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Disposition of lead (Pb) in saliva and blood of Sprague-Dawley rats following a single or repeated oral exposure to Pb-acetate

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Abstract

Biological monitoring for lead (Pb) is usually based upon a determination of blood Pb concentration; however, saliva has been suggested as a non-invasive biological matrix for assessing exposure. To further evaluate the potential utility of saliva for biomonitoring, the disposition of Pb was evaluated in whole blood (WB), red blood cells (RBC), plasma, parotid gland, bone, and saliva following either a single oral dose of 100 mg Pb-acetate/kg body weight in rats or ~1-week after 5 sequential daily oral gavage doses of 1, 10, or 100 mg Pb-acetate/kg/day. Saliva volume, pH, total saliva protein, and α-amylase activity were also determined. At specified times post-dosing groups of animals were anesthetized and administered pilocarpine to induce salivation. Saliva was collected, the animals were humanely sacrificed, and tissue samples were likewise collected, weighed, and processed for Pb analysis. Following a single dose exposure to Pb-acetate, Pb was detectable in all samples by 30 min post-dosing. For both the single and repeated dose treatments the concentration of Pb was highest in WB and RBC relative to plasma and saliva. However, the Pb rapidly redistributed (within 5-days post-treatment) from the blood into the bone compartment based on the substantial decrease in WB and RBC Pb concentration, and the concurrent increase in bone Pb following repeated exposure at all dose levels. Although there is clear variability in the observed Pb concentrations in plasma and saliva, there was a reasonable correlation ($r^2 = 0.922$) between the average Pb concentrations in these biological matrices, which was consistent with previous observations. The single oral dose of Pb-acetate resulted in a decrease in salivary pH which recovered by 24 h post-dosing and a decrease in α-amylase enzyme activity which did recover within 5-days of ceasing exposure. It is currently unclear what impact these slight functional changes may or may not have on Pb salivary clearance rates. These results demonstrate a feasibility to rapidly detect Pb in saliva and suggest that saliva may correlate best with plasma Pb concentration.

Keywords: Lead; Saliva; Oral exposure; Pharmacokinetics; Rats

1. Introduction

Although a considerable effort has been put forth to understand the health implications of lead (Pb) exposure and to identify and eliminate sources of Pb contamination, this metal still remains a significant public health concern (Juberg et al., 1997). Hence there is still an ongoing need to evaluate exposures, and biomonitoring has been an important component of this evaluation. In the case of Pb, biomonitoring has primarily focused on the measurement of blood Pb concentration, although hair, urine, and saliva have likewise been utilized to assess exposure (P'an, 1981; Revich, 1994; Pirkle et al., 1995).

Although blood measurements represent the most common strategy for Pb biomonitoring, several studies

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suggest that saliva is a viable alternative matrix. The use of saliva is particularly enticing since this represents a simple non-invasive method that has distinct advantages in the evaluation of new-born infants and young children (Gorodischer and Koren, 1992). The potential utility of saliva as a biomonitoring medium for assessing human body burden of Pb from ambient environmental exposure has previously been demonstrated (P'an, 1981; Brodeur et al., 1983; Gonzalez et al., 1997). Gonzalez et al. (1997) utilized saliva to biomonitor for Pb and cadmium in a limited population of young adults living in Mexico City. Their results suggest that saliva is a potential biomonitoring matrix for Pb and demonstrated that populations residing in Mexico City had elevated Pb levels. However more recently, Koh et al. (2003) reported a poor correlation between blood and saliva Pb in workers with blood Pb levels ranging from 10 to 50 μg/dl. P'an (1981) did suggest that saliva Pb concentration may correlate better with the diffusible plasma concentration because this fraction has been shown to be directly excreted into sweat, urine, and feces (Baloh, 1974). These previous studies suggest that saliva has potential utility as a biomonitoring matrix for Pb; however, a better understanding of the dose- and time-dependent relationship between blood, plasma, and saliva Pb concentration is needed to understand the strengths and limitations of saliva as a biological matrix.

In this regard, studies have been conducted using rats to better understand the relationship between saliva Pb concentration and the distribution of Pb in whole blood (WB), plasma, plasma ultrafiltrate, and tissues (Mobarak and P'an, 1984; Timchalk et al., 2001). The objective of the current study is to continue the evaluation by assessing the "early" tissue disposition of Pb and salivary elimination following a single and repeated exposure, and to conduct a preliminary assessment of the impact of these exposures on salivary gland function. These results are also being used to further demonstrate the feasibility of using saliva as a biological matrix for assessing Pb exposure, and to help guide in the design of future pharmacokinetic studies.

