stirred constantly. Blood samples, collected by intravenous puncture using heparinized evacuated blood collection tubes, are separated into plasma and blood cells by centrifugation. Placenta samples and duplicate 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 other trace elements

The instrumental neutron activation analysis is the analytical method used for the determination of total mercury and other trace elements. 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 2.6×10^{12} n·cm^{-2}·s^{-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 gamma spectrometry using high-purity germanium detectors with relative efficiency of about 20% 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 gamma spectra.

2.2.2. Determination of methylmercury

The levels of methylmercury are measured by the gas chromatographic method described by Zelenko and Kosta [1] modified in the extraction phase to obtain the separation of phospholipids. The final benzene extract is injected in a Perkin Elmer Sigma 300 gas-chromatograph equipped with electron capture detector.

3. RESULTS AND DISCUSSION

Figs 1, 2 and 3 show the age frequency distribution for all subjects, male and female subjects, respectively. The median age of all subjects is 39 years and the range is 5 - 76 years. The median age of the selected women is smaller than that for men, 32 and 41 years respectively. Also the number of females included in the study, up to now, is much smaller than that of male subjects. This is because hair samples are generally collected in working places, where men are the majority. Fig. 4 shows the box plots for the mercury concentration measured in hair samples of all subjects, male and female subjects respectively. The median Hg concentration in hair samples of male subjects, 3.8 μg/g, is significantly higher than the median value, 2.3 μg/g, found for female subjects. Also the range of values measured for men, 0.2 - 28.5 μg/g, is much wider compared to the range observed for women, 0.8 - 8.8 μg/g. These results can be explained with the higher fish consumption observed for male subjects. The median amount of fish consumption for men is, in fact, 1000 grams per week, while it is 521 grams for women. The data have been analysed separately for each of the three selected locations, the box plots are presented in Fig. 6. The median Hg concentration observed in hair samples of subjects from Ravenna, 3.0 μg/g, is slightly smaller than the values observed in Fiumicino and Bagnara Calabra, 3.7 and 3.8 μg/g respectively, but due to the small number of samples from Ravenna examined up to now, this difference is not statistically significant.

The data on Se concentration show a trend similar to that observed for Hg. Fig. 5 shows the box plots for Se concentration measured in hair samples of all subjects, male and female subjects respectively. The median Se concentration in hair samples from men, 0.59 μg/g, is higher than the value observed for women, 0.52 μg/g, but the difference is not statistically significant. The results for the three locations are presented in Fig. 7 in the form of box plots. The Se median value observed for Ravenna, 0.53 μg/g, is slightly smaller than the values observed for Fiumicino, 0.58 μg/g, an Bagnara Calabra, 0.56 μg/g. Also in this case the differences are not statistically significant. A few very high Se concentrations, (43.6, 27.7, 8.3, 5.8 μg/g) observed in subjects from Bagnara Calabra can

be explained with the use of special shampoos rich in Se to solve scalp problems.

4. QUALITY CONTROL

A quality control programme is carried out routinely by irradiating at each run standard reference materials (BCR, IAEA, NIST). The data on Hg and Se concentration measured in Human Hair BCR 397, Citrus Leaves NIST 1572, Oyster Tissue NIST 1566a and Copepod Homogenate IAEA MA-A-1 are presented in Figs 8 - 13. Looking at these results we can make the following remarks. When both the concentrations of Hg and Se are high as in standard reference material Human Hair BCR 397 (certified values: Hg = 12.3 ± 0.5 μg/g, Se = 2.00 ± 0.08 μg/g) the results, as shown in Figs 8 and 9, are satisfactory for both elements. If the levels of both Hg and Se are low as in standard reference material Citrus Leaves NIST 1572 (certified value Hg = 0.08 ± 0.02 μg/g, information value Se = 0.025 μg/g) the results for Hg, presented in Fig. 10, are still in good agreement with the certified value. Instead, when the concentration of Se is high and that of Hg is low as in standard reference material Oyster Tissue NIST 1566a certified values: Hg = 0.0642 ± 0.0067 μg/g, Se = 2.21 ± 0.24 μg/g and in Copepod Homogenate IAEA MA-A-1 (certified values: Hg = 0.28 ± 0.01 μg/g, Se = 3.0 ± 0.2) the results for Se, as shown in Figs 11 and 13, are satisfactory, but those for Hg, presented in Fig. 13, are not very good. The poor performance in this last case is determined by the interference of the Se peak at 279.5 KeV with the Hg peak at 279.2 KeV. In fact also a small error in the calculation of the Se contribution to the Hg peak at 279.5 KeV will result in a large error in the determination of the area of this peak.

