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Lead in teeth from lead-dosed goats: Microdistribution and relationship to the cumulative lead dose

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Abstract

Teeth are commonly used as a biomarker of long-term lead exposure. There appear to be few data, however, on the content or distribution of lead in teeth where data on specific lead intake (dose) are also available. This study describes the analysis of a convenience sample of teeth from animals that were dosed with lead for other purposes, i.e., a proficiency testing program for blood lead. Lead concentration of whole teeth obtained from 23 animals, as determined by atomic absorption spectrometry, varied from 0.6 to 80 $\mu\text{g g}^{-1}$. Linear regression of whole tooth lead ($\mu\text{g g}^{-1}$) on the cumulative lead dose received by the animal (g) yielded a slope of 1.2, with $r^2 = 0.647$ ($p < 0.0001$). Laser ablation inductively coupled plasma mass spectrometry was employed to determine lead content at micrometer scale spatial resolution in the teeth of seven goats representing the dosing range. Highly localized concentrations of lead, ranging from about 10 to 2000 $\mu\text{g g}^{-1}$, were found in circumpulpal dentine. Linear regression of circumpulpal lead ($\mu\text{g g}^{-1}$) on cumulative lead dose (g) yielded a slope of 23 with $r^2 = 0.961$ ($p = 0.0001$). The data indicated that whole tooth lead, and especially circumpulpal lead, of dosed goats increased linearly with cumulative lead exposure. These data suggest that circumpulpal dentine is a better biomarker of cumulative lead exposure than is whole tooth lead, at least for lead-dosed goats.

Keywords

Teeth; Lead; Laser ablation; Lead exposure; *Capra hircus*

1. Introduction

The use of teeth as a biomarker of lead exposure rose to prominence through to the work of Needleman et al. (1972), who later demonstrated a relationship between tooth lead concentration and classroom performance of children (Needleman et al., 1979). Recent studies suggest that even relatively low levels of lead exposure, measured as blood lead concentrations below 10 $\mu\text{g dL}^{-1}$, are associated with adverse health effects in children

(Lanphear et al., 2005). Monitoring exposure to lead thus remains important in the environmental health field.

In contrast to blood or urine lead measurements, which reflect recent exposure, teeth, and bone provide an indication of long-term or past exposure (Graziano, 1994), since lead accumulates in the hydroxyapatite matrix of teeth or bone. Measurement of bone lead for the purpose of exposure assessment has gained popularity due to the development of the non-invasive *in vivo* techniques, L-shell or K-shell X-ray fluorescence spectrometry (Todd and Chettle, 1994). The method detection limit of around $5 \mu\text{g g}^{-1}$ limits quantitative determinations of low-level exposure, and the necessity for the subject to remain motionless for 30 min makes the technique better suited to adults than to pediatric subjects.

Deciduous teeth are convenient samples that are readily available from pediatric subjects. It was recognized at an early stage that while useful information can be obtained from measurement of the whole tooth lead content (Needleman et al., 1972), more refined information can be obtained by focusing on specific structures within the tooth (Shapiro et al., 1973). Several workers have favored dentine or circumpulpal dentine (Shapiro et al., 1973; Grobler et al., 1985; Gulson, 1996). Circumpulpal dentine is the tissue that surrounds the pulp cavity of the tooth. It can be remodeled over time (secondary dentine) and is directly supplied with lead from the blood-filled, highly vascularized pulp cavity. Some studies have reported lead concentrations in circumpulpal dentine that are 10 times higher than those found in other tooth areas (Shapiro et al., 1973; Grobler et al., 1985).

