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TRENDS IN MERCURY CONCENTRATIONS IN THE HAIR OF WOMEN OF NOME, ALASKA EVIDENCE OF SEAFOOD CONSUMPTION OR ABIOTIC ABSORPTION?

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Trends in Mercury Concentrations in the Hair of Women of Nome, Alaska Evidence of Seafood Consumption or Abiotic Absorption?

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ABSTRACT

Eighty samples of hair from women of child-bearing age from Nome, Alaska, and seven control samples from women living in Sequim, Washington, were analyzed for mercury concentration by segmental analysis in an effort to determine whether seasonal fluctuations in mercury concentration in the hair samples can be correlated to seasonal seafood consumption. Full-length hair strands were analyzed in 1.1-cm segments representing 1 month's growth using a strong acid digestion and cold vapor atomic fluorescence analysis. It was assumed that the concentration of mercury in each segment is an indicator of the mercury body burden during the month in which the segment emerged from the scalp.

When mercury concentration versus growth month is plotted for each participant, a number of trends are seen. Forty of the hair samples, including one control, are either too short to show any particular trend or have steady concentrations between 0.2 and 4.5 ppm for all segments. Eighteen of the samples show seasonal variability, with five of the controls and one Nome resident showing winter highs while the remainder, all Nome residents, show summer highs. Twenty-six of the samples show an increase in mercury concentration toward the distal end of the strand regardless of month of growth. Fourteen of the 26 distally increasing samples, including 1 control, have a maximum of less than 3 ppm, while the remainder, all Nome residents have maximums as high as 16 ppm. The remaining three samples show a distal increase with a superimposed seasonal variation.

The 12 individuals with maximums over 3 ppm are of interest. These 12 individuals exceed normal levels for people consuming fish 1 to 4 times per month and in some cases 1 to 4 times per week, and some also exceed the commonly accepted levels of concern for fetal effects of mercury poisoning. However, the trend of increasing mercury concentrations toward the distal end of the hair strand regardless of month of emergence, and the documented presence of elevated levels of elemental mercury in the Nome area suggest that these elevated levels may actually be due to external contamination of the hair strands by adsorption and not due to ingestion of contaminated foodstuffs such as seafood.

INTRODUCTION

In the autumn of 1989, 200 samples of human hair from women of child-bearing age residing in Nome, Alaska, were analyzed for total mercury. The mercury analyses were conducted at Battelle/Marine Sciences Laboratory (MSL) as part of a baseline monitoring study undertaken by Minerals Management Service (MMS) during the preparation of an environmental impact statement evaluating the feasibility of off-shore gold dredging leases. There was concern that off-shore dredging could release elemental mercury, which is often associated with gold deposits, to the waters of Norton Sound. This mercury could then be accumulated by marine mammals and fish of the region that are, in turn, consumed by the population of Nome.

The results of the 1989 study[1] prompted MMS to pursue a more thorough investigation of the mercury levels. To that end, 80 full-length hair samples were collected in the autumn of 1990 from 27 participants of the original study, including 10 of 16 with relatively high mercury levels, plus 53 additional heavy users of subsistence foods. The goal of this study was to analyze the full-length hair samples in segments equivalent to 1 month of growth to ascertain whether variations occur in the levels of mercury in the hair as a function of dietary habits such as seasonal consumption of certain forms of marine life.

Samples were collected by personnel from Norton Sound Health Corporation using methods and equipment supplied by MSL. Samples were taken as close to the scalp as possible by a gloved staff member using clean scissors. The sample was carefully bound with tape within 2 cm of the scalp end to maintain the hair in a bundle and placed in labeled polyethylene bags for shipment to MSL. Upon arrival, each sample was carefully removed from the bag and sectioned into 1.1-cm lengths. This length has been determined to be equivalent to 1-month growth on average[2]. Each segment was placed in a labeled, pre-weighed, acid-cleaned glass vial. The portion of the sample that was in contact with the tape was discarded and the amount discarded was recorded. To reduce sample loss from static electricity, samples were wetted with distilled water during segmentation and dried prior to weighing. The number of segments generated from each sample has varied from 2 to 26, recording between 2 and 26 months of mercury exposure.

