TRACING FETAL AND CHILDHOOD EXPOSURE TO LEAD USING ISOTOPE ANALYSIS OF DECIDUOUS TEETH

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Abstract

We report progress in using the isotopic composition and concentration of Pb in the dentine and enamel of deciduous teeth to provide a high resolution time frame of exposure to Pb during fetal development and early childhood. Isotope measurements (total Pb and $^{208}Pb/^{206}Pb$, $^{207}Pb/^{206}Pb$ ratios) were acquired by laser ablation inductively coupled mass spectrometry at contiguous 100micron intervals across thin sections of the teeth; from the outer enamel surface to the pulp cavity. Teeth samples (n=10) were selected from two cohorts of children, aged 5-8 years, living in NE England. By integrating the isotope data with histological analysis of the teeth, using the daily incremental lines in dentine, we were able to assign true estimated ages to each ablation point (first 2-3 years for molars, first 1-2 years for incisors + pre-natal growth). Significant differences were observed in the isotope composition and concentration of Pb between children, reflecting differences in the timing and sources of exposure during early childhood. Those born in 2000, after the withdrawal of leaded petrol in 1999, have the lowest dentine Pb levels (<0.2µgPb/g) with $^{208}Pb/^{206}Pb$ (mean ±2σ: 2.126-2.079) $^{208}Pb/^{206}Pb$ (mean ±2σ: 0.879-0.856) ratios that correlate very closely with modern day Western European industrial aerosols (PM_{10}, $PM_{2.5}$) suggesting that diffuse airborne pollution was probably the primary source and exposure pathway. Legacy lead, if present, is insignificant. For those born in 1997, dentine lead levels are typically higher (>0.4µgPb/g) with $^{208}Pb/^{206}Pb$ (mean ±2σ: 2.145-2.117) $^{208}Pb/^{206}Pb$ (mean ±2σ: 0.898-0.882) ratios that can be modelled as a binary mix between industrial aerosols and leaded petrol emissions. Short duration, high intensity exposure events (1-2 months) were readily identified, together with evidence that dentine provides a good proxy for childhood changes in the isotope composition of blood Pb. Our pilot study confirms that laser ablation Pb isotope analysis of deciduous teeth, when carried out in conjunction with histological analysis, permits a reconstruction of the timing, duration and source of exposure to Pb during early childhood. With further development, this approach has the potential to study larger cohorts and appraise environments where the levels of exposure to Pb are much higher.

Keywords

Lead isotopes, Laser ablation ICP-MS, Biomarkers, Deciduous teeth, Childhood exposure, Source apportionment

1 INTRODUCTION

This paper describes our progress in reconstructing detailed chronologies of pre- and post-natal childhood exposure to Pb using the stable Pb isotope composition of dentine and enamel in deciduous teeth. The primary aim was to measure age-related changes of the biomarker that shed light on sources of exo- and endogenous Pb from '*in utero*' to several years after birth.

Subsequent to the publications of Gulson and Wilson (1994), Gulson (1996) and Farmer et al. (1994) documenting Pb exposure using the isotopic composition of Pb in deciduous teeth, comparatively few, more recent studies (Grobler et al., 2000; Gulson et al., 2004; Farmer et al., 2006; Robbins et al., 2010) have addressed the issue of variation in exposure source. With the exception of Grobler et al. (2000), these have used either large (mg) sub-samples of whole tooth (dentine+enamel) or transverse sections (mm slices) of dentine-free enamel. Enamel has tended to be the preferred tissue because it develops over a relatively short period of time and ceases to form once the tooth has erupted into the oral cavity. Neither of these types of sample is optimal for resolving fine, time-scale chemical variation accompanying pre- and post-natal tooth growth. Advances in instrumental analysis, most notably laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), now permit the acquisition of elemental and isotope data at high spatial resolution (less than 100µm) without the need for sample digestion. Of those papers detailing the concentration of Pb in dental tissues by LA-ICP-MS, we refer to Arora et al. (2004, 2006, 2014); Dolphin et al. (2005); Hare et al. (2011); Humphrey et al. (2008a); Kang et al. (2004); Shepherd et al. (2012). These papers raise the interesting question "If the micro-technology exists to measure the isotopic abundance of elements in very small samples, why are there so few publications relating to the isotope composition of Pb in children's teeth?" We argue that progress has been constrained by three main issues: lowered perceptions of health risk of Pb, analytical challenges and insufficient use of dental histology for chronological sampling.

With regard to the first issue, the phasing out of leaded petrol in Western Europe in the 1980-90's and abatement in the use of leaded paints and solders, environmental levels of Pb have fallen dramatically. Pb in air, for example, has decreased from 0.31 µg/m^3 prior to 1990 to 0.045 µg/m^3 in 2007 (Bierkens et al., 2011). Over the same period, blood Pb levels in European children have continued to decline. In Sweden for example, Stromberg et al. (2008) report a decrease from 5.8µg/dL in 1978–1982, 3.4 µg/dL in 1989 to less than 1.5µg/dL in 2005 for children living in an urban environment. This has led to a perception of lowering risk about the chronic health effects of exogenous Pb on children at low levels of exposure (Lamphear, 2007). Challenging this complacency, there is now a wealth of clinical evidence that documents the irreversible damage to cognitive development (Canfield et al., 2003; Lamphear et al., 2005; Chandramouli et al., 2009) and delayed

neurodevelopment in infants (Jedrychowski et al., 2008) with blood Pb values significantly lower than 10µg/dL. Of increasing concern is the pre-natal exposure to Pb through the placental transfer of endogenous blood Pb from mother to child during pregnancy, which can result in poor birth outcomes (Hu, 2002; Xie et al., 2013). Using a combination of blood Pb concentrations and Pb isotope ratios Manton et al. (2003) and Gulson et al. (2015) have demonstrated very convincingly the importance of maternal bone restructuring during pregnancy and lactation on the release of Pb from skeletal reservoirs and its transfer to the infant. Thus the intensity, timing and duration of low level exposure to Pb, especially during the first few years of life, are factors as important now as they were before the introduction of the major public health interventions.

The second issue concerns the analytical sensitivity and precision needed to identify individual sources of environmental Pb. Meaningful application of stable Pb isotopes to exposure studies depends on there being measurable differences in the isotopic composition of a limited number of anthropogenic and/or geogenic sources (Gulson et al., 2004). In Western Europe the markedly different isotopic composition of leaded petrol compared to other anthropogenic sources made it relatively easy to calculate its contribution to total body burdens (Campbell and Delves, 1989; Delves and Campbell, 1993). However, excluding base metal mining/smelter environments, the current situation in a post-leaded petrol era is very different. Sources are often little above elevated background concentrations and broadly similar in isotopic composition (Ayrault et al., 2012). If teeth are to be routinely used as reliable biomarkers of low level exposure, there is a need for better isotope discrimination.

The third issue relates to the temporal relationship between point analyses as performed by laser ablation micro-sampling and the development history of the tooth. As demonstrated by Humphrey et al. (2008b) and Shepherd et al. (2012), to extract temporal information on trace elements in dental tissue it is essential to assign an estimated age to the point of analysis as well as having an understanding of the processes controlling their incorporation into the tooth matrix.

Our study sought to assess these issues by acquiring Pb concentration and Pb isotope data for dentine and enamel in deciduous teeth for which we could apply histological control on the timing of tissue growth. In doing so we acknowledge the constraints on interpretation imposed by the small number of samples analysed and the analytical limitations of LA-ICP-MS.

2 MATERIAL AND METHODS

2.1 Materials

We analysed 6 deciduous incisors and 4 deciduous molars donated by children living in NE England for which ethical approval had previously been granted. On completion of the original projects, the teeth were entered into the Newcastle University Faculty of Medical Sciences Biobank and anonymised according to standard ethical procedures. The Biobank records include the name of the person who collected the tissues but do not permit identification of the donor. We therefore do not have postcode data for the sample but do know the general area in which the children who donated the teeth lived at the time of collection.

