Archives of Environmental Health: An International Journal
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/vzeh20

Mercury in Human Hair

Peter J. Nord PhD [a] , Murali P. Kadaba MS [a] & John R. J. Sorenson PhD [a]
[a] Department of Environmental Health , College of Medicine, University of Cincinnati , Cincinnati
Published online: 02 May 2013.

To cite this article: Peter J. Nord PhD , Murali P. Kadaba MS & John R. J. Sorenson PhD (1973) Mercury in Human Hair, Archives of Environmental Health: An International Journal, 27:1, 40-44, DOI: 10.1080/00039896.1973.10666305

To link to this article: http://dx.doi.org/10.1080/00039896.1973.10666305

Mercury in Human Hair

A Study of the Residents of Los Alamos, NM, and Pasadena, Calif, by Cold Vapor Atomic Absorption Spectrophotometry

Peter J. Nord, PhD; Murali P. Kadaba, MS; John R. J. Sorenson, PhD, Cincinnati

Rapid digestion and quantitative mercury recovery are obtained when hair is digested in a mixture of nitric acid, sulfuric acid, and potassium permanganate. The studies were done on replicate samples drawn from a large homogeneous hair sample used as a standard reference. The method was applied to hair samples from 80 men and 147 women, long-term residents of Los Alamos, NM, and 99 women, long-term residents of Pasadena, Calif. The geometric mean of the mercury concentration found in the hair samples was: Los Alamos, 18.0 μg/gm (men) and 18.9 μg/gm (women); Pasadena, 25.0 μg/gm. Values ranged from 5 μg/gm to over 100 μg/gm. The mean values found are higher than previously reported. The results suggest that there may be differences in the environmental exposure of the two populations to mercury.

In the past few years an intense interest has developed with regard to deposition and distribution of mercury in man's environment. Mercury content of body hair has been used as an indicator of environmental exposure. Scalp hair mercury content has been determined in patients with Minamata disease in Japan.[1] In another Japanese study,[2] which included Americans, Japanese, and Nepalese, a good correlation was found between the mercury content of scalp hair and the amount of fish consumed. In Sweden, mercury content of axillary and chest hair has been used to evaluate exposure in fishing communities.[3] Canadian forensic workers,[4,5] investigating the concentration of 18 metals in scalp hair, determined that the distribution of hair metal concentration is unique enough to permit the identification of an individual in a manner similar to identification by fingerprint analysis. The goal of the present work was to determine the range of scalp hair mercury levels using atomic absorption spectrophotometry and to identify geographical variations associated with industrialization within the United States.

Because mercury content of hair has been used as an indicator of exposure, we investigated the possibility of distinguishing differences in mercury exposure between an industrialized urban population and a nonindustrialized suburban population on the basis of hair mercury content. As part of this study, hair mercury concentration is being determined in eight urban-suburban locations in the United States. Hair samples were obtained by Tepper and Levin in conjunction with their survey of air and population lead levels in selected American communities. In this preliminary report, data on two western communities are presented to demonstrate that, with regard to this toxic nonessential metal, people have higher hair levels in industrial urban areas than in nonindustrial suburban areas.

Accurate determination of mercury, as well as other trace materials, is often very difficult due to losses of the material, which may occur during chemical treatment of the sample. This is particularly troublesome if a volatile compound of the material is formed during such treatment. Losses may be detected by adding a known amount of the metal to several samples of a standard sample. The results of analysis of these samples will show complete recovery of the added metal if there are no losses during the procedure. All careful work involving analytical methods should include recovery studies.

Often in analysis, a convenient precise method is chosen and relative differences between samples and blanks are reported without mention of recovery. Sumino[1] and Yamaguchi et al[2] used colorimetric and cold vapor atomic absorption methods, respectively, to determine total mercury in hair, but did not include an evalua-

Submitted for publication Sept 29, 1972; accepted Feb 19, 1973.

From the Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati.