ANALYTICAL METHODS

The samples were received and logged in at Battelle MSL on November 1, 1991. Prior to beginning analysis of the samples, preliminary experiments were performed to be certain the procedure was appropriate and would work as expected. An experiment to ascertain whether any significant contamination to the hair sample would occur during storage in polyethylene bags indicated an insignificant contamination level of 0.013 ng of mercury. Using samples of the Japanese certified hair standard, NIES-5, a series of digestions were performed to optimize the digestion method and time while still assuring complete digestion. This resulted in the HNO_3/H_2SO_4 digestion at 350°F for 3 hours as described below.

Each 1.1-cm hair segment was place in an acid cleaned, pre-weighed glass scintillation vial. 5.0 ml of a 70% HNO_3/30% H_2SO_4 solution was pipetted into the vial and swirled to mix. An acid-cleaned glass sphere was placed over the mouth of the vial, and the samples were predigested at room temperature for about an hour. Samples were then placed on a hot plate, and brought up to a refluxing boil in small temperature increments. This is to avoid excessive foaming, which is especially common with tissue samples. The samples were refluxed (hot plate temperature about 300°C) for 2-3 hours, or until all organic matter had dissolved, the solution looked almost colorless or light yellow, and the brown gas above the liquid had almost disappeared. The samples were allowed to cool on the hot plate. Each sample was then diluted to the neck of the vial with 1% BrCl, capped and thoroughly mixed prior to analysis. The final volume was determined to be 21.378 ± 0.183 ml for the lot of vials used for this study. Samples were digested in groups of 24 to 30 segments. This usually included two or three segmented samples (depending on the length), a blank, a NIES hair standard, a spiked NIES hair standard, and another tissue standard (usually DORM-1 dogfish muscle). Digestion batches were visually separated from each other using different colored labels so that the appropriate batch blank could be applied during analysis. Individual samples were identified by their participant number, and the segments were identified alphabetically, beginning with "a" at the scalp.

Samples were analyzed by a cold vapor atomic fluorescence technique, based upon the emission of 254 nm radiation by excited Hg° atoms in an inert

gas stream. Mercuric ions in the oxidized sample are reduced to Hg° with $SnCl_2$, and then purged onto gold-coated sand traps as a means of preconcentration and interference removal. Mercury vapor is thermally desorbed to a second "analytical" gold trap, and from that into the fluorescence cell. Fluorescence (peak area) is proportional to the quantity of mercury collected, which is quantified using a standard curve as a function of the quantity of sample purged. Due to the strong oxidation step, followed by dual gold amalgamation, there are no observed interferences with the method[3].

The instrumentation was calibrated daily using a four-point linear regression and a calibration check standard NBS-1641b. The average of the daily calibration checks was 1.51 ± 0.07 μg/ml, which compares very well with the certified value of 1.52 ± 0.04 μg/ml. Two tissue standards (NIES-5 human hair and DORM-1 dogfish muscle) were digested with each set and analyzed several times daily. A spiked NIES-5 hair sample was analyzed for matrix spike recovery as well.

A total of 828 hair segments were analyzed, not including duplicates, control samples, and standards. Seven samples from women of childbearing age living in the Sequim, Washington, area were segmented and analyzed as controls. Two of the samples were split prior to segmentation and analyzed as duplicates: control sample #7 was duplicated at MSL, and sample #62 was duplicated at another laboratory. The mean deviation between mercury concentrations in each segment of the duplicated sample was 6.5% for the sample duplicated in-house and 19% for the sample duplicated at another laboratory.