The incisors were a sub-sample of a larger collection of naturally exfoliated deciduous teeth acquired during the 2005 Newcastle 'Tooth Fairy' Study; a joint project between the University of Newcastle, Public Health England and Newcastle City Council with the aim of examining the relationship between parental socio-economic status, place of residence and Pb in children's teeth as a measure of environmental Pb exposure (Hodgson et al., 2015; County Durham and Darlington Local Research Ethics Committee: ref nº. 05/00904/10). The cohort comprised 69 children, aged 5-8 years, living in the city and inner urban areas of Newcastle upon Tyne. Though little evidence now remains of the city's once industrial past, its environs carry a legacy of environmental heavy metal pollution. By contrast, the molars had been surgically extracted and were a sub-sample of 15 children, aged 6-8 years, attending a dental clinic in Billingham, Teesside, in 2009 for treatment (Shepherd et al., 2012; County Durham & Tees Valley Research Ethics Committee: ref nº. 09/H0905/42). Though we lack residential postcode data for this cohort, they are inferred to reside primarily in the immediate urban and rural areas. Prior to the present study the teeth from both cohorts had been analysed by LA-ICP-MS and provisional data were acquired for the concentration of Pb in enamel and dentine (Shepherd et al., 2012; Hodgson et al., 2015). Comparison with published data indicated that the mean dentine Pb for the Newcastle cohort (0.26±0.16µg/g; n=69) and Billingham cohort (0.18±0.07µg/g; n=15) were significantly lower than the mean value of 2.23±1.32µg/g for deciduous teeth of children living in non-polluted areas of South Africa (Grobler et al., 2000) and the overall means reported for primary school children in Taipei and Boston of 4.4±3.5µg/g and 3.3±2.5µg/g respectively (Rabinowitz et al., 1991). From these comparisons we concluded that our cohorts are representative of low exposure populations. Samples were selected from the upper quartile of teeth having the higher dentine Pb levels. For the incisors this corresponded to a range 0.18-0.95 µgPb/g (n=6); for the molars 0.05-0.22 µgPb/g (n=4), excluding a maximum outlier of 0.69µgPb/g. Time frames of childhood exposure also differed (Newcastle 1997-2005; Billingham 2001-2009) (see Table 1). This disparity, though precluding absolute time comparisons, still permits between-cohort comparisons and a critical assessment of the analytical limitations at very low blood Pb levels.

2.2 Stable Lead Isotope Analysis

Analysis was performed directly on the tooth blocks remaining from teeth that had been used in the previous studies. These had been cut parallel to the long axis of the tooth through the cusp tips and dentine horn. Samples were ultrasonically cleaned in methanol to remove all traces of surface debris, especially the removal of fine particles trapped within exposed dentine tubules. Using a 100 µm diameter laser beam, contiguous point analyses were then made from the enamel surface to the pulp cavity, following the growth orientation of the dentine tubules. Each ablation transect (one per tooth) crossed the enamel/dentine junction (EDJ), continued across the neonatal line in dentine (Birth) and terminated at the dentine/pulp junction (DPJ) (Figure 1) Unlike our earlier work (Shepherd et al., 2012) we did not polish the samples, relying instead on a low power pre-ablation lasering of the cut surface. This procedure created a smooth and flat surface for isotope analysis and resulted in fewer spikes in ion intensity during data acquisition.

Measurement of Pb isotopes utilised a Nu Instruments Attom single-collector ICP-MS coupled to a New Wave Research 193nm excimer laser ablation system. Isotopic measurement was achieved in E-scan mode (i.e. with a fixed magnet position), measuring the following masses ^{206}Pb, ^{207}Pb and ^{208}Pb, with dwell times of 700ns, 700ns and 400ns, respectively. Each datum reflects 200 sweeps of this mass range, meaning a 30 second ablation is roughly the mean of 60 values. Pre-ablation was achieved using 150µm spots ablated at low power (2-3 J/cm^2) for 10 seconds. Ablation parameters for analyses were 100µm spots, 30 seconds ablation, using 5 J/cm^2 at 10 Hz. A 10 second washout was allowed between each ablation, and the gas blank was measured for 60 seconds before every set of ca. 15 ablations.

A standard-sample bracketing routine was used for normalisation, using the reference material NIST Glass 614 (Woodhead and Hergt, 2001) for Pb isotope ratios. Normalisation of Pb concentrations (µg/g) utilised NIST 614 (Jochum et al., 2011) and the abundance of ^{208}Pb, but since the matrix of this glass is different to that of the teeth, a correction factor (0.58 for enamel and 0.54 for dentine) was calculated and applied to the concentrations. This correction factor was determined by prior analysis of teeth dentine and enamel measuring both ^{208}Pb and stochiometric ^{44}Ca, and comparing the concentrations between internally standardised data and those that were simply normalised relative to NIST. This approach, compared to internal standardisation, probably adds a small (5-10%) degree of uncertainty to the absolute concentrations, but will have negligible impact on the relative concentrations between ablations. Visual analyses of the ablation pits revealed <10% variation in ablated volume.

All four stable isotopes of lead ^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb were measured in initial tests. However, because Pb concentrations in dentine and enamel were generally <0.5 µg/g, analytical errors associated with the minor isotope ^{204}Pb were too large for variation in $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{208}Pb/^{204}Pb$ ratios to be confidently interpreted, partly due to low intensity but also to the necessity to correct for ^{204}Hg interference that is present in the argon carrier gas. The sample analyses were thus obtained without measurement of ^{204}Pb, and are discussed with reference to the more robust $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios. Uncertainties on the reported $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios include measurement uncertainty and propagation of the reproducibility of the reference material analyses as excess scatter. The reproducibility of the reference material averaged 0.36% and 0.38% (2σ) for $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$, respectively.

2.3 Histological Analysis

Detailed histological analysis of dentine was performed on three teeth (Table 2: molars HT 05-54 and HT 12-84, incisor 033-04B). Because histological sections had already been prepared for our previous analyses, the current analysis was done by mirror imaging the ablation transects on the blocks to the appropriate area on the sections themselves. Measurements made along the enamel/dentine junction (EDJ) to the ablation transect were made on the block, and then transferred to the section. A line was marked across the section representing the ablation transect and divided into intervals representing each ablation pit. The age for each interval was calculated using the daily incremental lines (von Ebner lines), using the neonatal line as point zero (birth). The ages were then combined into age categories that encompassed the maximum and minimum age range of an interval. The mean daily secretion rate (DSR) in the two molars was 3.2-3.3µm/day then dropped postnatally to between 2.4-2.7 µm/day. The incisor dentine formed at a slightly higher rate postnatally ~3µm/day. For the molars, our ablation transects provide a continuous record for the first 2-3 years of childhood and our incisors the first 1-2 years. Histological analysis after ablation also avoids the risk of including analyses which contain secondary or tertiary dentine for which there is little or no age control.

3 RESULTS

A total of 162 individual point analyses were performed on the teeth; the exact number per tooth being by determined by the diameter of the laser ablation pit (100µm) and the width (thickness) of the dentine and enamel layers along the line of the transect. Table 2 summarises the mean $^{208}Pb/^{206}Pb$, $^{207}Pb/^{206}Pb$ ratios and error statistics for each tooth. For discussion, the analyses are presented as 3 variable graphs (Figures 2a-d) where the Y' and Y'' axes correspond to the $^{208}Pb/^{206}Pb$ and Pb concentration respectively, and the X axis displays the sequence of ablation analyses (1 to n)

along the transect from the enamel surface to the pulp cavity. Additionally, in Figures 2a-c, the ablation sequence in dentine has been converted into days at two fixed points after birth, as estimated by histological analysis. Finally, to illustrate source apportionment, Figure 3 is a bivariate plot of $^{208}Pb/^{206}Pb$ -$^{207}Pb/^{206}Pb$. Error bars where shown are the 2σ uncertainty estimates and vary according to Pb concentration (i.e. a function of ion signal/background ratios). For concentrations >1 µgPb/g the relative 2σ errors for $^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$ are 0.5-0.6%. For concentrations <1 µgPb/g and >0.2 µgPb/g the errors are typically 0.6-1.0% but increase rapidly to > 2.0% close to the limit of detection (~ 0.02 µgPb/g).

