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Pb enamel biomarker: Deposition of pre- and postnatal Pb isotope injection in reconstructed time points along rat enamel transect

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Abstract

Exposure to lead (Pb) as well as other heavy metals in the environment is still a matter of public health concern. The development of the enamel biomarker for heavy metal exposure assessment is designed to improve studies of dose–effect relationships to developmental anomalies, particularly embryonic dysfunctions, and to provide a time-specific recount of past exposures. The work presented in this paper demonstrates maternal transfer across the placental barrier of the enriched isotope ^{206}Pb tracer to the enamel of the rat pup. Likewise, injections of ^{204}Pb-eriched tracer in the neonate rat resulted in deposition of the tracer in the enamel histology as measured by secondary ion microprobe spectrometry. Through enamel, we were able to observe biological removal and assimilation of prenatal and postnatal tracers, respectively. This research demonstrates that enamel can be used as a biomarker of exposure to Pb and may illustrate the toxicokinetics of incorporating Pb into fetal and neonatal steady-state system processes. The biomarker technique, when completely developed, may be applied to cross-sectional and longitudinal epidemiological research.

Keywords: Isotopic tracer; Blood–enamel mineral exchange; Lead; Heavy metal biomarker; Amelogenesis

1. Introduction

Environmental exposure to lead (Pb) is a matter of continuing public health concern. Atmospheric Pb concentrations have decreased over the past 30 years due to reduced Pb production and elimination of Pb additives in gasoline in the USA, Europe, and other countries (Thomas et al., 1999; Nriagu, 1990). However the current use of leaded gasoline in other, often lesser-developed, countries throughout the world poses an important threat to the healthy development of children in those countries and also actively adds to the global Pb burden. The Center for Disease Control and Prevention (CDC) recommends that blood Pb levels not exceed 10 μg/dL (Lustberg and Silbergeld, 2002). Despite successful efforts to lower atmospheric Pb concentrations, the National Health and Nutrition Examination Survey (1999–2000) reports that approximately 434,000 children under 6 years old in the US have blood Pb levels of 10 μg/dL or more (Meyer et al., 2003).

Inhalation of Pb as airborne particles from automobile and industrial emissions used to be the dominant route of Pb exposure for children in the United States. As atmospheric Pb concentrations declined, ingestion of Pb from soil and food, in addition to inhalation from resuspended particulate matter (dust), has become the more prominent route of Pb exposure for children (Mielke et al., 1999; Mielke and Reagan, 1998; Lejano and Ericson, 2005).

Once deposited, Pb can remain trapped and concentrated in the upper horizons of undisturbed soil for up to 75 years (Bindler et al., 1999). Soil Pb concentrations accumulate due to resuspended particles from

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Pb-contaminated road services, continued automotive and industrial emissions, and soil erosion (Petrosyan et al., 2004; Kurkjian and Flegal, 2003). Pb washed into soil from painted surfaces and rooftops also contributes to the soil Pb burden near the perimeter of homes. This elevated level of Pb on surfaces near homes is often tracked into the home environment, adding to the total Pb exposure (Mielke and Reagan, 1998).

Fetal, neonatal, and adolescent development presents numerous time points during which organ-specific susceptibility to Pb exposure and toxicity may be critical. In evaluating the effects of Pb on health, it is important to determine when Pb exposure occurred, as this knowledge could greatly improve our ability to relate Pb exposures to specific neurological or physiological damage. Thus, reconstructing Pb exposure histories is important for assessing the ongoing potential health risks of Pb (Silbergeld, 2003, 1995). The current methods for measuring blood Pb and Pb-isotopic content from biological tissues are adequate for assessing recent exposures or for identifying source locations, but they are not adequate for reconstructing time-specific exposure histories. In this paper, we test the ability to reconstruct the time course of exposure to Pb during fetal and neonatal development using the enamel of the rat mandibular incisor.

The rat mandibular incisor is a continuously growing, occlusal tissue. Enamel only forms along the labial edge of the incisor where enamel at the incisor tip represents first formed enamel and enamel near the gum line (gingival margin) indicates the most recently formed enamel (see Fig. 1). The final stages of enamel maturation occur at the gingival margin just prior to eruption into the oral cavity (Sato et al., 1996) and the enamel is fully mature at time of eruption. Before eruption, mineral exchange can occur between the blood and the forming enamel, allowing minerals to incorporate into the maturing enamel crystalline structure. This blood–enamel mineral exchange decreases as the enamel matures, and after eruption there is minimal to no mineral exchange between the secreted enamel and the surrounding environment. Therefore, the Pb content of mature enamel should reflect the Pb concentration in the blood while that enamel was developing.

We therefore tested the hypothesis that we could expose pregnant animals at specific times and then reconstruct the time course of Pb exposures by determining Pb concentrations at specific locations along the tooth enamel that represent the developing enamel and the enamel–mineral exchange that occurred at the time of exposure. To test this hypothesis we exposed rats prenatally and postnatally to Pb by injection under controlled conditions using two different nonradioactive isotopically-enriched Pb tracers, ^{206}Pb and ^{204}Pb, to unambiguously differentiate between prenatal and postnatal exposures, respectively. Secondary ion mass spectrometry was used to longitudinally scan rat tooth enamel and to determine the concentration of these Pb isotopes at histological locations representing enamel formed at the time of prenatal and postnatal Pb exposure and that could be characteristic of different time points of the rats' development.