Initially, a problem was encountered with the mercury values in the tissue standards consistently running 10% to 15% high. This problem was finally resolved when it was discovered that when the sample vials were warmed to dry the samples after segmentation, the labels were actually losing weight as some of the adhesive evaporated. When the vials were reweighed following sample addition, the calculated weight difference was, therefore, too small, resulting in calculated mercury concentrations being too high. Because the weights of the segmented hair samples were very small, this weight difference is significant. This problem was rectified by heating the labeled vials briefly prior to the initial weighing. Because the "blank" vial was always weighed and treated exactly like the samples, its weight difference after

heating was used to correct the concentrations of the samples analyzed prior to identification of the cause of the problem. Once this was done, the tissue standards once again fell into their certified ranges.

Another problem related to the very low sample weights was that a small percentage of the segments at the distal end of the samples (where there were fewer strands than at the scalp) were so light that we were often working near the limits of the balance resulting in a potentially larger margin of error in the sample weight and therefore in the final concentration. Samples exhibiting this problem are flagged on the final graph. Two segments were lost in the course of the study: one caused by a vial rupture during digestion and one because of an apparent weighing error.

Throughout the course of analysis standard reference materials (NIES-5 hair and DORM-1) and matrix spikes of NIES-5 were analyzed. The mean value determined for NIES-5 (certified value = 4.4 ± 0.4 ppm) was 4.51 ± 0.44 (n=81) and the mean value determined for DORM-1 (certified value = 0.798 ± 0.074 ppm) was 0.864 ± 0.091 ppm (n=72). Spike samples of NIES-5 yielded a mean recovery of 96.6 ± 12.7% (n=47).

RESULTS AND DISCUSSION

A summary of minimum, maximum, and average concentrations for each participant, as well as the concentration determined in the previous study when applicable, is presented in Table 1. When two participant numbers are given, the first is for the present study and the second is for the 1989 study. Trend abbreviations are as follows: SVWH = seasonal variability summer high, SVWH = seasonal variability winter high, SSDI = superimposed seasonal and distally increasing, NT = not apparent trend, DIL = distal increase low concentration, and DIH = distal increase high concentration. No statistically significant correlation was found between mercury concentration and chemical hair treatments as indicated in the perm/color column. When mercury concentration is plotted versus growth month the data reveal several interesting trends.

Of the 87 hair samples that were segmented for analysis, (80 Nome residents and 7 controls), 40 exhibit no statistically significant trend, predominantly because the samples were too short (5 segments or fewer). None

of these trendless samples has an average mercury concentration greater than 3 ppm. In the remaining 47 samples, all but 12 samples have maximum concentrations less than 3 ppm. In the low level group (maximum [Hg]<3 ppm) several trends stand out: 1) seasonal increases, 2) distal increases, or 3) some combination thereof. In the 12 higher level samples (maximum [Hg]>3 ppm) only the latter two trends are seen.

Idealized examples of the common trends are presented in Figure 1. Example 1 illustrates the seasonal trends, both summer and winter highs. As illustrated, the samples with summer highs tend to have slightly higher concentrations overall, as well. Example 2 illustrates both large and small distal increases and Example 3 illustrates seasonal trends superimposed on a distal increase and samples that show no trend, predominantly from lack of length.

The most common trend in the "less than 3 ppm" group appears to be seasonal increases with or without a superimposed distal increase. Note that six of the seven control subjects fall into this category; however, the control subjects show winter increases while almost all of the Nome participants show seasonal variation peak during the summer months. None of the controls analyzed has an average concentration over 1 ppm.

In the remaining 12 samples, those with maximums in the 3 to 16 ppm range, all of the participants except participant #3 exhibit a nearly constant concentration for the first 3 to 5 months of emergence followed by a steady increase toward the distal end regardless of month of emergence. Participant #3 showed this general trend, but the values fluctuate somewhat because of low segment weights.

