Relation between lead in surface tooth enamel, blood, and saliva from children residing in the vicinity of a non-ferrous metal plant in Belgium

R Cleymaet, K Collys, D H Retief, Y Michotte, D Slop, E Taghon, W Maex, D Coomans

Abstract
Two groups of schoolchildren between seven and 12 years old residing in the vicinity of a non-ferrous industrial plant and exposed to lead (Pb) at a concentration that could cause health problems, were monitored. Concentrations of Pb in blood (blood-Pb), which were determined at regular six monthly intervals, were related to the Pb concentrations in surface tooth enamel (enamel-Pb). Acid etch biopsy samples of surface enamel were taken at the end of the five year study period in the first group (A) and after two years in the second group (B). Salivary Pb (saliva-Pb) concentrations were determined for the first study group on the same day that the enamel biopsies were performed. Calibration of the data was necessary—that is, blood-Pb concentration with respect to age and sex and enamel-Pb concentration with respect to etch depth and age. The blood-Pb concentrations declined with time. Surface enamel Pb concentrations correlated with blood-Pb concentration for the period starting with the pre-eruptive development of the incisors, related to blood-Pb concentration for a long time, and corresponded partly to the exposure at the time of pre-eruptive development and/or eruption. Through the correlation with enamel-Pb concentration, the seasonal behaviour of blood-Pb concentration became apparent. Saliva-Pb concentrations related to blood-Pb concentrations only in the short term.

Heavy metals (for example, lead (Pb)) contribute to the pollution of the environment and can affect human health.1-3 Lead intoxication in humans may lead to dysfunctions such as mental retardation, cerebral palsy, and hyperactivity. Exposures to this metal can be detected by analysis of blood, which is routinely used as a tool for monitoring and screening of the body burden. Because of the affinity of Pb for hydroxyapatite—even at low concentrations4—human teeth can provide further information about the past exposure and accumulation of Pb in the body.

For epidemiological studies, whole teeth are not routinely available so the method of sampling of surface tooth enamel by acid etch microbiopsy was developed.7 This method was recently reassessed by Cleymaet et al7 and used in several studies as a potential sampling medium for Pb accumulation.8,10,11 Lead in saliva can be detected but this sampling medium is said to provide only short term information,12 could be influenced by oral Pb contamination in the subject, and does not necessarily reflect the degree of environmental pollution.13

In this study Pb concentration was determined in blood, surface enamel, and saliva of children living in the vicinity of a non-ferrous industrial plant, and the correlations among these variables were established.

Materials and methods
STUDY GROUP
The study was performed with children in the age range seven to 12 years from a school located less than 500 m from a non-ferrous metal industrial plant. These children were divided into two groups.

Group A consisted of 23 children. Blood samples were taken from each member of the group every six
months from the spring of 1982 until autumn 1988. Acid etch enamel biopsy samples were taken in January 1987 together with saliva samples.

Group B consisted of 19 children. Blood samples were taken every six months from the spring of 1987 until autumn 1989. Etch biopsy samples were taken in January 1989. Saliva samples were not collected from this group.

Informed consent from the parents of the children and the approval of the Ethical Committee, Faculty of Medicine and Pharmacy, Free University of Brussels were obtained before the study started.

**SAMPLING PROCEDURE**

**Enamel**

*Group A*—The enamel biopsy procedure was a modification of the method described by Brudevold *et al.*6 Acid etch biopsy samples were taken from maxillary permanent right central incisors, free of caries and fillings, as described by Cleymaet *et al.*7 using the window technique. Samples were stored at 4 °C.

*Group B*—Two successive etch biopsies on the same spot were performed in this group. Samples were stored at 4 °C.

**Blood**

Blood samples (500 μl) were taken by finger pricking. Before pricking, the fingertip was cleaned with 2% HNO₃ (suprapure grade, E Merck, Darmstadt, Germany), disinfected with alcohol (70%), and rubbed with vaseline (pure grade) to facilitate droplet formation and to avoid contact between blood and skin. A lancet (blood lancets, Feather Industries Ltd, Japan) was used for pricking. The blood was stored at 4 °C in a microcontainer (Becton Dickinson No 5973) with ethylenediaminnetetra-acetic acid (EDTA) as anticoagulant.

