Toxicology Letters 231 (2014) 270–276

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Investigation of saliva as an alternative matrix to blood for the biological monitoring of inorganic lead

James F. Staff [a,*], Anne-Helen Harding [a], Jackie Morton [a], Kate Jones [a], Erica A. Guice [b], Thom McCormick [b]

[a] Health and Safety Laboratory, Harpur Hill, Buxton, SK17 9JN, UK
[b] Coventry Diagnostics LLC, 1197 Rochester Road, Troy, MI 48083, USA

HIGHLIGHTS

- Lead exposure is measured by invasive blood sampling. A non-invasive alternative is desirable.
- We determine lead in blood and saliva in 105 UK workers, presenting a new method for saliva analysis.
- Blood–saliva correlation improves at higher exposures; unchanged by history, smoking or age.
- StatSure device is effective for high levels. Contamination hinders lower-level measurements.
- Saliva lead may be effective as a surrogate for blood lead only in highly-exposed populations.

ARTICLE INFO

Article history:
Received 26 June 2014
Received in revised form 12 September 2014
Accepted 23 September 2014
Available online 26 September 2014

Keywords:
Lead
Saliva
Blood
Occupational exposure
Biological monitoring
ICP-MS

ABSTRACT

Introduction: Whole blood is the established matrix for biological monitoring of inorganic lead; however blood sampling is an invasive procedure. Saliva offers a potential non-invasive alternative. This study determines lead in whole blood and saliva. A novel method for saliva sampling and preparation is presented.

Methods: Paired blood and saliva samples were obtained from 105 occupationally exposed UK workers. Saliva was collected using a StatSure sampling device, and a nitric acid digestion step was incorporated. The utility of the device for this application was evaluated. Whole blood was obtained by venepuncture. Analyses were carried out by ICP-MS.

Results: The limit of detection for lead in saliva was 0.011 μg/L. Mean blank-corrected recovery from 10 μg/L spiked saliva was 65.9%. The mean result from blank saliva extracted through the StatSure device was 2.86 μg/L, compared to 0.38 μg/L by direct analysis. For the paired samples, median blood lead was 6.00 μg/dL and median saliva lead was 17.1 μg/L. Pearson's correlation coefficient for saliva lead versus blood lead was 0.457 (95% C.I. 0.291–0.596).

Conclusions: ICP-MS analysis allows sensitive determination of lead in saliva with low limits of detection. The StatSure device is effective for high occupational exposures, but contamination from the device could confound lower-level measurements. Saliva would only be effective as a surrogate for whole blood for highly-exposed populations, although with further work it may have applications as a biomarker of recent exposure.

1. Introduction

Workers in a wide range of industries are at risk of occupational exposure to lead. Although the adverse effects of acute lead poisoning are well-known, most incidences of lead toxicity occur through the accumulation of lead in the body by repeated exposures to small amounts (Thaweboon et al., 2005). Toxic effects of repeated low-level lead exposures include hypertension,

* Corresponding author at: Health and Safety Laboratory, Harpur Hill, Buxton, Derbyshire SK17 6HH, United Kingdom, Tel.: +44 1298 218522.
E-mail addresses: james.staff@hsl.gsi.gov.uk, jamesfrankstaff@googlemail.com (J.F. Staff).

http://dx.doi.org/10.1016/j.toxlet.2014.09.018

alteration of bone cell function and reduction in semen quality (Goyer, 1993). Lead is also classified by the International Agency for Research on Cancer (IARC) as a class 2B carcinogen, indicating that "the agent is possibly carcinogenic to humans" (IARC, 2013). However, the major risk of lead exposure is toxicity to the nervous system, with the most susceptible populations being children, infants and the foetus (Goyer and Clarkson, 2001).

Lead may be absorbed into the body by several different pathways. In the UK, biological monitoring for lead is mandatory under the Control of Lead at Work Regulations (2002) where a worker's risk of lead exposure is considered significant by inhalation, ingestion or dermal absorption (HSC/HSE 2002). Whole blood is currently the matrix most commonly used for the determination of inorganic lead exposure and has been used as such for over fifty years (Agency for Toxic Substances and Disease Registry, 2007). However, blood sampling is an invasive procedure. Sample collection requires a qualified phlebotomist, and therefore incurs expense. The procedure also causes discomfort, which may be a source of stress to workers participating in monitoring. A non-invasive alternative would therefore be desirable.

As well as occupational exposures, lead exposure from environmental sources is increasingly a matter of concern, especially involving populations living in low-income urban communities (Nriagu et al., 2006). A cheap, simple, non-invasive sampling technique would facilitate much more extensive studies of such environmental exposures.

Several studies have explored saliva as an alternative matrix for the biological monitoring of lead (Koh et al., 2003; Nriagu et al., 2006; Barbosa et al., 2006; Costa de Almeida et al., 2009). The use of saliva would have several potential advantages: its collection is non-invasive and therefore there are no concerns over discomfort to participants; collection is straightforward and cheap to carry out; sample storage and transport arrangements are less complex than those for blood; and in addition the ethical approval for sampling is more easily obtained (Nriagu et al., 2006; Morton et al., 2014).