2. Materials and methods

2.1. Animal studies

The experiments were conducted in adult male Sprague-Dawley rats (Charles River Laboratories, Inc., Raleigh, NC). Study I was focused on evaluating the early (0–24 h post-dosing) distribution of Pb and involved a single oral gavage dose of Pb-acetate (100 mg/kg). Study II focused on evaluating the distribution of Pb approximately 1-week after repeated oral exposures to Pb-acetate. Specifically, rats were given 5 sequential daily oral gavage doses of 1, 10, or 100 mg Pb-acetate/kg/day, and following the last dose the rats were maintained for 5 additional days prior to evaluation. For Study I, groups of rats (5 animals/time-point) were randomly assigned to 0 (control), 30 min, 1, 5, 12, and 24 h post-dosing groups. For Study II, groups of rats (5–7 animals/dose-group) were randomly assigned to 0 (control), 1, 10, or 100 mg/kg/day treatment groups. Prior to sacrifice all rat was anesthetized with an intraperitoneal (i.p.) injection of ketamine (87 mg/kg):xylazine (13 mg/kg), and given an i.p. injection of the cholinergic agonist pilocarpine (1 mg/kg) to induce salivation. Saliva was then collected from each rat, using a glass capillary tube, for approximately 30 min at which time the rats were exsanguinated by cardiac puncture as previously described (Timchalk et al., 2001). Whole blood and saliva specimens were collected from each animal and the blood was centrifuged for 10 min ($2000 \times g$) to separate plasma and RBCs, all samples were weighed prior to storage. In addition, the parotid salivary gland and a single femur bone from each rat was dissected, weighted, and stored for Pb analysis. An aliquot (~100 μl) of each saliva sample was used for protein determination using the BCA reagent (Pierce, Rockford, IL) with BSA as the standard, and for analysis of α-amylase activity using the Salimetric Salivary α-Amylase Assay Kit (Salimetric, Inc., State College, PA). All samples were stored frozen (−80 °C) until analyzed.

2.2. Analytical methods

The concentration of Pb in the specimens was determined by inductively coupled plasma/mass spectrometry (ICP/MS). Tissue samples were acid digested prior to ICP/MS analysis. To prepare the specimens for analysis they were carefully weighed into a tared AC (advanced composite) vessel and 1 ml each of deionized milli"Q" water and concentrated nitric acid, along with a known concentration of a Thallium (Tl-205) or Gold (Au-197) internal standard were added. The Teflon ACV vessels were capped and sealed into numbered stainless steel Parr Bomb sleeves, then transferred to a preheated oven and maintained at 140 °C for a minimum of 3 h. Upon removing and cooling of the samples, each vessel was opened and rinsed with ~2.0 ml of milli"Q" water, the rinse procedure was repeated three times. The contents of the vessel and rinses were transferred to a centrifuge tube which was brought up to volume (14.0 ml) with milli"Q" water. Internal standards were also added to each of the Pb standard solutions, which were likewise diluted to 14 ml (final nitric acid concentration ~7%). All glassware was washed in a leaching solution (10% (v/v) HNO_3/HCl) to remove potential Pb contamination.

The ICP/MS instrument used for the Pb analysis was a VG Plasma Quad, model PQ2 ICP/MS (VG Instruments Inc., Cherry Hill Drive, Mass). The Pb was analyzed using a scanning mode acquisition with the parameters set at 10.24 dwell (ms), 19 channels/amu, PC Detector mode, 0.5 time/sweep (s), and selected mass range of 99.6–210.4. The instrument used a Henry RF generator, the incident power was set at 1350 W for the analysis. Mass 208 was selected for the Pb analysis, mass

208 has a higher abundance (52.400) compared to the other atomic masses for Pb. Redistilled HNO_3 was used to rinse the tubing from the autosampler to the instrument, and double distilled HNO_3 was used for sample dilutions, standards, and background samples. Three replicates were done for each analysis.

2.3. Data analysis

The experimental data were analyzed with Microsoft® Excel to calculate descriptive statistics such as the means and standard deviations. The Pb concentration in WB, RBC, plasma, saliva, parotid, and bone were statistically analyzed for differences following the 1, 10, 100 mg/kg/day or 100 mg/kg (single) doses. Likewise the results from the saliva pH, total protein, and α-amylase activity analysis were also subjected to a statistical evaluation. The statistics entailed a one-way analysis of variance and Bonferoni multiple *t*-test was applied to evaluate the significance using GraphPad Prism®4 (GraphPad Software, Inc., San Diego, CA). The p value <0.05 was considered to be significant. In addition, linear regression analyses were also conducted using GraphPad Prism® 4 to compare the relationship between Pb concentrations in WB or plasma with saliva Pb concentration.