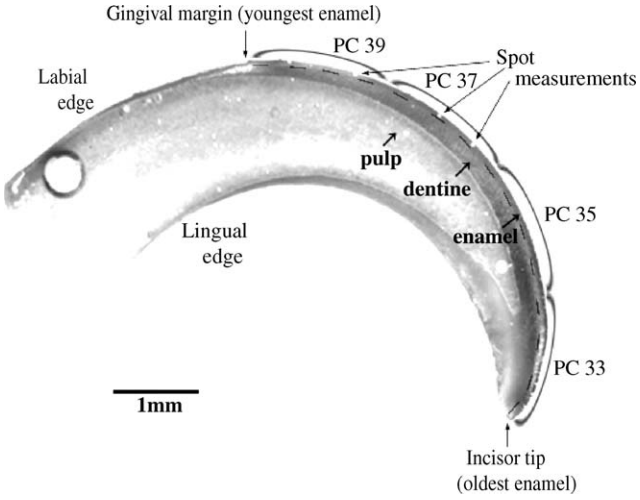

Fig. 1. Cross-section of a rat mandibular incisor (unstained, gold-coated, thick-sectioned specimen photographed under reflected light). The enamel transect is situated along the labial edge of the incisor. The dashed line indicates the histological division between enamel and dentine. The incisor tip, representing the first erupted and oldest enamel, and the gingival margin, representing the most recent erupted and youngest enamel, are located at the beginning and end of the enamel transect, respectively. The mandibular incisor is continuously growing outward from the gingival margin and enamel maturation is completed just prior to erupting into the oral cavity. According to respective proximity, spot measurements (white dots indicated by arrows) along the enamel transect were averaged into four equidistant locations corresponding to the postconception age (PC 33, 35, 37, and 39), in days, of enamel eruption for each spot. The mandibular incisor first erupted at PC 33 and enamel growth continued through PC 39, when the tooth was excised for data acquisition.

2. Materials and methods

Four timed-pregnant Sprague Dawley rats (Charles River, Inc., Hollister, CA) were randomized into control and exposure groups. Litters ranged from 10 to 13 pups, though only male progeny were used. The rats and dams with littermates were individually housed under barrier conditions in an AAALAC-accredited vivarium using a 12-h light/dark cycle. Standard rat chow and distilled water were provided ad libidum. Animal husbandry was conducted in strict accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and the research protocol was approved by the Institutional Animal Care and Use Committee at the University of California, Irvine (Protocol No. 1998–1992).

2.1. Isotopic Pb dosing

To differentiate between prenatal and postnatal Pb exposure, injection of ^{206}Pb and ^{204}Pb tracers, respectively, were administered to induce enriched blood Pb ratios relative to endogenous (dietary) Pb. Common Pb contains four stable isotopes: ^{208}Pb, ^{207}Pb, ^{206}Pb, and ^{204}Pb and comprise 52.4%, 22.1%, 24.1%, and 1.4%, respectively, of the natural composition of Pb; though these concentrations may vary depending on geographical region and nutrient composition. Incorporation of Pb tracers into enamel was identified due to significantly altered Pb isotope ratios when compared to enamel from control animals. For injection, Pb compounds were dissolved into solution using 0.5 N HNO_3. The solutions were neutralized with dilute NaOH to pH 6.5 and sterile filtered.

2.1.1. Prenatal Pb exposure

Pregnant rats were injected intraperitoneally (i.p.) with 0.25 cm^3 of 0.9% sterile filtered saline ($n = 2$) or 2 mg/kg ^{206}Pb in saline ($n = 2$) (Lead-206 Oxide, >98% chemical purity, 94% enrichment; Cambridge Isotope Laboratories, MA). Exposures were administered on 2 consecutive days during the third gestation week, a time at which fetal enamel development has commenced (Navia and Narkates, 1980).

2.1.2. Postnatal Pb exposure

Postnatal dosing was administered on male progeny (see details below) during the first week after birth, a time when the first formed enamel that will become the incisor tip is histologically visible (Navia and Narkates, 1980), allowing sufficient growth to ensure tracers incorporation. Rats were euthanized during the third week postdelivery by lethal pentobarbital injection (65 mg/kg bodyweight, i.p.) and exsanguination. One central mandibular incisor was removed from each pup and processed for ion probe microscopy.

2.1.3. Saline control pups

Pups from saline-exposed dams received a total of 0.3 ($n = 2$) or 0.6 cm^3 ($n = 2$) of 0.9% sterile-filtered saline split into three daily injections (i.p.) over a period of 4 days.