It is thought that the lead content of saliva may be related to the unbound fraction in the plasma (Nriagu et al., 2006), and as the plasma composition closely reflects that of the extracellular fluid, measuring salivary lead may therefore indicate the level of exposure to which most bodily cells are subjected (Costa de Almeida et al., 2009). However, using saliva does present some problems, particularly in the collection and preparation of the sample: the flow and ion content of saliva can vary significantly throughout the day; whole saliva may contain other substances such as food debris, bacteria and epithelial cells; and hand-to-mouth behaviour prior to sample collection could cause sample contamination (Barbosa et al., 2006). There is also no widely agreed method to adjust for how dilute/concentrated the saliva collected is (such as creatinine-correction for the analysis of urine).

The literature does not present a standard method for the collection and preparation of saliva samples. The use of stimulants to increase saliva flow, collection of whole saliva versus particular components of saliva, the choice of sampling device and the treatment of the saliva before analysis have been approached very differently by different authors (Koh et al., 2003; Nriagu et al., 2003 Barbosa et al., 2006; Costa de Almeida et al., 2009; Thaweboon et al., 2005; Morton et al., 2014).

Past studies have also produced very different results when comparing lead levels in blood and saliva. The saliva lead: blood lead ratio has varied from <1% (Barbosa et al., 2006) up to 271% P'an AYS, 1981. The correlation reported between saliva lead and blood lead has also varied: P'an AYS, 1981 and Morton et al. (2014) reported good correlations ($r = 0.80$ and $r = 0.69$ respectively) between log(blood lead) and log(saliva lead), Koh et al. (2003) reported a weaker correlation ($r = 0.41$) between log(saliva lead) and blood lead, whereas others have reported poorer correlations (Barbosa et al., 2006; Nriagu et al., 2006; Thaweboon et al., 2005).

In this study, paired samples of whole blood and saliva were collected from UK workers occupationally exposed to inorganic lead, as part of their routine biological monitoring schedule. The authors present a novel method for the collection and preparation of saliva for analysis, using a StatSure (StatSure Diagnostics Systems, Inc., New York, USA) saliva collection device and incorporating a nitric acid digestion preparation step, prior to dilution with an acid diluent. Whole blood was collected by venepuncture and diluted with an alkaline diluent. Analyses of both matrices for lead were carried out by inductively-coupled plasma mass spectrometry (ICP-MS).

The recovery of lead from a 10 μg/L spiked saliva sample using the StatSure device was evaluated, and components of the device tested individually for any lead emanating from them. The correlation between blood lead and saliva lead measurements in an occupationally-exposed cohort was calculated, and multiple regression analyses carried out to explore whether this relationship was affected by age, smoking status or the history of previous lead exposure.

2. Methods

2.1. Study cohort

This study determines lead levels in paired blood and saliva samples from a cohort of 105 UK workers routinely monitored for occupational exposure to inorganic lead. The study was approved by the National Research Ethics Service Committee East Midlands – Nottingham 1 (12/EM/0217). Consenting workers were asked to provide a saliva sample at the same time as their routine blood sample. Descriptive statistics of the sample cohort are provided in Table 1.

Table 1
Descriptive statistics of the sample cohort.

	All samples	History				
		1	2	3	Fluctuating history	No history
Number of paired samples	105	27	42	44	21	40
Number of smokers	53	11	19	19	10	24
Number of non-smokers	52	16	23	25	11	16
Age range (years)	18–65	19–65	19–65	19–65	21–55	18–58
Mean age (years)	37	40	42	42	33	32
Median age (years)	35	44	43	43	33	30
Mean Δ^a (μg/dl)	0.63	−0.07	−0.53	−0.50	3.01	N/A
Standard deviation Δ^a (μg/dl)	9.49	0.79	1.19	1.34	16.60	N/A
Median Δ^a (μg/dl)	−1.00	0.00	−0.80	−0.80	−4.00	N/A
Δ^a interquartile range (μg/dl)	−2.00–1.00	−1.00–0.89	−1.50–0.00	−1.53–0.19	−7.71–11.00	N/A

[a] Δ = the difference between the result of the study Pb(B) value and the mean of the historical Pb(B) observations.

2.2. Sample collection:

Saliva samples were collected using the StatSure sampling device (Fig. 1). The mouth was not rinsed prior to sampling. The collector paddle was positioned under the tongue until the indicator at the opposite end turned blue (as per the manufacturer's guidelines). This indicates that a volume of at least 1 mL of saliva has been collected by the device. The collector was then removed from the mouth and inserted into the tube, so that the paddle end was immersed in the buffer solution. The cap was placed over the top of the collector stem and pushed to close. The tube was then gently shaken to mix the saturated collector with the buffer.

Whole blood samples were collected by venepuncture, in an ethylenediaminetetraacetic acid (EDTA) coated Vacutainer (BD, Oxford, UK).

The paired samples were transported immediately to the Health and Safety Laboratory, Buxton (HSL). Upon receipt the blood samples were refrigerated and analysed within 5 working days. The saliva samples were stored at −20 °C and analysed as a single batch once all samples had been received. The devices were stored intact (i.e. with the sampling paddle immersed in the buffer solution).

2.3. Analytical methods

2.3.1. Analysis of blood samples

The blood samples were analysed for lead according to HSL's standard operating procedure. Whole blood was diluted 1 in 50 with an alkaline diluent (1 g/L EDTA (Fisher Scientific, Loughborough, UK), 0.1% v/v Triton X-100 (Fisher Scientific, Loughborough, UK), 1% v/v ammonia (Romil Ltd., Cambridge, UK) and 80 μg/L platinum (VWR Standards, Lutterworth, Leicestershire, UK) as an internal standard. Standard solutions were prepared from a 1000 mg/L lead standard solution (VWR Standards, Lutterworth, Leicestershire, UK). The final calibration range was 10–80 μg/dL.