3. Results

All animals tolerated the oral dosing of the Pb-acetate and the i.p. administration of the anesthesia and pilocarpine needed to induce salivation. The time-course for Pb in WB, RBC, plasma, and saliva following an oral dose of 100 mg/kg through 24 h post-dosing is presented in Fig. 1 (Study I). Overall the relative concentration of Pb followed the order: WB > RBC ≫ plasma ≈ saliva. Gastrointestinal (GI) tract uptake of Pb into the WB was rapid since an average peak Pb concentration of 8.8 ± 4.5 μg/ml WB was attained between 0.5 and 1 h post-dosing. Between 5 and 24 h post-dosing WB Pb concentration remained substantially elevated with average concentrations ranging from 3.3 ± 1.3 to 4.6 ± 1.4 μg Pb/ml. The RBC accounted from the majority of the Pb present within the WB (67–85%), with the average Pb concentration in RBC ranging from 6.7 ± 3.6 μg/ml (1 h post-dosing) to 2.4 ± 0.5 μg/ml (12 h post-dosing). Lead was also rapidly detected in the plasma fraction although the Pb concentrations were 2-orders of magnitude lower (30 ± 19 to 156 ± 152 ng/ml) and demonstrated substantially greater variability as illustrated by the larger standard deviations (see Fig. 1C). In saliva the highest average Pb concentration was 800 ± 665 ng/ml and was obtained at 30 min post-dosing. For the remaining time-points the average Pb concentration ranged from 92 ± 26 to 220 ± 184 ng/ml. As with the plasma there was greater variability observed in the saliva than with the WB or RBC.

The concentration of Pb in WB, RBC, plasma, and saliva following repeated oral doses of 1, 10, or 100 mg/kg (Study II), and for comparison the Pb concentration following a single 100 mg/kg dose at 24 h post-dosing (Study I) are illustrated in Fig. 2. Analysis of Pb concentrations 5-days following the repeated administration of Pb-acetate indicated that the relative order of Pb tissue concentration was different than that observed following the single dose since: RBC > WB ≫ saliva > plasma. The slightly greater Pb concentration in RBC vs. WB may be related to the

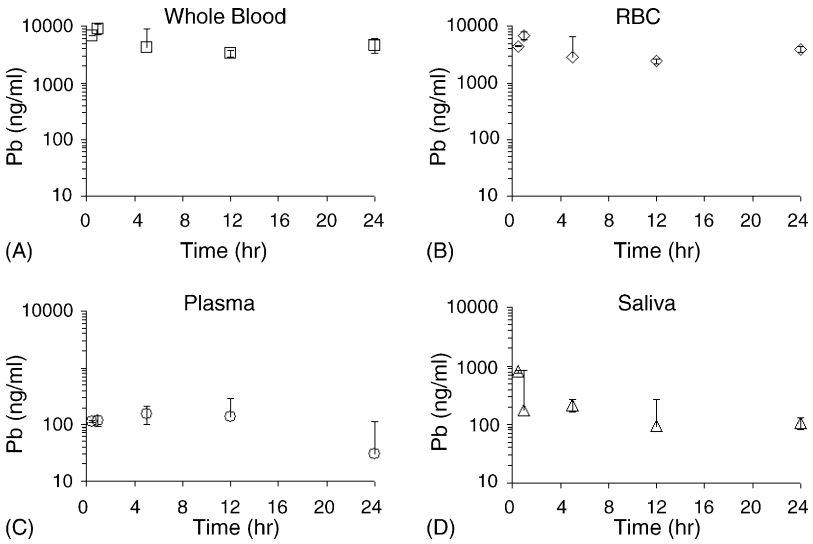

Fig. 1. Time course of Pb concentration in (A) whole blood (WB), (B) RBC, (C) plasma, and (D) saliva following oral gavage administration of 100 mg Pb-acetate/kg of body weight to adult male Sprague-Dawley rats. The values represent the mean ± S.D. of four to five animals per time-point.