Other tooth areas that have received attention include the enamel, which is fixed early in tooth growth and may thus represent lead exposure at that time. Several studies have examined enamel in deciduous teeth that is located on either side of the neonatal line, to contrast *in utero* exposure with newborn exposure (Lee et al., 1999; Lochner et al., 1999; Dolphin et al., 2005). Enamel has also been examined for archaeological purposes (Budd et al., 1998). A significant number of workers have applied laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for *in situ* analysis with spatial resolution on the micrometer scale (Evans et al., 1995; Cox et al., 1996; Uryu et al., 2003). In comparison with techniques requiring physical separation, LA-ICP-MS requires less extensive sample preparation and offers improved spatial resolution. Analysis of small tooth fragments typically requires specialized handling and measurement procedures (Grünke et al., 1996). LA-ICP-MS is minimally destructive, allowing samples to be retained for further studies. In addition to lead, other trace metals such as zinc and strontium have been examined in teeth, in particular for the purpose of providing information on dietary intake (Kang et al., 2004; Dolphin et al., 2005).

Despite this quantity of work, there appear to be few data on the content or distribution of lead in teeth where data on specific lead intake (dose) are available. Since the 1970s, the New York State Department of Health (NYSDOH) has maintained a herd of goats that are dosed with variable levels of lead for the primary purpose of creating blood pools for the NYSDOH proficiency testing (PT) program for blood lead measurements. Details regarding the organization of the PT program for blood lead have been described elsewhere (Parsons, 1992; Parsons et al., 2001). Briefly, lead is administered orally as lead acetate, several weeks prior to a test event. Typically, three test events are performed in a calendar year, as required for PT programs approved under the Clinical Laboratory Improvement Amendments of 1988 (CLIA' 88). Animals are used in this program until they reach the end of their working lives, after which they are euthanized under veterinary supervision. Following euthanasia, major organs, long bones, and teeth are harvested for research studies.

This study describes the analysis of a convenience sample of teeth derived from animals that were dosed with lead for other purposes, i.e., the NYS PT program for blood lead. The purpose was to compare the lead content in whole teeth, and in individual tooth structures, to the dose of lead administered. Specifically, we wished to identify and validate the most appropriate tooth tissue for use in exposure assessment studies. To our knowledge, this is the first study of the relationship between tooth lead and lead dose in animals with a known lead dosage administered over several years.

2. Materials and methods

2.1. Lead dosing

The NYSDOH's Wadsworth Center has maintained a herd of goats (*Capra hircus*) for its blood lead PT program since the 1970s. The animals are periodically dosed with sub-toxic amounts of lead acetate ((CH₃COO)₂Pb), to provide a source of blood containing elevated levels of physiologically bound lead. All experimental work was conducted in accordance with an active protocol (number 01-096) approved by the Wadsworth Center's Institutional Animal Care and Use Committee (IACUC). The dosage and the timing of dosing were determined by the requirements of the PT program rather than specifically for the present study. About 1 g of lead acetate from commercial sources was packed into a gelatin capsule and administered orally. Daily doses did not exceed 60 mg kg⁻¹, or 4.2 g for a typical 70 kg goat, and were typically 1–2 g of lead per animal. Dosing was performed periodically throughout the year and sometimes on consecutive days. The maximum, cumulative lifetime dose received by a particular animal was 92.5 g lead acetate, equivalent to 50.5 g lead, over a 10-year period. A number of animals in the herd were not dosed with lead and thus served as undosed controls.

2.2. Sample collection

Teeth have been collected *post mortem* since 1994. Lower incisors (goats lack upper incisors) were removed *post mortem* due to death from natural causes or veterinarian recommended euthanasia. Up to eight teeth were obtained per subject. Molars were not collected, since these are excessively worn through rumination. The lower jaw section containing incisors was stored at –80 °C. The samples were thawed, and teeth were removed from the jaw using disposable surgical scalpels. Further cleaning was conducted with the scalpel, and through immersion in 30% (m/v) hydrogen peroxide solution (J.T. Baker; Phillipsburg, NJ). The cleaned teeth were thoroughly rinsed with deionized water (18.2 MΩ cm, MilliQ; Millipore; Billerica, MA) and then freeze dried to remove any residual moisture. The teeth were subsequently stored in cleaned containers at room temperature.

