Measurement by ICP-MS of lead in plasma and whole blood of lead workers and controls

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Abstract

Objectives—To test a simple procedure for preparing samples for measurement of lead in blood plasma (P-Pb) and whole blood (B-Pb) by inductively coupled plasma mass spectrometry (ICP-MS), to measure P-Pb and B-Pb in lead workers and controls, and to evaluate any differences in the relation between B-Pb and P-Pb between people.

Methods—P-Pb and B-Pb were measured by ICP-MS in 43 male lead smelter workers and seven controls without occupational exposure to lead. For analysis, plasma and whole blood were diluted 1 in 4 and 1 in 9, respectively, with a diluted ammonia solution containing Triton-X 100 and EDTA. The samples were handled under routine laboratory conditions, without clean room facilities.

Results—P-Pb was measured with good precision (CV = 5%) even at concentrations present in the controls. Freeze storage of the samples had no effect on the results. The detection limit was 0.015 µg/l. The P-Pb was 0.15 (range 0.1–0.3) µg/l in controls and 1.2 (0.3–3.6) µg/l in lead workers, although the corresponding B-Pbs were 40 (24–59) µg/l and 281 (60–530) µg/l (1 µg Pb/l = 4.8 nmol/l). B-Pb was closely associated with P-Pb ($r = 0.90$). The association was evidently non-linear; the ratio B-Pb/P-Pb decreased with increasing P-Pb.

Conclusions—By means of ICP-MS and a simple dilution procedure, P-Pb may be measured accurately and with good precision down to concentrations present in controls. Contamination of blood at sampling and analysis is no major problem. With increasing P-Pb, the percentage of lead in plasma increases. In studies of lead toxicity, P-Pb should be considered as a complement to current indicators of lead exposure and risk.

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Lead is a common pollutant of the general environment and many workplaces. Exposure to lead occurs through inhalation of particles containing lead and ingestion of food, drinking water, settled indoor dust, and soil contaminated with lead. Lead is absorbed through the

lungs or the gastrointestinal tract into the blood.

After entering the plasma most (about 99%) of the lead is taken up into the erythrocytes.¹ In vitro experiments indicate a very rapid equilibration of lead between plasma and erythrocytes (within minutes).^{1–5} Most of the lead remaining in the plasma (P-Pb) may be bound to albumin.^{6,7}

Studies of P-Pb or serum lead (S-Pb) have given varying results; values between 0.02⁸ and 550 µg/l⁹ have been reported for single subjects without occupational exposure to lead. When considering only results obtained by analytical techniques capable of reaching adequate detection limits—such as electrothermal atomisation atomic absorption spectrometry (ETA-AAS) and mass spectrometry (MS)—the variation still ranges over one to two orders of magnitude in people without any occupational exposure.

Most studies did not show any association between B-Pb and P-Pb,^{10–13} although some studies showed a significant correlation.^{1, 14, 15} Furthermore, two of the studies clearly indicated a curvilinear relation between B-Pb and P-Pb.^{1, 15} Interestingly, similar non-linear relations with B-Pb have also been found for lead uptake,^{16–22} urinary lead excretion,²³ various biological indicators,^{13, 23–29} and health effects.^{30–33} Accordingly, it can be hypothesised that some of these exponential associations will become rectilinear when B-Pb is exchanged for P-Pb.

Thus P-Pb may be a more relevant index than B-Pb of exposure, distribution, and health risks. Also from a physiological point of view, it is reasonable to assume that toxic effects of lead are primarily associated with P-Pb, which should reflect the most rapidly exchangeable fraction of lead in the bloodstream. Despite this, no attempts seem to have been made to introduce P-Pb in the monitoring of lead uptake and health risks. Analytical problems at the low concentrations found are major reasons why P-Pb has not been used. However, the development of analytical techniques has proceeded, and especially MS in combination with inductively coupled plasma (ICP-MS) has developed into a very powerful technique for the measurement of trace elements in samples such as biological fluids.

The aims of the present study were to test a simple procedure for preparation of samples combined with ICP-MS to measure P-Pb, to evaluate the effect of freeze storage on P-Pb results, to study the P-Pb in groups of lead workers and controls, to investigate the rela-

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tion between B-Pb and P-Pb at varying values of P-Pb, and to evaluate variation between people.