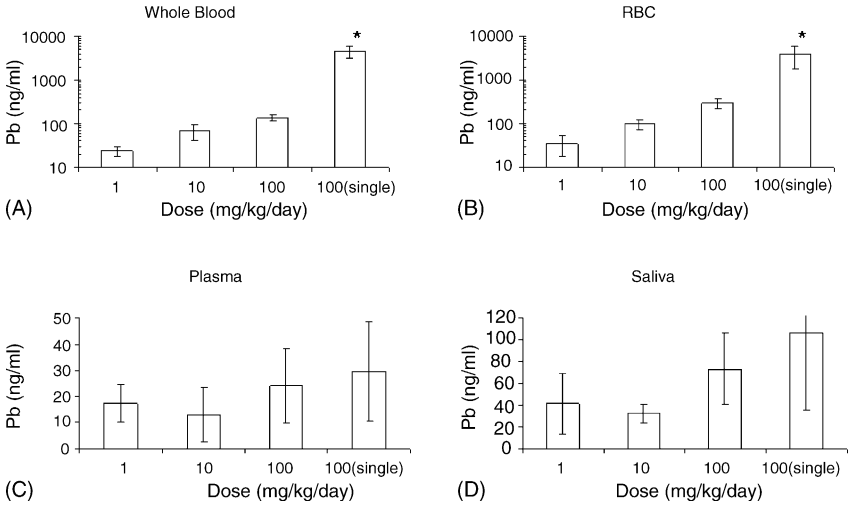

Fig. 2. Concentration of Pb in (A) whole blood (WB), (B) RBC, (C) plasma, and (D) saliva 5 days following oral exposure of adult male Sprague-Dawley rats to 5 daily doses of 1, 10, or 100 mg Pb-acetate/kg/day ($n = 4$–6), or 24 h after a single oral dose of 100 mg/kg ($n = 3$–5). The values represent the mean ± S.D. and the asterisk (*) denotes statistical significance ($p < 0.001$) relative to all other treatment groups.

lower observed plasma Pb concentrations (Fig. 2C) relative to those observed following the single dose exposures (Fig. 1C). Only the RBCs demonstrated a clear dose-dependent increase in Pb concentration at 1, 10, and 100 mg/kg/day. Whereas, in WB, plasma, and saliva there was no observable increase in Pb concentration within these tissues at doses of 1 and 10 mg/kg/day; yet all tissues demonstrated an increasing Pb concentration at 100 mg/kg/day. It is of interest to note that following the 100 mg/kg single dose of Pb-acetate the WB and RBC 24 h post-dosing Pb concentrations were 2- to 3-orders of magnitude greater than following the repeated dose (see Fig. 2A and B). In contrast, the Pb concentrations in both the plasma and saliva following the 100 mg/kg single and repeated doses were reasonably comparable (see Fig. 2C and D).

A comparison of the Pb concentration ratios in WB, RBC, or plasma versus saliva as a function of time post-dosing (Study I) and following a repeated administration of 100 mg/kg/day (Study II) are presented in Table 1. Overall, there was substantial variability observed in the Pb concentration in saliva relative to WB, RBC, or plasma. For the single dose study, the comparison was done starting at 1 h post-dosing to minimize any influence of cross contamination of saliva due to the oral gavage dosing. The ratio of Pb in Saliva:WB ranged from an average of 0.01 to 0.06, with the majority of the ratios across all time-points being less than 0.02. Similarly, the average Pb ratio in Saliva:RBC ranged from 0.02 to 0.12. The higher average ratio in the 5 h post-dosing group was associated with two samples where the ratio was 0.20 and 0.40 due to relatively higher Pb concentrations in saliva and lower concentrations in the WB/RBC. In contrast, the average Saliva:WB and Saliva:RBC ratios following the repeated exposures (Study II) were substantially higher. For the 10 and 100 mg/kg/day dose groups the average Saliva:WB and Saliva:RBC Pb ratios ranged from 0.35 to 0.68; whereas, following the 1 mg/kg/day dose the average ratios in Saliva:WB or Saliva:RBC were both 1.75. The overall observed increase in the ratios was primarily associated with lower WB and RBC Pb concentrations in these treatment groups. For the single dose exposure (Study I) the average Saliva:Plasma Pb concentration ratios ranged from 0.53 to 5.38 suggesting that the Pb concentration in saliva was comparable or slightly

Table 1
Comparison of the Saliva:WB, Saliva:RBC, and Saliva:Plasma Pb concentration ratios in Sprague-Dawley rats (Study I) administered 100 mg Pb-acetate/kg of body weight at selected time points post-dosing or (Study II) administered 1, 10, or 100 mg/kg/day for 5 days then sacrificed 5 days following the last dose of Pb-acetate

	Saliva:WB ratio	Saliva:RBC ratio	Saliva:Plasma ratio
Study I: time post-dosing (h)			
1	0.014 ± 0.005	0.020 ± 0.008	1.32 ± 1.19
5	0.060 ± 0.093	0.119 ± 0.202	2.02 ± 2.27
12	0.017 ± 0.010	0.028 ± 0.025	0.53 ± 0.64
24	0.012 ± 0.009	0.019 ± 0.012	5.38 ± 3.22
Study II: 5 days post-dosing (mg/kg/day)[a]			
100	0.68 ± 0.24	0.35 ± 0.15	5.89 ± 4.14
10	0.68 ± 0.41	0.50 ± 0.21	3.58 ± 3.37
1	1.75 ± 1.25	1.75 ± 1.63	3.14 ± 2.75

Values are mean ± S.D. for four to five animals.
[a] Five-repeated daily doses.