Reprint requests to the Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati 45219 (Dr. Sorenson).

tion of recovery. Tejning[3] and Perkins and Jervis[4,5] used neutron activation to determine the concentration of mercury in hair. Perkins and Jervis[4] calculated the accuracy of this method to be about 2% to 10% for mercury. They obtained values ranging from 1.1μg/gm to 55.3μg/gm in a small group of individuals. One individual varied from 8.0μg/gm to 53.3μg/gm in the time from 1947 to 1961. Later Perkins and Jervis[5] analyzed 600 Canadian hair samples and found a concentration range of 0μg/gm to 5μg/gm.

We used the cold vapor atomic absorption spectrophotometric adaptation described by Hatch and Ott.[6] In our first efforts, we used several metals for the determination of samples that had been digested by heating with concentrated nitric acid. The result was very poor recovery. However, using a mixture of nitric acid, sulfuric acid, and potassium permanganate, we obtained satisfactory recovery and accuracy. We have demonstrated that this method is particularly useful for hair mercury analysis in that excellent recovery of added metal has been achieved even when the additions are made prior to digestion of the hair.

For development and recovery work one requires a standard sample with homogeneous metal content. Our standard sample was composed of hair from one individual finely cut and blended. This hair sample is referred to as the standard reference hair sample. It was used as the sample in the method development and recovery studies and was analyzed once a day during the routine analysis. Portions of the same standard reference hair sample were used in the work of Sorenson et al in the previous paper (see page 36).

Applying these two digesting methods to the standard reference hair we have shown that the concentration of mercury obtained with the nitric acid method was only 10% to 30% of that obtained with the permanganate method. This method has also been successfully used to determine mercury content of National Bureau of Standards, Standard Reference Material 1571, Orchard Leaves SRM (1571), as a demonstration of accuracy.

Table 1.—Recovery of Mercury Added to Standard Hair Samples*

μg Added	μg ± SE Recovered	% Recovery
0.20	0.209 ± 0.024	104.5 ± 12.3
0.50	0.564 ± 0.055	112.8 ± 11.1
0.70	0.746 ± 0.023	106.5 ± 3.3

*Ten samples were used at the 0.20μg and 0.50μg levels and five samples were used at the 0.70μg level.

Table 2.—Duplicate Determinations of Mercury in Hair

Sample No.	μg Hg/30 mg
C-0511	0.69, 0.87
C-0608	0.27, 0.27
C-0548	1.35, 1.20
C-0505	0.75, 0.81
C-0478	0.27, 0.33
C-0604	0.48, 0.51
C-0642	0.21, 0.21
C-0524	0.75, 0.84
C-0545	0.96, 0.99
C-0676	0.15, 0.18

Methods

Theory.—In the cold-vapor method of mercury determination, the sample is chemically digested. Mercury in the sample solution is reduced to metallic mercury by stannous chloride. A stream of air is passed through the sample solution carrying the volatile metallic mercury to a gas cell. The spectrophotometer passes a beam of ultraviolet light of wavelength 253.65 nm through the cell. The beam is absorbed by the mercury vapor. The absorbance due to mercury in the sample is compared with the absorbance due to mercury in standard solutions of known concentrations. The concentration of mercury in the sample is found by interpolating the sample absorbance with that due to the known solutions. This is known as the method of interpolation.[7]

Sample Collection.—Hair samples were collected during the first half of 1969 from 80 men and 147 women residents of Los Alamos, NM, and 99 women residents of Pasadena, Calif. The individuals had been residents of the same general neighborhood in their respective cities for at least five years prior to sample collection. This residency was required to ensure that the sample collected would reflect only exposure from the test area. Samples were not collected by our interviewer, but were mailed to Kettering Laboratory at the individual's convenience. The average sample weight was about 1 gm.