2.3. ETAAS analysis

Twenty-seven secondary incisor teeth from 23 animals (i.e., four duplicates) were selected from the tooth repository. Twenty-two of these animals had been dosed with different amounts of lead and one was undosed. Typical tooth weight was 0.4±0.1 g. Samples were digested with 5mL high-purity concentrated nitric acid (Ultrex Ultrapure Reagent; J.T. Baker, Phillipsburg, NJ) in 50-mL tubes (Sarstedt, Newton, NC) with moderate heating in a microwave digestion system (MDS 2100; CEM, Matthews, NC) at atmospheric pressure and subsequently diluted to 20 mL with deionized water. The samples were analyzed for lead content using an electrothermal (graphite furnace) atomic absorption spectrometer (ETAAS) (Z5100, Perkin-Elmer, Shelton, CT), via a method developed by Zong et al. (1996) for determination of lead in bone. Samples were diluted either 1 + 9 or 1 + 19 with a modifier solution containing 2 g L⁻¹ NH₄H₂PO₄, and were analyzed using duplicate injections. The effective method detection limit (i.e., three times the standard deviation of the blank) was 0.6 µg g⁻¹ (dry mass). NIST SRMs 1400 Bone Ash and 1486 Bone Meal (National Institute

of Standards and Technology, Gaithersburg, MD) were analyzed along with tooth samples for quality control and validation purposes. Statistical analysis of the data was carried out using Origin version 7.5 (OriginLab Northampton, MA) and included simple linear regression analysis, calculation of correlation coefficients and *p* values.

2.4. LA-ICP-MS analysis

Seven primary (I_1) incisors were selected from each of six lead-dosed animals and one undosed animal, and were embedded in epoxy resin in a cuboid mold; the resin was allowed to set overnight. Each tooth was subsequently sectioned along the longitudinal axis using a low-speed Isomet saw with diamond tipped blade, which was cooled and lubricated with deionized water. The surface of each section was etched for 10 s in 1 M hydrochloric acid before quenching with purified water. The sections were analyzed using a LA sample introduction system (LSX100, CETAC Industries, Omaha, NE) coupled to an ICP-MS (ELAN 5000; Perkin-Elmer, Shelton, CT). LA parameters followed those previously developed for tooth analysis on this instrument by Kang et al. (2004) and for bone analysis by Bellis et al. (2006). Analysis was performed using a continuously firing, 10-Hz, 20- μ m diameter laser beam of about 1.3-mJ energy (energy level 13/20) beam. The sample stage of the LA system was moved at a speed of 20 μ m s^{-1} along the *X*-axis, to create an ablation track along the horizontal axis of the tooth section. The track was positioned so as to start and finish at opposing edges of the tooth, in either enamel or cementum, for the crown and root, respectively. The track thus passed through dentine and the centrally located pulp cavity.

The isotopes ^{43}Ca , ^{46}Ca , ^{88}Sr , ^{64}Zn , ^{206}Pb , ^{207}Pb , and ^{208}Pb were monitored by the ICP-MS using time-resolved analysis, for a total of 350 s. Each mass was sequentially detected for 100 ms, creating a measurement cycle of 700 ms. The laser scan was manually initiated after about 30 s of ICP-MS data acquisition and was automatically ended at least 30 s before the end of the data acquisition (350 s), depending on the track length.

As a calibration standard for the LA-ICP-MS measurements, approximately 4 g of NIST SRM 1400 Bone Ash (National Institute of Standards and Technology, Gaithersburg, MD), which is certified to contain 38.18 ± 0.13 wt% calcium, 9.07 ± 0.12 $\mu\text{g g}^{-1}$ lead, 181 ± 3 $\mu\text{g g}^{-1}$ zinc, and 249 ± 7 $\mu\text{g g}^{-1}$ strontium, was pressed into a pellet using a manual pellet press. The pellet was measured in triplicate using ablation lines of about 250 s duration and the instrumental parameters described above at the start and end of the analytical run. Lead and zinc concentrations were estimated from the mean $^{208}\text{Pb}/^{43}\text{Ca}$ and $^{64}\text{Zn}/^{43}\text{Ca}$ ratios, respectively. Statistical analysis of the data was carried out using Origin version 7.5 (OriginLab Northampton, MA) and included simple linear regression analysis, calculation of correlation coefficients, and *p* values.