REFERENCES

[1] ZELENKO, V., KOSTA, L., Talanta 20 (1973) 115

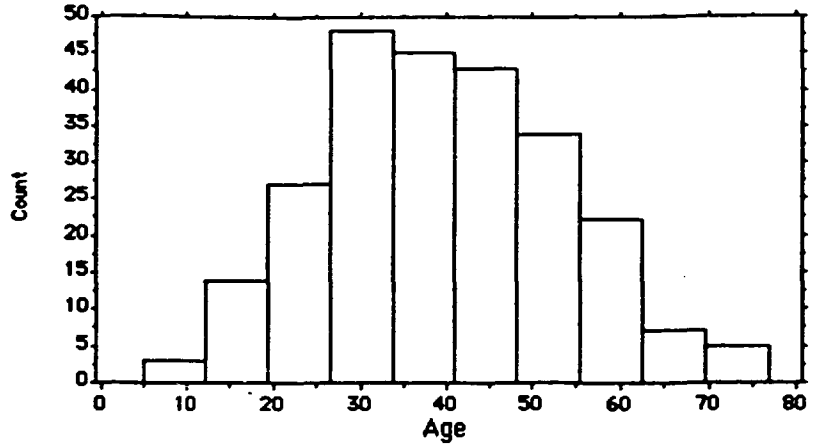

Figure 1. Age frequency distribution for all subjects

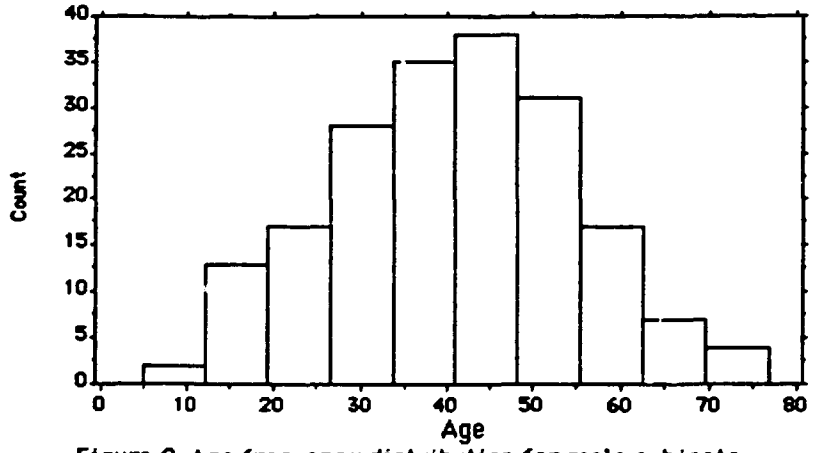

Figure 2. Age frequency distribution for male subjects