**SALIVA**

Before performing the acid etch biopsy, whole saliva from each child in group A was collected at 10 am in a graduated polyethylene tube (Falcon 2070, Becton Dickinson) during ten minutes. The flow rate was measured unstimulated, and stimulated by chewing on a piece of parafilm (American Can Company, Dixie/Marathon, Greenwich, CT, USA). Samples were stored at 4 °C.

**CHEMICAL ANALYSIS**

**Enamel**

The samples were analysed for calcium (Ca), phosphorus (P), and Pb concentrations according to the procedures described by Cleymaet *et al.*7 Calcium content was determined with a flame atomic absorption spectrophotometer (Perkin Elmer, ADS 373, Norwalk, CT, USA). Phosphorus was determined with the colorimetric method described by Itaya and Uj.14 Calcium concentration was used for the calculation of the amount of etched enamel and etch depth. Phosphorus concentration was used as a control assuming that the Ca/P ratio in enamel is fairly constant.5 Lead was analysed by graphite atomic absorption spectrometry (Perkin Elmer AAS 5000 System). The instrumental conditions have been described previously by Cleymaet *et al.*15 The Pb concentrations in surface enamel were expressed as μg Pb/g enamel.

**Blood**

Lead concentration in blood was determined by furnace atomic absorption spectrophotometry (Perkin Elmer AAS-Z380, Norwalk, CT, USA) equipped with autosampler (PE AS40), furnace programmer (PE, HGA 400), and recorder (PE 023). Commercially available control blood samples (BCR, EC Directorate General for Science, Research and Development, Brussels, Belgium) with certified Pb concentrations were used to construct the calibration curve. Blood standards and samples were diluted ×6 with 1/1000 triton X-100 (alkylaryl polyether alcohol) (E Merck, Darmstadt, Germany) before analysis.

**Saliva**

Saliva samples were analysed for Pb concentration by graphite furnace atomic absorption spectrometry (Perkin Elmer AAS 500 atomic absorption spectrophotometer, a HGA 500 graphite furnace, an AS40 autosampler, a PC 7500 data station with a PR–210 matrix printer, and R 100 A recorder). Pyrolytically coated graphite furnace tubes equipped with an L'Vov platform were used. Before analysis 3 ml of saliva was treated with 1 ml (or more if necessary) of a 1 : 4 mixture of HClO₄ : HNO₃ of suprapure grade (E Merck, Darmstadt, Germany) and heated at 250 °C to 300 °C until destruction was complete. The destruction residue was dissolved in 1 ml ultrapurified water and heated again to remove traces of perchloric acid. The residue was transferred quantitatively and diluted into a 5 ml volumetric flask with 0-2% HNO₃. Samples were analysed for Pb content against a calibration curve of 0, 10, 30, 50, and 80 μg/ml Pb after correction for blank destruction.

All glassware and plasticware were checked and found to be Pb free.

**STATISTICAL ANALYSIS**

**Enamel**

The variation in Pb content of enamel is not only due to differences in uptake but is also significantly dependent upon tooth type, etch depth, and age. This was reported in a previous study.15 It was also reported that sex and dental arch quadrant were non-significant contributing factors. In the present study.
the tooth types were identical. For the significant factors age and etch depth a calibration was performed. An improved standardisation of Pb in enamel was feasible using linear models after outlier detection by multiple least median of squares regression (LMS). The factors were fitted to the logarithm of the Pb content in enamel using reweighed ordinary least squares regression analysis (RLS).

**Blood**

The variation in blood-Pb concentration in young children is due in part to differences in Pb uptake, and is also affected by the non-environmental factors, age and sex. Therefore, decorrelation of blood-Pb concentrations with respect to factors such as age and sex was necessary to remove part of the undesired variation in Pb content. Again RLS was used for this purpose.

**Saliva**

Lead concentrations of Pb in saliva showed a large variation among subjects. Part of this variation was due to factors other than the level of exposure to Pb.

**Comparisons**

Considering the undesirable variation in enamel-Pb, blood-Pb and saliva-Pb concentrations, and to obtain unbiased Pb concentrations, it was decided to compare these variables with each other at two levels:

1. Including the influence of external factors without decorrelating.
2. After decorrelating the external factors using an LMS–RLS scheme.