3. Results and discussion

Adult goats have eight lower incisors that erupt between 1 and 3 years of age, replacing deciduous teeth (Constanttinescu, 2001). They are considered 'true' teeth, since they reach full size early in life and do not exhibit further growth and they have the same basic structure as human incisors. External layers of enamel cover the crown, and cementum covers the root. The bulk of the tooth is dentine, with circumpulpal dentine surrounding the centrally located pulp cavity. Some teeth analyzed here also showed a large growth of cementum around the root, indicating an abnormal condition known as hypercementosis. The pair of primary incisors located at the front of the mouth are the earliest forming and largest incisors. In animals studied here these teeth were typically 2–2.5 cm in length and 0.5 cm in width. Goats lack upper incisors, and instead have a bony pad. The goat molars are not 'true' teeth, since they grow continuously over time, to offset wear through rumination.

3.1. ETAAS analysis of goat teeth

The bulk lead concentration in the caprine whole incisors studied here varied from 0.6 to 80 $\mu\text{g g}^{-1}$. Whole tooth analysis has previously been employed as an indicator of human lead exposure (Needleman et al., 1972). The lead content of whole human teeth varies according to the exposure level and subject age, but is typically from 0.1 to 100 $\mu\text{g g}^{-1}$ in the general population, and several hundred $\mu\text{g g}^{-1}$ in highly exposed populations (Gulson, 1996). Reported circumpulpal lead content, using physical separations has shown a similar range, although the values for this content are up to 10-fold higher than the respective whole tooth content (Shapiro et al., 1973; Grobler et al., 1985). Fig. 1 compares ETAAS data for whole tooth lead with the cumulative lifetime dose. The slope of a linear regression was 1.2 $\mu\text{g g}^{-1}$ tooth-Pb g^{-1} Pb-dose, and r^2 was 0.647 ($p < 0.0001$), indicating a statistically significant correlation between increasing tooth lead content and lead dose. However, if goat 89-7 is eliminated as an outlier, then the slope becomes 1.1, with $r^2 = 0.722$ ($p < 0.0001$).

3.2. LA-ICP-MS analysis of goat teeth

Incisors from seven goats representing the dosing range were selected for detailed analysis by LA-ICP-MS. Detailed information regarding the dosing history of these seven animals is given in Table 1. The whole tooth lead content in these seven goats did not uniformly increase with the cumulative lead dose; for example the highest dosed goat with 50.5 g Pb (82-17) had lower whole tooth lead content than goats dosed with 45.1 and 37.4 g Pb (89-4 and 89-7, respectively).

Fig. 2 shows photographs of the primary incisor collected from a 16-year-old animal that received total of 50.5 g lead (82-17) on an annual basis over a 10-year period. The photograph shows the various tooth structures and the tracks resulting from the LA-ICP-MS analysis. Fig. 3 shows the lead concentration recorded in the laser ablation line shown in the magnified view of Fig. 2(b). The direction of scanning was left to right as viewed. A localized lead content approaching 1600 $\mu\text{g g}^{-1}$ was measured in the circumpulpal dentine adjacent to the pulp cavity. There was relatively little lead detected in the enamel and primary dentine. The observation is consistent with the deposition of lead from blood, supplied from the pulp cavity. The tooth also exhibited clear signs of hypercementosis (Fig. 2b), i.e., the abnormal condition that results in increased growth of cementum surrounding the root. Fig. 4 shows that the crown of the tooth had similar lead concentration in circumpulpal dentine. Zinc was also found in the circumpulpal dentine and cementum.

Fig. 5 shows LA-ICP-MS analysis of the primary incisor collected from undosed animal (95-10), and Fig. 6 shows an incisor from a goat dosed with 18 g lead dentine at about 20 $\mu\text{g g}^{-1}$. Although not intentionally dosed with lead, the animal (89-3). In the undosed goat, a small amount of lead was detected in circumpulpal would have been exposed to background levels of lead in the environment. In the animal dosed with 18 g lead the circumpulpal dentine contained around 600 $\mu\text{g g}^{-1}$ lead. There was some elevation of lead found in the root cementum. Zinc was found in the areas that showed high lead, but the concentration was not substantially different from that found in the undosed goat's tooth.