External certified reference materials (CRM) used were Lyphochek Whole Blood Metals Control levels 1 and 3 (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK) and were analysed at the start and end of every run. A matrix-matched 40 μg/dL standard check was run at the start, end and after every 10 samples to monitor drift over the course of the run. If drift exceeded ±10%, the run was repeated.

The method is accredited by the United Kingdom Accreditation Service (UKAS) and routine external quality assurance schemes are successfully participated in (United Kingdom National External Quality Assessment Service (UK-NEQAS) monthly and the German External Quality Assessment Scheme for analyses in biological materials (G-EQUAS) annually).

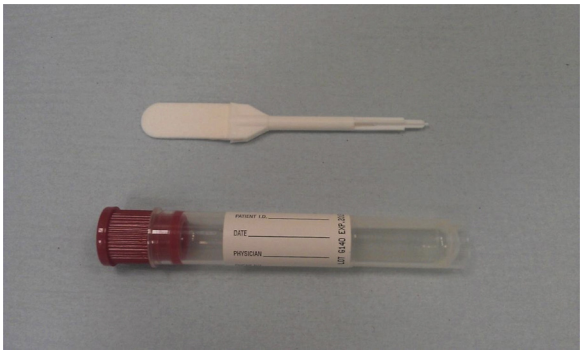

Fig. 1. StatSure saliva sampling device – collection paddle (above) and tube containing buffer solution (below).

2.3.2. Analysis of saliva samples

The sampling devices were thawed at room temperature, placed on rollers for 1 h and then vortex-mixed for 10 s each. The paddle was then removed and discarded. In screw-cap 5 mL polypropylene tubes (Sarstedt Ltd., Leicester, UK) 0.15 mL of the saliva/buffer mixture was added to 0.15 mL concentrated nitric acid (Romil Ltd., Cambridge, UK). The tubes were capped, vortex-mixed and heated for 1 h at 100 °C. The tubes were cooled and vortex-mixed. The acid-digested sample was then diluted 1 in 10: each sample contained 0.25 mL of the digest, 0.75 mL ultrapure water (Millipore, Watford, UK) and 1.50 mL acid diluent (1% v/v conc. nitric acid, 10 μg/L platinum as internal standard). Standard solutions were prepared in 5% v/v nitric acid, from a 1000 mg/L lead standard solution. The final calibration range was 0.05–10 μg/L. A 0.5 μg/L standard was run at the beginning and end, and after every 10 samples, in order to check for drift over the course of the run.

Quality control (QC) samples were prepared using blank saliva (Innovative Research, Novi, MI, USA) which was analysed both as a blank, and spiked with 10 μg/L lead. For "Device" QCs, 1 mL of saliva was sampled from a plastic beaker using the StatSure sampling device. The device was stored overnight at −20 °C and then prepared as the samples were. For "Fresh" QCs, 1 mL spiked saliva was added to 1 mL ultrapure water (to replicate the volume of buffer in the device) and mixed. This mixture was then analysed as the device contents were. "Fresh" and "Device" QCs (blank and 10 μg/L spike) were analysed at the beginning and end of the analysis and after every 20 samples. An external CRM, Lyphochek Urine Metals Control level 1 (no saliva CRM material is commercially available), lot 69151 (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK) was prepared as the "Fresh" QC was, and also analysed at the beginning and end of the analysis and after every 20 samples.

2.3.3. ICP-MS analyses

The diluted blood and saliva samples were analysed using a Thermo X7 Series 2 ICP-MS instrument (Thermo-Fisher Scientific, Hemel Hempstead, UK). The instrument was tuned on a daily basis to ensure optimisation. The instrument was set up with direct nebulisation in normal mode with optimised conditions. Extraction voltage was typically – 100 V, Rf Power 1400 W, focus voltage 12.0 V and nebuliser gas flow rate (using a Burgener Miramist nebuliser (Burgener Research International, Kingston-upon-Thames, UK)) 0.83 L/min. Dwell times were 50 ms for ^{208}Pb for blood analysis and 100 ms for ^{208}Pb for saliva analysis, both methods had a dwell of 10 ms for ^{195}Pt. 3 replicates per sample were carried out. For blood analysis there were 100 sweeps per replicate, for saliva analysis, 50 sweeps per replicate.

2.4. Additional testing of sampling devices

2.4.1. Blank saliva

An additional investigation was carried out, to investigate whether any contamination of the sample could occur from the StatSure sampling device, and if so, whether the freezing/thawing process had any effect.

Four sample types were prepared using blank saliva:

- A) 1 mL of refrigerated blank saliva – prepared as the "Fresh" QC above.
- B) 1 mL of frozen and thawed blank saliva – prepared as the "Fresh" QC above.
- C) 1 mL of refrigerated blank saliva – sampled using device; filled device refrigerated overnight before analysis.
- D) 1 mL of refrigerated blank saliva – sampled using device; filled device frozen overnight before analysis.

Ten of each sample type were prepared and analysed using the same ICP-MS method as specified above.