This trend suggests that the participants showing the distal increase (particularly those greater than 3 ppm) are exposed to some source of mercury that results in hair strand uptake by adsorption rather than ingestion. In order for this trend to be related to the ingestion of contaminated foodstuffs, the contamination would have to be continuously decreasing with an onset sometime prior to the sampling and an end coinciding with the sampling (because all of the participants exhibiting this trend had normal levels (<3 ppm) at the scalp). This is highly unlikely. This type of trend would also be expected to some degree from exposure of the hair strand to airborne

contaminants. The longer the hair strand has been exposed to the environment, the greater the degree of external contamination. Most of the hair samples that were long enough to exhibit any trend at all exhibit this distal increase, but the overall Hg concentrations are still at or below normal levels of 1.9 ± 0.9 ppm (derived from an average of 559 samples from 13 industrialized countries from individuals consuming fish 1 to 4 times per month)[4]. However, 12 of the samples exhibit this trend to a greater extent, with distal end concentrations approaching 16 ppm. Because level of concern is generally considered to be 10 ppm[4], it is important to determine whether these high concentrations are truly representative of body burden in these 12 individuals.

An "adsorption factor" was calculated based on the slope of a least-squares regression of concentration versus month of growth (excluding the first four constant level segments) to examine the rate of mercury concentration increase. Assuming the increase can be entirely attributed to external adsorption, the rate of adsorption varies from 0.034 to 0.090 ppm/month (mean = 0.072 ppm/month) for the samples exhibiting superimposed seasonal variation and distal increase. The rate varies from 0.063 ppm/month to 0.223 ppm/month (mean = 0.123 ppm/month) for the distally increasing samples with maximum concentrations less than 3 ppm. Finally, for the samples with maximum concentrations greater than 3 ppm, the rate varies from 0.206 ppm/month to 1.850 ppm/month (mean = 0.583 ppm/month).

There was initial suspicion that the distal increases could be an analytical artifact caused by processing contamination or weighing error in the distal ends of the hair strands. These segments were sometimes up to 50% lighter than segments near the scalp from the same subject because of layered haircuts or breakage. However, examination of the numerous replicate analyses of the 31 digestions of the NIES-5 hair standard varying in weight from 0.0032 to 0.0156 g (a total of 81 analyses) shows that this is not probable (Figure 2). This graph shows the correlation between sample weights of 31 digestions of the NIES hair standard, ranging from 0.003 and 0.0156 g, and the corresponding analytical result. The range of NIES-5 digestion weights bracket the sample weights with the exception of a few that were flagged as somewhat unreliable because of low weights. The graph shows that there is little or no

correlation between sample size and analyzed concentration, except for sample weights less than 0.004 g. However, even the slight correlation seen at weights less than 0.004 g does not account for the order-of-magnitude increases seen in many of the samples.

Similar trends were seen by Dr. Tom Clarkson of the University of Rochester in the hair of infants and their families exposed to diapers containing phenylmercury and by Wilson et al.[5] in a family using a shampoo containing an unusually high concentration of mercury. In these cases, it is probably the first few segments (those most recently emerged from the scalp) that are indicative of the true body burden of the participant. These are the segments which, in this study, exhibited the lowest, most constant concentrations.

CONCLUSIONS

Because 65 of the 80 participants of the study had maximum mercury in hair concentrations of less than 3 ppm, it can be inferred, assuming that these subjects consume quantities of marine life representative of the population of Nome as a whole, that consumption of marine life from Norton Sound does not contribute levels of mercury that are above normal levels of concern. Apparent seasonal variations were seen only in the samples having lower maximum concentrations (<3 ppm). The apparent absorption may be masking seasonal effects in those with higher, probably non-dietary, concentrations. Possible sources of this adsorbed mercury are airborne mercury (such as vapor from latex paints containing mercury as a mildew retardant), water, or sediment tracked into buildings and contributing mercury to the vapor phase. However, the fact that most of the Nome participants who showed seasonal variations had peak levels in the summer may indicate that the contamination may have an outdoor source. It is known that, in the early part of this century, when mercury was heavily used in the gold ore purification process, large amounts of elemental mercury were released to the environment in the vicinity of Nome and soil levels in the range of 350 to 1000 ppm have been measured within the city limits[2]. The relatively high mercury concentrations measured in the hair of the individuals exhibiting this trend may be indicative of this rather large source of mercury contamination in the Nome area.