The mean lead content in circumpulpal dentine recorded in three LA-ICP-MS transects was calculated for each of the seven teeth and plotted against the known cumulative lifetime lead dose of the animal (Fig. 7). Unlike the whole tooth lead concentrations, circumpulpal lead in these teeth increased proportionately with lead dose. A linear regression of the localized lead data yielded a slope of 23 $\mu\text{g g}^{-1}$ tooth-Pb g^{-1} Pb-dose with $r^2 = 0.961$ ($p = 0.0001$), indicating a statistically significant correlation between increasing circumpulpal lead and lead dose. Furthermore, the correlation was much better than that found for whole tooth analysis. Measurement uncertainty for the LA-ICP-MS data was substantial due to sampling

in-homogeneity. This variability reflects the small sample volume ablated for each run (approximately 20- μm laser diameter) and the relatively small number of replicates that were performed. It was apparent that measurement of lead in circumpulpal dentine by LA-ICP-MS has the potential to indicate the level of cumulative lead dose. The zinc concentration in circumpulpal dentine remained almost constant across all animals. A linear regression of the localized zinc data yielded a slope of -1.6 , with $r^2 = 0.279$ which was not statistically significant ($p = 0.224$).

These data, though based on a relatively small sample set, indicated a statistically significant correlation between tooth lead concentration and the cumulative lead dose. There is a well-established relationship between lead uptake and retention, and nutritional status (ATSDR, 1999). Dietary deficiencies of both calcium and phosphorus are associated with increased lead absorption, and the severity of adverse health effects, and it has been suggested that calcium and lead compete for similar binding sites in the body (ATSDR, 1999). There was benefit in analyzing specific structures in the tooth, namely circumpulpal dentine, rather than the whole tooth. In whole teeth, the circumpulpal accumulation of lead is diluted by the relative mass of the dentine and other structures. Larger teeth may therefore show lower lead content. The amount of cementum present in the tooth will also impact the whole tooth concentration. Circumpulpal dentine is thus the preferred choice for long-term exposure assessment.

Lead is associated with adverse effects on human dental health, including an increase in the prevalence of dental caries (Gil et al., 1996). Our data show a considerable accumulation of lead in circumpulpal dentine and in cementum that could play some role in the development of the adverse effects. The relationship between lead and hypercementosis, in particular, warrants further study. It was previously noted that Barbados slave populations with high skeletal lead exhibited frequent signs of hypercementosis (Corrucini et al., 1987).

4. Conclusion

The lead content of whole incisors obtained from goats, and measured using ETAAS, ranged from $0.6 \mu\text{g g}^{-1}$ for an undosed animal to $80 \mu\text{g g}^{-1}$ for lead-dosed animals. Further analyses at micrometer scale spatial resolution using LA-ICP-MS identified localized accumulation of lead in circumpulpal dentine and in cementum related to hypercementosis. Localized lead concentrations found in some teeth approached $2000 \mu\text{g g}^{-1}$. The amount of lead in circumpulpal dentine increases linearly with the cumulative lifetime lead dose given to the animals. These data would suggest that circumpulpal dentine is a better biomarker of cumulative lead exposure than is whole tooth lead, at least for lead-dosed goats. Further studies are required to determine whether human teeth show a similar accumulation of lead to that observed here in caprine teeth from dosed animals, and whether a similar dose-response relationship can be expected.