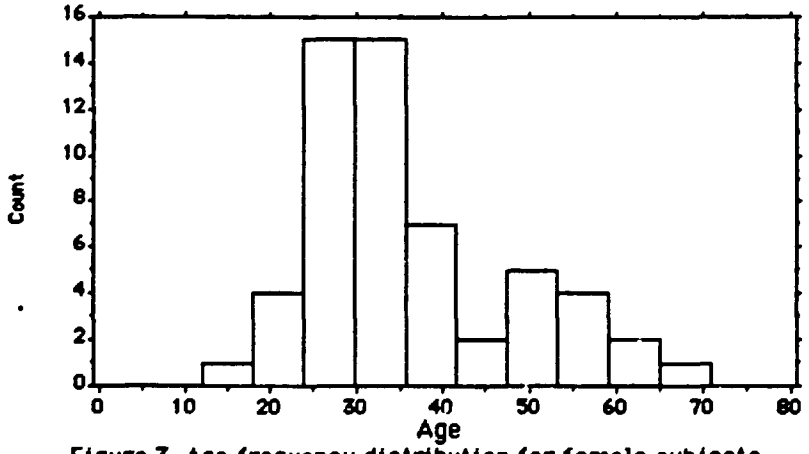

Figure 3. Age frequency distribution for female subjects

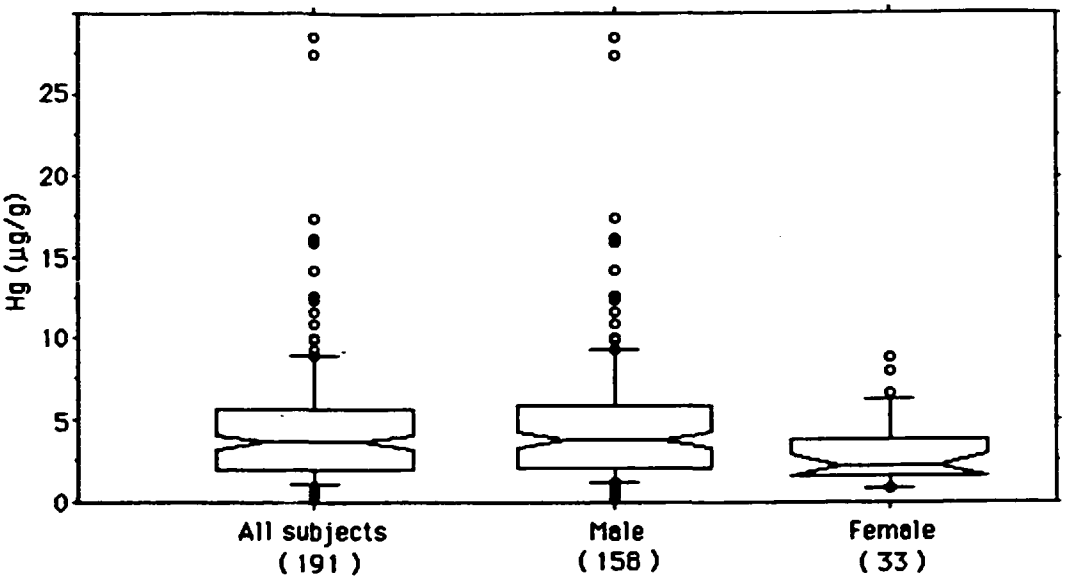

Figure 4. Hg concentration in hair samples of subjects from three selected areas.