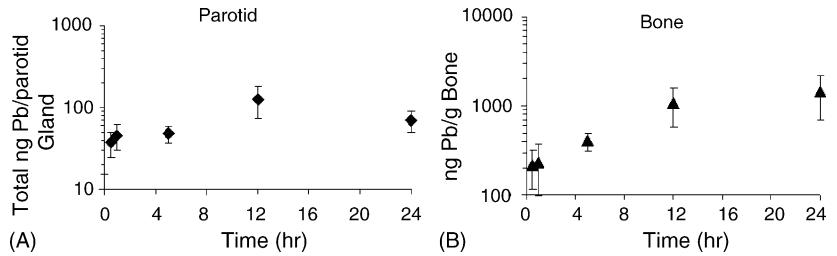

Fig. 3. Time course of Pb in (A) parotid saliva gland, and (B) concentration in bone following oral gavage administration of 100 mg Pb-acetate/kg of body weight to adult male Sprague-Dawley rats. The values represent the mean ± S.D. of four to five animals per time-point.

higher than the observed concentration in plasma. Similar ratios were observed following the repeated 1, 10, or 100 mg/kg/day exposures (Study II) where the average Saliva:Plasma ratios ranged from 3.14 to 5.89. These results suggest clear differences in the Pb disposition between blood components and saliva both as a function of dose and how soon sampling was conducted after exposure (i.e. immediate versus 5-day post-dosing).

The time-course of Pb in both the parotid salivary gland and in a sample of femur bone of rats following a single oral exposure to 100 mg Pb-acetate/kg of body weight (Study I) are presented in Fig. 3. In the parotid the average amount of Pb detected within 30 min post-dosing was 36 ± 12 ng and the Pb concentration in the femur bone was 140 ± 93 ng/g. In both tissues, the Pb burden increased over time, and appeared to plateau between 12 and 24 h post-dosing. The amount of Pb detected in the parotid following repeated exposures at 1, 10, and 100 mg/kg/day were 6.44 ± 4.01, 10.8 ± 6.1, and 24 ± 20 ng, respectively (see Fig. 4A), and did not increase proportionally with dose. In addition the total amount of parotid Pb following the single 100 mg/kg oral Pb-acetate dose relative to the repeated 1, 10 and 100 mg/kg/day doses was statistically elevated at 69 ± 20 ng ($p < 0.001$). Whereas, the amount of Pb detected in the bone following a repeated exposures of 1, 10, or 100 mg/kg/day were 0.37 ± 0.30, 3.9 ± 2.5, and 25 ± 13 μg/g bone, respectively (see Fig. 4B), and demonstrated a clear dose-dependent increase in concentration. The repeated 100 mg/kg/day dose group was significantly elevated ($p < 0.001$) relative to the concentrations seen following the repeated 1 or 10 mg/kg/day doses. In contrast, the amount of Pb sequestered in the bone 24 h following a single oral dose of 100 mg/kg was also significantly ($p < 0.001$) less than the amount of Pb deposited in the bone 5-days after receiving five-repeated daily doses of 10 or 100 mg/kg/day, which is consistent with the known accumulation of Pb in the bone.

In addition to assessing the blood and tissue burden of Pb following the single and repeated dose treatments, the effects of these exposures on saliva pH, total protein levels, and α-amlyase activity were determined. The results for the single oral 100 mg/kg dose (Study I) are presented in Table 2. The stimulation of salivation by pilocarpine administration resulted in substantial (average 0.61–1.1 ml) amounts of saliva being collected within 30 min of induction. The pH of the control saliva was 8.45 ± 0.20 and demonstrated a decreasing trend to a minimum of 8.12 ± 0.12 ($p < 0.01$) by 12 h post-dosing, but returned to control levels (8.48 ± 0.20) by 24 h post-dosing. The saliva total protein concentrations ranged from 0.58 ± 0.10 to 0.86 ± 0.18 mg/ml and there were no observable trends as a function of time post-dosing. In contrast, salivary α-amylase activity decreased as

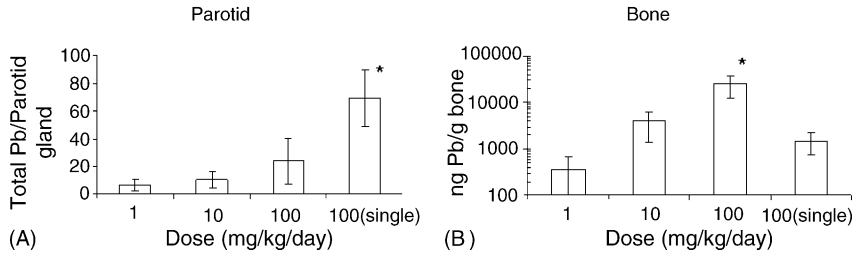

Fig. 4. The amount of Pb in (A) parotid saliva gland, and (B) concentration in bone, 5 days after oral exposure of adult male Sprague-Dawley rats to 5 daily doses of 1, 10, or 100 mg Pb-acetate/kg/day ($n = 5–7$), or 24 h after a single oral dose of 100 mg/kg ($n = 4–5$). The values represent the mean ± S.D. and the asterisk (*) denotes statistical significance ($p < 0.001$) relative to all other treatment groups.