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References

- Agency for Toxic Substance Disease Registry (ATSDR). Toxicological Profile for Lead (update). US Department of Health and Human Services; Atlanta, GA: 1999.
- Bellis DJ, Hetter KM, Jones J, Amarasiriwardena D, Parsons PJ. Calibration of laser ablation inductively coupled plasma mass spectrometry for quantitative measurements of lead in bone. *J Anal At Spectrom.* 2006; 21:948–964. [PubMed: 22833692]
- Budd P, Montgomery J, Cox A, Krause P, Barreiro B, Thomas RG. The distribution of lead within ancient and modern human teeth: Implications for long-term and historical exposure monitoring. *Sci Total Environ.* 1998; 220:121–136. [PubMed: 9810721]
- Constanttinescu, GM. Guide to Regional Anatomy Based on Dissection of the Goat. Iowa State University press; Ames, IA: 2001.
- Corrucini RS, Jacobi KP, Handler JS, Aufderheide AC. Implications of tooth root hypercementosis in a Barbados slave skeletal collection. *Am J Phys Anthropol.* 1987; 74:179–184. [PubMed: 3322025]
- Cox A, Keenan F, Cooke M, Appleton J. Trace element profiling of dental tissues using laser ablation-inductively coupled plasma mass spectrometry. *Fresenius J Anal Chem.* 1996; 354:254–258.
- Dolphin AE, Goodman AH, Amarasiriwardena DD. Variation in elemental intensities among teeth and between pre- and postnatal regions of enamel. *Am J Phys Anthropol.* 2005; 126:878–888. [PubMed: 16118782]
- Evans RD, Richner P, Outridge PM. Micro-spatial variations of heavy-metals in the teeth of walrus as determined by laser-ablation ICP-MS. The potential for reconstructing a history of metal exposure. *Arch Environ Contam Toxicol.* 1995; 28:55–60. [PubMed: 7717762]
- Gil F, Facio A, Villanueva E, Perez ML, Tojo R, Gil A. The association of tooth lead content with dental health factors. *Sci Total Environ.* 1996; 192:183–191. [PubMed: 8956526]
- Graziano JH. Validity of lead exposure markers in diagnosis and surveillance. *Clin Chem.* 1994; 40:1387–1390. [PubMed: 8013124]
- Grobler SR, Rossouw RJ, Kotze D. Lead levels in circumpulpal dentine of children from different geographical areas. *Arch Oral Biol.* 1985; 30:819–820. [PubMed: 3868971]
- Grünke K, Stärk HJ, Wennrich R, Franck U. Determination of traces of heavy metals (Mn, Cu, Zn, Cd and Pb) in microsamples of teeth material by ETV-ICP-MS. *Fresenius J Anal Chem.* 1996; 354:633–635.
- Gulson BL. Tooth analysis of sources and intensity of lead exposure in children. *Environ Health Perspect.* 1996; 104:306–312. [PubMed: 8919769]
- Kang D, Amarasiriwardena D, Goodman AH. Application of laser-ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS) to investigate trace metals spatial distributions in human tooth enamel, dentine growth layers and pulp. *Anal Bioanal Chem.* 2004; 378:1608–1615. [PubMed: 15214424]
- Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HJ, Schnaas L, Wasserman G, Graziano J, Roberts R. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect.* 2005; 113:894–899. [PubMed: 16002379]
- Lee KM, Appleton J, Cooke M, Keenan F, Sawicka-Kapusta K. Use of laser ablation inductively coupled plasma mass spectrometry to provide element versus time profiles in teeth. *Anal Chim Acta.* 1999; 395:179–185.
- Lochner F, Appleton J, Keenan F, Cooke M. Multi-element profiling of human deciduous teeth by laser ablation-inductively coupled plasma-mass spectrometry. *Anal Chim Acta.* 1999; 401:299–306.
- Needleman HL, Tuncay OC, Shapiro IM. Lead levels in deciduous teeth of urban and suburban American children. *Nature.* 1972; 235:111–112. [PubMed: 4550400]
- Needleman HL, Gunnoe C, Leviton A, Reed R, Peresie H, Maher C, Barrett P. Deficits in psychologic and classroom performance with elevated dentine lead levels. *N Engl J Med.* 1979; 300:689–695. [PubMed: 763299]
- Parsons PJ. Monitoring human exposure to lead: an assessment of current laboratory performance for the determination of blood lead. *Environ Res.* 1992; 57:149–162. [PubMed: 1568437]

