

Lead in Human Hair: Relation to Age, Sex and Environmental Factors

by R. D. REEVES, K. W. JOLLEY, and P. D. BUCKLEY
*Department of Chemistry, Biochemistry and Biophysics
Massey University
Palmerston North, New Zealand*

Several recent studies have dealt with various aspects of the lead content of human hair, both in 'normal' individuals and in those showing various symptoms of lead poisoning. KOPITO *et al.* (1967) found that the hair of a presumably normal control group of 41 children under the age of eight years contained 2-95 $\mu\text{g Pb/g hair}$, with a mean of 24 $\mu\text{g/g}$. Significantly higher lead levels (42-975 $\mu\text{g/g}$) were found in 16 children with chronic lead poisoning. From subjects in the latter group, analyses were carried out on hair segments proximal to the scalp and on distal portions. A much higher lead level in the proximal segment was taken as evidence of abnormal lead intake during a period of several weeks prior to sampling.

SCHROEDER and NASON (1969) reported lead levels in the hair of 125 people representing a sample of normal individuals. No significant difference between levels in male and female subjects were found. Mean values of lead in male hair showed no significant dependence on the age-group of the subject, but a significant decrease was found in the mean lead content of the hair of female subjects, from 24.5 $\mu\text{g/g}$ in the 1-30 year age-group to 8.4 $\mu\text{g/g}$ in the 40-70 year age-group. These findings differ from those of SHABEL'NIK (1968) who reported that female subjects had higher hair lead levels than male subjects, and that the lead content increased with age.

The investigation of WEISS *et al.* (1972) showed that the lead levels in the hair of present-day subjects were considerably lower than those of 50-100 years ago. This was attributed to an overall decrease in general exposure to lead from a variety of sources. It was also indicated that adult hair contained significantly less lead than that of children, implying an age-related decrease in the ability of hair to take up lead from the body.

HAMMER *et al.* (1971) eliminated possible effects of age and sex, focusing on the differences arising from different degrees of environmental exposure. The hair of 4th-grade boys in five cities representing a range of exposure levels (as indicated, for example, by average airborne lead data) showed a strong correlation between hair lead and environmental lead.

In the present work lead analyses have been carried out on hair from 250 subjects, male and female, with ages ranging from 1 to 87 years. The sample represented as closely as

possible a cross-section of the population of a community of about 60,000 people and its neighboring rural areas. The wide range of occupations included those related to education, farming and farm trading, general commerce and light industry. The sample was large enough that many statistically-large subgroups could be derived from it. Analysis of the data was made in terms of age, sex and occupational differences.

ANALYTICAL METHODS

Hair samples from the sides and back of the head, generally representing a distal portion of the hair about 3 cm in length, were collected in plastic bags for washing and analysis. Dyed, tinted or bleached hair was not included. Little attention was paid to hair color, as more than 80% of the samples showed a continuous gradation from fair through light brown to dark brown; more distinctive color groups (red, black, gray, blond) were each represented by only a small number of samples.

The washing stage is one of the most critical in the analysis of hair samples, as the procedure used may influence the lead concentrations found. There is no general agreement on the most satisfactory washing procedure. BOWEN (1966) notes that some tissues, including mammalian hair, may continue to lose some ions through an indefinite number of washes. Although lead is not mentioned specifically by Bowen in this context, SCHROEDER and NASON (1969) minimised this possibility by the use of a carbon tetrachloride wash. However, KOPITO et al. (1967) quote claims (BAGCHI et al. 1940; NISHIYAMA 1957) that lead is not removed from hair itself by boiling water, dilute acids, alcohols, soaps or detergents. It is also reported (BATE and DYER 1965) that, as most trace elements are very tightly bound to the follicular proteins, washing with organic solvents and with aqueous non-ionic detergents are equally satisfactory. The work of HARRISON et al. (1969) and WEISS et al. (1972) made use of non-ionic detergent washing.