Hair samples were washed, air-dried, and placed in glassine bags in a cardboard box as standard procedure (see page 36). The standard reference hair sample consisted of 10 gm of hair donated by a woman volunteer from our laboratory. It was finely cut (2 to 3 mm), blended by stirring in water, dried, washed by the standard procedure (see page 36), and stored in a covered acid-washed beaker. The drying procedure supplied by the National Bureau of Standards was followed in the preparation of SRM 1571.

Reagents.—Standard analytical grade reagents and deionized distilled water were used throughout this procedure. A stock solution of 100 ppm of mercury was made from mercuric nitrate. From this a 1 ppm solution was made fresh daily.

Apparatus.—An atomic absorption spectrophotometer was adapted by replacing the burner with a gas cell made of glass tubing 9.5 cm long with an outside diameter of 2.4 cm. Quartz windows cut from old hollow cathode lamps were cemented on each end. Side arms attached 1 cm from the ends of the tube served as entrance and exit for the gaseous sample.

Ambient air was pumped in polyvinyl chloride tubing through a rotameter and needle valve to regulate the flow to 4 ml/sec. The air passed to the reaction vessel, which was a 250-ml widemouth Erlenmeyer flask. The flask was fitted with a rubber stopper containing air inlet and outlet tubes as well as a burette, so that the reducing agent could be added while the system was closed to prevent loss of mercury vapor. The air entered the sample solution through a fritted glass gassing tube. When the stannous chloride was added from the burette, the bubbling air provided adequate mixing of the sample solution and reagents.

Air from the reaction flask carried mercury vapor through a drying tube made from glass tubing 10 cm by 2.5 cm in diameter. The drying tube prevented contamination of the gas cell with water from the reaction vessel. Two drying agents were tried, and it was found that anhydrous magnesium perchlorate was more satisfactory than anhydrous calcium sulfate.

The air containing mercury vapor passed from the drying tube through the plastic tubing to the gas cell where the photometric measurements were made. The exit tube of the gas cell was vented to the hood of the atomic absorption spectrophotometer.

Analytical Procedure.—Hair samples of about 0.03 gm were accurately weighed, placed in 250-ml wide-mouth Erlenmeyer flasks, and treated with 10 ml of concentrated sulfuric acid. One gram of crystal-

line potassium permanganate was added, followed by 15 ml of concentrated nitric acid, which was added directly onto the hair sample with an automatic pipette. Cautious addition of the acid prevents splashing while providing a gentle mixing of the reagents. To avoid loss of hair onto the flask's sides and resultant incomplete digestion, samples were not shaken.

Digestion of the sample was complete in two hours. This digestion was done without heating. It is important to note that mercury was not recovered from samples digested with nitric acid alone, aqua regia, a commercially available toluene solution of a quaternary ammonium hydroxide compound, and an aqueous solution of tetramethylammonium hydroxide. Sanning (oral communication, March 1972) suggests that, in the present method, mercury is not lost as long as the permanganate purple color is maintained in the sample prior to analysis. It is absolutely essential that there be an excess of permanganate added to the digest solution. One gram of SRM 1571 required slightly more time to digest and about 2 gm more of potassium permanganate than the hair samples. After digestion, the samples were diluted to about 125 ml with water.

While the samples were being diluted, the atomic absorption spectrophotometer was warmed up. A flask filled with 125 ml of water was connected to the apparatus. The absorbance due to air bubbling through this flask was used to set the base line absorbance. Excess permanganate in the first sample flask was reduced with 2 ml of 20% hydroxylamine. The sample flask was connected to the apparatus, replacing the flask filled with water. Two milliliters of 20% stannous chloride was added to the sample solution with the burette. The metallic mercury formed from the reduction of the sample by the stannous chloride was swept by the air stream through the drying tube and into the gas cell where the ultraviolet absorption of mercury at the 253.65 nm line was measured.

When maximum absorption at the 1X scale expansion occurred, a flask of water was substituted for the sample. Air bubbling through the water flushed the system, reestablishing base line absorption. Using this procedure, we have been able to conveniently analyze 50 hair samples a day. It was noted that base line drift occurred after a large number of samples. The drift could be minimized if the sample cell was washed with a detergent solution, rinsed with deionized water, and air-dried after every 100 determinations.