Owing to the very low Pb concentrations and correspondingly high 2σ errors, the $^{208}Pb/^{206}Pb$ ratios for individual laser ablation points in enamel are not discussed but have been included in Figures 2a-d for completeness.

4 DISCUSSION

Blood Pb isotope analyses have been widely used for identifying sources of Pb exposure (Rabinowitz, 1995; Gulson et al., 1996, Gulson et al., 2006; Gwiazda and Smith, 2000; Glorennec et al., 2010). However, because blood Pb has a short half life of ~30-40 days (Barbosa et al., 2005), single samples are unsuitable for detecting changes in near recent or previous high-level, short duration exposure events. To provide an effective and complete picture of exposure requires serial blood samples, which are impractical in most circumstances. For retrospective studies therefore, LA-ICP-MS techniques applied to the analysis of teeth offer a means of reconstructing childhood exposure at high temporal resolution (weeks, months). To realise the full potential of this approach, the age of individual laser ablation points must be known.

4.1 Histology

An important conclusion to be drawn from the detailed histological analysis of the Newcastle and Billingham teeth is that the secretion of primary dentine, defined as that dentine secreted before apical closure of the tooth root (Arana-Chavez and Massa, 2004), ceases well before the age of 8 years, irrespective of whether the teeth are surgically extracted or naturally exfoliated. This observation is supported by an earlier study of 15 molars from the Billingham cohort which demonstrated that primary dentine (as estimated by daily growth increments) is a true record of the first 2-3 years of tooth development (Shepherd et al., 2012). There is slight variation in the initiation and completion of the four types of deciduous incisors and therefore the position of the neonatal line as well as slight variation between individuals in its position due to variation in gestation length. Studies reviewed by Hillson (2014) show a standard deviation of 0.07 yrs for the age at crown

completion in the lower second deciduous incisor, 0.20 for the lower first, and 0.24 for both upper incisors. The mean age at apical closure of the root occurs between 1.98 and 2.58 yrs for all deciduous incisors. We believe the data for Incisor 033-04, albeit a single sample, are in keeping with this generalised sequence of growth and that the incisors analysed in this study are a faithful record of the first 1-2 years of childhood.

4.2 Lead Concentration

For all 10 children, Pb levels are consistently higher in dentine than in enamel (Newcastle: enamel mean 0.05µg/g, dentine mean 0.26µg/g; Billingham: enamel mean 0.03µg/g, dentine mean 0.09µg/g). As noted by other researchers (Arora et al., 2006; Hare et al., 2011, Shepherd et al., 2012) this is also accompanied a distinctive step wise change in concentration at the EDJ (see Figures 2a-c). The marked difference in mean dentine Pb levels between the Newcastle and Billingham children indicates that the former were exposed to higher levels of environment lead during the first 2-3 years (1997-1999) of early childhood (see Table 1). Constrained by the small number of samples however, we are unable to test for statistically meaningful differences between pre-natal and early post-natal Pb concentrations. One of the advantages of LA-ICP-MS is the ability to screen out analyses close to the DPJ (i.e. the lead enriched 'circum-pulpal zone') without the need for the physical removal of dental tissue. When used in conjunction with histological analysis this allows for better estimation of cumulative exposure and a more informed understanding of time-averaged dentine Pb differences. With regard to cumulative exposure, dentine-only analyses are difficult to compare with analyses obtained for the whole tooth or different parts of the tooth (crown, root, enamel). However, if we assume that primary dentine Pb concentrations tend to be 16% higher than whole tooth concentrations (Grobler et al., 2000), the children of Newcastle and Billingham provide compelling evidence of a major reduction in environmental lead since the 1970-80's, in good agreement with UK blood Pb data (Delves et al., 1996). The magnitude of this reduction can be judged by the decrease in whole tooth Pb (mean 5.1µg/g) for London in the 1970's (Smith et al., 1983) compared with the 1997-99 estimated mean (0.24µg/g) for this study. Table 3 summarises some of the extensively published information for lead in modern and historical deciduous teeth. Clearly evident is the decrease in whole tooth Pb from a high level in the 1960'-70's (~5-15µg/g) to lower values in the 1980's (~2-6µg/g), culminating in a sharp drop in the 1990's (~0.25-2µg/g). These changes are directly attributable to sustained governmental measures to reduce the sale of leaded petrol and its final withdrawal from domestic markets in the USA and most of western Europe by the late 1990s. Looking further back in time, one has to return to Prehistoric periods to match values for

enamel as low as those determined for children presently living in NE England (Montgomery et al., 2010).

4.3 Lead Isotope Ratios

Unlike previous Pb isotope studies that have used carefully selected mg samples of dental tissue to identify and quantify the sources of environmental Pb, this study sought to explore the possibility of acquiring similar high precision data by laser ablation without the need for sample dissolution. By combining this approach with histological analysis we hoped to map Pb concentration-Pb isotope data onto a high resolution time frame for each tooth; thus providing an exact record of pre-natal and post-natal exposure. Overall the results are encouraging but due to statistical constraints we have been unable to demonstrate differences between exposure to endogenous Pb during fetal development and exogenous Pb after birth. The problem is a consequence of low dentine Pb concentrations. For concentrations <0.5µg/g the calculated 2σ errors are too high to allow statistical discrimination between points along a transect. As demonstrated in Figure 2a there is total overlap of the 2σ error bars. At higher dentine Pb levels (>0.5µg/g) the error bars are significantly smaller (Figure 2d) and we confidently predict that LA-ICP-MS performed on teeth from higher exposure cohorts, would have the sensitivity and precision comparable to that afforded by whole tissue analysis. Two important outcomes of our study are shown in Figures 2a and 2b. Firstly, the methodology is capable of capturing short, high intensity exposure events. In Figure 2a the 2µgPb/g peaks at ablation points 11 and 12 (an approximately 2 month interval) are not accompanied by a change in $^{208}Pb/^{206}Pb$ ratios. This indicates that the source of Pb to which the child was exposed did not change significantly throughout early childhood but was temporally high at about 200 days after birth. This was an event specific to that child. The second outcome relates to the use of circum-pulpal dentine (i.e. immature dentine). Whereas total Pb concentrations rise exponentially on approaching the DPJ making it difficult to draw comparisons with mean dentine concentrations (Shepherd et al., 2012), the related isotope signals are unaffected by dentine maturity (Figure 2b). This observation was noted for all 10 teeth. Assuming no change in the source of lead, circum-pulpal dentine affords a reliable isotope proxy for blood Pb at the cessation of primary dentine secretion.