Materials and methods

At a secondary lead smelter in southern Sweden, 43 male workers and seven male hospital laboratory staff were studied. On the day of sampling, the lead workers were on duty at the smelter. Before blood sampling, they had a shower and changed into clean clothes. The samples from the lead workers were obtained at the healthcare centre at the plant in connection with regular blood sampling for B-Pb monitoring. The median (range) age was 35 (20–63) for the lead workers and 41 (35–59) for the controls.

Venous blood was drawn from the cubital vein into an evacuated, heparinised 10 ml Venoject (Terumo Europe NV, Leuven, Belgium) tube. A set of the sampling tubes had been checked for lead contamination by leaching with 10 ml 1% nitric acid for 16 hours. A mean (range) lead content of 1.0 (0.3–1.9) ng per tube ($n = 10$) was obtained, corresponding to 0.10 (0.03–0.19) $\mu\text{g/l}$ whole blood. Plasma was separated by centrifugation (15 minutes, 1500 g) and transferred by acid washed Pasteur pipettes into acid washed, screw capped polypropylene tubes. In 9 of the samples from the lead workers, a “slight” or “obvious” haemolysis was recorded on the sample protocol. The samples were analysed fresh. Forty plasma samples were reanalysed after three months’ storage in a freezer (-20°C). Before analysis of the frozen samples, they were thawed, treated in an ultrasonic bath for 60 s, and centrifuged (10 minutes, 200 g). The clear supernatant fluid was used for analysis.

CONTROL SAMPLES

A commercial reference sample of lyophilised serum, without any data on lead concentration (seronorm trace elements, batch 010017, Nycomed AS, Oslo), and two samples of whole blood, with recommended lead concentrations (seronorm, batches 205052 and 203056), were included in all analytical series.

SAMPLE PREPARATION

Blood plasma (1.00 ml) was transferred into acid washed, screw capped polypropylene tubes and diluted with 4.0 ml deionised Millipore water containing ammonia (0.04 M), Triton-X 100 (100 mg/l), and disodium ethylenediaminetetra-acetate dihydrate ($\text{Na}_2\text{-EDTA}$; 200 mg/l). A similar sample preparation was used by Lutz *et al* for whole blood analysis by ICP-MS.³⁴ Bismuth nitrate (25 ng ^{209}Bi in 100 μl) was added to each sample as an internal standard to compensate for fluctuations in instrument stability. The normal concentration of Bi in human plasma has been reported to be very low (<0.25 ng/ml),³⁵ which is in accordance with pilot studies performed at our laboratory. Thus an interference with the internal standard is very unlikely.

Whole blood was homogenised by 10 min-

utes of mechanical shaking, then 0.50 ml aliquots were diluted with 4.5 ml deionised water containing ammonia (0.07 M), Triton-X 100 (500 mg/l), and $\text{Na}_2\text{-EDTA}$ (500 mg/l); 50 ng ^{209}Bi was added to the whole blood samples.

MEASUREMENT OF LEAD

Lead was measured by ICP-MS (PlasmaQuad 2+, Fisons Instruments, Winsford, UK). The samples were introduced with a segmented flow technique (1.0 ml/min; 10 s for plasma and 14 s for whole blood) through a Meinhard nebuliser and a chilled spray chamber. No flow injection valve was used; the autosampler was simply programmed to stay in the sampling position for the specified time. The argon plasma conditions were: forward power 1350 W; reflected power < 5 W; nebuliser gas flow rate 0.9, intermediate and outer gas flow rates 0.8 and 13.0 l/min, respectively. Interface cones of nickel were used. The instrument was run in peak jumping mode, with ^{206}Pb , ^{207}Pb , ^{208}Pb , and ^{209}Bi monitored at three points per peak. The sum of the three lead isotopes was used for calculations. For plasma analysis, the dwell time was 70 ms/point for lead and 20 ms/point for Bi, and the acquisition time was eight seconds. For whole blood analysis, the dwell time was 15 ms/point for lead and 10 ms/point for Bi, and the acquisition time was 20 seconds. The instrument was calibrated against spiked plasma (+5 and +10 $\mu\text{g/l}$) and whole blood (+100 and +500 $\mu\text{g/l}$) samples.

All samples were analysed in duplicate. The entire sample handling and analysis was carried out in a laboratory designed for routine measurements of trace elements, but without clean room facilities.

The instrument capacity was about 50 P-Pb measurements an hour. Thus, despite a high initial instrument cost, the measurement of P-Pb is not expensive when the samples are run in a large series.