Mercury standard solutions were prepared by addition of 0.2, 0.5, and 0.7 ml of

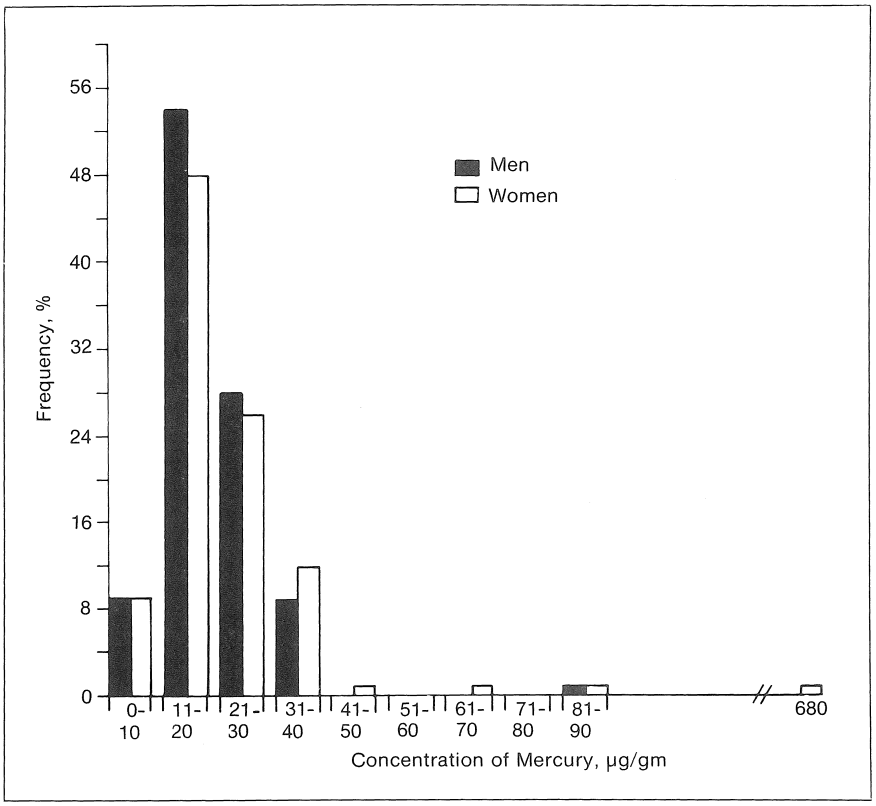
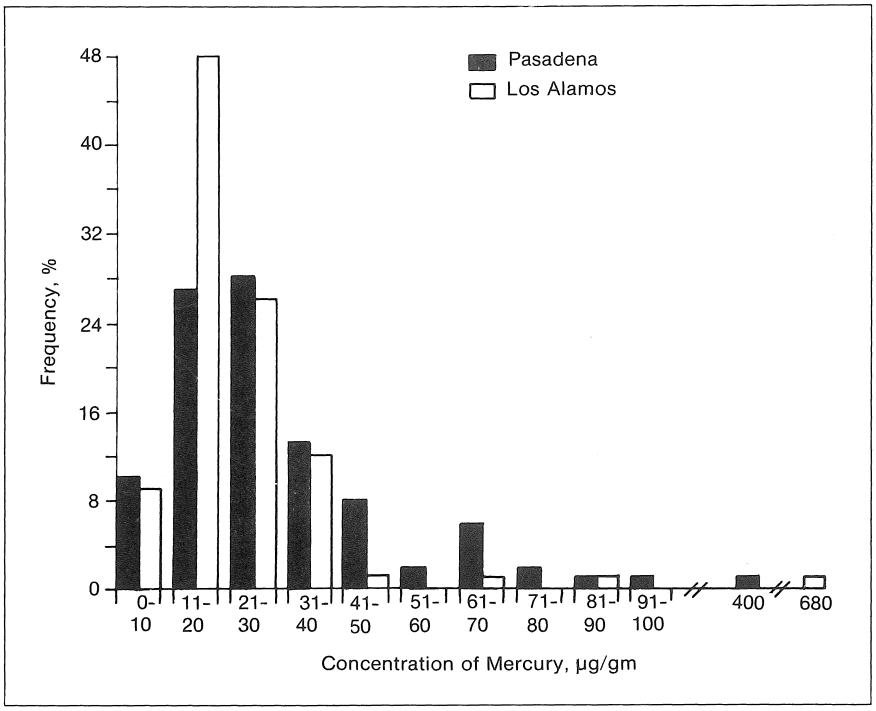


