Lead levels in deciduous teeth of children from selected urban areas in the Cape Peninsula

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Summary

The lead levels in shed deciduous teeth of children from two selected urban regions in the Cape Peninsula were compared. The average levels in the teeth of children living in the vicinity of two large industrial plants were: whole teeth 20,419 ppm, enamel 10,952 ppm, and dentine 22,733 ppm. The lead levels in teeth from children living in the vicinity of light industries were: whole teeth 16,556 ppm, enamel 2,919 ppm, and dentine 19,926 ppm. These differences were significant at the 1% level (teeth and enamel) and 5% level (dentine).

S Afr Med J 1983; **63**: 559-562.

Considerable attention has been paid during recent years to the presence of lead in the human body, and there is general agreement that this level can be influenced by *inter alia* industrialization.[1] An important source of lead is air pollution.[2] Although the body levels of lead in such circumstances may not cause obvious plumbism, it has been found that poor scholastic performance can be related to subclinical lead levels in the body,[3] and that this is apparently the result of an intake of small doses over long periods. However, identification of chronic low-grade lead poisoning presents some difficulties. Blood and urine levels are markers of recent exposure and only bone and teeth contain levels which represent chronicity. Teeth are superior to bones because dental structures once formed are not replaced and one can determine the time that the exposure occurred from the affected teeth.[4]

In the RSA we have regions such as the Cape Peninsula which are relatively highly industrialized and may have high indices of lead pollution. Because of the distribution of industrial plants, however, it would seem reasonable to suppose that air pollution will not be uniform. One can expect therefore that the body lead levels of subjects in the Peninsula will vary depending on living circumstances and residential areas. For these reasons we decided to compare the lead levels in the teeth of children living in the vicinity of large industrial plants with those in the teeth of children living in an area where there are only light industries.

Materials and methods

Two locations were selected for comparison, one a residential area in the vicinity of an oil refinery and a fertilizer plant (Bothasig) and the other a residential area in the vicinity of Tygerberg Hospital (Parowvallei).

One hundred and twenty-eight shed deciduous teeth were collected from children attending primary schools in these areas who had lived there since birth. In order to rule out the possibility of contamination, all teeth with fillings or carious defects were discarded. Finally 59 teeth were selected, 21 from 21 children from the Bothasig area and 38 from 27 children from the Parowvallei area. Eleven children in the Parowvallei group donated a pair of usable teeth each.

The following investigations were made: (*i*) a pilot study to compare the lead content of teeth which were cleaned by acid etching with teeth which were not etched; and (*ii*) determination of the lead levels in etched teeth from both communities. Atomic absorption spectrometry (AAS) was employed for the lead determinations.

Reagents. Origins and grades of reagents were as follows: nitric acid (BDH Chemicals) for AAS; perchloric acid (60% Fisher reagent ACS); bromoform (Fisher Spectro grade); acetone (Merck). To prepare lead nitrate solutions, a standard solution for AAS (BDH) was used to prepare a stock solution of 20 ppm of lead in 0,1M nitric acid, and further diluted to working standard solutions to cover a range from 0,3976 ppm to 0,0100 ppm of lead. These were freshly prepared as required. All other standard solutions were made up in nitric acid and perchloric acid so as to match the acid concentrations of the sample solutions.

Water. The water used for preparations was de-ionized and redistilled from glass. No lead detectable by AAS was present in the water.

Glassware was cleaned by soaking it in tetrasodium ethylenediamine tetra-acetic acid solution, rinsing thoroughly, soaking in 50% (v/v) reagent grade nitric acid, rinsing at least 3 times with demineralized de-ionized water, and finally air-drying. All caps were free of any inserts (e.g. rubber or cardboard) capable of contaminating samples. Borosilicate tubes were used during the wet-ashing procedure.

Apparatus. Flameless analyses were carried out with a Pye-Unicam Model SP9 atomic absorption spectrophotometer equipped with a strip chart recorder, a Model SP9 video graphite furnace, an autosampler, a Model SP9 computer and a deuterium arc background corrector. Optimum time and temperature settings were as follows: for drying, 30 seconds at 100°C; for charring, 30 seconds at 550°C; and for atomizing, 3 seconds at 2 200°C. Uncoated graphite tubes were used in the furnace and purged with argon at a flow rate of 0,75 ml/min. The absorbance peaks were recorded at 283,3 nm for lead. The hollow cathode lamp for lead was operated at 6 mA.

Procedures

The selected teeth were stored in plastic bags at -7°C. Before analysis remnants of roots (if present) were separated from the crowns with a diamond disc, the crowns were etched with 1,0N HCl solution for 15 seconds, washed 4 times with de-ionized glass-distilled water, dried at 105°C and ground to a fine powder

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Date received: 1 June 1982.

in an agate mortar. About half of the powder of each specimen was then weighed and analysed for lead levels in whole teeth.