The calibrated variables obtained are indicated by calibrated enamel-Pb, calibrated blood-Pb, and calibrated saliva-Pb concentrations. Calculations were done using the software package SPSS/PC and software program Progress. For the calculation of the relations between the variables, the non-parametric Spearman correlation coefficient was used because enamel-Pb, blood-Pb, and saliva-Pb concentrations have a skewed distribution and the enamel-Pb and blood-Pb values tend to a log normal distribution.

Mean values were used to represent the location of the sample distribution of the different data. Because of the skewed distributions, the data would normally be presented by the median value or by the inversely transformed mean value of the logarithmically transformed values. We decided, however, to present the arithmetic mean values to enable a comparison with existing published data. Where necessary, further calculations were done on logarithmically transformed data.

**Results**

**ETCH BIOPSY SAMPLES OF SURFACE TOOTH ENAMEL AND Pb CONTENT**

The table shows the basic statistics for the variables evaluated in the present study. The mean age of the children in group B was somewhat higher than that of group A. In both groups the sexes were roughly equal. The etch biopsies were well standardised as shown by the coefficient of variation of the etch depths for the first layer of enamel removed in both groups, as well as for the second layer removed in group B. The mean etch depth in the first layer was comparable for both groups and the mean etch depth for the second biopsy sample in group B was of the same order of magnitude as the mean etch depth for the first biopsy sample. The Ca/P ratio for group A was somewhat higher than for group B. The mean Pb concentrations in the etch biopsies were different. The enamel-Pb concentration in the first layer was 30% higher in group A than in group B. The largest difference was found within group B in which enamel-Pb concentration in the first layer was much greater compared with enamel-Pb concentration in the second layer. A steep decrease in Pb concentrations from the outer to inner enamel was the reason for this difference. The correlation coefficients between enamel-Pb concentrations for the children in group A and the factors etch depth, Ca/P ratio of enamel, and age were \(-0.40\) (0.10 > \(p > 0.05\), 0.21 (non-significant), and 0.29 (non-significant) respectively.

### Basic statistics for variables determined for groups A and B

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>CV*</td>
</tr>
<tr>
<td>Age (y)</td>
<td>9.5 (1.6)</td>
<td>16-8</td>
</tr>
<tr>
<td>Etch depth (µm; first enamel layer)</td>
<td>3.1 (0-6)</td>
<td>19-3</td>
</tr>
<tr>
<td>Etch depth (µm; second enamel layer)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ca/P in first enamel layer</td>
<td>2-43 (0-12)</td>
<td>4-9</td>
</tr>
<tr>
<td>Ca/P in second enamel layer</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Enamel-Pb (µg/g; first enamel layer)</td>
<td>2985 (2269)</td>
<td>76-0</td>
</tr>
<tr>
<td>Enamel-Pb (µg/g; second enamel layer)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Blood-Pb (µg/100 ml; autumn 1986)</td>
<td>19.7 (6-4)</td>
<td>32-5</td>
</tr>
<tr>
<td>Saliva-Pb (µg/100 ml)</td>
<td>2.02 (1-57)</td>
<td>77-7</td>
</tr>
</tbody>
</table>

*Coefficient of variation expressed as a percentage.
†Calcium/phosphorus ratio
Thus decorrelation procedure according was values scheme stepwise regression analysis to respect tor sex was entered one age, depth, and saliva-Pb values for different time points (\(\ast\)). Spearman rank correlation coefficient \(r\) for blood-Pb values for different time points (\(\circ\)), enamel-Pb values in the second biopsy sample for one time point and uncalibrated blood-Pb values for different time points (\(\ast\) ... \(\ast\)), and saliva-Pb values at one time point and blood-Pb values for different time points (\(\ast\) ... \(\ast\)) \(v\) time. The dotted horizontal line corresponds to the \(p = 0.05\) (2 sided) significance level for \(r\) calculated for a study group (A) of 20 children. \(^1\) Indicates the time at which the etch biopsy samples and saliva samples were taken. \(S = \) Spring; \(A = \) Autumn.