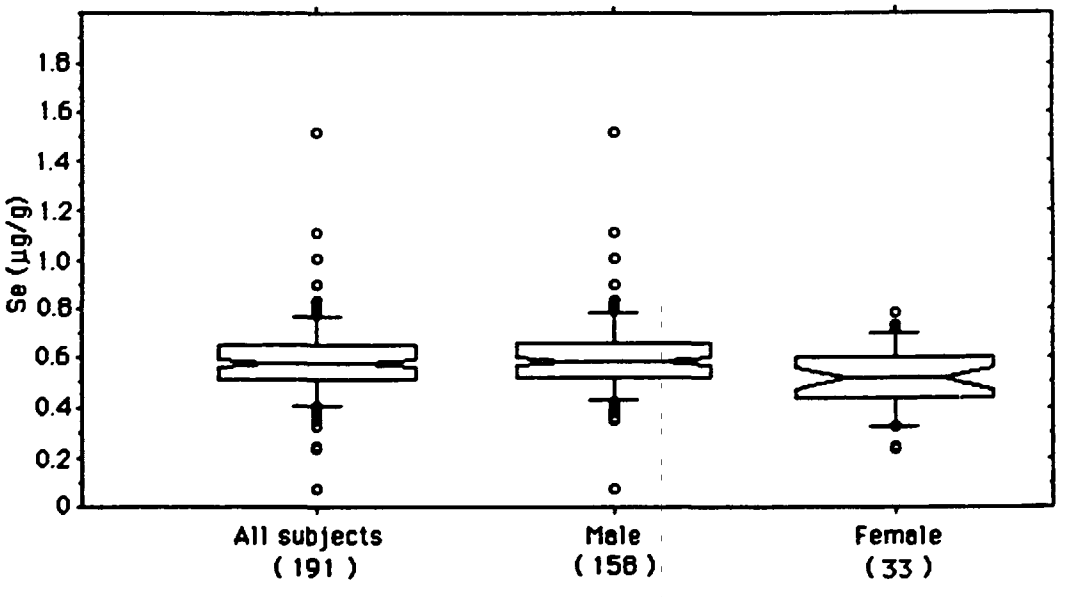

Figure 5. Se concentration in hair samples of subjects from three selected areas.

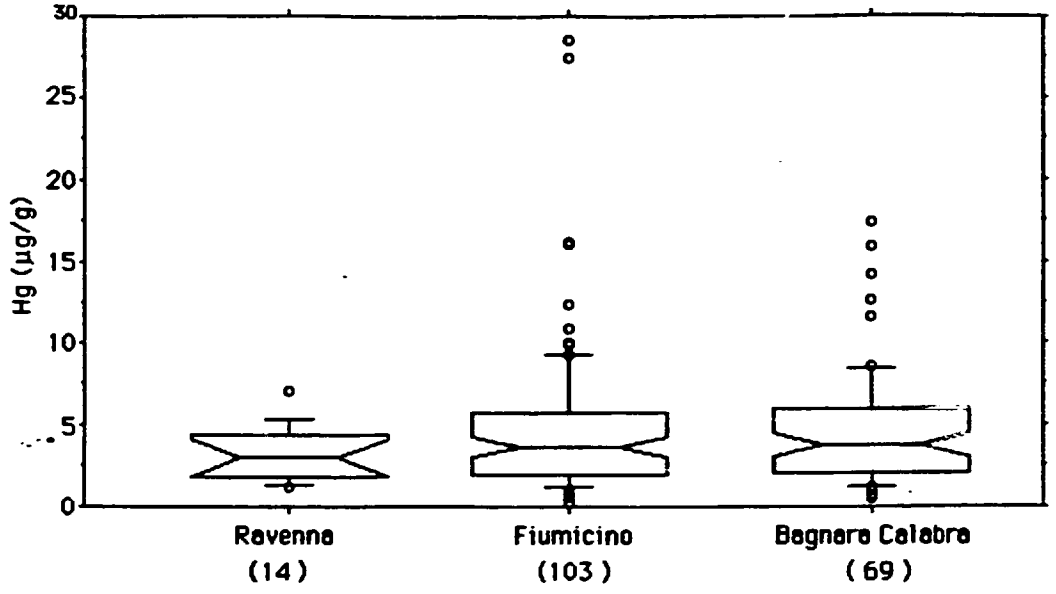

Figure 6. Hg concentration in hair samples of subjects from three selected areas.