2.1.4. Postnatal ^{204}Pb-dosed pups

Eight pups from ^{206}Pb-exposed dams were tagged according to three postnatal exposure groups: those receiving a total of (a) 0.3 cm^3 ($n = 2$) or (b) 0.6 cm^3 ($n = 2$) of 0.26 mg/mL ^{204}Pb in saline (Lead-204 Metal, >98% chemical purity, 66.5% enrichment; Cambridge Isotope Laboratories) split into three daily injections (i.p.) over a period of 4 days or (c) or no ^{204}Pb injection ($n = 4$). The pups were allowed to remain with their dam and littermates.

Pups from saline-exposed dams were not administered postnatal ^{204}Pb injection due to the limited number of male progeny. As postnatal exposures were administered prior to weaning, we could not combine litters. Pups from each dam were treated equally in order to control for potential maternal effect. We felt it important to maintain a saline control for each dose–response group (0.3 or 0.6 cm^3) and to obtain data for natural endogenous ^{206}Pb and ^{204}Pb received from maternal transfer (dietary) and the immediate ambient environment. Pups from the ^{206}Pb-injected dams in the "no-^{204}Pb-injection" group did not receive saline injection in order to control for any potential effect of the injection procedure to alter dietary ^{206}Pb and ^{204}Pb in animals not receiving the postnatal dose. Due to limited litter size, we determined it more pertinent to maintain the dose response in postnatal injected animals and that the saline-injected pups from saline-injected dams and the no-^{204}Pb-injected pups from Pb-injected dams provided sufficient markers of comparison to determine significant alteration in enamel isotopic Pb composition due to prenatal and postnatal Pb treatment.

2.2. Ion probe mass spectrometry

Rat mandibular incisors were placed flat on double-sided tape within a metal ring, into which molten epoxy was poured. After curing for 24 h under vacuum, the epoxy disc was removed from the ring. The epoxy surface was carefully ground down to expose a cross-section of the enamel along the labial edge between the incisor tip and the gingival margin (Fig. 1).

A Cameca IMS 1270 high-resolution, high-sensitivity ion microprobe optimized for Pb isotope measurement (Schumacher et al., 1994) was used to measure Pb isotope composition at specific points (spots) along the enamel transect (Fig. 1). Concentrations of ^{208}Pb, ^{206}Pb, ^{204}Pb, and ^{48}Ca were determined in situ by ablating each spot with a focused $^{16}O^-$ primary ion beam and measuring (in counts per second) the positively charged ions discharged from the ablated enamel surface. ^{48}Ca, an isotope naturally abundant in calcified enamel, was used to normalize beam intensity between spot analyses of each transect and to control for interindividual variation of enamel. As the most abundant isotope in common Pb, ^{208}Pb was measured as a marker for background endogenous Pb.

2.3. Data interpretation

Pb and Ca isotopic concentrations were measured along the enamel transect, scanning from the incisor tip (oldest mature enamel) to the gingival margin (the most recently matured enamel). Approximately 6–8 spots 50 μm in diameter were analyzed along each transect. Spots were then grouped according to proximity along

the enamel transect into four equidistant locations corresponding to the postconception age (PC 33, 35, 37, and 39), in days, of enamel eruption for each spot (Fig. 1) and thus representing the mineral Pb content present in blood and consequently incorporated into the enamel structure during that stage in both enamel maturation and prenatal and postnatal development.

The number of spots measured on each incisor tooth varied depending on tooth length and useable enamel data. With the width of the enamel transect as wide as the ion detection beam (approximately 50 μm), if the ion beam was not precisely centered over the enamel transect, the enamel data samples were contaminated with material from the adjacent dentine or epoxy structures. The data measurements from these possibly contaminated spots were not used in our computations.

All valid enamel isotopic data were normalized against ^{48}Ca. Isotopic data used to represent the recovery of ^{206}Pb and ^{204}Pb tracer doses were corrected against endogenous Pb ratios: 2.26 and 17.9 for $^{208/206}$Pb and $^{208/204}$Pb, respectively, as determined by concentrations in the enamel of control animals. Concentrations of Pb isotopes at each of the four locations were compared with concentrations in the control teeth using single-factor ANOVA. Significance was attributed at the $P \leqslant 0.05$ level and the 95% confidence interval displayed for all data.

2.4. Calculation of enamel age

Eruption of the mandibular incisor generally occurs on Postnatal Day 7, or PC 33 (Navia and Narkates, 1980). Assuming a constant eruption rate, we estimated the day posteruption for each spot analyzed according to the transect eruption rate and the distance of each spot from the first erupted enamel at the tip of the incisor. Data are presented in days PC in order to establish a standard time reference between prenatal and postnatal exposures. With a gestation time of 26 days for Pb-injected animals and eruption of the first mature enamel occurring on PC Day 33 and that the time Pb-exposed rats were euthanized on PC Day 39, we estimated the age of enamel in each of the four selected regions as follows:

$$\text{enamel age} = \text{age at time of initial tooth eruption} + \text{posteruption age of each location, } A_{\text{erupt}},$$

where A_{erupt} = distance of location from tip/transect growth rate, T_{gr}; T_{gr} = total transect length/days of transect growth, T_{g}; T_{g} = age at time sacrifice–age of initial tooth eruption.