- Parsons PJ, Reilly AA, Esernio-Jenssen D, Werk LN, Mofenson HC, Stanton NV, Matte TD. Clin Chem. 2001; 47:322–330. [PubMed: 11159782]
- Shapiro IM, Dobkin B, Tuncay OC, Needleman HL. Lead levels in dentine and circumpulpal dentine of deciduous teeth of normal and lead poisoned children. Clin Chim Acta. 1973; 46:119–123. [PubMed: 4745356]
- Todd AC, Chettle DR. *In vivo* X-ray fluorescence of lead in bone: review and current issues. Environ Health Perspect. 1994; 102:172–177. [PubMed: 8033846]
- Uryu T, Yoshinaga J, Yanagisawa Y, Endo M, Takahashi J. Analysis of lead in tooth enamel by laser ablation-inductively coupled plasma-mass spectrometry. Anal Sci. 2003; 19:1413–1416. [PubMed: 14596408]
- Zong YY, Parsons PJ, Slavin W. Accurate and precise measurements of lead in bone by electrothermal atomic absorption spectrometry with Zeeman-effect background correction. J Anal At Spectros. 1996; 11:25–30.

Fig. 1. Lead content of whole teeth ($[Pb] \mu g g^{-1}$) from 23 individual goats (two teeth were analyzed from four animals) as a function of cumulative lead dose. Line is a linear regression fit of the data; dotted lines are the 95% confidence intervals ($r^2 = 0.647$, $p < 0.0001$).

Fig. 2. Longitudinal section of a primary incisor from a 16-year-old goat having a cumulative lifetime Pb dose of 50.5 g. The image shows (a) an entire tooth view and (b) a close-up view following LA-ICP-MS analysis. Key: LA, laser ablation track; PC, pulp cavity; F, fracture; (i) cementum; (ii) dentine; (iii) circumpulpal dentine surrounding pulp cavity; (iv) hypercementum; and (v) enamel. Tooth dimensions are 26 mm length and 7 mm width.

Fig. 3. Laser ablation ICP-MS measurements showing the spatial distribution of lead in a transect across a longitudinally sectioned primary incisor from a goat dosed with 50.5 g lead, as shown in Fig. 2b. (i) Cementum; (ii) dentine; (iii) circumpulpal dentine surrounding pulp cavity; and (iv) hypercementum.

Fig. 4. Laser ablation ICP-MS measurements showing the spatial distribution of lead and zinc in (a) the root and (b) the crown of a longitudinally sectioned primary incisor from a lead-dosed goat. The animal had a lifetime dose of 50.5 g of Pb. $[M]$ represents the concentration of either Pb or Zn in $\mu\text{g g}^{-1}$.

Fig. 5. Laser ablation ICP-MS measurements showing the spatial distribution of lead and zinc in (a) the root and (b) the crown of a longitudinally sectioned primary incisor from an undosed goat. $[M]$ represents the concentration of either Pb or Zn in $\mu\text{g g}^{-1}$.

Fig. 6. Laser ablation ICP-MS measurements showing the spatial distribution of lead and zinc in (a) the root and (b) the crown of a longitudinally sectioned primary incisor from a lead-dosed goat. The animal had a lifetime dose of 18.0 g of Pb. $[M]$ represents the concentration of either Pb or Zn in $\mu\text{g g}^{-1}$.

Fig. 7.

Plot of (a) lead and (b) zinc content ($\mu\text{g g}^{-1}$) in caprine circumpulpal dentine as a function of cumulative lifetime lead dose (g). Each data point represents the mean $\pm$ standard deviation of the maxima (i.e., peak height) of three laser ablation tracks. Solid lines are the linear regression fits of the data and dotted lines are the 95% confidence intervals. For lead, $r^2 = 0.961$ ($p = 0.0001$). For zinc, $r^2 = 0.279$ ($p = 0.224$).

Table 1
Details of lead-dosed goats selected for the LA-ICP-MS study

Goat ID	Age (years)	Cumulative lead dose (g)	Whole tooth lead ($\mu\text{g g}^{-1}$)
82-17	15	50.5	67
89-3	7	18.0	17
89-4	11	45.1	69
89-7	12	37.4	88
93-4	10	20.8	36
93-10	6	1.6	2.4
95-10	10	0.0	0.6