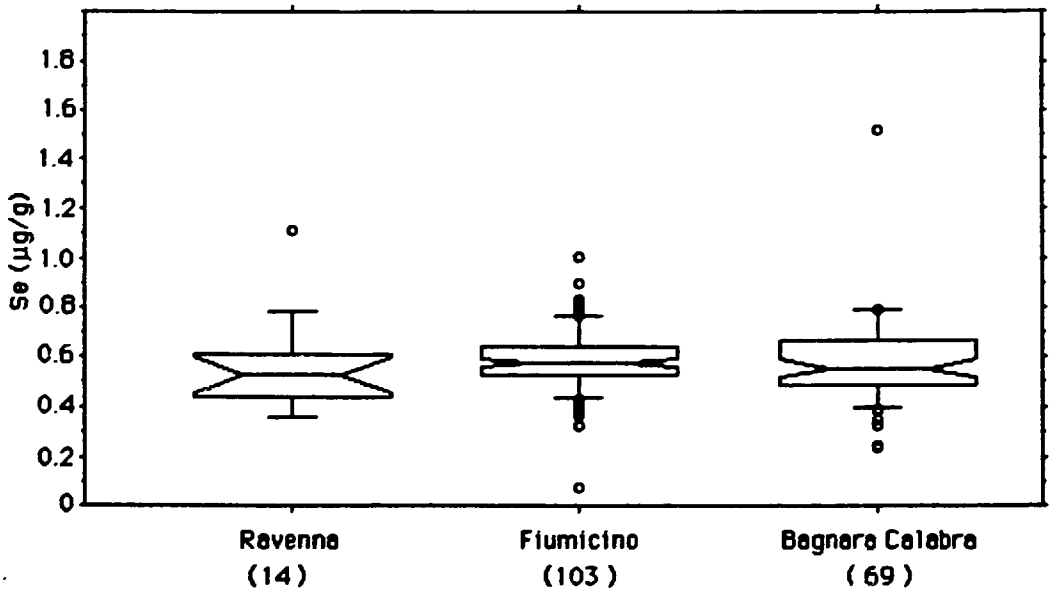

Figure 7. Se concentration in hair samples of subjects from three selected areas.

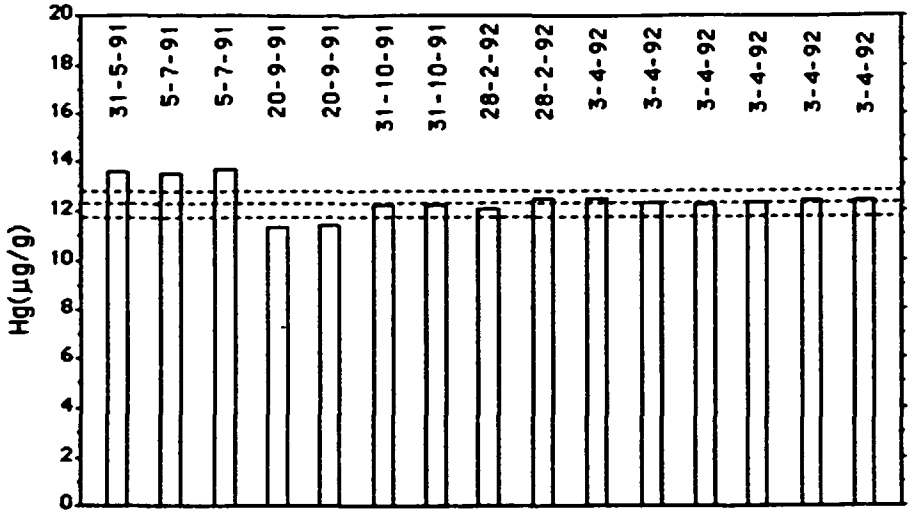

Figure 8. Pattern of Hg concentration measured in Human Hair BCR 397 in various irradiation runs (cerified value 12.3±0.5)