Table 2
Saliva volume, pH, total protein, and α-amylase activity determined in Sprague-Dawley rats orally administered a single dose of 100 mg Pb-acetate/kg of body weight

	Saliva			
	Volume (ml)	pH	Total protein (mg/ml)	α-Amylase (U/ml)
Study I: time post-dosing (h)				
0 (control)	0.71 ± 0.40	8.45 ± 0.20	0.66 ± 0.28	18.1 ± 1.70
0.5	0.61 ± 0.21	8.23 ± 0.11	0.59 ± 0.19	16.3 ± 4.79
1	0.94 ± 0.31	8.18 ± 0.07*	0.59 ± 0.11	14.4 ± 3.96
5	0.86 ± 0.45	8.28 ± 0.12	0.75 ± 0.18	13.5 ± 6.03
12	1.08 ± 0.16	8.12 ± 0.12**	0.58 ± 0.10	12.2 ± 6.33
24	0.88 ± 0.53	8.48 ± 0.20	0.86 ± 0.18	10.5 ± 2.19

Values are mean ± S.D. for four to five animals per time-point.
* $p < 0.05$.
** $p < 0.01$ compared to controls.

Table 3
Saliva volume, pH, total protein, and α-amylase activity determined in Sprague-Dawley rats orally administered five repeat doses of 0, 1, 10 or 100 mg Pb-acetate/kg /day, and sacrificed 5 days after the last dose

	Saliva			
	Volume (ml)	pH	Total protein (mg/ml)	α-Amylase (U/ml)
Study II: dose (mg/kg/day)				
0 (control)	1.12 ± 0.50	8.09 ± 0.19	0.71 ± 0.19	19.5 ± 7.92
1	1.05 ± 0.53	8.09 ± 0.30	0.71 ± 0.23	32.7 ± 12.4
10	1.38 ± 0.31	7.92 ± 0.33	0.84 ± 0.30	38.6 ± 20.0
100	1.24 ± 0.33	8.16 ± 0.29	0.67 ± 0.27	20.3 ± 8.68

Values are mean ± S.D. for six to seven animals per dose level.

a function of time, with control activity averaging 18.1 ± 1.70 U/ml while the 24 h post-dosing saliva was decreased ~58% and averaged 10.5 ± 2.19 U/ml.

Likewise, similar salivary functional assessments were made for the animals that were exposed to five-daily doses of 1, 10, and 100 mg/kg/day then allowed 5 days to recover prior to evaluation (Study II). These results are presented in Table 3. As with the single dose experiments substantial amounts of saliva were collected (average 1.1–1.4 ml) within each treatment group. Also there were no statistically significant differences in the pH, total protein, or α-amylase activity of the saliva across treatment groups. The average pH ranged from 7.92 ± 0.33 to 8.16 ± 0.29, while the saliva protein concentrations ranged from 0.67 ± 0.27 to 0.84 ± 0.3 mg/ml, with no observable trends as a function of dose. Compared to the control α-amylase activity (U/ml) the average for the 1 and 10 mg/kg/day dose groups, were elevated (32.7 ± 12.4 and 38.6 ± 20 U/ml, respectively), but not statistically different that controls (19.5 ± 7.0 U/ml). Consistent with this observation, the enzyme activity for the 100 mg/kg/day treatments were comparable to controls (20.3 ± 8.7 U/ml).

4. Discussion

There is considerable interest in the utilization of saliva as a diagnostic fluid including its utility as a biological monitoring medium for drugs and chemical exposures (Pichini et al., 1996; Streckfus and Bigler, 2002). To advance the potential utility of saliva for Pb biomonitoring, sensitive and portable analytical tools are being developed to assess exposure in real-time (Timchalk et al., 2001, 2004; Lin et al., 2001). However, before saliva can be confidently utilized for biomonitoring, the kinetic relationship between Pb concentration in blood and saliva needs to be more fully understood to interpret a saliva Pb measurement and how it may or may not relate to systemic dosimetry (Timchalk et al., 2004). In this regard, the current study was designed to evaluate the saliva Pb disposition in rats immediately following a single oral exposure or several days after a repeated exposure where Pb has an opportunity to redistribute to other tissue compartments.