Thus decorrelation of enamel-Pb concentration with respect to etch depth was considered appropriate. A stepwise regression analysis based on the LMS-RLS scheme with the natural log transformation of the enamel-Pb values as dependent variable and etch depth, age, and Ca/P ratio as independent variables was performed. The independent variables were entered one by one in the stepwise regression procedure according to the absolute value of the Spearman rank correlation coefficient. Also, the factor sex entered as last variable. Decorrelation with respect to etch depth reduced the variation of logarithm of enamel-Pb significantly. Although age seemed to be of borderline importance (\(r = 0.29\)), it was kept in the regression equation. The two other variables (Ca/P and sex) did not contribute to the variance reduction at all. Group B showed similar results and the calibration of the logarithm of the enamel-Pb values proceeded in the same way as for group A.

**CONCENTRATION OF Pb IN BLOOD**

The table presents the blood-Pb concentrations determined in the autumn of 1986. The concentra-
tions were used as a reference to compare groups A and B and to determine correlation coefficients with other data variables. The table shows that the mean blood-Pb concentration and the variability of the blood-Pb values were much the same for the two groups for the autumn of 1986. The coefficient of variation for blood-Pb concentration was not very different for the different blood collection times over the period of investigation and was in line with the values given in the table for blood-Pb concentrations in the autumn of 1986 for groups A and B (the standard deviation coefficients for the Pb concentrations at different time intervals were within the range 5.1 to 7.2). For the group A children a decreasing trend in mean blood-Pb concentration was seen from the autumn of 1983 to the autumn of 1987 (fig 1A), beyond which the blood-Pb concentrations tended to increase. The number of data points were insufficient, however, to show whether these trends were significant and persistent. The mean blood-Pb pattern for group B was similar to that of group A for the same time span (fig 2A). Blood-Pb concentration correlated with age. For instance for our reference in the autumn of 1986, blood-Pb concentration had a correlation coefficient of r = 0.30 (0.10 > p > 0.05) with age. There was no correlation between blood-Pb values and etch depth (r = 0.09), as expected. Using the same regression strategy as with enamel-Pb concentration with inclusion of sex at the end, it was considered worthwhile to decorrelate the logarithm of blood-Pb values with respect to both age and sex. The regression coefficient was significant for age (p < 0.05) and sex (p < 0.05). The sign of the regression coefficient of sex indicates that higher values for blood-Pb concentration could be expected in boys.