For the measurement of lead in enamel and dentine the other half of each powdered specimen was subjected to a flotation procedure, by which dentine and enamel can be separated because of their differences in density.[5] When 91 ml bromoform is added to 9 ml acetone a flotation solution with a density of 2,70 is achieved. The tooth powder is centrifuged in this solution; the enamel sinks to the bottom and the dentine floats to the surface. These are collected separately, washed until free from bromoform and acetone, dried overnight at 80° C, weighed and then analysed for lead content.

In order to compare the lead content of etched and unetched teeth the 11 pairs of teeth of the children from the Parowvallei area were used. Half were etched and treated as described above while the other half were not cleaned in acid before analysis.

The ground samples were wet-ashed in borosilicate glass tubes by the addition of 1 ml concentrated nitric acid and 0,25 ml concentrated perchloric acid. The ashing process was completed by heating the specimens at 80° C overnight and then diluting the solution to 5,0 ml with water. For the AAS 10 μl of each sample was injected by automatic means into the furnace and analysed by the standard addition method.

In the direct determination of lead, matrix interferences were found. The absorbance values were suppressed to an extent and varied from sample to sample, so that aqueous standards could not be used for calibration; the variation between samples prevented the use of matrix-matched calibration standards. The method of standard additions was therefore employed to compensate for the matrix effect.

The concentrations of lead are expressed as ppm of the dry weight.

Results

The lead content differences in enamel and dentine of the 11 pairs of teeth (acid-etched and unetched) from Parowvallei were as follows: the enamel and dentine of the etched teeth contained a mean ($\pm$ SD) of 3,51 $\pm$ 3,1 ppm and 17,8 $\pm$ 7,1 ppm lead respectively, while the unetched teeth contained 11,1 $\pm$ 4,1 ppm and 17,0 $\pm$ 8,2 ppm lead respectively. These differences are significant at the 1% level for enamel but not for dentine.

Table I compares the lead levels in teeth from the two areas. The mean lead levels in enamel, dentine and whole teeth from Parowvallei were 2,919 ppm, 19,926 ppm and 16,556 ppm respectively. The mean lead levels in teeth from Bothasig were 10,952 ppm for enamel, 22,733 ppm for dentine and 20,419 ppm for whole teeth. Because of the non-normal distribution of the results they were subjected to the Mann-Whitney test for statistical analyses. The differences in lead values in enamel, dentine and whole teeth from the two areas were significant at the 1%, 5% and 1% levels respectively.

TABLE I. LEAD LEVELS (ppm) IN ENAMEL, DENTINE AND WHOLE TEETH FROM PAROWVALLEI AND BOTHASIG

		Parowvallei (N = 27)	Bothasig (N = 21)
Enamel	Mean	2,919	10,952
	Median	3,000	9,900
	SD	2,081	3,714
Dentine	Mean	19,926	22,733
	Median	18,600	22,200
	SD	8,296	4,423
Whole tooth	Mean	16,556	20,419
	Median	15,200	20,200
	SD	6,840	3,429

Discussion

There are several reasons why deciduous teeth rather than permanent teeth were used. The lead burden of children is more pronounced than that of adults[6] and higher lead levels have been reported in deciduous teeth than permanent teeth.[6] Also deciduous teeth were easier to obtain in the selected areas. The available permanent teeth were usually filled or carious and the only sound permanent teeth were premolars extracted for orthodontic purposes, but they were scarce. Another advantage of deciduous teeth is that they belong to young children and there is a better chance of getting samples from subjects who have lived in one location only. A distinct disadvantage of permanent teeth is that the lead level in these teeth increases with age. Derise and Ritchey[7] claimed significantly higher levels of lead in teeth of subjects over the age of 25 years than in younger age groups. In other words, such accumulation may give inflated values.

Since lead levels are extremely low in biological material, reliable analytical procedures are necessary. In addition contamination by the loss of lead causes difficulties and there are interferences from various elements. For this reason several methods have been used for the determination of lead in teeth, such as high-resolution gamma spectrometry, X-ray emission spectrography, mass spectrometry, AAS and anodic stripping voltametry. Of these, AAS has received wide attention because of its sensitivity (especially graphite furnace AAS[8]). As a result of developments in this field over the last few years, AAS may now be considered as one of the most reliable techniques for the analysis of trace elements, especially when the apparatus is equipped with a video furnace, autosampler, strip chart recorder and computer. Matrix interference can mainly be overcome by the method of standard additions.

Contamination is another problem. In equipment, it can be overcome by special cleaning methods; once cleaned the glassware must be reserved for lead determinations only. Special care must be taken not to contaminate the sample and the sample solutions during experimental procedures. In addition the collected samples, especially those removed by special procedures such as dental procedures, should be regarded as possibly contaminated and results must be interpreted accordingly.