If these data are to be used as a reliable indicator for exposure assessment, it is important to determine whether the steady distal increase seen in many of the samples is indeed due to abiotic adsorption from the environment. If that is the case, there are implications with respect to the interpretation of this and other mercury in hair data, both segmental and total hair. If adsorption is taking place, it would be in the form of elemental mercury, as opposed to methylmercury, which is the predominant species of mercury found in marine mammals and fish. It would be possible to resolve the question of the origin of the mercury increase by analyzing segmented hair from the same subject for both methylmercury and total mercury (methylmercury + elemental mercury). If the increase is an accurate reflection of the mercury ingested by the subject, the trend should be the same for both types of mercury. If the increase is due to abiotic adsorption of airborne elemental mercury, the methylmercury levels should remain relatively constant while the total mercury level increases toward the distal end of the hair. By analyzing hair in this way, the results would be indicative of both body burden as a result of ingestion of organomercury compounds common in fish and marine mammals and abiotically adsorbed mercury.

ACKNOWLEDGEMENT

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Table 1 Tabulation of Data for All 80 Participants of the Study

PARTICIPANT ID	NUMBER OF SEGMENTS	MINIMUM (Hg) PPM	MAXIMUM (Hg) PPM	AVERAGE (Hg) PPM	FACTOR	TREND TYPE	NHB STUDY AVERAGE (Hg) PPM	LENGTH	PERM/COLOR
CONTROL 2	15	0.880	1.572	0.983		SVWH			
CONTROL 4	14	0.427	1.341	0.874		SVWH			
26/67	22	0.727	1.278	1.056		SVWH	0.99		
CONTROL 1	19	0.403	0.726	0.605		SVWH			
CONTROL 6	14	0.249	0.807	0.475		SVWH			P
CONTROL 5	10	0.483	1.272	0.764		SVWH			
17	12	0.531	1.470	0.881		SVSH			P
27/27	13	0.413	1.028	0.537		SVSH	0.58		P
68	12	0.885	1.402	0.828		SVSH			P
64	21	0.712	1.232	0.873		SVSH			
41	22	0.892	1.288	1.061		SVSH			P
44/110	16	0.483	0.880	0.669		SVSH	0.85		P
52	13	0.908	1.613	0.850		SVSH			P
32	12	0.802	1.700	1.186		SVSH			
53	13	0.209	0.360	0.270		SVSH			P
13	12	0.423	1.130	0.637		SVSH			P
80	17	0.516	1.19	0.885		SVSH			
76	20	0.852	1.537	1.098		SVSH			
84	24	0.429	1.342	0.830	0.006	SSO			
25/20	16	1.049	2.09	1.810	0.006	SSO	2.15		P
34	26	0.821	2.859	1.781	0.000	SSO			
57	12	1.185	2.324	1.547		NT			
59	4	2.025	2.123	2.068		NT		SHORT	
65	8	1.176	2.387	1.755		NT			P