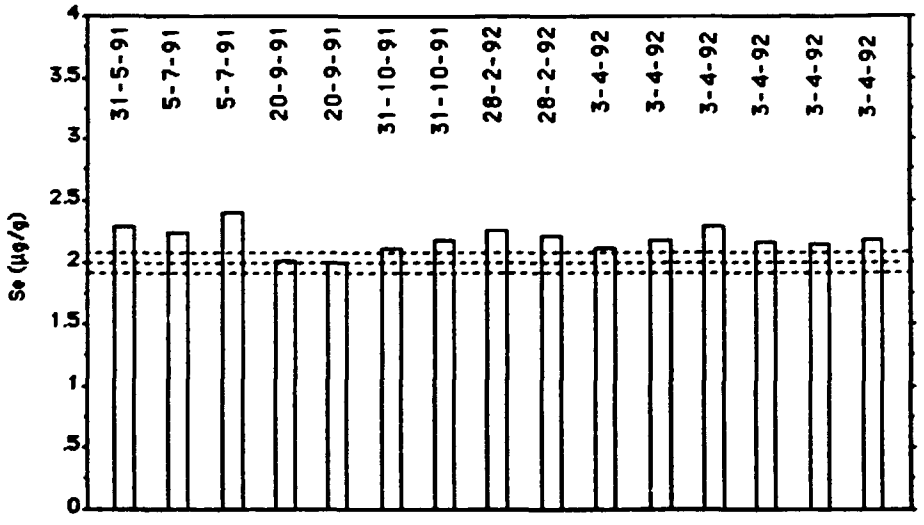

Figure 9. Pattern of Se concentration measured in Human Hair BCR 397 in various irradiation runs (cerified value 2.0±0.08)

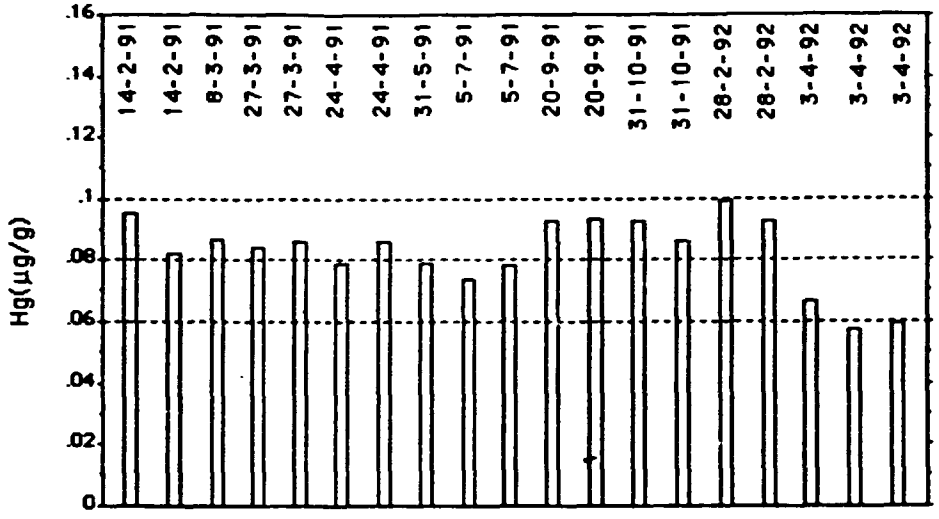

Figure 10. Pattern of Hg concentration measured in Citrus Leaves NIST 1572 in various irradiation runs (certified value 0.08±0.02)

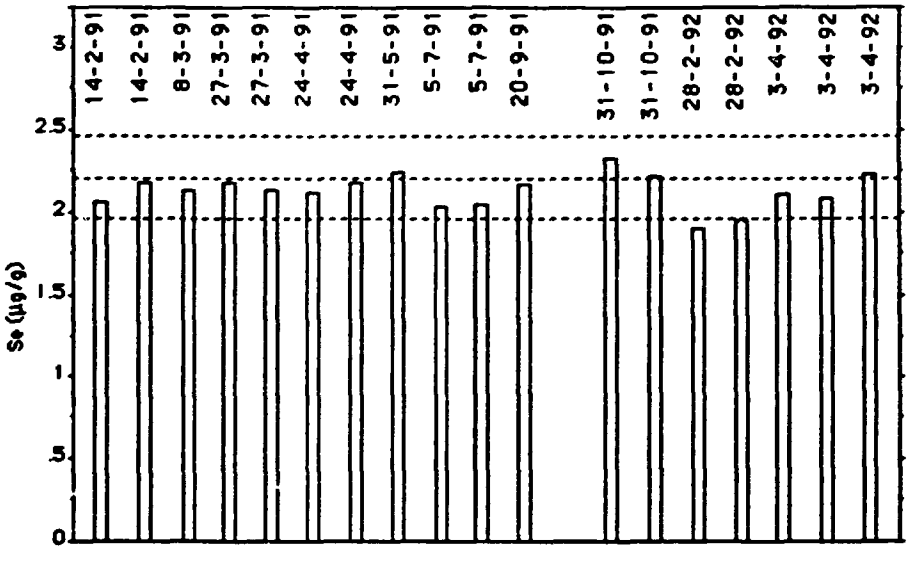

Figure 11. Pattern of Se concentration measured in Oyster Tissue NIST 1566a in various irradiation runs (certified value 2.21±0.24)