Over and above the limitations imposed by 2σ errors, there appear patterns and changes in lead isotope composition with age that invite comment; albeit very speculative. For example, all 4 molars as typified by HT 05-54 (Figure 2a) including incisors 040-07, 067-23 and 053-23 (Figure 2d) display mean $^{208}Pb/^{206}Pb$ ratios for individual laser ablation points in both pre-natal and post-natal dentine that occupy a relatively narrow range of $^{208}Pb/^{206}Pb$ ratios (~2.12-2.09). Since the principal source of cross-placental Pb is linked to the restructuring of the mother's bones (Gulson et al., 1999, 2015;

Chuang, 2001; Hu et al., 2002; Tellez-Rojo, 2004) we tentatively suggest this may be an indication that mother and child were exposed to the same ambient source of environmental lead for an extended period of time. In contrast, the 3 other incisors (033-04B, 009-09, 006-15) display a different age-related pattern. From birth, the $^{208}Pb/^{206}Pb$ ratios appear to increase steadily from ~2.12 at birth to peak between 2.16 and 2.18 during the first year of tooth development, before decreasing again to ~2.12 at the DPJ. This pattern is illustrated by incisor 033-04B (Figure 2b). Another feature that cannot readily be described as a random analytical artefact is the apparent decrease in $^{208}Pb/^{206}Pb$ ratios 100-200 days after birth as shown in Figure 2a. This pattern is very similar to that shown by molar HT 12-84 (Figure 2c) and whilst we cannot validate this apparent decrease due to overlapping 2σ errors, the similarity shown by 2 of 4 molars poses the possibility 'Is there an underlying process' worthy of further investigation.

We wish to emphasise that the above comments, based on the mean dentine values at each ablation point, are speculative but draw attention to the potential application of higher precision laser ablation studies. One way of increasing precision would be to use a larger diameter laser beam, thereby increasing the signal/background ratio but only at the expense of poorer age resolution. Another way would be to use laser ablation coupled to a multicollector ICP-MS. It comes down to a trade off between precision (typically <0.1%) obtained for the analysis of solutions prepared by the dissolution of dental tissue by thermal ionization mass spectrometry or multicollector ICP-MS (Kamenov and Gulson, 2014), and a lower precision (>0.2%) obtained by single collector LA-ICP-MS (this study) for detailed time-scale chronologies.

4.4 Source Apportionment

Environmental Pb is a multi-component mix of discrete sources some of which may have greater or lesser numerical control on the bulk isotopic composition. To deconvolute the individual sources requires high precision analyses and a reference database of the most likely sources. Ideally this is undertaken using all four Pb isotopes; expressed conventionally as $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{206}Pb/^{204}Pb$ ratios. This affords a reliable test for multiple sources and mixing relationships (Ellam, 2010). Without the minor ^{204}Pb isotope, the resultant three isotopes, and ratios $^{208}Pb/^{206}Pb$, $^{207}Pb/^{206}Pb$, can only used to model binary mixing with isotopic fields distributed along linear arrays. This is the case for our study. Nevertheless, as discussed below, one can eliminate possible sources and conclude 'best fit' scenarios. Figure 3 brings together data for the Newcastle and Billingham children, data for the most likely sources of environmental Pb and relevant historical data.

From this simple bivariate graph, it can be seen that the Newcastle and Billingham teeth occupy different isotopic fields. Of possible anthropogenic sources, coal is unlikely to account for the

observed range of dentine values. For more than 150 years, NE England has been an important coal mining region and until recently, mines supplied several local coal-fired power stations. Since 2000, UK coal production has declined dramatically and more than 50% of the coal now used is imported from Columbia, USA, Australia and Russia (Kerai, 2013). In general, both local and imported coals have $^{208}Pb/^{206}Pb$ ratios <2.08 and $^{207}Pb/^{206}Pb$ ratios <0.85 (Shepherd et al., 2009; Farmer et al., 1999; Diaz-Somoano et al., 2007).

All 4 Billingham molars (including 3 Newcastle incisors) plot within or strongly overlap the isotopic domain for UK and western European airborne particulates; sometimes referred to as 'diffuse pollution'. Following the withdrawal of leaded petrol, the importance of industrially generated air borne particulates as a source of Pb has been documented by several European studies. National statistics compiled by MacCarthy et al. (2012) indicate that from 2001-2003 industrial processes and industrial combustion accounted for >80% of total lead emissions with transport emissions reduced to <2%. In France, industrial aerosols sampled in 2004 had $^{208}Pb/^{206}Pb$, $^{207}Pb/^{206}Pb$ ratios of 2.112-2.093 and 0.874-0.858 respectively (Widory et al., 2004). A slightly wider range ($^{208}Pb/^{206}Pb$ 2.125-2.106, $^{207}Pb/^{206}Pb$ 0.885-0.866) was reported by Bollhofer et al. (2001) for 1994-1998; the higher values corresponding to a tail-off in the use of leaded petrol. Whilst in central London (2000-2001), Noble et al. (2008) found values ($^{208}Pb/^{206}Pb$ 2.123-2.109, $^{207}Pb/^{206}Pb$ 0.881-0.868) for airborne particulates indistinguishable from those in western Europe. We contend therefore that the similarity between airborne particulates and the mean (±2σ) range of $^{208}Pb/^{206}Pb$ ratios (2.126-2.080) and $^{207}Pb/^{206}Pb$ ratios (0.879-0.856) for the Billingham molars suggests that regional diffuse pollution was probably the principal source of Pb to which this cohort was exposed.

By contrast, analyses for the Newcastle incisors are distributed along an array projecting from the field for airborne particulates to the field for UK leaded petrol (Sugden et al., 1993; Monna et al., 1997). In the absence of significant contributions of Pb from other sources, we believe the analyses lie on a mixing line between industrial aerosols and leaded petrol; the cluster having the higher $^{208}Pb/^{206}Pb$, $^{208}Pb/^{206}Pb$ ratios corresponding to a petrol Pb component of 30-45%. Because early childhood for this cohort (1997-99) corresponds to the final sales of leaded petrol in the UK, we are unable to say whether this is an environmental legacy of leaded petrol (MacKinnon et al., 2011) or concurrent exposure to leaded petrol emissions. Whichever pathway applies, the evidence implies exposure to a significant component of petrol Pb within the children's residential environment. The variability shown by the Newcastle cohort (a subset of the Tooth Fairy study) is in keeping with the conclusions of Hodgson et al. (2015) which drew attention to the wide range of differing exposure levels and/or exposure sources across this population.

For children exposed to environmental Pb in the 1980's, contemporary blood Pb data provide a useful comparison. Delves and Campbell (1993) report a mean blood Pb $^{207}Pb/^{206}Pb$ ratio of 0.889 for children living in inner London (1981-82). This value, as seen in Figure 3, falls within the upper range for the Newcastle cohort and which, according to their modelling, equates to a petrol contribution of 30% to total blood Pb; a percentage in good agreement with our own estimate. From a greater historical perspective, analyses of human dental enamel (1-19thcen AD) define a very narrow range of $^{207}Pb/^{206}Pb$ ratios (0.855-0.840) consistent with exposure to lead derived almost exclusively from indigenous UK lead ores (Farmer et al., 2006; Millard et al., 2014; Montgomery et al., 2010; Rohl, 1996). These low ratios then persist until the introduction of leaded petrol in the mid 20th century.

Lacking data for the most common sources of Pb within the home environment (household dust, diet, drinking water) and limited by the small number of samples included in this pilot study, we are unable to critically assess possible exposure pathways. However, given that the isotopic ratios for both cohorts can be linked to domains characterised by airborne Pb emissions, it is highly likely that inhalation and/or ingestion of particulates is a major factor in determining the concentration and isotopic composition of Pb in the dentine of their deciduous teeth. This conclusion is consistent with the extensive study of lead in modern teeth from Europe, North and South America, and Australia carried out by Kamenov and Gulson (2014) which demonstrated an unequivocal isotopic link between tooth enamel and leaded petrol emissions. Together, these two studies reinforce the view that inhalation and ingestion of airborne particulates constitute exposure pathways equal to, if not more important than traditional pathways such as diet and drinking water.