2.4.2. Comparison of device components

The individual components of the sampling device were also investigated, in order to elucidate from which part of the device any possible contamination originated. Samples were prepared from the buffer contained within the device, the paddle with which the saliva is collected, and the outer tube. From each device, the buffer was decanted into a 5 mL screw-cap polypropylene tube. The outer tube component was then rinsed thoroughly with ultrapure water and dried, before adding 2 mL ultrapure water and capping the tube. The head of the paddle component was cut from its stick and placed in a screw-cap 5 mL polypropylene tube. All tubes were vortex-mixed for 10 s and then rolled for 1 h before being stored overnight at −20 °C. The samples were then thawed at room-temperature, rolled for a further hour and vortex-mixed for a further 10 s. The contents of each tube were then diluted 1 in 10 and analysed by ICP-MS as per the method specified above. Five of each sample type were analysed.

2.5. Statistical analyses:

2.5.1. Categorisation of exposure history

In order to ascertain whether a worker with a "steady" history of lead exposure would produce differing results to one whose lead exposure had fluctuated, it was necessary to quantify the degree to which each worker's historical exposure had fluctuated. Over 90% of the lead content of whole blood is contained in the erythrocytes (Goyer, 2001). The average survival time of erythrocytes in the bloodstream is 120 days (Dessypris, 1999). To account for this, the mean of all blood lead values acquired since January 2009, and recorded ≥120 days prior to the measurement of the study sample, was calculated for each individual. The difference between the result of the study blood lead value and the mean of the historical observations (Δ) was then calculated. The median Δ was −1 μg/dL, and the 25th and 75th percentiles −2 μg/dL and +1 μg/dL respectively. However, the presence of a small number of large Δ values produced an overall standard deviation of 9.49 μg/dL. It was decided to categorise the samples for their exposure history according to the magnitude of Δ. History "1" included all samples where $\Delta \leq \pm 1$ μg/dL; history "2" all samples where $\Delta \leq \pm 2$ μg/dL; history "3" all samples where $\Delta \leq \pm 3$ μg/dL. Samples where $\Delta > \pm 3$ μg/dL were categorised as "fluctuating history". Samples with no blood lead values recorded ≥120 days prior to the measurement of the study sample were categorised as "no sample history".

2.5.2. Regression analysis

Neither the blood lead nor the salivary lead data were normally distributed, with the salivary lead data more skewed than the blood lead data. Both datasets could be much more closely approximated to a log-normal distribution; therefore the relationship between log(saliva lead) and log(blood lead) was investigated. Log(saliva lead) was plotted against log(blood lead) and the Pearson's correlation coefficient (r) was calculated, for the entire dataset and for the various history categories.

Multiple regression analyses were also carried out to investigate whether smoking status or the age of the participant had any effect on the saliva or blood lead levels, or on the relationship between the two.

3. Results

3.1. Analytical performance

For the blood lead analysis, all CRM results were within the certified range. Values obtained for the CRMs were as follows: level 1 lot 36741 (certified range 9.39–14.1 μg/dL): $n = 91$ mean 11.1 μg/dL, standard deviation (SD) 0.63 μg/dL; level 3 lot 36743 (certified range 43.7–65.5 μg/dL): $n = 91$, mean 52.5 μg/dL, SD 2.81 μg/dL.

The limit of detection (LOD) for the saliva analysis for the study was 0.011 μg/L, based on the mean of all the blank samples, plus three times the standard deviation of the mean (McNaught and Wilkinson, 1997). All results were greater than the LOD and therefore no non-detects were observed. A urine-based CRM (Lyphochek Urine Metals Control level 1, lot 69151) was analysed with the saliva samples. All results from this CRM fell within the acceptable range (8.82–13.2 μg/L).

The results of the prepared QC saliva samples were used to calculate percentage recoveries for the 10 μg/L spiked sample, corrected for the lead level present in the blank, for both the "Fresh" and "Device" QCs. For the "Fresh" QCs, recovery of 107.7% was observed. For the "device" QCs, recovery was 65.9%.

3.2. Summary statistics

Descriptive statistics of the sample cohort are provided in Table 1. The cohort comprised 105 paired blood and saliva samples. All participants were male (this was not an intentional discrimination by the authors, but due to the presence of very few female workers in the industries studied). There were 53 samples provided by smokers and 52 by non-smokers. The age range of participants was 18–65 years, with a mean age of 37 years old and a median age of 35 years old. Forty of the individuals sampled were categorised as having "no sample history". History category 1 ($\Delta = \pm 1$ μg/dL) included 27 samples; category 2 ($\Delta = \pm 2$ μg/dL) included 42 samples; and category 3 ($\Delta = \pm 3$ μg/dL) included 44 samples. The remaining 21 samples had $\Delta > \pm 3$ μg/dL and were classified as "fluctuating history".

Summary statistics of the lead levels observed in both the blood and in the saliva samples are presented in Table 2. There were no significant differences in blood lead values between the history categories 1–3 (mean: 5.59 μg/dL, 5.40 μg/dL and

Table 2
Summary statistics for lead levels in blood and saliva samples.