Fig 1.—Hair mercury levels, Los Alamos men and women.

Fig 2.—Hair mercury levels, Los Alamos and Pasadena women.



a 1-ppm mercury standard solution to flasks containing 125 ml of 10% nitric acid. A micropipette was used to make these additions. The plot of absorbance vs concentration for the standard solutions became nonlinear when more than 1μg of mercury was present in the reaction vessel. We found that a 30-mg sample of hair usually gave an absorbance in the linear range of our standard curve. In the event that the absorption was not in the linear range, a sample smaller than 30 mg was analyzed. Reagent blank values were determined with each set of samples and were subtracted from sample values. However, blank values rarely differed from zero.

Results

To evaluate the precision of this method, recovery of added mercury was studied by adding 0.2, 0.5, and 0.7 ml of 1-ppm mercuric nitrate solution to 30-mg samples of the standard reference hair. It is important to point out that these additions were made prior to digestion. Results of this study are given in Table 1. These data demonstrate the value of this method for mercury analysis. We obtained essentially quantitative recovery of the added mercury. Mercury recovery is very often poor due to losses during the digestion and subsequent chemical treatment of the sample. In our experience with this method, recovery is consistently good.

Additional evidence for the precision of this method has been obtained by repeated analysis of hair samples from different individuals. Ten samples of the standard reference hair were analyzed by the method of additions[9] described above. The concentration obtained with this method was 0.35μg of mercury per 30 mg of hair, with a standard deviation (SD) of 0.06μg. As anticipated, analysis by the interpolation method[10] gives the same value. Duplicate analysis, with use of the interpolation method, of hair samples from different individuals in our study have provided the data presented in Table 2. These are samples selected at random and subjected to the digestion and analysis procedure on different days. A SD for the duplicate analyses was computed, using the method given by Kaiser.[9] The SD of the double analyses listed in Table 2 was 0.06μg. This clearly indicates the precision of hair mercury determination, and suggests that the hair clippings from an individual are relatively homogeneous with regard to metal content.

To evaluate the accuracy of our method, duplicate 1-gm samples of the SRM 1571 were analyzed by the same procedure. The mercury concentration in each was determined to be 0.13μg/gm of sample. The SRM 1571 carried a provisionally certified mercury concentration of 0.155μg/gm of sample.

To further substantiate our accuracy, we have evaluated possible interferences associated with this spectrophotometric method of analysis. It has been clearly established that light scatter and nonspecific molecular absorption interferences are absent. Background absorption due to these two types of interferences were evaluated by sample irradiation with a silicon lamp, which produces a wavelength of 250 nm, in place of the mercury lamp, which produces a wavelength of 254 nm. No absorption of the shorter wavelength was observed. There was also no difference in absorption when an instrument equipped with a continuous-source compensation system[7] was used to measure concentration. Finally, the lack of chemical interferences was indicated by the excellent recovery of mercury in our study. Further evidence that the absorption measure in each sample was due solely to the presence of mercury in the cell was obtained in the following manner: With the mercury lamp in place and air bubbling through a sample, no absorption was recorded until the stannous chloride was added.