5. Conclusions

Our study successfully demonstrates that the LA-ICP-MS analysis of deciduous teeth when combined with dental histology constitutes a powerful tool for acquiring information on the intensity and fine scale chronology (1-2 monthly intervals) of pre-natal and early life exposure to Pb. Dentine is the preferred tissue, having a regular and measurable rate of secretion allowing age-related isotope ratio measurements to be made from '*in utero*' to the cessation of primary dentine growth depending upon the precision and degree of discrimination required. Short duration-high intensity exposure events can easily be identified and assessed with respect to possible changes in the source of lead. The study also confirms that dentine maturation has no influence on the isotopic composition of Pb and that $^{208}Pb/^{206}Pb$, $^{207}Pb/^{206}Pb$ measurements provide robust proxies for blood Pb. Problems we encountered are not considered an intrinsic weakness of the methodology; simply

a consequence of the analysis of teeth having very low concentrations of Pb. For children exposed to higher levels of environmental Pb, the analytical errors will be correspondingly less, thus giving greater confidence to the interpretation of changes during the fetal and early years of childhood. Whilst unable to prove isotopic differences between pre- and post-natal dentine for the same child, there are significant 'between-cohort' differences in the source apportionment of Pb. The post-2001 Billingham cohort have an isotope signature consistent with exposure to industrially generated airborne particulates whereas the earlier post-1997 Newcastle cohort are characterised by exposure to pollution comprising a mix of leaded petrol emissions and industrial particulates. Lacking evidence for a petrol lead component in the dentine of children from Billingham, we conclude that 'legacy lead', if present, is very minor. Extending this methodology to larger cohort studies will depend on improvements in the ease of laser ablation (e.g. software programmable ablation) and developing a protocol that simplifies the histological analysis. Both lines of research are currently under investigation. The importance and value of a high resolution time frame cannot be understated. It allows for better assessment of the association between dentine Pb levels, residential, dietary and lifestyle characteristics, and in doing so focuses attention on the most likely exposure pathways.

ACKNOWLEDGMENTS

We wish to thank Dr Charuwan Manwee for allowing use of teeth collected as part of her PhD study of children living in Billingham, Northeast England. Publication for NMWR is by permission of the Director, British Geological Survey, NERC.

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TABLES

Table 1. Timeframe of exposure for Newcastle and Billingham cohorts with reference to UK leaded petrol

Table 2. Summary statistics for isotope measurements in enamel and dentine

Table 3. Modern and historical changes in lead concentrations in dentine, enamel and whole teeth

FIGURES

Figure 1.

Illustrative cross section through a deciduous molar showing the major tissue compartments and histological features referred to in the text. Key: E enamel; D dentine; PC pulp cavity; EDJ enamel-dentine junction; DPJ dentine-pulp junction; NLe neonatal line in enamel. Note: The corresponding neonatal line in dentine, though less prominent than the NLe in this specimen, generally follows the contours of the EDJ some 10 to several hundred microns beneath the EDJ.

Figures 2a-d.

Graphs showing co-variation in $^{208}Pb/^{206}Pb$ and Pb concentration (µg/g). Ablation sequence 1-n along the x axis. Vertical grey lines are the $^{208}Pb/^{206}Pb$ 2σ error bars. Filled circles $^{208}Pb/^{206}Pb$ ratios in dentine, open circles in enamel. Filled squares Pb concentrations (µg/g) in dentine, open squares in enamel.

2a. Molar HT 05-54 Billingham cohort. Enamel/Dentine junction is between points 4 and 5.

2b. Incisor 033-04B Newcastle cohort. Enamel/Dentine junction is between points 2 and 3.

2c. Molar HT 12-84 Billingham cohort. Enamel/Dentine junction is between points 6 and 7.

2d. Incisor 053-23 Newcastle cohort. Enamel/Dentine junction is between points 1 and 2.

Figure 3.

Source apportionment graph ($^{208}Pb/^{206}Pb$ - $^{207}Pb/^{206}Pb$) showing the relationship between deciduous tooth dentine and major sources of UK environmental lead.
Solid filled diamonds - Newcastle cohort (incisors). Open circles - Billingham cohort (molars). Light grey errors bars are the 2σ uncertainty limits. Lead ores - indigenous lead ores from the north of England Pennine orefields (Shepherd et al., 2009; Rohl, 1996). Coal – local and imported coal (Shepherd et al., 2009; Diaz-Somoano et al., 2007). Range $^{207}Pb/^{206}Pb$ for historic tooth enamel (Montgomery et al., 2010). Range $^{207}Pb/^{206}Pb$ for blood Pb for children living in inner London from 1981-82 (Delves and Campbell, 1993).

Table

		Newcastle	Billingham
1997	1	D.O.B.	
1998			
1999	2		
2000	3		
2001			D.O.B.
2002			
2003			
2004			
2005		Exfoliated	
2006			
2007			
2008			
2009			Extracted

1. From 1990 onwards sales of leaded petrol decreased
2. Leaded petrol withdrawn from UK market in 1999 but not fully banned until mid 2000
3. 1990 to 2000 UK traffic-related atmospheric lead emissions decreased by >99% (MacCarthy et al., 2012)

D.O.B. Date of birth

Exfoliated Deciduous incisors naturally exfoliated

Extracted Deciduous molars surgically extracted

Table 1 Timeframe of exposure for Newcastle and Billingham cohorts with reference to UK leaded petrol

Table

Cohort	Tooth		Enamel $^{207}Pb/^{206}Pb$ mean	Enamel 2σ %	Enamel $^{208}Pb/^{206}Pb$ mean	Enamel 2σ %	Dentine $^{207}Pb/^{206}Pb$ mean	Dentine 2σ %	Dentine $^{208}Pb/^{206}Pb$ mean	Dentine 2σ %
Newcastle	033-04B	I	0.880	1.9	2.096	2.0	0.895	1.0	2.142	1.0
Newcastle	040-07	I	0.871	1.7	2.107	1.5	0.874	0.8	2.115	0.8
Newcastle	009-09	I	0.895	1.5	2.145	1.6	0.900	1.0	2.148	1.0
Newcastle	053-23	I	0.866	1.5	2.097	1.6	0.871	0.6	2.108	0.6
Newcastle	006-15	I	0.875	1.4	2.131	1.3	0.891	0.7	2.138	0.8
Newcastle	067-23	I	0.873	1.5	2.094	1.6	0.865	0.8	2.107	0.8
Billingham	HT-14-74	M	0.875	2.1	2.102	1.8	0.867	1.5	2.103	1.4
Billingham	HT-12-84	M	0.874	2.4	2.099	2.2	0.870	1.6	2.108	1.5
Billingham	HT-13-84	M	0.865	1.7	2.109	1.7	0.868	1.0	2.096	0.8
Billingham	HT-05-54	M	0.859	3.1	2.087	2.5	0.865	1.2	2.094	1.2

I Incisor M Molar 2σ % 2 sigma errors

Table 2 Summary statistics for isotope ratio measurements in enamel and dentine

Table

Location	Childhood Exposure	Dentine mean Pb (µg/g)	Enamel mean Pb (µg/g)	Whole tooth mean Pb (µg/g)	Ref
Boston, USA	1960's	16.9		*14.6*	1
Iceland (rural)	1960's	5.4		*4.6*	1
Boston, USA	early 1970's	12.7		*10.9*	2
London, UK	1970s			5.1	3
Dusseldorf, Germany	1970's			6.2	4
Edinburgh, Scotland	late 1970's			9.3	5
Norway (urban)	1970's			3.8	6
Sassuolo, Italy	early 1980's			6.1	7
Boston, USA	1980's	2.8		*2.4*	8
Broken Hill, Australia	late 1980's		1.2**		9
Germany	1980's			2.1	10
Edinburgh, Scotland	1980's			3.2	11
Wupperthal, RSA (rural)	1990's	2.2	0.33	*1.9*	12
Norway	1990's			1.6	13
Broken Hill, Australia	late 1990's	0.39[b]**	0.11[b]**	*0.33*	14
Mexico City, Mexico	late 1990's	0.27[b*]		*0.25*	15
England, UK	1997-1999[a]	0.26	0.05	*0.24*	16
England, UK	2001-2003[a]	0.09	0.03	*0.08*	16
Britain	14-15th cen		4.69*		17
Britain	1-11th cen		1.78*		17
Britain	Prehistoric		0.07*		17