		All samples	History					Smoking status	
			1	2	3	Fluctuating history	No history	Smoker	Non-smoker
Blood Pb	Number of samples	105	27	42	44	21	40	53	52
	Mean (μg/dl)	8.34	5.59	5.40	5.91	17.62	6.15	7.94	8.75
	Median (μg/dl)	6.00	4.00	4.00	4.00	15.00	5.00	6.00	5.50
	Interquartile range (μg/dl)	3.00–11.00	3.00–6.50	3.00–6.00	3.00–7.00	10.00–18.00	2.75–7.25	3.00–10.00	3.00–11.25
	Sandard deviation (μg/dl)	7.99	4.16	3.72	4.32	11.31	5.14	6.21	9.53
–									
Saliva Pb	Mean (μg/l)	40.2	19.8	27.8	29.0	66.2	38.9	43.5	36.9
	Median (μg/l)	17.1	15.5	15.7	15.9	48.8	15.2	17.0	17.8
	Interquartile range (μg/l)	11.5–43.9	11.3–23.8	10.4–29.1	10.9–30.6	22.4–79.4	9.8–24.6	11.5–53.7	12.2–33.8
	Standard deviation (μg/l)	56.1	14.2	31.9	32.2	66.3	66.9	57.1	55.3

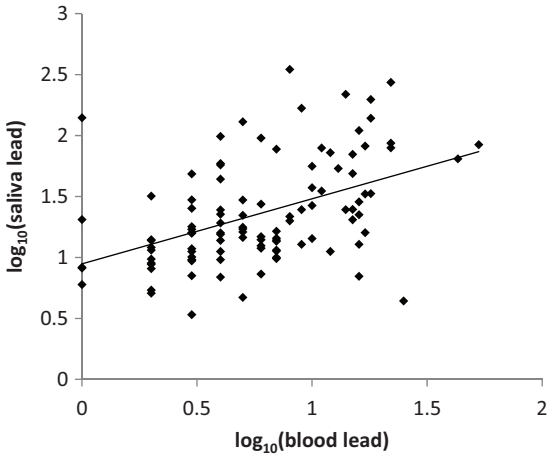

Fig. 2. Linear regression plot of log10(saliva lead) versus log10(blood lead) for all data. Pearson's correlation coefficient (r) = 0.457.

5.91 μg/dL respectively; median: all 4.00 μg/dL). Variability was also very similar for the three categories (standard deviation: 4.16 μg/dL, 3.72 μg/dL and 4.32 μg/dL, respectively). However, the blood lead values for the "fluctuating history" category were much higher (mean: 17.62 μg/dL; median: 15.00 μg/dL). Variability was also much greater in this category (standard deviation: 11.31 μg/dL).

For the saliva lead values, the mean and 75th percentile values are substantially lower for history category 1 than for categories 2 and 3 (mean: 19.8 μg/L, 27.8 μg/L and 29.0 μg/L, respectively; 75th percentile: 23.8 μg/L, 29.1 μg/L and 30.6 μg/L, respectively). The variability is also lower in category 1 than the other two categories (standard deviation: 14.2 μg/L, 31.9 μg/L and 32.2 μg/L respectively). However the median values do not demonstrate any significant difference (15.5 μg/L, 15.7 μg/L and 15.9 μg/L, respectively). Similarly to the results in blood, the salivary lead values for the "fluctuating history" category were much higher (mean: 66.2 μg/L; median 48.8 μg/L). Variability was also much higher (standard deviation: 66.3 μg/L) than for categories 1, 2 or 3.

There were no substantial differences in the blood lead values between smokers and non-smokers. For the saliva lead values, the mean and 75th percentile values were higher (not statistically significant) in smokers than non-smokers (mean: 43.5 μg/L and 36.9 μg/L, respectively; 75th percentile 53.7 μg/L and 33.8 μg/L, respectively). However, the median saliva lead values for smokers and non-smokers were very similar (17.0 μg/L and 17.8 μg/L, respectively), and variability was only very slightly higher (not statistically significant) in smokers than non-smokers (57.1 μg/L and 55.3 μg/L, respectively).

3.3. Regression analysis

Fig. 2 shows log(saliva lead) plotted against log(blood lead) for all of the 105 paired samples. A Pearson's correlation coefficient (r) of 0.457 (95% C.I. 0.113–0.723; $p = 0.0128$) was observed between the two datasets. The correlations between log(saliva lead) and log (blood lead) for the various history categories are shown in Table 3.

Only the "no history" category showed any substantial difference in the r-value, with a much lower Pearson's r (0.159, C.I. −0.161 to 0.448) than the other categories. The correlations for all other history categories were very similar, with no significant differences in Pearson's r from one another, or from that of the whole dataset.

Regression of log(saliva lead) and log(blood lead) on smoking showed no evidence of any significant effect due to smoking (coefficient 0.0446, $p = 0.598$ and coefficient 0.0713, $p = 0.108$ respectively). Regression of log(saliva lead) on age showed no evidence of a significant effect due to age (coefficient −0.00577, $p = 0.099$); however there was evidence of an inverse relationship between age and log(blood lead) (coefficient −0.0128, $p = 0.000$).

The correlations between log(saliva lead) and log(blood lead), unadjusted and adjusted for smoking or for age (see Table 4a) indicate that neither smoking nor age has a significant effect on the correlation between log(saliva lead) and log(blood lead). The Pearson's r values when adjusted for smoking status ($r = 0.445$ among smokers; $r = 0.476$ among non-smokers) or for age ($r = 0.474$) all remain very similar to the unadjusted value ($r = 0.457$). Regression of log(saliva lead) on log(blood lead), adjusted for smoking status or for age (see Table 4b) confirms this – the coefficients for smokers compared to non-smokers and for age are both small and with high p-values, indicating that they are not statistically significant (coefficient = 0.036, $p = 0.632$; and coefficient = −0.004, $p = 0.153$ respectively).