3. Results

Enamel growth rate varied significantly between exposure groups. Transects from saline-injected (control) animals were 5.5 ± 0.4 mm in length on average. Transects for the Pb-exposed rats were 6.8 ± 1.0 mm ($P = 0.03$ vs. controls). The enamel eruption rates for control and Pb-exposed rats were 0.50 ± 0.03 and 0.85 ± 0.13 mm/day ($P \leqslant 0.0001$ vs. controls), respectively.

3.1. Prenatal exposure

Fig. 2 shows ^{206}Pb content in the enamel of prenatally exposed rats significantly above that of the controls ($P \leqslant 0.001$). The enamel at the gingival margin shows a significantly smaller concentration of ^{206}Pb compared to the ^{206}Pb concentration at the incisor tip ($P \leqslant 0.001$). This is consistent with a prenatal ^{206}Pb exposure, where we expect Pb in blood to decrease over time and the enamel Pb concentrations to subsequently reflect this: highest at the first matured enamel located at the incisor tip and lowest at the last matured enamel located near

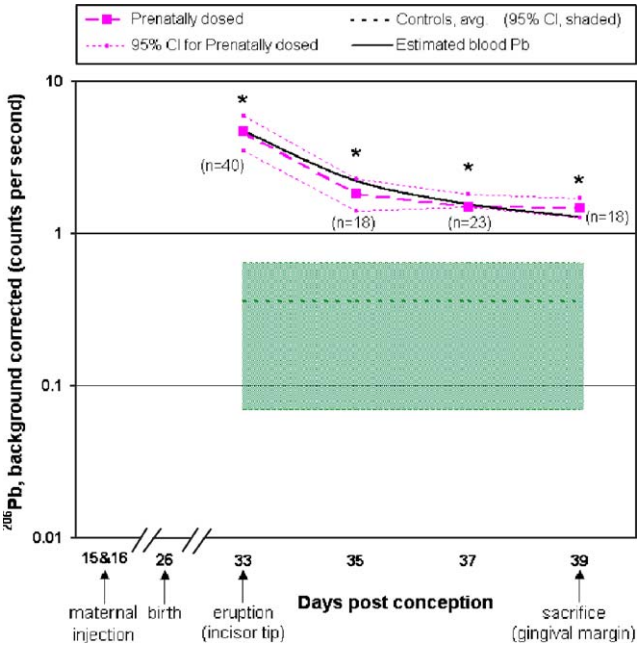

Fig. 2. Recovery in enamel of ^{206}Pb prenatal injection. ^{206}Pb data per spot for all prenatal- and control-exposed rats were averaged (±95% confidence interval (CI)) into four equidistant locations along the enamel transect corresponding to the postconception age (PC 33, 35, 37, and 39), in days, of enamel eruption for each spot. *n* indicates the number of spots averaged per location. Data have been corrected for endogenous (dietary) ^{206}Pb and normalized to ^{48}Ca. We estimated the trend of Pb in blood from the time of injection to the time of enamel maturation (solid line) using the expected half-time of Pb in rat blood ($t_{1/2\ \text{fast}} = 21$ and $t_{1/2\ \text{slow}} = 280$ h (USEPA, 1999)) and found that our enamel data (dashed line) fit closely with this model. *$P \leqslant 0.0001$ vs. controls, single factor ANOVA.

the gingival margin. We estimated the trend of Pb in blood from the time of injection to the time of enamel maturation (solid line, Fig. 2), assuming a biological half-time for Pb in rats to be 21 and 280 h for fast and slow clearance rates, respectively (US EPA, 1999). As shown in Fig. 2, ^{206}Pb concentrations along the enamel transect are consistent with the computed relative ^{206}Pb concentrations in blood at the time of enamel maturation.

3.2. Postnatal exposure

Tooth enamel from rats injected with ^{204}Pb on Days PC 30, 31, and 33 show significant levels of ^{204}Pb above background levels in animals receiving no ^{204}Pb injection and also above ^{204}Pb levels in control animals ($P \leqslant 0.05$) (Fig. 3). Endogenous ^{204}Pb from the no-^{204}Pb-injected group was higher compared to controls; however, the 95% CI for these groups overlap making the difference insignificant. As expected for a postnatal dose, ^{204}Pb concentrations in enamel nearest the gingival margin are higher than those measured at the incisal tip. Enamel secretion is complete in 6.6 days (Smith and Warshawsky, 1975), the time it takes the mineralized crystalline structure to replace the organic proteinaceous mixture formed during the earlier stages of enamel development. Thus, enamel secretion increasingly restricts the exchange of Pb between blood and enamel. Since postnatal Pb injections were completed immediately prior to eruption of the incisors, the nearly mature and first formed enamel at the incisor tip (erupting on Day 33 PC) and the enamel erupted on Day 35 PC were approximately 95% and 65% secreted, respectively, at the time of ^{204}Pb injection. Pb exchange was expected to be restricted in the more mature enamel, which is consistent with our findings of lower ^{204}Pb in spots closer to the incisal tip of the tooth.