* median values; ** low exposure population; [a] first 3years of childhood; [b] post-natal; Values in italics calculated as [dentine x 0.86] after Grobler et al. 2000

1 Shapiro et al. 1973; 2 Needleman et al. 1979; 3 Smith et al. 1983; 4 Winneke et al. 1983; 5 Fulton et al. 1989; 6 Fosse and Justesen 1978; 7 Bergomi et al. 1989; 8 Rabinowitz et al. 1989; 9 Gulson 1996; 10 Begerow et al. 1994; 11 Farmer et al. 1994; 12 Grobler et al. 2000; 13 Tvinnereim et al. 1997; 14 Arora et al. 2006; 15 Arora et al. 2014; 16 This study; 17 Montgomery et al. 2010.

Table 3 Modern and historical changes in lead concentration in dentine, enamel and whole teeth

Figure

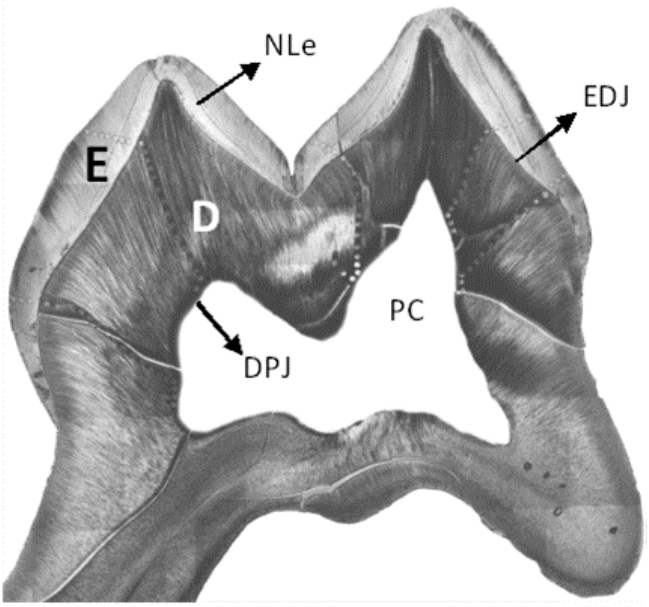

Figure 1. Illustrative cross section through a deciduous molar showing the major tissue compartments and histological features referred to in the text. Key: E enamel; D dentine; PC pulp cavity; EDJ enamel-dentine junction; DPJ dentine-pulp junction; NLe neonatal line in enamel. Note: The corresponding neonatal line in dentine, though less prominent than the NLe in this specimen, generally follows the contours of the EDJ some 10 to several hundred microns beneath the EDJ.

[Graph is a Picture GIF file]

Figure

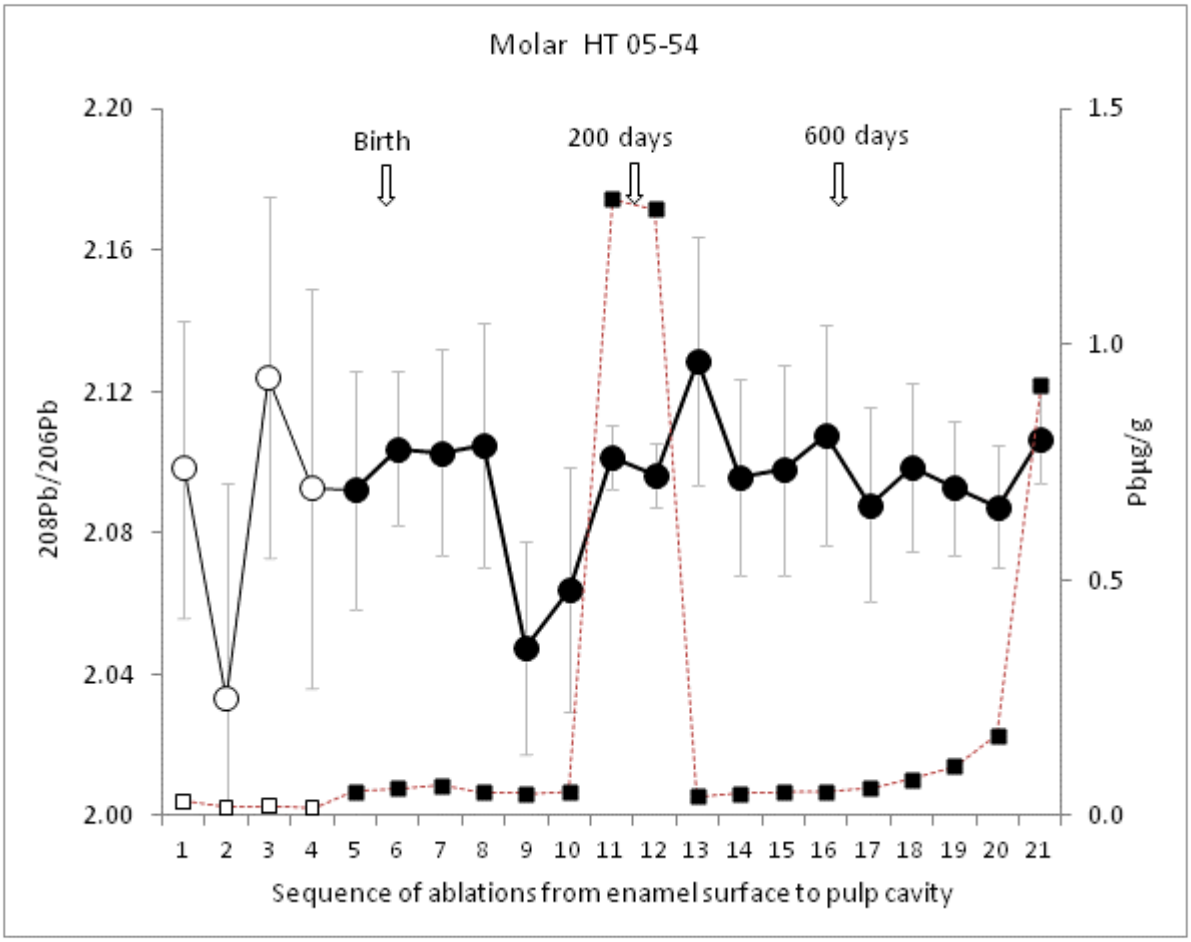

Figure 2a. Molar HT 05-54 Billingham cohort. Enamel/Dentine junction is between points 4 and 5.

Figures 2a-d.

Graphs showing co-variation in $^{208}Pb/^{206}Pb$ and Pb concentration (µg/g). Ablation sequence 1-n along the x axis. Vertical grey lines are the $^{208}Pb/^{206}Pb$ 2σ error bars. Filled circles $^{208}Pb/^{206}Pb$ ratios in dentine, open circles in enamel. Filled squares Pb concentrations (µg/g) in dentine, open squares in enamel.