There is probably a fine difference between surface contamination and surface adsorption. A portion of the lead content of unwashed hair consists of particulate matter from external sources, embedded in lipid material. Although this in itself may be a useful guide to environmental exposure from airborne sources, it is generally of more interest to attempt to determine that part of the lead content which is chemically bound or otherwise strongly adsorbed to the hair protein. The analyses of HAMMER et al. (1971) followed a multiple washing with detergent, distilled water, ethanol, and boiling EDTA solution. Lead appeared in the detergent wash solution, but this may have been classifiable as surface contamination. No further lead was removed by the organic solvent, but lead was extracted by the boiling EDTA solution. This treatment is likely to remove weakly-adsorbed Pb^{2+} ions and probably explains why the lead values found in cities with 'low' environmental exposure were considerably lower than the means reported for other 'normal' samples (KOPITO et al. 1967;

SCHROEDER and NASON 1969; WEISS et al. 1972). A low mean of 9.4 $\mu\text{g/g}$ was also found in a 'normal' control group by EL-DAKHAKHNY and EL-SADIK (1972) who included a washing with hot 1% nitric acid in the sample processing.

In the present work a detergent-washing technique similar to those of HARRISON et al. (1969) and WEISS et al. (1972) was used. This should approximate in severity the treatment given to growing hair in the course of its normal management. Hair samples weighing 2-4 g were cut into 1 cm lengths and washed in polythene bottles with 150 cm^3 of 0.2% detergent solution. The bottles were shaken for 30 minutes with a mechanical shaker. Samples extracted were washed down with 100 cm^3 of deionized water, placed on a Buchner funnel and washed with eight further 100 cm^3 portions of deionized water. During preliminary trials, lead was undetectable ($< 0.05 \text{ mg/l}$) in the detergent solution both before and after the washing procedure.

After drying for 3 hours at 110°C samples of 0.5-2.0 g were weighed into borosilicate glass beakers and dry-ashed in a muffle furnace, the temperature being gradually raised to 490°C over a period of 4 hours, and maintained at this level overnight. No measurable loss of lead occurred during a period of 12-36 hours provided the temperature remained below 500°C . The amount of sample available allowed for duplicate analyses in most cases. Two samples that were incompletely ashed gave lead values 10-30% lower than properly-ashed duplicates; the low values were therefore discarded.

After cooling, the ash dissolved rapidly in 5 cm^3 of 2M HCl, prepared from redistilled (constant-boiling) analytical-grade acid. Analyses were carried out with a Varian AA-5 atomic absorption spectrophotometer, using standard solutions in 2M HCl. Replicate analyses showed the standard deviation of the whole analytical procedure to be approximately 3.8-5% at all lead levels between 2-100 $\mu\text{g Pb/g hair}$.

RESULTS AND DISCUSSION

The hair lead concentration was measured in 254 subjects. In 4 cases (all male) values of 1050, 1160, 2280 and 2410 $\mu\text{g/g}$ were found. For two of these cases, follow-up enquiries were possible and both subjects proved to be users of a proprietary hair preparation containing 1.2% lead acetate. All four of these extremely high values were removed from subsequent consideration and data analysis.

TABLE I
LEAD CONTENT OF HAIR - TOTAL SAMPLE
AND VARIOUS AGE, SEX AND OCCUPATIONAL SUBGROUPS

Sample	N	\bar{x} *	Geometric mean Pb ($\mu\text{g/g}$)	95% Confidence limits of G.M. ($\mu\text{g/g}$)	Range ($\mu\text{g/g}$)
Total	250	1.108 \pm 0.026	12.8	11.4-14.4	2.0-360
Male (M)	133	1.135 \pm 0.039	13.6	11.4-16.3	2.1-360
Female (F)	117	1.079 \pm 0.033	12.0	10.4-13.9	2.0-145
Age 1-10	28	1.114 \pm 0.068	13.0	9.5-17.7	2.5-68.5
Age 1-21	83	1.123 \pm 0.042	13.3	11.0-16.0	2.0-219
Age 22-42	87	1.094 \pm 0.044	12.4	10.2-15.2	2.3-283
Age 43-87	80	1.105 \pm 0.048	12.7	10.3-15.8	2.1-360
Age 1-21(M)	36	1.200 \pm 0.079	15.8	11.1-22.6	2.5-219
Age 1-21(F)	47	1.072 \pm 0.047	11.8	9.6-14.6	2.0-99.5
Age 22-42(M)	51	1.130 \pm 0.060	13.5	10.3-17.7	2.3-283
Age 22-42(F)	36	1.042 \pm 0.064	11.0	8.3-14.7	3.3-86.6
Age 43-87(M)	46	1.089 \pm 0.069	12.3	9.0-16.8	2.1-360
Age 43-87(F)	34	1.126 \pm 0.063	13.4	10.1-17.8	3.1-145
Occup. I**	28	1.516 \pm 0.090	32.8	21.8-49.3	3.4-360
Occup. II**	44	1.018 \pm 0.050	10.4	8.3-13.1	2.5-82.8
Other male**	61	1.045 \pm 0.055	11.1	8.6-14.2	2.1-121