**COMPARISON BETWEEN BLOOD-Pb AND ENAMEL-Pb CONCENTRATIONS**

In the group A children, the correlation between enamel-Pb and blood-Pb concentrations in the autumn 1986 before calibration with respect to the external factors was significantly positive (r = 0.50, p < 0.05). The significance remained after decorrelation by external factors (r = 0.55, p < 0.05). Figure 1B shows the curve of the Spearman rank correlation coefficient between enamel-Pb and blood-Pb concentration as a function of time for the period from the autumn of 1983 to the autumn of 1988. The correlation coefficients were calculated between enamel-Pb concentrations from the etch biopsy samples taken in the first half of the year 1987 and each group of half yearly blood-Pb measurements over the period 1983-8. Some interesting observations can be made from the curve. From the autumn of 1983 until the autumn of 1985 a steady increase occurred in the correlation. From the spring of 1985 to the autumn of 1988 the correlation was more or less stable with, however, an up and down fluctuation. This cyclic behaviour showed higher values for the autumn measurements of each year starting in 1985. These correlation coefficients were significant (p < 0.05). The spring blood-Pb measurements correlated less well with enamel-Pb concentrations, all falling below the significance limit. When using the uncalibrated values together with the calibrated enamel-Pb values with respect to external factors, some changes occurred in the curve but the overall picture remained similar to the uncalibrated situation. It must be noted that from a theoretical point of view, it is sufficient to calibrate only one of the two variables in order that the correlation between these two variables of interest (here blood-Pb and enamel-Pb concentrations) would be free of influence by undesirable external factors. So we calibrated enamel-Pb and not blood-Pb concentration. This gives the same correlation coefficient as when blood-Pb is also calibrated. The fluctuations in the correlation reflects cyclic time dependent variations in the blood concentrations that are not evident in the mean blood-Pb values for the period from the spring of 1985 to the autumn of 1988 as shown in fig 1A. This cyclic behaviour would not have been detected in the correlation coefficients if the enamel-Pb concentrations also showed such a cyclic behaviour. The fact that the enamel-Pb data of group A were not correlated at all with the blood-Pb concentrations in 1983 and 1984, and that the correlation increased thereafter, may be related to the somewhat steeper decrease of the mean blood-Pb concentrations in the 1983-4 period. It also indicates that the enamel-Pb values in 1987 do not contain any information about blood-Pb values for the period 1983-4. From then on the information increases and becomes significant in the spring of 1985, when the children of group A have a mean age of about 7.5 years. The building up of the blood-Pb related information in surface enamel clearly starts when the incisor was pre-eruptively mineralised. During the spring of 1987 when the etch biopsies of group A were taken, the mean age of the children was about 9.5 years (table), which corresponds to a mean age of 6.5 years in the spring of 1984. At that stage the pre-eruptive enamel mineralisation of the children's permanent central incisors under study was completed. For both sexes the eruption of the maxillary permanent central incisors in the oral cavity starts at a mean age of 6.0-6.5 years. The blood-Pb related information does not alter much from 1985 onwards, except for blood related seasonal variations, which means that the Pb concentration in the few µm of surface enamel does not increase with time. This is supported by the finding that blood-Pb correlates with sex whereas enamel-Pb concentration does not. Only a part of the Pb concentration in surface enamel, however, contains blood-Pb related information as the correlation
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is at most 0.6, which is smaller than the blood-Pb correlations (up to 0.85 for group A). Figure 1B indicates the correlations between blood-Pb concentrations and the remaining measurements from other collection times. Blood-Pb concentration in the autumn of 1986 was chosen as the reference because this time more closely corresponded with the data of the enamel biopsy samples. The correlations among the blood-Pb values were higher than the correlations with enamel-Pb (fig 1B). Moreover, no decrease in correlation occurred when going back in time from the autumn of 1985. Figure 2B shows a large effect for the group B children figure of calibration of enamel-Pb and blood-Pb concentrations on the Spearman rank correlation time profiles. The uncalibrated profiles for enamel-Pb concentrations in the first biopsy samples and enamel-Pb concentrations in the second biopsy samples show no correlation with the blood-Pb concentrations. The calibrated profiles for enamel-Pb concentrations in the first biopsy samples and enamel-Pb concentrations in the second biopsy samples are clearly significant. Lead in the first biopsy sample correlates in the same way as Pb in the second one. The correlations with respect to Pb in surface enamel (calibrated) are not much different from the correlations among the blood-Pb concentrations at different time points (fig 2C). Moreover, we found that the calibrated enamel-Pb values in the first biopsy samples were correlated significantly with the ones of the second biopsy sample \( r = 0.621; p < 0.01 \) at the level that they were correlated most of the time with the calibrated blood-Pb values. This is surprising as the second biopsy samples contain six times less Pb than the first biopsy samples (table).