Figure

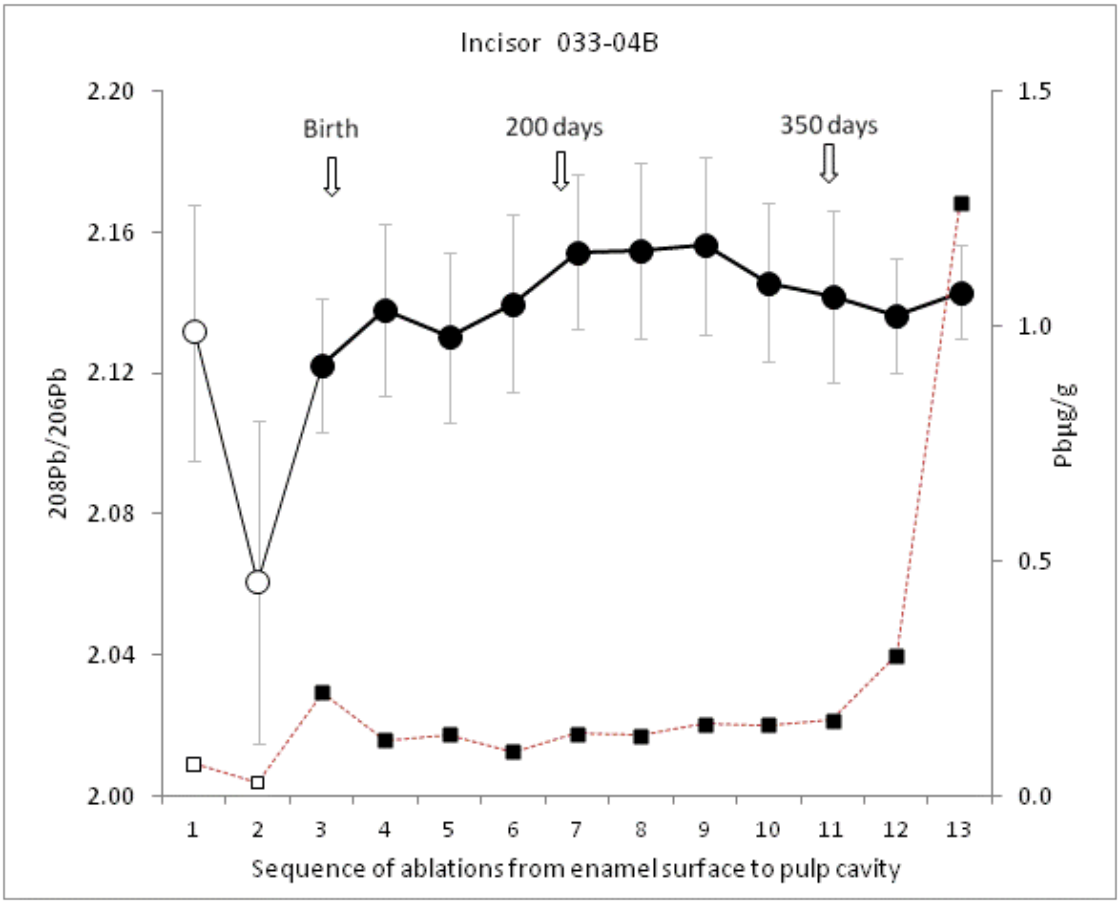

Figure 2b. Incisor 033-04B Newcastle cohort. Enamel/Dentine junction is between points 2 and 3.

Figures 2a-d.

Graphs showing co-variation in $^{208}Pb/^{206}Pb$ and Pb concentration (µg/g). Ablation sequence 1-n along the x axis. Vertical grey lines are the $^{208}Pb/^{206}Pb$ 2σ error bars. Filled circles $^{208}Pb/^{206}Pb$ ratios in dentine, open circles in enamel. Filled squares Pb concentrations (µg/g) in dentine, open squares in enamel.

Figure

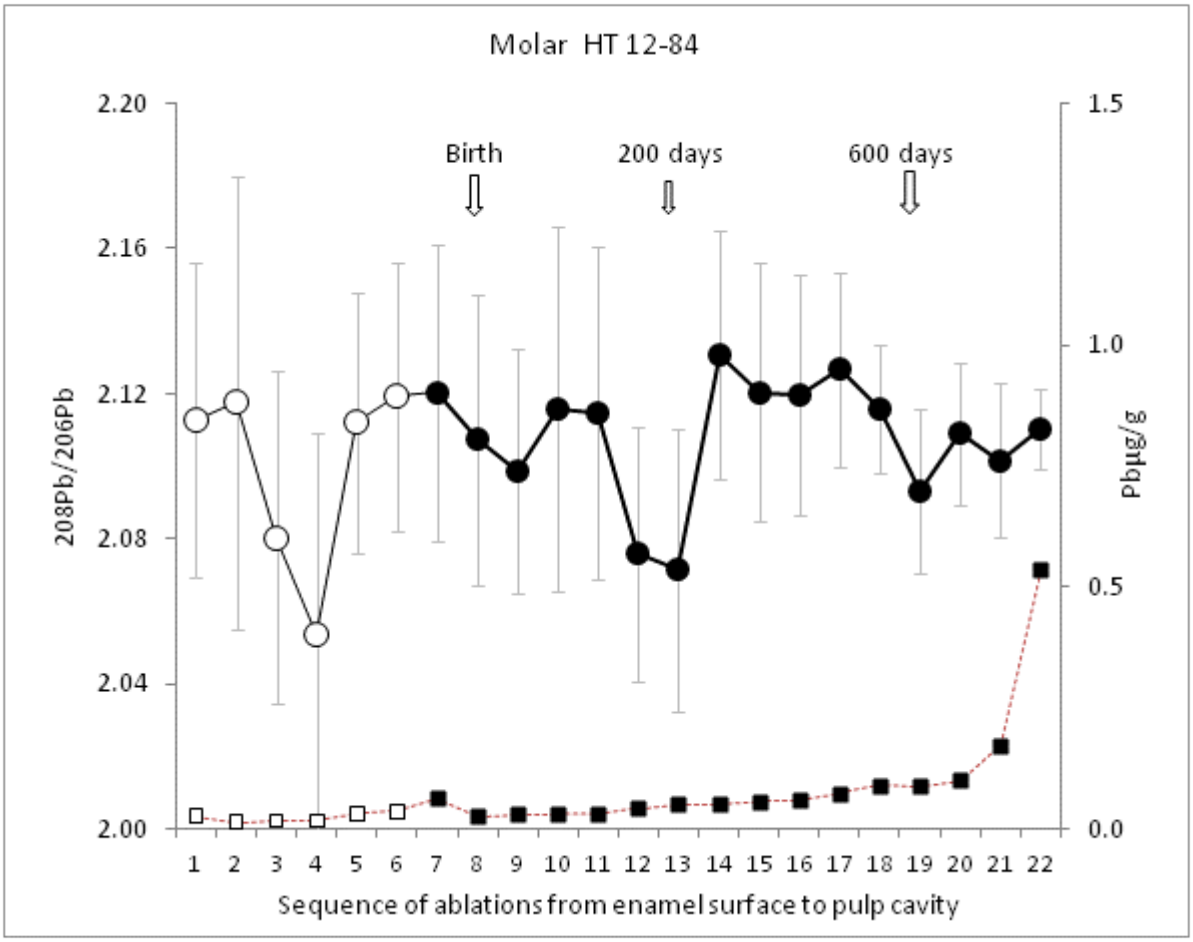

Figure 2c. Molar HT 12-84 Billingham cohort. Enamel/Dentine junction is between points 6 and 7.

Figures 2a-d.

Graphs showing co-variation in $^{208}Pb/^{206}Pb$ and Pb concentration (µg/g). Ablation sequence 1-n along the x axis. Vertical grey lines are the $^{208}Pb/^{206}Pb$ 2σ error bars. Filled circles $^{208}Pb/^{206}Pb$ ratios in dentine, open circles in enamel. Filled squares Pb concentrations (µg/g) in dentine, open squares in enamel.