* $x = \log_{10}(\text{Pb concentration}/\mu\text{g g}^{-1})$

** 'Occupation group I' includes males 15-65 who are printers, mechanics, drivers, metalworkers, machinists, and in other similiar occupations.

'Occupation group II' includes males 15-65 who are students, laboratory staff, clerical and other office workers, teachers and various other professions, and businessmen.

'Others' includes all males outside the 15-65 age range, together with those in a variety of occupations - farmers, salesmen, carpenters, policemen.

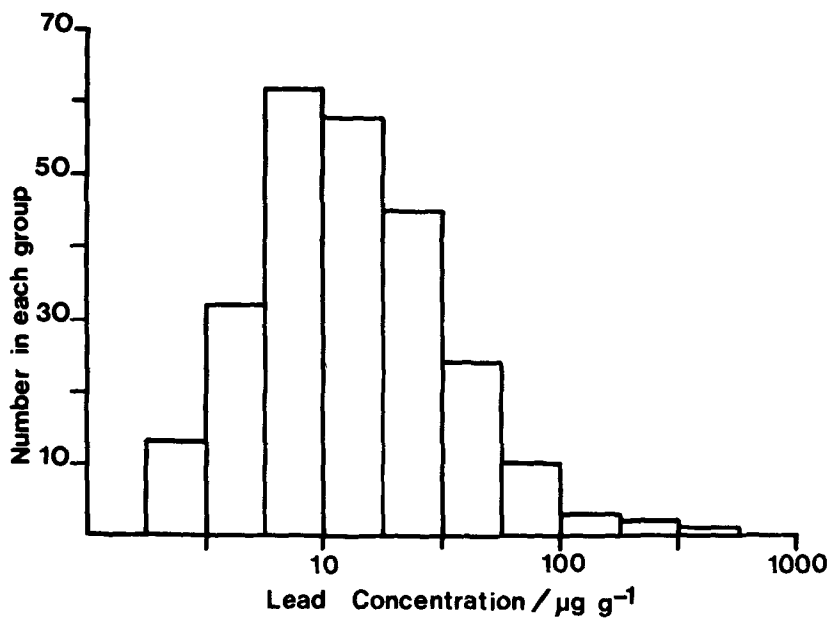


Figure 1. Histogram of Hair Lead Concentrations

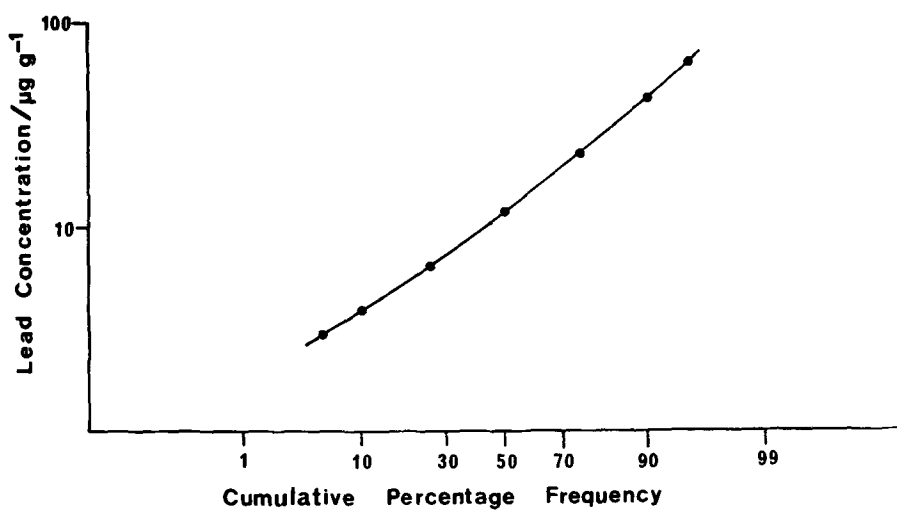


Figure 2. Cumulative Percentage Frequency Plot

The other 250 subjects all had hair lead levels in the range 2.0-360 $\mu\text{g/g}$, the value at the high extreme being that of a man employed as a printer. Preliminary examination of the lead concentrations showed a distribution with a very marked positive skew, which has been noted previously (SCHROEDER and NASON, 1969; HAMMER *et.al.* 1971). The distribution is very close to lognormal, as indicated by the histogram (Fig. 1). The extent to which the lognormal distribution itself shows a slight positive skew is shown by the upward curvature of the cumulative percentage frequency plot of Fig. 2.

For the purpose of subsequent statistical testing, all data were transformed logarithmically. Following HAMMER *et.al.* (1971) the means tabulated here are therefore geometric means of the original data, and are rather lower than arithmetic means which have apparently been quoted by most previous investigators. It should be noted that the arithmetic mean of the present sample, 21.8 $\mu\text{g/g}$, is strongly influenced by the small number of very high values (nine in the range 80-360 $\mu\text{g/g}$), the arithmetic mean of 241 results being 16.5 $\mu\text{g/g}$.

The sample size allowed significance tests to be carried out for a variety of subgroups, generally with tests appropriate to large samples, i.e. $N > 30$. In particular, differences were sought (i) between male and female subjects, (ii) among subjects of three age-groups of about equal size, (iii) between male and female subjects in each age-group, (iv) among various occupational groups which might differ in the extent of their environmental exposure to lead.