3.4. Additional testing of sampling devices

3.4.1. Blank saliva

The mean lead concentration and its standard deviation were calculated for each blank saliva sample type. Sample types A (refrigerated blank saliva, directly analysed) and B (frozen and thawed blank saliva, directly analysed) both showed very low blank results (0.238 ± 0.063 μg/L and 0.376 ± 0.130 μg/L respectively), with the frozen saliva producing slightly higher results. This difference was found to be significant using a Student's t-test (95% confidence), and may have occurred due to the extra preparation step in freezing and thawing the blank saliva.

Sample types C (refrigerated blank saliva, refrigerated in device) and D (refrigerated blank saliva, frozen in device), which passed through the StatSure device, showed much higher mean results (3.512 ± 1.352 μg/L and 2.861 ± 1.128 μg/L, respectively) than A and/or B. This indicates that a significant amount of lead contamination emanated from the device itself. This contamination was also highly variable, with the standard deviation for both C and D approximately 40% of the mean. Although these levels of contamination were small in comparison to the results obtained from the occupationally-exposed lead workers participating in this study; measurements of lower-level environmental exposures could be over-estimated.

Using a Student's t-test (95% confidence), sample types C and D were not found to differ significantly from one another, indicating

Table 3
Pearson's correlation coefficients (r) for log(saliva lead) with log(blood lead) for different history categories.

	All samples	History of blood lead				
		Stability 1	Stability 2	Stability 3	Fluctuating history	No history
Number of samples	105	27	42	44	21	40
Pearson's correlation coefficient (r)	0.457	0.473	0.494	0.531	0.498	0.159
95% confidence interval	0.291, 0.596	0.113, 0.723	0.224, 0.694	0.278, 0.715	0.085, 0.765	−0.161, 0.448
p-value	<0.0001	0.0001	0.0009	0.0002	0.0216	0.3276

Table 4a
Pearson's correlation coefficients (r) for log(saliva lead) with log(blood lead), unadjusted and adjusted for smoking status or for age.

		Pearson's r between log(saliva lead) and log (blood lead)
	Unadjusted	0.457 ($p < 0.0001$)
Adjusted for smoking (yes/no)	Smokers	0.445 ($p = 0.0008$)
	Non-smokers	0.476 ($p = 0.0004$)
–		
Adjusted for age (continuous variable)		0.474 ($p < 0.0001$)

that the process of freezing the sample inside the device does not affect the blank result.

3.4.2. Comparison of device components

The mean and standard deviation were calculated for each blank saliva sample type. The water samples from the outer tube showed consistently low lead levels (mean: 0.027 μg/L; standard deviation 0.051 μg/L). The buffer solution showed slightly higher lead levels (mean 0.293 μg/L; standard deviation 0.055 μg/L); however, they were reasonably consistent, and at a low enough level to be of minimal concern for the routine analysis of biological samples. The paddle however, showed significantly higher levels of lead contamination, with a high degree of variability (mean 1.643 μg/L; standard deviation 0.661 μg/L). This contamination could reduce the reliability of low-level environmental exposures using the device.

4. Discussion

This study presents a sensitive method for the determination of lead in saliva by ICP-MS. The LOD for this ICP-MS method was extremely low (0.011 μg/L), allowing effective detection of lead at trace levels. This is comparable to the sensitivity previously achieved by Morton et al. (2014) (0.024 μg/L); and overcomes the problems faced by researchers such as Wilhelm et al. (2002), where a less sensitive method (LOD: 1.5 μg/L) led to a high proportion of non-detects in the data. In this study, detectable lead levels were found in all samples.

The lead levels detected in the saliva were lower than those detected in blood, with the mean saliva lead value at 48.2% of the mean blood lead value. As noted by Koh et al. (2003), the process of saliva collection is inherently more prone to contamination than that of obtaining a blood sample. It is possible that oral contamination could have caused some of the highest saliva lead measurements, and thereby skewed the mean saliva lead value upwards. Therefore, a comparison of medians is perhaps more valid–the median saliva lead value being 28.5% of the median blood lead. The likelihood of oral contamination may have been reduced by rinsing the mouth prior to sample collection; however, for this sample collection, logistical constraints made it impracticable to implement any further sampling procedures. Rinsing of the mouth prior to sample collection may be beneficial to reduce oral contamination in future studies.

Previous studies have observed widely differing values of the saliva lead: blood lead ratio, ranging from <1% (Barbosa et al., 2006) up to 271% in P'an's most highly-exposed subset (1981).

Table 4b
Regression of log(saliva lead) on log(blood lead) adjusted for smoking status or for age.

	Coefficient
Log(saliva lead) on log(blood lead) adjusted for smoking:	
Coefficient for log(blood lead)	0.533 ($p < 0.001$)
Coefficient for smoker compared to non-smoker	0.036 ($p = 0.632$)
–	
Log(saliva lead) on log(blood lead) adjusted for age:	
Coefficient for log(blood lead)	0.522 ($p < 0.001$)
Coefficient for age	−0.004 ($p = 0.153$)

Although the very high values obtained by P'an may be explained by the rapid increase in saliva lead levels at blood lead levels >50 μg/L (Koh et al., 2003), there is still a great deal of unexplained variation between studies in the literature. This is most likely due to the lack of a standardised sample collection or preparation method for the analysis of lead in saliva; the wide variety of different procedures employed.