This problem is well illustrated in the results obtained for the etched and unetched teeth in this study. Etching removes about 5 μm of the surface enamel. The significant difference of lead found in the enamel of the experimental teeth can therefore be due to superficial contamination of the enamel by lead from ingestion, inhalation, extraction and/or storage of teeth. However, a disadvantage of the cleaning procedure is that the enamel lead levels obtained after cleaning might be lower than the real value, as the lead gradient in the surface enamel is steep. Since the lead contamination factor may vary widely from one tooth to another, we feel that the cleaning procedure is advisable before analysis is undertaken. This problem can be overcome if the dentine levels only are interpreted.

In all our experimental work we found that dentine contains more lead than enamel (Table I), which is in agreement with the majority of other workers. According to Shapiro *et al.*[9] the lead contained in dentine is 6 times that in enamel, whereas Al-Naimi *et al.*[10] and Needleman *et al.*[2] found it to be 4 times and 3,8 times greater respectively. Additional work has shown that circumpulpal dentine is especially rich in lead (Table II). It would appear therefore that lead is laid down in enamel and dentine during tooth formation but continues to be deposited in

TABLE II. COMPARISON OF RESULTS FROM VARIOUS WORKERS

Area	Type	Dentine lead (ppm)	Enamel lead (ppm)	Whole-tooth lead (ppm)	Remarks
Birmingham					
Mackie *et al.*[10]	Deciduous			11,8 ± 6,6	
Jones *et al.*[10]	Deciduous			12,0 ± 7,6	
Jones *et al.*[10]	Permanent			8,2 ± 2,7	
Al-Naimi *et al.*[10]	Permanent	34,2 ± 17,0 pulpal		8,5 ± 4,2	
Philadelphia					
Needleman *et al.*[2]	Deciduous	42,6 ± 2,9 pulpal		11,2 ± 2,9	Low-pollution area
	Deciduous	144,6 ± 20,1 pulpal		34,4 ± 5,5	High-pollution area
Cambridge					
Brudevold *et al.*[12]					
High Pb group			2 360 (biopsies)		High
Low Pb group			2 120 (biopsies)		Low
Cincinnati					
Altshuller *et al.*[13]	Deciduous			15,1	Normal Pb exposure
	Deciduous			159,0	Children died
	Deciduous			116,6	Children survived
Richmond					
De la Burdé and Shapiro[14]	Deciduous		194,5		Lead exposure
	Deciduous		103,2		Control group
Norway					
Fosse and Justesen[15]	Deciduous			3,73 ± 5,0	
Iceland					
Shapiro *et al.*[9]	Deciduous	5,4; 35,5 circumpulpal			
Boston					
Shapiro *et al.*[9]	Deciduous	16,9; 84,4 circumpulpal			Suburban
	Deciduous	54,8; 606,8 circumpulpal			Lead-poisoned
Brudevold *et al.*[12]	Permanent		200 - 3 550		Within 4 μm
	Permanent		260 - 520		Thicker surfaces
Chelsea and Somerville					
Needleman *et al.*[3]		< 6			Low lead level
		> 24			High lead level
Oslo					
Attramadal and Jonsen[6]	Deciduous			1,5 - 13,4	
	Permanent			0,9 - 7,8	
Langmyhr and Sundli[16]	Permanent			2,39 ± 1,5	
Finland					
Lappalainen [11]	Permanent	≈ 40	65,3		Rural
	Permanent	≈ 50	56,6		Urban
Virginia					
Derise and Ratchey[7]	Permanent	43,3	45,2		
Northern Ireland					
Lindvall and Radford[17]	Deciduous			9,7	Urban
	Deciduous			8,3	Suburban
	Deciduous			4,8	Rural

normal and abnormal secondary dentine. A similar process can be expected in the cementum of a tooth.

Derise and Ritchey[7] and Lappalainen and Knuuttila,[11] however, demonstrated a higher lead content in enamel than in dentine. The reasons for the differences between their results and the findings mentioned earlier cannot be explained. One wonders how far enamel contamination might have influenced their findings.

From Table II it can be seen that our findings correspond to the findings of some workers, while they differ significantly from those of others. Such differences can be accounted for by the existing lead levels in the regions examined and the various methods used for lead determination.

However cautiously one may view the findings of the present study, three significant factors remain. The first is that the lead levels differ significantly between areas in the same geographical

region. Average levels for regions such as the Cape Peninsula are therefore meaningless. Problem areas may develop in parts of the Cape Peninsula. Secondly, lead was found in all the teeth analysed, which indicates that chronic lead uptake is widespread in the Peninsula. Finally, the lead content of teeth from one of the areas studied here compares unfavourably with the high lead content of teeth from a series reported on by Needleman *et al.*[3] This should give cause for reflection.

We wish to thank Dr D. Kotze of the Department of Statistics of the University of the Western Cape for the statistical analyses and Miss G. S. Townshend of the Institute for Medical Literature of the South African Medical Research Council for proof-reading the article.

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