Table I shows for the whole sample and for each subgroup, the sample size, the mean and standard error in $x = \log (\text{Pb concentration}/\mu\text{g g}^{-1})$, and for convenience, certain data transformed back into lead concentrations - the geometric mean Pb concentration together with the 95% confidence limits of this mean, and the range of lead concentrations found.

The application of standard statistical tests (BAILEY 1959) for significance of differences between the means of various subgroups, yields the following results.

- (1) Male/female: the difference between the means is not significant at the 90% confidence level.
- (2) Age groups: the differences between the means for any pair of the three age-groups (1-21, 22-42, 43-87 years) are not significant at the 90% confidence level, nor is the mean for the 1-10 year age-group significantly different from that of the remainder of the population.

- (3) Within each age-group there is no male/female difference that is significant at the 90% confidence level.
- (4) The mean for occupational group I (printers, mechanics, drivers, metalworkers, machinists and other related technical occupations) is very significantly higher (> 99.9% confidence) than the mean for occupational group II (students, laboratory staff, clerical and other office workers, businessmen, teachers, accountants and other professional occupations). The latter group does not differ significantly from the remainder of the male population, which includes subjects outside the 15-65 age range, as well as those in a variety of occupations such as farming, sales, carpentering, and the police force.

The results of the present work agree with those of SCHROEDER and NASON (1969) insofar as no significant male/female difference was found over a large and fully-representative sample. We can not, however, support any of the age-related differences found by other investigators (SHABEL'NIK 1968; SCHROEDER and NASON 1969; WEISS et al. 1972). The exposure related differences found here support the conclusions of HAMMER et al. (1971). In the present work, such differences are apparent even within a single geographical area, in which a general uniformity might be presumed in factors such as diet and water-supply, and in the atmosphere of the environment away from the places of employment.