The method presented in this study, using a new sampling device and a nitric acid digestion step to release protein-bound lead in the matrix, obtained a mean blank-corrected recovery from 10 μg/L spiked saliva of 65.9% (SD: 1.83 μg/L, $n = 13$). This demonstrates an improvement on the recovery of 30–35% reported by Morton et al. (2014), where a comparable ICP-MS method was used, but with a different sampling device and no acid-digestion step. This may account for the higher levels of saliva lead observed by this study than Morton et al. (2014) (median: 17.1 μg/L and 7.3 μg/L, respectively), despite the sample cohort reported in this paper showing lower blood lead levels (median: 8.34 μg/dL and 20 μg/dL, respectively).

However, the StatSure device did exhibit a drawback – a significant level of lead contamination was shown to emanate from the device, with a mean result from blank saliva of 2.86 μg/L (SD: 1.13 μg/L, $n = 10$) using the device, compared to 0.38 μg/L (SD: 0.36 μg/L, $n = 10$) by direct analysis, *i.e.* the device contributed 2.48 μg/L of lead to the saliva result. The results from blank saliva aliquots sampled using the device also showed a higher degree of variation than those analysed directly. An investigation of the lead concentration of the device components showed that this contamination originated in the sampling paddle. For this study of occupationally-exposed lead workers, the median saliva lead was 17.1 μg/L, and therefore the effect of this contamination would be relatively small. However, this would be of concern for the measurement of lower-level environmental exposures. The manufacturers of the device have been made aware of the authors' findings and will endeavour to ensure that this contamination is not present in future batches. Additional analyses will be necessary to confirm this.

A weak but significant correlation ($r = 0.457$) was observed between the log(saliva lead) and log(blood lead) results from the 105 paired samples analysed. This is a stronger correlation than that observed between the same variables by Barbosa et al. (2006) ($r = 0.277$) or by Nriagu et al. (2006) ($r = 0.156$), and slightly stronger than the correlation observed by Koh et al. (2003) between log (saliva lead) and blood lead results ($r = 0.41$). A further study by Thaweboon et al. (2005) reported a poor correlation between saliva lead and blood lead. However, stronger correlations between log(saliva lead) and log(blood lead) were observed in studies by Morton et al. (2014) ($r = 0.69$) and by Pan AYS (1981) ($r = 0.80$).

The inconsistencies in the strength of the correlation between blood and saliva measurements in these studies may perhaps be explained by the degree of lead exposure received by the participants, with higher lead exposures appearing to produce a stronger correlation. The strongest correlation ($r = 0.80$) was found in Pan AYS (1981), in which the majority of the individuals concerned were highly occupationally exposed to lead, with a mean blood lead value of 35.5 μg/dL. The studies by Morton et al. (2014) and by Koh et al. (2003) also studied workers with

moderately high occupational lead exposure (mean blood lead: 20 μg/dL and 26.6 μg/L, respectively) and both produced significant correlations between blood and saliva lead ($r = 0.69$ and 0.41 respectively); whereas the studies by Barbosa et al. (2006), that measured individuals with lower environmental exposures (mean blood lead: 8.77 μg/dL) and by Nriagu et al. (2006), that measured an unexposed population (mean blood lead: 2.7 μg/dL), produced weaker correlations ($r = 0.277$ and 0.156 respectively). This pattern was however contradicted by the Thaweboon et al. (2005) study, which comprised 29 moderately-exposed individuals (geometric mean blood lead: 24.03 μg/dL) from a village in which the water supply was contaminated due to lead mining, but reported a poor correlation (Goodman–Kruskal $\gamma = -0.025$).

Using a multiple regression model for log(saliva lead) on log (blood lead), adjusted for smoking status and for age; neither term was shown to have a statistically significant effect on the correlation (smoking status: $p = 0.632$, age: $p = 0.153$). These findings are in agreement with previous work by Morton et al. (2014) using a similar model (smoking status: $p = 0.451$, age: $p = 0.207$). However, Nriagu et al. (2006) reported a much stronger correlation in participants aged 46 and older ($r = 0.49$), than in participants age ≤ 25 ($r = 0.11$) or age 26–45 ($r = 0.15$). This effect may be significant at the low exposure levels present in the unexposed population studied by Nriagu et al. (2006), but insignificant in an occupationally-exposed population with a higher degree of lead exposure. A further study could use multiple regression to investigate the effects of smoking status and age in an unexposed UK population.

The history of the individual's previous lead exposure was not found to significantly affect the correlation between log(blood lead) and log(saliva lead). History categories 1 ($\Delta = \pm 1$ μg/dL), 2 ($\Delta = \pm 2$ μg/dL), 3 ($\Delta = \pm 3$ μg/dL) and "fluctuating history" produced Pearson's correlation coefficients of $r = 0.473$ (C.I. 0.113–0.723), $r = 0.494$ (C.I. 0.224–0.694), $r = 0.531$ (C.I. 0.278–0.715) and $r = 0.498$ (C.I. 0.085–0.765), respectively. None of these differ significantly from one another, or from the value for all samples of $r = 0.457$ (C.I. 0.291–0.596). At the time of writing, the authors are unaware of any comparable studies that have taken this factor into consideration.