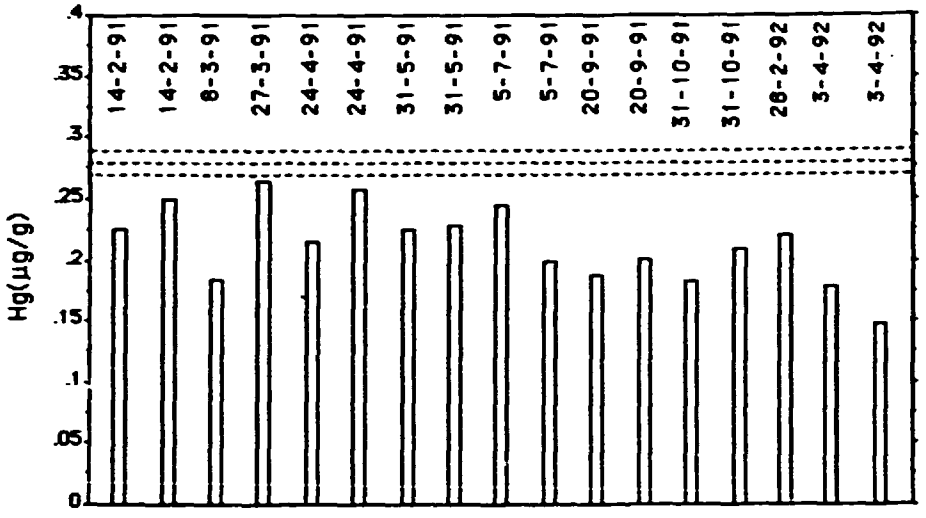

Figure 12. Pattern of Hg concentration measured in Copepod homogenate IAEA MA-A-1 in various irradiation runs (certified value 0.28±0.1)

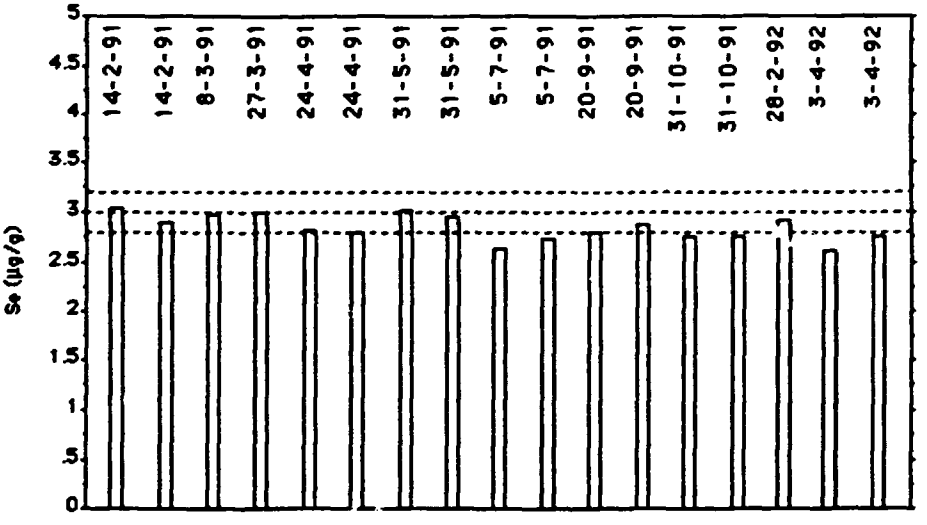

Figure 13. Pattern of Se concentration measured in Copepod homogenate IAEA MA-A-1 in various irradiation runs (certified value 3.0±0.2)