Having established that this is an accurate method, we then undertook the analysis of hair samples from Los Alamos and Pasadena. The objective of this work was to evaluate the possibility that the industrialized Pasadena area could be distinguished from the nonindustrialized Los Alamos area based upon the hair mercury content. The initial assumption was that the residents of nonindustrial areas would have a relatively lower exposure to mercury than those of industrial areas. If this assumption was correct, then mercury hair concentration in the Los Alamos residents would be less than that of the Pasadena residents.

Populations sampled in this study were derived from women volunteers in Pasadena as well as men and women volunteers in Los Alamos. It was expected that the hair mercury concentration would be representative of the total intake from air, food, and water. Husbands of 80 women participants in Los Alamos were sampled to evaluate a possible sex difference. Most of these men were employed by the Los Alamos Scientific Laboratory. The ambient air of the Los Alamos Scientific Laboratory probably did not vary from that of the suburban community of Los Alamos. It was also assumed that both husband and wife in this group consumed food and water from the same source and in general had the same exposure to mercury.

The data presented in Fig 1 compare the concentration of mercury found in hair with frequency from Los Alamos men and women. Frequency is the fraction of the samples occurring in the concentration ranges indicated, expressed as percent. The average hair mercury concentration for the 80 men sampled was 20.1μg/gm while that of the 146 women was 20.8μg/gm. The geometric mean for the men was 18.0μg/gm and 18.9μg/gm for the women. A sample containing 680μg/gm from an individual in the group of Los Alamos women was excluded from the calculation of the arithmetic mean but was included in the geometric mean. There were no samples of less than 5μg/gm obtained from either city. The frequency distribution is similar in all concentration ranges for both the men and women of the Los Alamos sample. The occurrence of more samples with greater than 40μg/gm found for the women probably is related to the larger number of women sampled. The conclusion is that these men and women cannot be distinguished on the basis of their hair mercury content.

Figure 2 is the histogram obtained when hair mercury concentrations found in women from Los Alamos are compared with the sample population

of 98 Pasadena women. The concentration of 410μg/gm obtained for one of the Pasadena women was discarded when the average hair mercury concentration, 29.6μg/gm was calculated. The geometric mean for all values, including the 410μg/gm value, obtained for the Pasadena women was 25.0μg/gm. This value, compared to those obtained for the other areas studied, was the largest. Comparison of the average hair mercury concentration obtained for the women of Los Alamos, 20.8μg/gm with that obtained for the women of Pasadena, 29.6μg/gm strongly suggests that there is a large difference with regard to exposure in these two communities. Closer inspection of the histogram in Fig 2 reveals that there are interesting differences in frequency at the various concentration levels. The comparison of 0μg/gm to 10μg/gm, 21μg/gm to 30μg/gm, and 31μg/gm to 40μg/gm illustrates an absence of any differences in frequency in these ranges. That is, these two populations do not differ with regard to percent of the population having these concentrations of mercury in their hair. More importantly it can be seen that there are a greater number of Los Alamos samples than of Pasadena samples, occurring in the 11μg/gm to 20μg/gm range, 48% vs 27%. In addition 20% of the Pasadena samples contain greater than 40μg/gm while only 3% of the Los Alamos samples occur in this range. In summary, analysis of hair samples from Los Alamos and Pasadena shows that the percent of men and women from Los Alamos in each concentration range is the same, but different from that of Pasadena women. Los Alamos women have been distinguished from Pasadena women with regard to average hair mercury concentration. The Los Alamos women had a higher frequency in the lower concentration range, while Pasadena women had a higher frequency in the higher concentration range. Since hair samples were not collected from Pasadena men, we have limited our interarea comparison to the women populations.