Figure

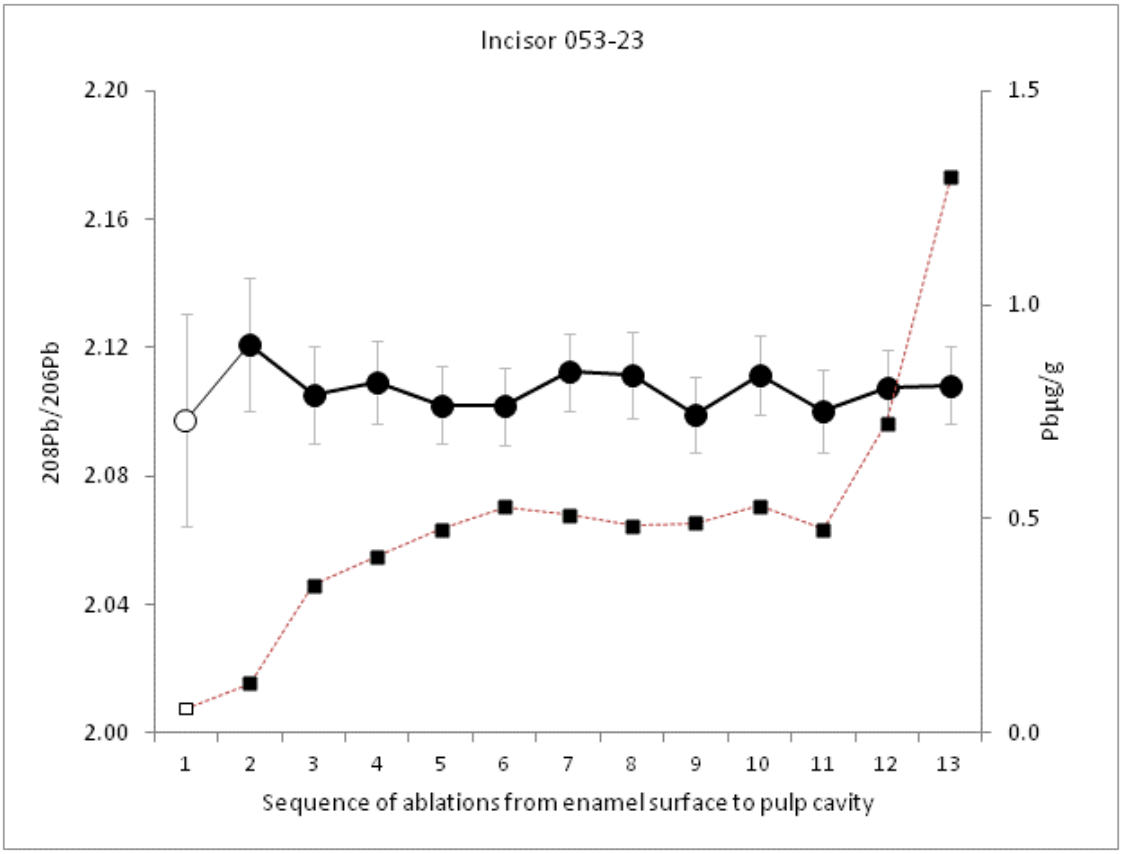

Figure 2d. Incisor 053-23 Newcastle cohort. Enamel/Dentine junction is between points 1 and 2. [Graph is a Picture GIF file]

Figures 2a-d.

Graphs showing co-variation in $^{208}Pb/^{206}Pb$ and Pb concentration (µg/g). Ablation sequence 1-n along the x axis. Vertical grey lines are the $^{208}Pb/^{206}Pb$ 2σ error bars. Filled circles $^{208}Pb/^{206}Pb$ ratios in dentine, open circles in enamel. Filled squares Pb concentrations (µg/g) in dentine, open squares in enamel.

Figure

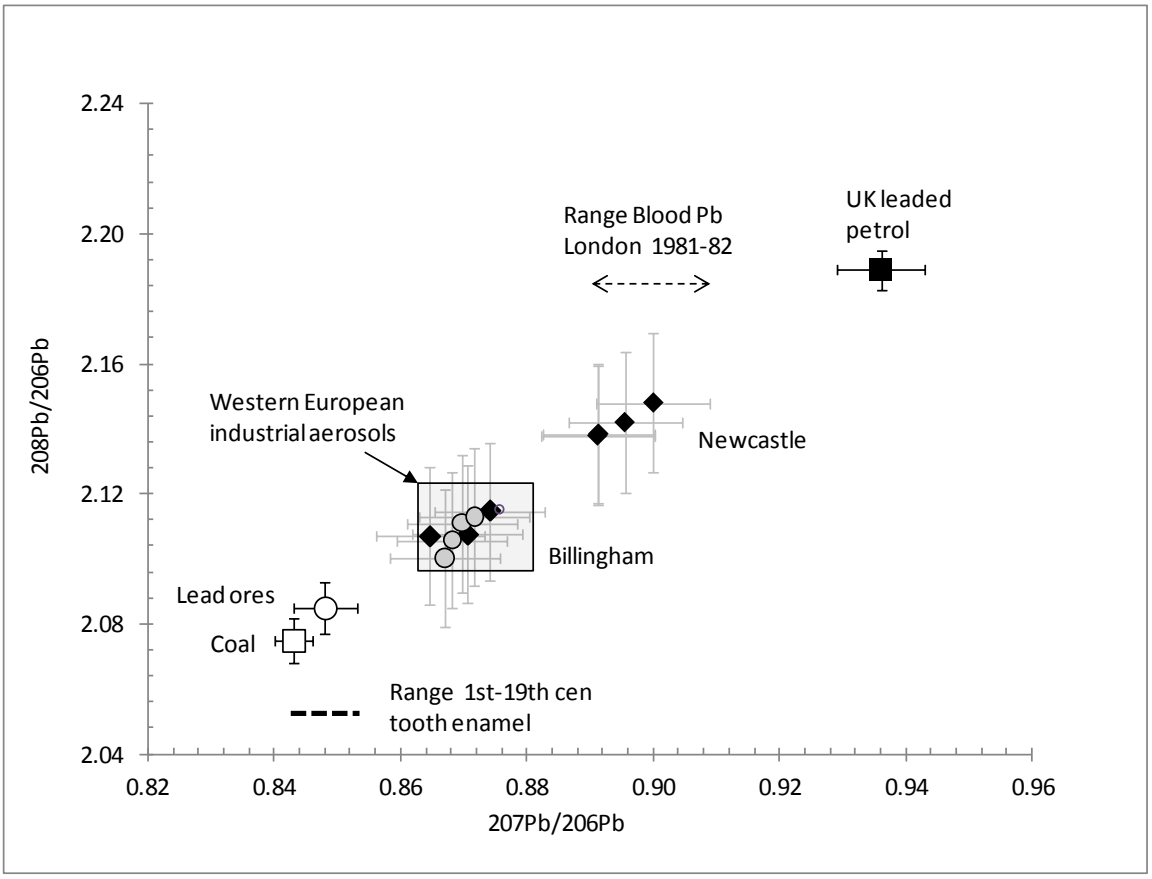

Figure 3. Source apportionment graph showing the relationship between deciduous tooth dentine and major sources of UK environmental lead.
Solid filled diamonds - Newcastle cohort (incisors). Open circles - Billingham cohort (molars). Light grey errors bars are the 2σ uncertainty limits. Lead ores - indigenous lead ores from the north of England Pennine orefields (Shepherd et al. 2009; Rohl 1996). Coal – local and imported coal (Shepherd et al. 2009; Diaz-Somoano et al. 2007). Range $^{207}Pb/^{206}Pb$ for historic tooth enamel (Montgomery et al. 2010). Range $^{207}Pb/^{206}Pb$ blood Pb - children living in inner London from 1981-82 (Delves and Campbell 1983).

[Graph is a Picture Windows metafile] preferably as a 2 Column Figure