**Discussion**

In this study, blood-Pb concentrations were considered as a reference as most body burden follow up studies have used this as an indicator. In this context much data have been published on children and adults resident in the vicinity of a non-ferrous industrial plant and in less exposed urban and rural regions in Belgium. The decreasing blood-Pb concentration in group A in the period from the autumn of 1983 to the autumn of 1987 could be due to more than one cause. In the period 1983–8, the Pb concentration in ambient air near the industrial plant decreased only slightly, which was in fact the exponential extension of a former large decrease seen during the period 1977–83. This was the result of several pollution control measures imposed by authorities upon the industrial plant. The average annual Pb concentration in air at the reference station 700 m from the plant decreased from 3.79 µg Pb/m\(^3\) in 1974 to 2.27 µg Pb/m\(^3\) in 1978, to 1.25 µg Pb/m\(^3\) in 1982, and to 1.1 µg Pb/m\(^3\) in 1987. The fallout of heavy Pb containing particles did not decrease substantially however during the past years. The mean blood-Pb concentration remained above 18 µg/100 ml, which was higher than mean values found in an urban area (9–12.9 µg/100 ml) and a rural area (9.1–11.2 µg/100 ml). The continuous high Pb deposition and Pb found in dust may be responsible for several children having excess Pb absorption and for the persisting high blood-Pb concentrations. We believe that the decrease in mean blood-Pb concentrations found in our study may be partly related to the Pb concentration in ambient air. The decrease in the blood-Pb concentrations could be caused partly by the fact that in extremely contaminated areas a peak in the blood-Pb concentrations in children has been observed. This was found in other studies. The peak was seen between the ages of three to five whereafter a decrease occurred up to 15 years of age. The peak can also extend up to 10 years of age before a decrease in blood concentrations occurs. The mean age of the children in group A in January 1986 was 9.5 years so that a decrease in the blood-Pb concentrations from the autumn of 1983 may be explained and may be related to specific sources to which the children have been exposed. Oral intake is one of these possibilities. The seasonal variations in blood-Pb concentrations detected through the correlations with the enamel-Pb content were also found by Hunter. Factors that may contribute to this phenomenon are seasonal variation of vitamin D, change of
exposure, and the dietary Ca and P intake. From our data it seems that blood-Pb related information is transferred to enamel-Pb in the pre-eruptive mineralisation phase and remains stable for at least two years. It suggests that surface enamel in these children contains historic information about the concentration of blood-Pb from the period of pre-eruptive mineralisation—that is in the case of the upper permanent central incisor, the period which starts just after birth to 6-5 years of age for both sexes. This was also suggested by Brudevold et al. The blood-Pb related information is only partial as the correlation between enamel-Pb and blood-Pb concentration is smaller than the within blood-Pb correlations.

Post-eruptive influence on the content of Pb in surface enamel may also play a part. The saliva-Pb concentrations and the 1:10 ratio with blood-Pb concentrations obtained in our study accord with the findings of Brudevold et al who also investigated schoolchildren from a community where exposure to Pb was a health hazard. Much lower Pb concentrations in saliva than in blood were also found by Brodeur et al and Fung et al. Brodeur et al showed that blood and salivary Pb respond in different ways to exposure to and removal from environmental Pb. The largest portion of absorbed Pb in blood is bound to erythrocytes. Plasma-Pb concentration seems to be in equilibrium with Pb present in erythrocytes. Salivary-Pb arises from the diffusible fraction of plasma-Pb. Each of these compartments seems, much more than total blood, to reflect recent exposure to Pb. The fact that in our study a significant correlation was only found with blood-Pb concentration for the period when the saliva samples were taken, might support the idea that salivary-Pb represents the portion of circulating Pb that is readily available for distribution to soft and hard tissues.

To discover relevant information it is often necessary to calibrate the data with respect to undesirable influencing factors other than the environmental factors under study. This was illustrated in our study for enamel-Pb concentration group B. Earlier studies indicated the influence of etch depth, age of the child, and tooth type on enamel-Pb concentrations. Blood-Pb concentrations in children may be related to age and sex. The presence of a significant negative association between Pb in saliva and the amount of enamel removed by the etch biopsy procedure may indicate a spurious correlation. It might be based, however, on a real physiological reason. Relations are assumed between surface enamel state (caries formation, surface enamel softening, de and remineralisation processes) and the oral environment in which saliva might play a major part. It may be like fluoride, steady small amounts of Pb in saliva contributing in one way or another to the protection against acid attacks of surface enamel. The inhibitory properties of Pb on caries formation have been described. Some authors tend to believe the reverse, whereas other investigators did not find any correlation between prevalence of caries and concentration of Pb in teeth.

In conclusion, our study further supports the concept that the surface enamel-Pb concentration may be a valid addition to the indicators in use for the assessment of the body burden to environmental Pb. It may add further information to that gained by blood-Pb measurements. The use of whole teeth or circumpulpal dentine is more frequently used but suffers from the disadvantage that extracted teeth from young children are not readily available. Although it appeared that saliva is not a suitable sampling medium for evaluating exposure to Pb, our study suggests that it might provide other short term information. More research is needed to further establish whether surface enamel-Pb, or saliva-Pb concentration, or both may find a place in monitoring or screening studies.

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