Results

In samples without haemolysis the median (range) P-Pb was 1.2 (0.23–3.6) $\mu\text{g/l}$ ($n = 34$) in lead workers and 0.15 (0.10–0.17) $\mu\text{g/l}$ in controls. The corresponding B-Pbs were 281 (60–531) and 40 (24–59) $\mu\text{g/l}$.

There was a very close association ($r = 0.90$, $P < 0.001$; haemolysed samples not included) between B-Pb and P-Pb (fig 1A). The data indicate a non-linear association between the variables. Logarithmic conversion of P-Pb (with haemolysed samples excluded) gave a roughly rectilinear relation (fig 1B; $r = 0.96$). Samples with a notation of slight haemolysis did not differ in P-Pb from the non-haemolysed ones. However, those with obvious haemolysis had slightly higher concentrations (fig 1A).

The table shows the results for the control samples. Our results for the whole blood samples are in good agreement with the recommended values.

The method precision, calculated as the

Figure 1 Relation between lead concentrations in plasma (P-Pb) and in whole blood (B-Pb) in 43 secondary lead smelter workers and seven controls. Open circles = samples without visible haemolysis; filled circles = slight haemolysis; crosses = obvious haemolysis. (A) Linear scale. (B) Logarithmic scale; rectilinear regression line: $\log P-Pb = 0.00277 \times B-Pb - 0.77$ ($r = 0.96$; haemolysed samples excluded). Considering the metabolism of lead, it seems logical to choose P-Pb as the independent variable (x axis), although other authors have chosen B-Pb as independent.

Lead concentration ($\mu\text{g/l}$) in lyophilised seronorm trace element control samples from Nycomed AS, Oslo, Norway

Sample	n	Obtained mean (range)	Recommended mean (range)
Serum, batch: 010017	8	2.5 (2.2–2.8)	—
Blood, batch: 205052	10	31.8 (30.1–32.7)	35 (31–41)
203056	10	385 (372–397)	383 (361–396)

n = Number of measurements.

Figure 2 Relation between lead concentrations in plasma (P-Pb), as measured in samples both fresh and after deep frozen storage for three months. The linear regression line is shown ($y = 1.003x + 0.007$; $r = 0.995$).

coefficient of variation from duplicate measurements, was 5.1% for fresh plasma, 1.3% for frozen plasma, and 1.8% for whole blood. The detection limit, calculated as three times the SD of repeated measurements ($n = 10$) of a reagent blanks, varied from day to day between 0.012 ng/l and 0.015 ng/l.

A very close association was found between the results for frozen and fresh plasma samples, without any systematic differences (fig 2).

Discussion

The present study shows that it is now possible to measure P-Pb with ICP-MS under routine laboratory conditions with good precision and a detection limit of about a 10th of the concentration among controls without occupational exposure to lead. A good precision in the measurements is verified by the close asso-

ciation between B-Pb and P-Pb, as well as between P-Pb in fresh and frozen samples. Also, the samples can be stored frozen without any effect on the results.

As there are no certified quality assurance samples for lead in plasma, the accuracy of the method for P-Pb could not be verified with certainty. It is possible that the values are slightly too high due to the fact that no correction was made for contamination of the blood sampling tubes. However, taking into consideration the very rapid absorption of lead by erythrocytes,^{1,3-5} such a correction is not likely to be necessary.

The P-Pb values in the present study are considerably lower than those reported in studies with atomic absorption spectroscopy techniques. Moreover, they are, together with the data reported by Everson and Patterson,⁸ the lowest values obtained in studies involving MS techniques. Accordingly, the present ratio B-Pb/P-Pb is higher than most earlier results.

Most of the data on P-Pb hitherto reported have been obtained by ETA-AAS techniques. However, in studies with ETA-AAS without any enrichment step, the detection limits, if reported, were $\geq 5 \mu\text{g/l}$ and no associations between B-Pb and P-Pb were found.^{10,13,36-39} To increase the sensitivity and minimise the non-specific absorption from the sample matrix components at atomisation, ETA-AAS detection has been combined with a liquid/liquid extraction step.^{12,14,15} The detection limits in these studies were reported to be 0.5 $\mu\text{g/l}$ –2 $\mu\text{g/l}$, and the average P-Pb/B-Pb ratio 1%–3%, within a B-Pb range up to about 1000 $\mu\text{g/l}$. To obtain the highest instrumental sensitivity, the lead resonance line of 217 nm has been used in these studies. However, at this wavelength, the non-specific background absorption is substantial, and was certainly difficult to compensate for with older ETA-AAS apparatus. This has, most probably, made these results less reliable.