The apparent increase in the strength of the correlation between saliva lead and blood lead with increasing exposure, and the fact that this correlation is unaffected by age or smoking status, suggests that biological monitoring of salivary lead may be useful as a non-invasive surrogate for blood lead, but only at high exposure levels.

The kinetics of lead within the body are complex and not yet entirely understood. Nriagu et al. (2006) found that the isotopic ratios ($^{208}Pb/^{206}Pb$ and $^{207}Pb/^{206}Pb$) were almost identical in blood and in saliva, suggesting that the lead content of saliva must be derived from that in the bloodstream. Brodeur et al. (1983) showed that blood and salivary lead respond differently during and after lead exposure; moreover that salivary lead arises from the diffusible fraction in the blood plasma, and that it reflects much more recent exposure than blood lead. Therefore saliva lead measurement may be useful in this context as a biomarker of recent lead exposure – for example as a screening tool for workers undergoing work such as demolition, which involves a risk of acute exposure. However, before saliva lead measurement could be utilised for the assessment of individuals; further work would need to be carried out to understand how saliva lead levels respond to exposure, and for how long after an exposure that the saliva lead levels remain elevated. It may also be beneficial to obtain data on the variability of saliva lead measurements from the same worker, by studying multiple repeat samples in quick succession.

5. Conclusions

The ICP-MS method proposed by this study allows sensitive determination of saliva lead with low detection limits and high recovery. The StatSure sampling device is currently effective for high occupational exposures, but contamination from the device could confound measurements at lower environmental levels. The correlation between saliva lead and blood lead was found to be stronger at higher levels of exposure. In an occupationally-exposed cohort, this correlation was not found to be significantly affected by age, smoking status or the history of the individual's previous lead exposure. Further work could investigate the effects of these factors at lower environmental exposure levels. Despite its advantages as a non-invasive matrix, saliva lead measurement could only be useful as a surrogate for blood lead for highly-exposed populations. However, saliva lead may be useful in certain applications as an alternative biomarker for recent lead exposure.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

This publication and the work it describes were funded by the Health and Safety Executive (HSE). Its contents, including any opinions and/or conclusions expressed, are those of the authors alone and do not necessarily reflect HSE policy.

References

Agency for Toxic Substances Disease Registry, 2007. Toxicological Profile for Lead (Online). (accessed 7.02.14.). http://www.atsdr.cdc.gov/toxprofiles/tp13.pdf.
Barbosa Jr., F., Rodrigues, M.H.C., Buzalaf, M.R., Krug, F.J., Gerlach, R.F., Tanus-Santos, J.E., 2006. Evaluation of the use of salivary lead levels as a surrogate of blood lead or plasma lead levels in lead exposed subjects. Arch. Toxicol. 80, 633–637.
Brodeur, J., Lacasse, Y., Tablor, D., 1983. Influence of removal from occupational lead exposure on blood and saliva lead concentrations. Toxicol. Lett. 19, 195–199.
Costa de Almeida, G.R., Umbelino de Freitas, C.U., Barbosa Jr., F., Tanus-Santos, J.E., Gerlach, R.F., 2009. Lead in saliva from lead-exposed and unexposed children. Sci. Total Environ. 407, 1547–1550.
Dessypris, E.N., et al., 1999. Erythropoiesis, In: Lee, C.R. (Ed.), Wintrobe's Clinical Haemotology. tenth ed Lippincott, Williams and Wilkins, Philadelphia, USA, pp. 169–192.
Goyer, R.A., 1993. Lead toxicity: current concerns. Environ. Health Persp. 100, 177–187.
Goyer, R.A., Clarkson, T.W., 2001. Casarett and Doull's Toxicology: The Basic Science of Poisons. In: C.D, Klaassen (Ed.), McGraw-Hill, USA, pp. 827–834.
HSC/HSE, 2002. Control of Lead at Work, third ed. HSE Books, UK ISBN: 9780717625659.
IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 2013. [Online] (accessed 7. 02.14.). http://monographs.iarc.fr/ENG/Classification.
Koh, D., Ng, V., Chua, L.H., Yang, Y., Ong, H.Y., Chia, S.E., 2003. Can salivary lead be used for biological monitoring of lead exposed individuals? Occup. Environ. Med. 60, 696–698.
McNaught, A.D., Wilkinson, A., 1997. IUPAC Compendium of Chemical Terminology, second ed. Blackwell Scientific Publications, Oxford UK.
Morton, J., Leese, E., Harding, A.-H., Jones, K., Sepai, O., 2014. Saliva as a matrix for biomonitoring of occupational and environmental exposure to lead. Biomonitoring 1, 75–84.
Nriagu, J., Burt, B., Linder, A., Ismail, A., Sohn, W., 2006. Lead levels in blood and saliva in a low-income population of Detroit, Michigan. Int. J. Hyg. Environ. Health 209, 109–121.
P'an AYS, 1981. Lead levels in saliva and blood. J. Toxicol. Environ. Health A 7 (2), 273–280.
Thaweboon, S., Thaweboon, B., Veerapradist, W., 2005. Lead in saliva and its relationship to blood in the residents of Klity village in Thailand. Southeast Asian J. Trop. Med. Public Health 36 (6), 1576–1579.
Wilhelm, M., Pesch, A., Rostek, U., Begerow, J., Schmitz, N., Idel, H., Ranft, U., 2002. Concentrations of lead in blood, hair and saliva of German children living in three different areas of traffic density. Sci. Total Environ. 297, 109–118.