56	4	1.984	2.036	2.073		NT		SHORT	P
CONTROL 7DUP	11	0.902	1.149	0.946		NT			
49	4	1.348	1.710	1.532		NT		SHORT	P
48	6	1.457	4.532	2.611		NT			
50	12	1.402	1.802	1.626		NT			
78/83	2	1.381	1.804	1.493		NT	0.54	SHORT	P
CONTROL 7	11	0.430	1.178	0.672		NT			
51	13	1.197	1.844	1.403		NT			
67	3	1.037	1.780	1.460		NT		SHORT	
66	8	2.051	3.113	2.306		NT			
85	7	0.854	1.764	1.186		NT			
74	4	1.028	1.358	1.207		NT		SHORT	P
72	7	1.700	2.883	1.693		NT			P
73	3	0.420	0.784	0.612		NT		SHORT	P
70	5	0.221	0.770	0.455		NT		SHORT	P
62	8	0.836	1.215	1.031		NT			
61	5	0.847	2.823	1.365		NT		SHORT	
60	6	0.864	1.084	0.748		NT			
82 DUP	6	0.480	1.240	0.873		NT			
63	5	1.854	2.284	1.990		NT		SHORT	
75	2	0.329	0.322	0.316		NT		SHORT	P
76	6	0.530	0.895	0.712		NT			P
21	7	0.891	1.306	1.120		NT			
20	3	0.467	0.811	0.646		NT		SHORT	P
22	3	1.520	2.xxx	2.083		NT		SHORT	P
33	3	1.847	3.333	2.364		NT		SHORT	
29	4	0.761	1.296	0.940		NT		SHORT	P
16/166	10	0.252	0.881	0.507		NT	3.80		C
7/165	4	0.853	0.742	0.897		NT	0.39	SHORT	
4/146	6	1.712	1.917	1.783		NT	3.16	SHORT	P
8	2	0.699	0.842	0.720		NT		SHORT	
11	6	0.532	0.896	0.857		NT		SHORT	P
10	5	0.430	0.842	0.573		NT		SHORT	P
46/116	8	0.457	0.814	0.611		NT	0.37		P
38/186	6	0.867	1.581	0.997		NT	0.70		P
40	4	0.727	0.984	0.853		NT		SHORT	
42	17	0.365	0.807	0.578		NT			P
45/85	2	0.715	0.85	0.775		NT	1.38	SHORT	P
39/32	16	0.828	0.824	0.717		NT	0.58		P
69/155	8	0.983	1.224	0.872	0.105	DIL	0.20		P
71/105	9	0.332	1.469	0.842	0.223	DIL	0.66		
77	12	0.254	1.787	0.577	0.154	DIL			P
43	18	0.617	2.525	1.385	0.123	DIL			
CONTROL 3	13	0.184	0.805	0.359	0.074	DIL			P
8/84	16	0.755	2.527	1.425	0.125	DIL	3.82		P
29/70	13	0.709	2.596	1.150	0.187	DIL	1.12		
36	6	0.280	0.783	0.440	0.086	DIL			P
54	10	0.315	0.704	0.417	0.057	DIL			
1	11	1.078	2.744	1.384	0.195	DIL			
47	10	0.800	1.806	1.255	0.146	DIL			
37/183	11	0.388	1.091	0.560	0.084	DIL	0.53		P
23/30	16	0.272	1.105	0.656	0.063	DIL	0.57		
19/194	18	0.362	1.680	0.769	0.094	DIL	2.05		P
2/36	22	1.290	15.194	4.979	0.565	DIH	0.89		
5/152	16	1.335	8.575	2.884	0.445	DIH	3.75		P
3/95	16	2.901	12.743	7.423	0.850	DIH	2.15		P
30	9	0.786	3.286	1.643	0.463	DIH			
24/85	18	1.027	3.896	1.831	0.208	DIH	3.70		P
35	13	0.569	3.153	1.535	0.203	DIH			P
31	15	1.259	4.386	1.944	0.224	DIH			P
18/117	5	0.945	3.283	1.585	0.797	DIH	0.22	SHORT	
12/6	6	1.081	6.198	2.898	1.650	DIH	1.18		
9	11	0.729	7.535	2.853	0.660	DIH			
15/170	16	1.129	8.424	3.450	0.513	DIH	3.01		
[illegible]	[illegible]	0.782	[illegible]	[illegible]	0.312	DIH	0.85		P