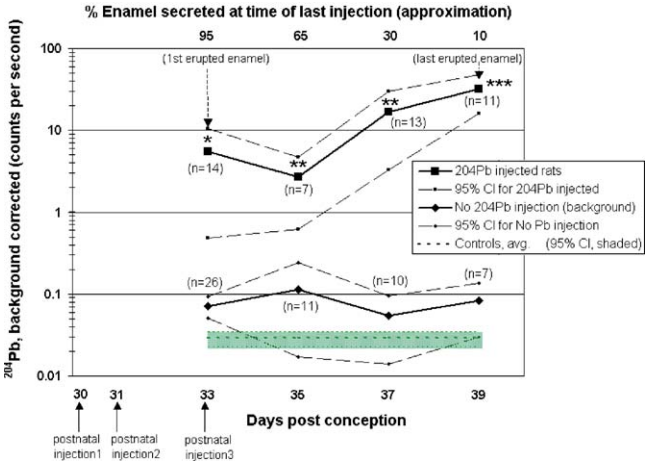

Fig. 3. Detection in enamel of ^{204}Pb postnatal dose above control and background levels. According to respective proximity, ^{204}Pb data per spot for all postnatally, no Pb injected, and control-treated rats were averaged ($\pm$95% confidence interval (CI)) into four equidistant locations along the enamel transect corresponding to the postconception age (PC 33, 35, 37, and 39), in days, of enamel eruption for each spot. *n* indicates the number of spots averaged per location. ^{204}Pb was administered on PC 30, 31, and 33 and injections were complete just prior to tooth eruption and when enamel was approximately 95% secreted (matured). Lesser-secreted enamel at time of injection was able to incorporate increasing amounts of ^{204}Pb. As expected for a postnatal dose, ^{204}Pb concentrations in enamel nearest the gingival margin are higher than those measured at the incisal tip. $^{*}P<0.05$, $^{**}P \leqslant 0.01$,** $P \leqslant 0.0001$ vs. controls, single-factor ANOVA.

Table 1
Postnatal dose–response relationship of ^{204}Pb along enamel transect (^{204}Pb, counts per second $\pm$ mean standard error, averaged over entire transect)

Injected dose	Saline-injected	^{204}Pb-injected[a]
No ^{204}Pb injection	No data	0.08 ± 0.09
Low-dose (0.3 mL)	0.03 ± 0.04	9.80 ± 9.12^*#
High-dose (0.6 mL)	0.02 ± 0.01	31.5 ± 8.97^^*

^{204}Pb concentration was measured in tooth enamel from rats injected with a total dose of 0.3 or 0.6 mL saline or ^{204}Pb. ^{204}Pb levels were significantly higher in ^{204}Pb-injected rats than in saline controls and in rats receiving ^{206}Pb prenatal dose but no ^{204}Pb postnatal dose ("no ^{204}Pb injection"). High-dose ^{204}Pb levels were significantly higher than that of low-dose injected animals.
$^{\wedge}P \leqslant 0.05$ and $^{\wedge\wedge}P \leqslant 0.001$ vs. controls. $^{*}P \leqslant 0.0001$ vs. no ^{204}Pb injection. $\#P \leqslant 0.0001$ vs. 0.6 cm^3.
[a]Note: 0.26 mg/mL ^{204}Pb.

To determine whether higher doses of Pb were reflected by greater concentrations of Pb in rat enamel, we injected a small number ($n = 2$) of rats with levels of ^{204}Pb that were twice those used in the other pups. As shown in Table 1, the ^{204}Pb concentration in teeth from animals receiving the higher dose (1.56 mg ^{204}Pb total dose) was greater than that in teeth from animals receiving the lower dose (0.78 mg ^{204}Pb total dose) ($P < 0.0001$ vs. lower dose). Both low- and high-dose ^{204}Pb levels in enamel were significantly above endogenous (dietary) levels measured in the enamel of control rats ($P \leqslant 0.05$) and the no-^{204}Pb-injected rats, which represent ^{204}Pb background levels in the enamel of rats receiving ^{206}Pb prenatal dose but no ^{204}Pb postnatal dose ($P < 0.0001$).

4. Discussion

Pb exposure remains an issue of environmental concern, particularly in Nigeria and China, among other countries, where lead additives are currently used in gasoline (Silbergeld, 1995). The continued Pb exposure can increase risks of health consequences, especially in children. Silbergeld (2003) suggests that past and present exposures to Pb may increase sensitivity to carcinogens as well as causing dysfunction of biological processes associated with development,

reproduction, and aging. In older children, decreased attention span, reduced academic achievements, reading disabilities, and failure to graduate from high school have been associated with increased blood Pb levels during infancy and early childhood (Needleman et al., 1990).