In previous studies of P-Pb with MS techniques, the number of people was generally very small; sometimes only one or two subjects. Only two of the studies give clear information on detection limits: 0.05 $\mu\text{g/l}$, obtained by ICP-MS,³⁵ and 0.001 $\mu\text{g/l}$, combining elec-

trothermal with isotope dilution ICP-MS.⁴⁰ Everson and Patterson⁸ reported the P-Pb for one control person and a lead worker to be 0.02 µg/l and 2 µg/l, respectively, the ratios for P-Pb/B-Pb were 0.02% and 0.25%. Possibly, the P-Pbs were too low due to overestimation of the effect of heparin blanks. Manton and Malloy described a decreasing S-Pb/B-Pb,⁴¹ from 1.6% to 0.3% in a lead worker whose B-Pb fell from 1160 µg/l to 310 µg/l. A median (range) S-Pb of 0.5 (0.2–1.2) µg/l in six controls was reported by Manton and Cook.¹

The relatively higher P-Pbs reported in most earlier studies may also have explanations other than analytical problems—for example, extensive haemolysis at sampling or during storage before the separation of plasma, and contamination at sampling or sample preparation. However, the present data show that slight haemolysis gives no significant increase in P-Pb, and even obvious haemolysis only has a limited effect. Thus haemolysis can scarcely explain all the difference in the published data. Further, contamination of the samples before separation of plasma would, most probably, hardly influence the P-Pb,¹ as traces of inorganic lead would rapidly be absorbed by the erythrocytes.^{1–5, 42} The use of the very strong chelator EDTA as an anticoagulant may, however,¹⁴ give falsely high P-Pbs due to extraction of lead from the erythrocytes.^{14, 15} Data from experiments with radiolabelled lead show that heparin does not extract lead from erythrocytes,⁴ although such an effect has been suggested by some authors.^{5, 7}

The association between B-Pb and P-Pb obtained in this study is much closer than reported in any other study. Moreover, this is the first study in which an association has been shown in the B-Pb range most relevant for exposures of today in western countries (< 600 µg/l). Possibly, the association would have been even closer if the B-Pbs had been corrected for differences in packed cell volume.

It has been suggested that the large scattering in the data of DeSilva¹⁵ and Manton and Cook¹ were caused by a short term effect on P-Pb by ongoing absorption of inhaled or ingested lead. However, in the present study, the workers were exposed shortly before blood sampling, and they certainly had varying loads of particles containing lead in their lungs. Further, the controls were sampled at varying times relative to the intake of meals. The close association between B-Pb and P-Pb obtained thus contradicts the explanations by these authors.

Although the present study indicates that the variation between people in the distribution of lead between erythrocytes and plasma is limited, it may still be of toxicological relevance. Moreover, the maximum B-Pb in our study was only 530 µg/l, and none of the subjects had any symptoms or signs of intoxication. Accordingly, the considerable variation reported between symptoms and signs and B-Pb^{30–33} may be caused by exceptional differences between people in the distribution of lead between erythrocytes and plasma. In such instances, B-Pb has only limited value as an

indicator of health risks.

The association between B-Pb and P-Pb was evidently non-linear; the ratio B-Pb/P-Pb decreased with rising P-Pb. This agrees with some previous studies.^{1, 15, 41} A possible explanation of the increasing fraction of lead in plasma with increasing B-Pb may be a gradual saturation of the binding sites in erythrocytes with highest affinities to lead. For example the enzyme δ -aminolevulinic acid dehydratase (ALAD) has been shown to bind lead^{43, 44}; the activity of ALAD is known to decrease immediately after a moderate increase in B-Pb.²³ Other explanations may be that haemolysis caused by lead results in release of lead that may be bound to a protein,⁶ or that lead induces an increase of protein that binds lead in plasma. The presence of such a protein has been indicated in erythrocytes of lead workers with B-Pbs > 1.9 µmol/l (400 µg/l).^{45, 46}

The present study shows that P-Pb can be measured routinely and precisely by ICP-MS without advanced clean room facilities. Although the association found between B-Pb and P-Pb is very close, it may include a variation in P-Pb large enough to be of toxicological significance. This should be considered as a possible explanation of differences in susceptibility to toxic effects of lead. It is thus time to generate data on the relation between P-Pb and lead uptake and effects.

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