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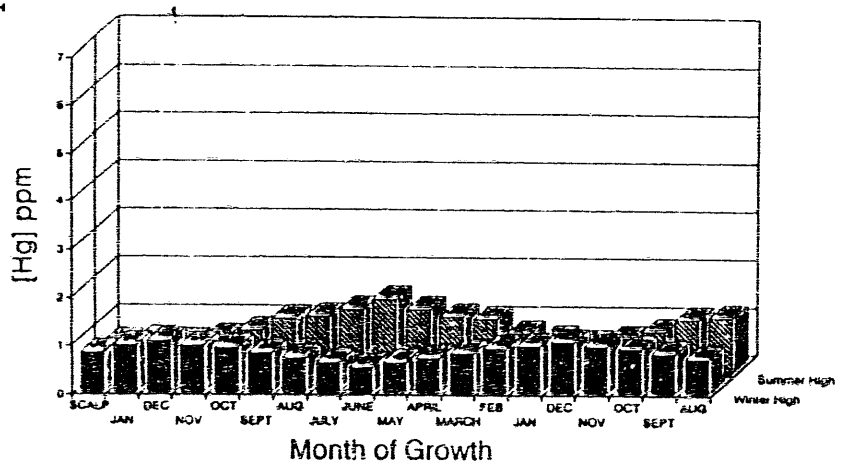
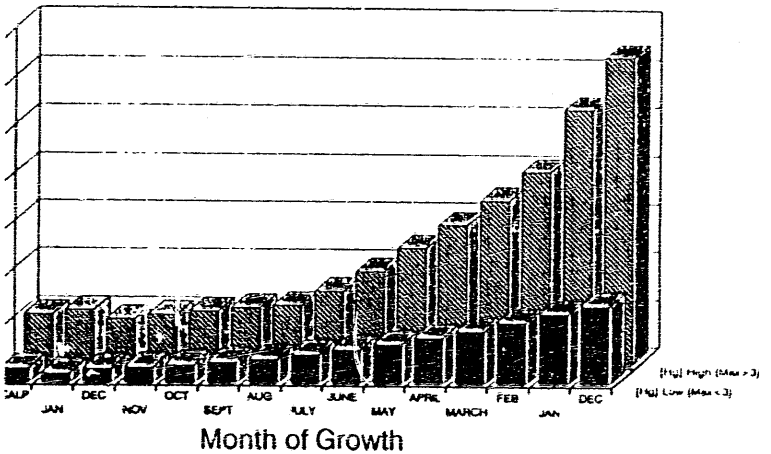
Example 1 Seasonal Variability

Summer Highs and Winter Highs



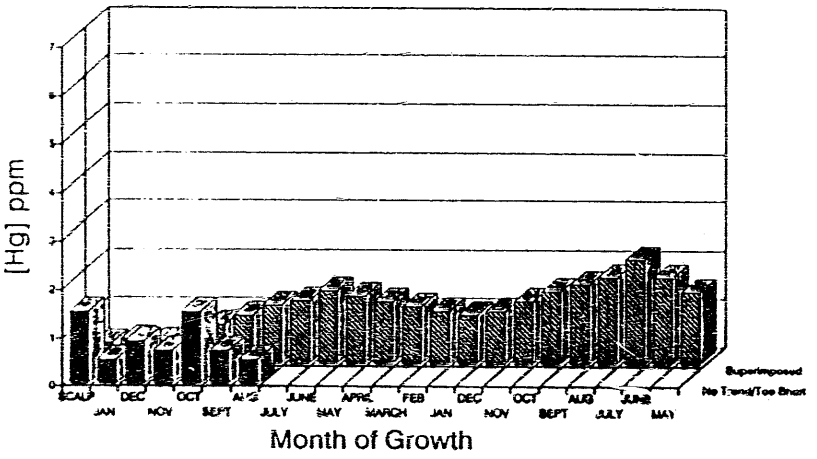
Example 2 Distal Increases

Overall [Hg] High vs Low



Example 3 - Other Trends

Superimposed Seasonal and No Trend



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Measured Hg Concentration vs Sample Wt.