The results suggest that Pb-acetate when administered by oral gavage is rapidly absorbed, since peak blood Pb concentrations were attained within 30 min to 1 h post-dosing. This rapid absorption in the rat following an

oral gavage dose is consistent with the absorption (peak 2 h post-dosing) seen in a human that ingested 100 mg Pb-acetate on two separate occasions (Marcus, 1985), or following the clinically reported acute ingestion of elemental Pb objects (McKinney, 2000). Within the blood, Pb is rapidly partitioned between RBC and plasma with >95% of the blood Pb being associated with the RBC component. This is comparable to the blood distribution reported by Mobarak and P'an (1984) in rats repeatedly administered (i.p. injection) 100 mg Pb-acetate/kg of body weight. In addition, the relative distribution of Pb in WB and plasma was consistent across species. For example, in humans following a single acute Pb-acetate exposure the maximum blood and plasma Pb concentrations were 450–790 ng/ml and 18–48 ng/ml, respectively (Marcus, 1985). This indicates that ~95% of the blood Pb is associated with RBC, which is comparable to the response observed in the rat following the single or repeated Pb-acetate exposures.

The current study provides important experimental evidence that absorbed Pb is rapidly transferred from blood to saliva following a single oral exposure. However, the first saliva sample obtained at 30 min post-dosing averaged 740 ng Pb/ml, exceeding the concentration in all other saliva and plasma samples by a factor of ~5. This high Pb saliva concentration may be associated with cross contamination resulting from residual dose within the oral cavity, since the mouth was not rinsed prior to saliva collection. However, within 1–5 h post-dosing the saliva Pb concentrations were substantially reduced and reasonably comparable to the plasma values, suggesting that most Pb residue in the mouth had been swallowed.

The results of this study are also consistent with Pb undergoing a rapid redistribution (within 5-days post-treatment) from the blood into the bone compartment based on the substantial decrease in WB and RBC Pb concentration, and the concurrent increase in bone Pb, at all dose levels. These results are clearly consistent with previous pharmacokinetic studies in the rat that suggest that Pb undergoes substantial sequestration into the bone from the blood (Morgan et al., 1977; Miller et al., 1983; Bankowska and Hine, 1985; O'Flaherty, 1991).

The average ratio of Pb in Saliva:WB in the single dose experiment (Study I) ranged from 0.01 to 0.06, (see Table 1) and was comparable to the 0.08 that Timchalk et al. (2001) previously reported following an acute Pb-acetate exposure in F344 rats and the 0.05 that Mobarak and P'an (1984) observed in Sprague-Dawley rats. In contrast, the increase in the Saliva:WB and Saliva:RBC ratios following the repeated exposure experiment (Study II) is consistent with the sequestering of Pb from blood into the bone compartment. With the exception of the results from the 30 min post-dosing time-point the concentrations of Pb in both saliva and plasma were relatively low compared to the WB/RBC. In the current study, the average Saliva:Plasma ratio following the single or repeated dose of Pb-acetate ranged from 0.53 to 5.89 which suggest that the Pb concentration in saliva and plasma were reasonably comparable at all times post-dosing (Study I) and across dose groups (Study II). Mobarak and P'an (1984) also reported that the saliva and plasma Pb were substantially lower than in WB; however, in their study the salivary Pb concentrations were consistently less than the plasma concentrations following i.p. administration.

Regression plots of the average Pb concentrations in saliva versus plasma for the results obtained in the current study are presented in Fig. 5. It has been noted that the saliva Pb content may be proportional to the diffusible component of plasma Pb rather than WB Pb (P'an, 1981; Koh et al., 2003). Although there is substantial variability in the observed Pb concentrations in plasma and saliva,