Neurological, cardiovascular, developmental, endocrine, and reproductive disruptions have even observed in adults and children exhibiting blood Pb levels *below* the CDC-allowable limit of 10 μg/dL (Bellinger et al., 1992; US EPA, 1999; Lanphear et al., 2000; Canfield et al., 2003; Ryan et al., 2004), suggesting that we need to expand our understanding of Pb toxicity and also that we may need to reconsider whether current guidelines provide adequate protections for susceptible children. Knowledge of past Pb exposure, especially in utero, is critical to improving our understanding the role of developmental Pb toxicity (Silbergeld, 1995; Gulson, 1996). Our data show that location-specific Pb analysis using IMS provided us the ability to localize Pb retention in rat tooth enamel in order to distinguish between prenatal or postnatal administration of isotopic Pb tracers. Our findings support the hypothesis that location of Pb in tooth enamel can be used to reconstruct the time course of potentially toxic exposures to Pb during fetal and neonatal development.

The data presented in this current study demonstrate the use of tooth enamel to reconstruct prenatal exposure history and clearly indicate that Pb enamel concentrations can be used as a biomarker. Previous work in using enamel to define Pb exposure history has been partially successful. Gulson and Wilson (1994) demonstrated the use, in bulk analysis, of deciduous tooth enamel to evaluate in utero Pb exposures. However, human enamel continues to mature after birth and, when analyzed in bulk measurement, can only generalize the time of exposure to early developmental stages and not exclusively prenatal association. The findings in this paper provide an association between the location of Pb along the enamel transect and a comparative exposure time according to the growth pattern of enamel and the development of the rat, placing us one step closer in assigning a time reference of toxic exposure to neurological development of the central nervous system and consequences that may manifest later in life.

This study demonstrates our ability to recover the dose history in the enamel of the continuously growing rat mandibular incisor of prenatal exposures and exposures to Pb that occur within 1 week after birth. Dental lamina in fetal pups is first visible on the 12th to 15th gestational day (Navia and Narkates, 1980) and is thought to precede enamel developmental by several days in the rat. We injected ^{206}Pb into the pregnant dam during Gestation Days 15 and 16 in order to incorporate the tracer into fetal blood prior to development of first mature enamel. First formation of histologically visible enamel occurs near the 3rd postnatal day (Navia and Narkates, 1980) while the tooth is still below the gingival margin. We administered the postnatal ^{204}Pb tracer on Days 4, 5, and 7 (PC Days 30, 31, and 33; Fig. 3) after birth to measure its incorporation into the enamel just prior to the eruption of the tooth on Day 7. ^{204}Pb was detected above background levels in the first formed enamel at the incisal tip, suggesting the enamel, even at the latter stages of maturation, can incorporate some metal from the blood, albeit at a restricted rate. Observing this time-dependent incorporation into enamel of pre- and postnatal tracers could be used to refine the existing knowledge of enamel amelogenesis. For example, an increase in mineral (or heavy metal)-to-calcium ratio may enhance, or speed up, the enamel growth rate, as observed in our data.

There are differences of rat tooth development compared with that in humans. For example, the rat incisor continues to grow even in adults. On the other hand, human enamel pattern has developmental similarities to the presecretion, secretion, and maturation stages in the continual growing maxillary rat incisor (Deutsch and Pe'er, 1982). Human enamel is 72% mature at birth, whereas primary rat enamel is 75% mature at birth (Navia and Narkates, 1980). The experiments presented in this paper, although using a rat model, suggest that measurement of Pb in human enamel can be used as a time-specific biomarker of exposure (Ericson, 2001). In fact, the human deciduous tooth has at least two distinct histological markers (zeitgebers) of enamel formation: (1) the birth line, a disorder in the enamel histology due to birth trauma (Provenza, 1988), and (2) the diurnal lines that indicate the daily growth of enamel formation (Avery and Steele, 1994), making the human tooth an easier model than the rat tooth with which to define the time of exposure. We were unable to identify any such histological markers in rat enamel.

This study also demonstrates that maternal exposure to Pb does, in fact, transfer Pb across the placental barrier into the fetal system and that through enamel, the toxicokinetics of fetal exposure to Pb can be examined. This study in rats is consistent with results in primates (Franklin et al., 1997; Inskip et al., 1992) and in humans (Gulson et al., 1998; Manton et al., 2003) that also showed the ability to use stable Pb isotope enrichment to detect the transfer of maternal Pb across the placental barrier and into the fetal system. An improvement of this study would have been to sample blood Pb concentrations at the time represented by each enamel spot location. However, whole-tooth Pb concentrations have been found to correlate in a direct manner with blood Pb (Rabinowitz et al., 1993), though dentin and cementum interchange more readily with minerals in the blood than enamel and serve to better indicate Pb levels in blood at the time of tooth removal