Standard NIES-5 [Hg]=4.4 microgram/gram

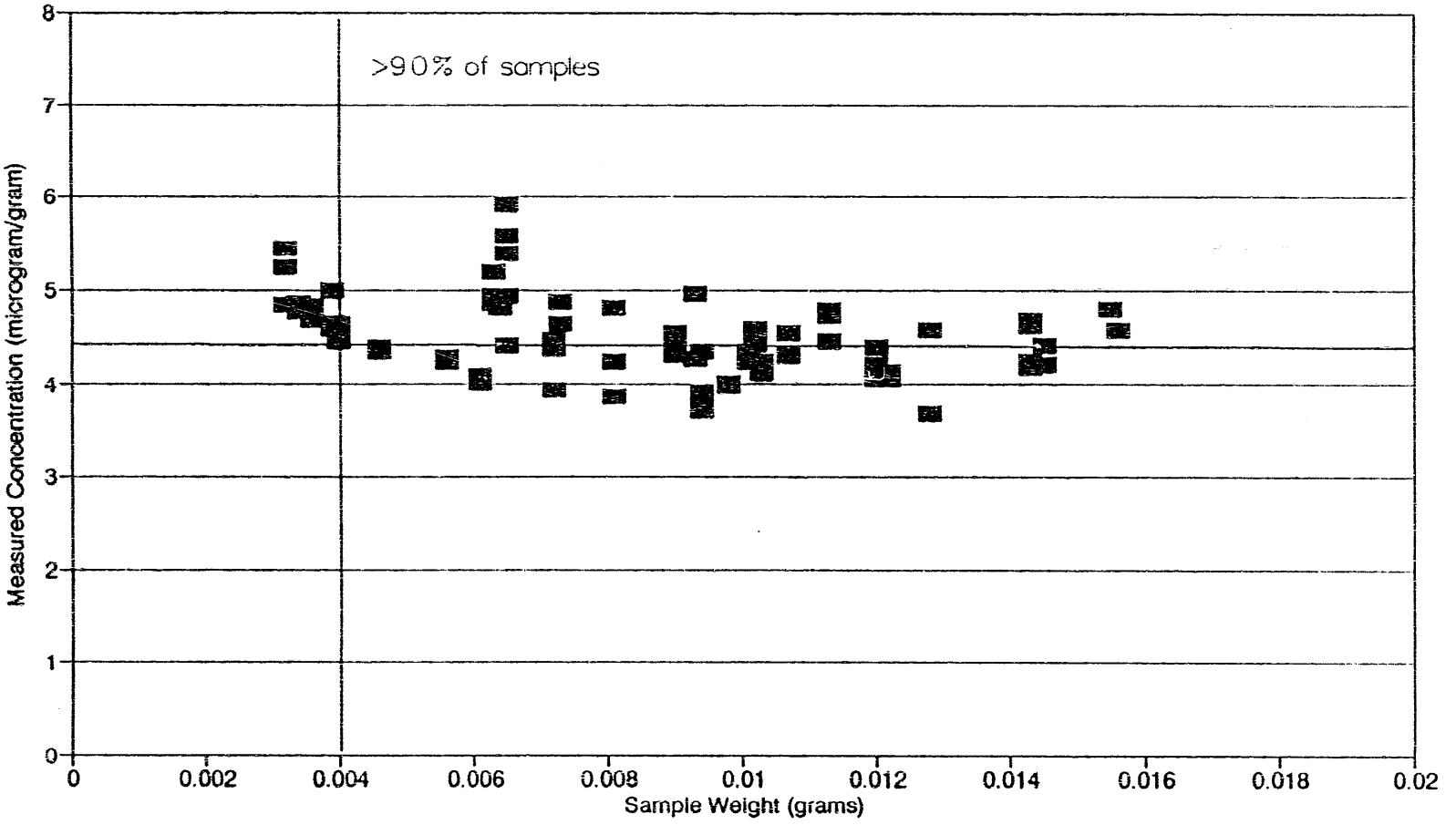

Figure Captions

Figure 1 Idealized examples of the trends common in the segmented hair samples.

Figure 2 Correlation of sample weight versus measured mercury concentration.

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