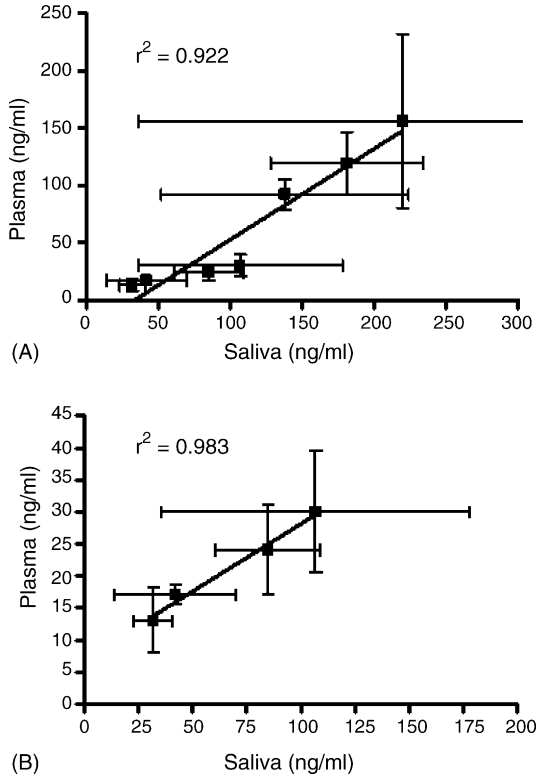

Fig. 5. Regression analysis of concentration of Pb in saliva versus plasma in adult male Sprague-Dawley rats. (A) Regression analysis includes ratio for single oral doses (1–24 h post-dosing) and 5 days following oral exposure to 5 daily doses of 1, 10, or 100 mg Pb-acetate/kg/day, and (B) regression analysis excluding the data from the single oral doses at 1, 5, and 12 h post-dosing.

there is a reasonable correlation ($r^2 = 0.922$) between the average Pb concentrations in these biological matrices, which is consistent with the observations of P'an (1981). The correlation was slightly improved if only those samples with a Saliva:Plasma ratio >3 were considered (see Fig. 5B; $r^2 = 0.983$). In contrast, there was no reasonable correlation between the WB or RBC and saliva Pb concentrations for either the single or repeated dose results (data not shown). These observations are consistent with the lack of a good correlation between blood and saliva Pb concentration in humans where blood Pb levels ranged from 10 to 50 μg/dl (Koh et al., 2003).

Mobarak and P'an (1984) reported that Pb concentration within the submaxillary gland (μg/g dry weight) exceeded the saliva Pb concentrations and proposed that plasma Pb is initially complexed to salivary acinar cell components prior to being released into the salivary excretory ducts. Consistent with previous reports, in the current study Pb was readily detected in the parotid salivary gland within 30 min of the oral dose and by 12 h post-dosing the amount of Pb in the parotid slightly exceeded the observed amount secreted in the saliva (data not shown). An important consideration is the understanding that a correlation between blood or plasma levels and saliva depends on the concentration range of interest (Timchalk et al., 2001). In this regard, the current blood Pb action levels for exposure in children and occupational exposure to workers are 10 and 40 μg/dl (100–400 ng/ml), respectively (ATSDR, 2005; CDC, 1997; NIOSH, 1999). In the current study, the blood Pb levels attained in rats 5 days after the repeated 100 mg/kg/day dosing were within the range of interest with regard to Pb action levels in humans.

O'Flaherty (1991) suggested that the majority of non-linear responses associated with dose, blood, or tissue Pb concentrations in vivo result from non-linearities in the relationship between plasma and RBC Pb concentrations. Hence, to be able to use saliva Pb measurements to estimate internal dosimetry requires a good understanding of the overall pharmacokinetic properties of Pb. To this end physiologically based pharmacokinetic models that have been developed for Pb are being adapted to predict blood Pb concentration from a saliva Pb measurement (O'Flaherty, 1991; Timchalk et al., 2001). This modeling approach should facilitate more accurate quantitative predictions of dosimetry over a very broad range of exposure conditions, and may be of particular importance if it is not possible to directly correlate the saliva and blood Pb concentrations.

In considering the utility of saliva for Pb biomonitoring, it is important to consider the impact that Pb exposure may have on salivary gland function, since altered function could modify Pb clearance rates. In this regard, several studies have evaluated the impact of longer term (28 day) Pb-acetate exposure via drinking water (0.01–0.05%, w/v) on rat submandibular gland secretory function (Abdollahi et al., 1997, 2000). They reported that Pb-acetate exposure resulted in a dose-dependent decrease in salivary flow rates, a decrease in both total salivary protein and α-amylase activity, decreased calcium and a reduced secretion of the lysosomal enzyme *N*-acetyl-β-D-glucosaminidase. In the current study the single oral exposure to Pb-acetate resulted in a decrease in salivary pH which recovered by 24 h post-dosing and as previously reported following sub-chronic exposure, a decrease in α-amylase enzyme activity which did recover within 5 days of ceasing exposure (see Table 3). However, it is currently unclear what impact these slight functional changes may or may not have on Pb salivary clearance rates; in this regard, future saliva Pb clearance studies should further evaluate the impact of saliva gland functional changes on Pb secretion.

In summary, the in vivo kinetics of Pb in blood, limited tissues, and saliva were evaluated following a single and repeated oral exposure to Pb-acetate in the rat. Lead was rapidly detected in all the matrices with the Pb concentration in WB and RBC substantially exceeding the plasma and saliva concentration. These results demonstrate the feasibility to rapidly detect Pb in saliva, after a recent Pb exposure, and the saliva Pb concentrations correlate best with the plasma Pb. These results are also being used to help design future studies that will evaluate the kinetics of Pb saliva clearance following repeated exposures to Pb over a broader dose-range.

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