rather than past exposures. Enamel, on the other hand, serves to record the Pb content in blood during the developmental stages of both enamel and human growth, that is not otherwise represented by blood Pb levels at the time of tooth removal. The trend of ^{206}Pb prenatal exposure in enamel approached the expected background level for enamel (Fig. 2), but did not reach background levels in the time frame of this study. Mobilization of Pb from maternal stores and dietary input could play a factor in the elimination of ^{206}Pb from fetal and neonatal system equilibrium. We assumed that Pb in blood followed an exponential removal rate. If our hypothesis was correct then the Pb content of the tooth enamel should reflect the blood concentration at the time the enamel was forming. We, therefore, estimated the concentration of ^{206}Pb remaining in the blood corresponding to the postconception age of the enamel using a log-normal regression model (solid line, Fig. 2) fit to the expected half-time of Pb in blood using a fast component of 21 h and a slow component of 280 h (US EPA, 1999) and found that our data (dashed line, Fig. 2) fit closely with this model. Morgan et al. (1977) report an effective half-time for blood Pb in rats after injection to be about 100 h based on a single exponential fit, which seems to "average" both the slow and the fast removal components. The ability to differentiate through tooth enamel the fast and slow compartmentalization of Pb body burden and removal over time is a unique and beneficial method to using tooth enamel as a biomarker for Pb and other heavy metal exposures. Thus, our results also show that it was possible to estimate the effective biological half-time for Pb using Pb isotope observations in enamel. In reconstructing a trendline for Pb in enamel and considering the known growth-time sequence of enamel development, it may be possible to back-calculate to the time of exposure.

Our research has shown isotopically-eriched tracers can be measured in directed regions of tooth enamel using IMS. This technique can be used to assess the impact of environmental heavy metal exposure sources that have different elemental and isotopic compositions (Ericson, 2001; Ericson et al., 2001), as has also been demonstrated using laser ablation-inductively coupled plasma-mass spectrometry LA-ICP-MS (Kang et al., 2004; Goodman et al, 2003; Budd et al., 1998). Our laboratory has used both IMS and LA-ICP-MS to achieve isotopic resolution for Pb and other minerals in rat and human enamel transects. Due to its multi-collection capability and high mass resolving power of up to 5000, we found the IMS technique to provide optimal resolution of complex isotope distributions and precision in preserving spatial resolution with data retrieval for each spot analyzed. In our experience, the IMS serves to be the optimal method in recovering isotopic exposure history in tissues with relatively narrow surface area and a nonlinear trajectory, such as the rat enamel transect. The ability to associate the age or maturation date of tooth enamel with a time and intensity of exposure, as we have shown in this paper, presents us with a potential new method for reconstructing the Pb exposure histories for children.

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References

Avery, J.K., Steele, P.F., 1994. Oral Development and Histology, second ed. Thieme, New York.

Bellinger, D.C., Stiles, K.M., Needleman, H.L., 1992. Low-level lead exposure, intelligence and academic achievement: a long-term follow-up study. Pediatrics 90, 855–861.

Bindler, R., Brännvall, M., Renberg, I., 1999. Natural lead concentrations in pristine boreal forest soils and past pollution trends: a reference for critical load models. Environ. Sci. Technol. 33, 3362–3367.

Budd, P., Montgomery, J., Cox, A., Krause, P., Barreiro, B., Thomas, R.G., 1998. The distribution of lead within ancient and modern human teeth: Implications for long-term and historical exposure monitoring. Sci. Total Environ. 220, 121–136.

Canfield, R.L., Henderson, C.R., Cory-Slechta, D.A., Cox, C., Jusko, T.A., Lanphear, B.P., 2003. Intellectual impairment in children with blood lead concentrations below 10 μg per deciliter. N. Engl. J. Med. 348, 1517–1526.

Deutsch, D., Pe'er, E., 1982. A development of enamel in human fetal teeth. J. Dent. Res. 61, 1543–1551.

Ericson, J.E., 2001. Enamel Pb biomarker for prenatal exposure assessment. Environ. Res. A 87, 136–140.

Ericson, J.E., Rinderknecht, A.R., Kleinman, M.T., Gonzalez, E.J., Crinella, F.M., 2001. Measurement of manganese in histological enamel cross sections: toward a tooth manganese biomarker. Environ. Res. A 86, 46–50.

Franklin, C.A., Inskip, M.J., Baccanale, C.L., Edwards, C.M., Manton, W.I., Edwards, E., O'flaherty, E.J., 1997. Use of sequentially administered stable lead isotopes to investigate changes in blood lead during pregnancy in a nonhuman primate (*Macaca fascicularis*). Fund. Appl. Toxicol. 39, 109–119.

Goodman, A.H., Dolphin, A.E., Amarasiriwardena, D.D., Klein, R., Backstrand, J.R., Reid Jr., J.B., Outridge, P., 2003. Tooth rings: dental enamel as a chronological biomarker of elemental absorption from pregnancy to adolescence. J. Child. Health 1, 203–214.

Gulson, B.L., 1996. Tooth analyses of sources and intensity of lead exposure in children. Environ. Health. Perspect. 104, 306–312.

Gulson, B.L., Wilson, D., 1994. History of lead exposure in children revealed from isotopic analyses of teeth. Arch. Environ. Health. 49, 279–283.