Frequently, a question is raised concerning possible changes in metal content due to direct contact of external agents with hair. Metals in air are usually associated with particulate matter or aerosols. Both particulates and aerosols are unlikely to release metals to binding sites in the hair protein, due to a protective coating of natural oils on the hairshaft, and should be removed easily by washing. Inhalation of particulates and aerosols offers a more likely route for incorporation of atmospheric air metal into hair. There is certainly more risk of exogenous contamination of hair in occupational exposure. However, it would require frequent contact of hair with solutions of high metal content, a most unusual occupational exposure.

Absorption of metals from cosmetic exposure is a possibility. Cosmetic exposure is likely to occur in any area with equal probability and would not bias a survey. However, those who wish to use hair for the assessment of exposure of one individual should be cautioned to sample new growth from the nape of the neck where cosmetic exposure may be minimized.

Comment

The values we have obtained for the concentration of mercury in hair are somewhat larger than that reported by Eyl et al[10] as "normal" (10μg/gm). In addition, some of these values are much larger than those reported by Eyl[11] as the highest total of mercury found to have no symptomatic effects in adults, 96μg/gm to 185μg/gm. A known fatal level of 500μg/gm has been reported by Eyl[12] but we have obtained values of 410μg/gm and 680μg/gm in our selected populations.

Since it is well known that a number of procedures for mercury analysis are associated with losses of this metal, we wonder whether or not the previously reported "record values" found in asymptomatic individuals might be low values. Recovery studies are sometimes not done to aid in the evaluation of the method or are done and not reported.

It is essential that analytical methods requiring sample digestion be evaluated by recovery of the analyte from a standard reference material. This would allow interlaboratory comparison of data, and would permit a more meaningful conclusion with regard to the question(s) being considered.

This investigation was supported by National Institute of Occupational Safety and Health grant OH-00337 and National Institute of Environmental Sciences grants ES-00159 and ES-00042. In addition this work was supported in part by a contract from the National Institute of Occupational Safety and Health, HSM-99-OSH-114 (2), Study of factors affecting removal of dust from the lungs. Dr. L. B. Tepper obtained hair samples and Drs. H. G. Peterint and E. Binfham supplied the equipment.

References

1. Sumino K: Analysis of organic mercury compounds by gas chromatography. *Kobe J Med Sci* 14:131-148, 1968.

2. Yamaguchi S, et al: Relationship between mercury content of hair and amount of fish consumed. *HSMHA Health Rep* 86:904-909, 1971.

3. Tejning S: *Mercury Content of Blood Corpuscles, Blood Plasma, and Hair in Heavy Fish Eaters From Different Areas of Lake Vanern and the Relation Between the Mercury Content of These Tissues and the Mercury Content of Fish and a Suggestion Regarding the International Food and Health Limit Value and Its Use for Fish and Food Products.* Fisheries Research Board of Canada Translation. Series 1362, Commercial Fisheries, Office of Foreign Fisheries (translation), U.S. Department of the Interior, Room 8015, Washington, D.C., 1970.

4. Perkins AK, Jervis RE: Application of radio-activation analysis in forensic investigations. *J Forensic Sci* 7:449-464, 1962.

5. Perkins AK, Jervis RE: Trace elements in human head hair. *J Forensic Sci* 11:50-63, 1966.

6. Hatch WR, Ott WL: Determination of submicrogram quantities of mercury by atomic absorption spectrophotometry. *Anal Chem* 40: 2085-2087, 1968.

7. Kahn HL: A background compensation system for atomic absorption. *Atomic Absorption Newsletter.* 7:40-43, 1968.

8. Slavin W: *Atomic Absorption Spectroscopy.* New York, Interscience Publishers, 1968.

9. Kaiser H: Quantitation in elemental analysis. *Anal Chem* 42:37A, 1970.

10. Eyl TB, Wilcox KR, Jr, Reizen MS: Mercury, fish and human health. *Mich Med* 69:873-880, 1970.

11. Eyl TB: Methyl mercury poisoning in fish and human beings. *Mod Med* 38:135-141, 1970.

12. Eyl TB: Organic-mercury food poisoning. *N Engl J Med* 284:706-709, 1971.