The probable cause of the higher hair lead levels of those in occupational group I is, in some cases, obvious - the two printers, for example, had 360 and 78.1 $\mu\text{g/g}$. In other cases, abnormal exposure may arise from the handling of metallic lead, or from exposure to lead tetraethyl and the particulates resulting from its combustion in automobile engines. In this connection it is worth noting that urban dusts are relatively rich in lead. Dust samples collected at various locations in the city of Palmerston North over a one-year period almost invariably contained 500-1000 $\mu\text{g Pb/g}$ (WARD 1974), a level which is consistent with values reported from cities in other parts of the world. Greater occupational exposure to urban dust may well be a factor contributing to the higher lead levels among those in occupational group I, compared to group II which consists of those in indoor white-collar and professional occupations.

Blood-lead levels were not measured in the present work, but detailed studies have been reported (HERNBERG et al. 1970) which show a strong correlation between occupational lead exposure and blood-lead levels, these factors being in turn negatively correlated with the activity of the enzyme δ -aminolevulinic acid dehydratase. Because elevated hair

levels can arise from both external and internal routes, the correlation between hair lead and other indices of the body lead burden (e.g. lead in blood) is not always a strong one. However, EL-DAKHAKHNY and EL-SADIK (1972) have shown positive correlations between lead in hair and lead in blood or coproporphyrin in the urine of occupationally-exposed workers, and hair lead levels above 30 $\mu\text{g/g}$ were suggested as giving a strong indication of excessive exposure. Values in the range 30-110 $\mu\text{g/g}$ were considered by SUZUKI *et al.* (1958) to be 'normal' for workers occupationally exposed to lead, but abnormal for other persons.

Each of the subgroups examined in the present work shows at least one person with hair lead above 50 $\mu\text{g/g}$. It is clear that some of these elevated levels are due to unusual personal habits or factors. These might include occasional cases of pica in young children, other mild forms of accidental ingestion (e.g. from lead paints or lead-glazed pottery), or metabolic differences leading to an abnormal proportion of the body lead burden being excreted via the hair.

In summary, this study has shown that, among a fully-representative urban/suburban/rural population sample of 250 subjects, there were no specific age- or sex-related hair-lead levels. Highly significant elevations were found only in a group of occupations which would be expected to involve above-normal exposure to metallic lead or to motor-vehicle exhaust particulates and urban dusts.

ACKNOWLEDGEMENTS

We thank the six hairdressers who assisted us with the collection of the hair samples, and Ms Deborah Rumsey for her substantial help in preparing the samples for analysis.

REFERENCES

- BAGCHI, K.N., H.D. GANGULY and J.N. SIRDAR: Indian J. Med. Res. 27, 777 (1940).
- BAILEY, N.T.J.: Statistical Methods in Biology. London: English Universities Press 1959.
- BATE, L.C. and F.F. DYER: Nucleonics 23, 74 (1965).
- BOWEN, H.J.M.: Trace Elements in Biochemistry. New York: Academic Press 1966.
- EL-DAKHAKHNY, A. and Y.M. EL-SAKIK: Amer. Ind. Hyg. Ass. J. 33, 31 (1972).
- HAMMER, D.I., J.F. FINKLEA, R.H. HENDRICKS, C.M. SHY and C.J.M. HORTON: Amer J. Epidemiol. 93, 84 (1971).
- HARRISON, W.W., J.P. YURACHEK and C.A. BENSON: Clin. Chim. Acta 23, 83 (1969).
- HERNBERG, S., J. NIKKANEN, G. MELLIN and H. LILIUS: Arch. Environ. Health 21, 140 (1970).
- KOPITO, L., R.K. BYERS and H. SHWACHMAN: New England J. Med. 276, 949 (1967).
- NISHIYAMA, K.: Shikoku acta med. 11, 164 (1957).
- SCHROEDER, H.A. and A.P. NASON: J. Invest. Dermatol. 53, 71 (1969).
- SHABEL'NIK, D.Y.: Mikroelem. Med. 1, 184 (1968).
- WARD, N.I.: Lead Pollution in the New Zealand Environment, M.Sc. Thesis, Massey University, Palmerston North, N.Z. 1974.
- SUZUKI, Y., K. NISHIYAMA and Y. MATSUKA: Tokushima J. Exper. Med. 5, 111 (1958).
- WEISS, D., B. WHITTEN and D. LEDDY: Science 178, 69 (1972).