Gulson, B.L., Mahaffey, K.R., Jameson, C.W., Mizon, K.J., Korsch, M.J., Cameron, Eisman, J.A., 1998. Mobilization of lead from the

skeleton during the postnatal period is larger than during pregnancy. J. Lab. Clin. Med. 131, 324–329.
Inskip, M.J., Franklin, C.A., Subramanian, K.S., Blenkinsop, J., Wandelmaier, F., 1992. Sampling of cortical and trabecular bone for lead analysis: method development in a study of lead mobilization during pregnancy. Neurotoxicology 13, 825–834.
Kang, D., Amarasiriwardena, D., Goodman, A.H., 2004. Application of laser ablation-inductively coupled plasma-mass spectrometry (LA–ICP–MS) to investigate trace metal spatial distributions in human tooth enamel and dentine growth layers and pulp. Anal. Bioanal. Chem. 378, 1608–1615.
Kurkjian, R., Flegal, A.R., 2003. Isotopic evidence of the persistent dominance of blood lead concentrations by previous gasoline lead emissions in Yerevan, Armenia. Environ. Res. 93, 308–315.
Lanphear, B.P., Dietrich, K., Auinger, P., Cox, C., 2000. Cognitive deficits associated with blood lead concentrations $<10\,\mu g/dL$ in US children and adolescents. Pub. Health Rep. 115, 521–529.
Lejano, R., Ericson, J.E., 2005. Enduring tragedy of the commons: soil bound lead and the anachronicity of risk. J. Environ. Plan. Manage. 48 (2), 299–318.
Lustberg, M., Silbergeld, E., 2002. Blood lead levels and mortality. Arch. Intern. Med. 162, 2443–2449.
Manton, W.I., Angle, C.R., Stanek, K.L., Kuntzelman, D., Reese, Y.R., Kuehnemann, T.J., 2003. Release of lead form bone in pregnancy and lactation. Environ. Res. 92, 139–151.
Meyer, P.A., Pivetz, T., Dignam, T.A., Homa, D.M., Schoonover, J., Brody, D., 2003. Centers for Disease Control and Prevention: surveillance for elevated blood lead levels among children—United States, 1997–2001. MMWR Surveill. Summ. 52, 1–21.
Mielke, H.W., Reagan, .L., 1998. Soil is an important pathway of human lead exposure. Environ. Health Perspect. 106, 217–229.
Mielke, H.W., Gonzales, C.R., Smith, M.K., Mielke, P.W., 1999. The urban environment and children's health: soils as an integrator of lead, zinc, and cadmium in New Orleans, Louisiana, USA. Environ. Res. 81, 117–129.
Morgan, A., Holmes, E., Evans, J.C., 1977. Retention, distribution and excretion of lead by the rat after intravenous injection. Br. J. Ind. Med. 34, 37–42.
Navia, J.M., Narkates, A.J., 1980. Dental research. In: Baker, H.J., Lindsey, J.R., Weisbroth, S.H. (Eds.), The Laboratory Rat, vol. II. Academic Press, New York, pp. 59–74.
Needleman, H.L., Schell, A., Bellinger, D., Leviton, A., Allred, E.N., 1990. The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. N. Engl. J. Med. 322, 83–88.
Nriagu, J.O., 1990. The rise and fall of leaded gasoline. Sci. Total Environ. 92, 13–28.
Petrosyan, V., Orlova, A., Dunlap, C.E., Babayan, E., Farfel, M., von Braun, M., 2004. Lead in residential soil and dust in a mining and smelting district in northern Armenia: a pilot study. Environ. Res. 94, 297–308.
Provenza, D.V., 1988. Fundamentals of Oral Histology and Embryology, second ed. Lea & Fibiger, Philadelphia.
Rabinowitz, M.B., Leviton, A., Bellinger, D., 1993. Relationships between serial blood lead levels and exfoliated tooth dentin lead levels: models of tooth lead kinetics. Calcif. Tissue Int. 53, 338–341.
Ryan, J.A., Scheckel, K.G., Berti, W.R., Brown, S.L., Casteel, S.W., Chaney, R.L., Hallfrisch, J., Doolan, M., Grevatt, P., Maddaloni, M., Mosby, D., 2004. Reducing children's risk from lead in soil. Environ. Sci. Technol. 38, 18A–24A.
Sato, K., Hattori, M., Aoba, T., 1996. Disturbed enamel mineralization in a rat incisor enamel. Adv. Dent. Res. 10, 216–224.
Schumacher, E., McKeegan, K.D., Harrison, T.M., Migeon, H., 1994. In situ dating of zircon with the CAMECA IMS 1720. In: Benninghoven, A., et al. (Eds.), A Secondary Ion Mass Spectrometry SIMS IX, pp. 919–922.
Silbergeld, E.K., 1995. The international dimensions of lead exposure. Int. J. Occup. Environ. Health. 1, 336–348.
Silbergeld, E.K., 2003. Facilitative mechanisms of lead as a carcinogen. Mutat. Res. 533, 121–133.
Smith, C.E., Warshawsky, H., 1975. Cellular renewal in the enamel orhan and the odontoblast layer of the rat incisor as followed by radioautography using 3H-thymidine. Anat. Rec. 183, 523–561.
Thomas, V.M., Socolow, R.H., Fanelli, J.J., Spiro, T.G., 1999. Effects of reducing lead in gasoline: an analysis of the international experience. Environ. Sci. Technol. 33, 3942–3948.
US Environmental Protection Agency (EPA), 1999. Toxicological profile for lead. Public Health Services, ATDSR. US Department of Health and Human Services.