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Stability of Alkyl-lead Compounds in Blood

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In order to investigate occupational exposure to various chemicals, blood samples from exposed subjects may be used. Such samples are usually collected locally for transport to an analytical laboratory. It is therefore essential that the necessary transport is performed under such conditions that an adequate stability of the analytes in the samples is maintained. The stability of trimethyl- and dimethyl-lead species in blood at various storage temperatures, as well as the insulation capacity of different transport packages for blood samples, were determined. The results showed that alkyl-lead compounds in spiked blood stored at room temperature were stable for 6 h and in a refrigerator for up to one week. Only in spiked blood stored at −20 °C were the alkyl-lead compounds found to be stable for more than one month. Blood samples from exposed subjects stored at −20 °C were found to be stable for at least two months. One transport package used, found to maintain this temperature of prefrozen samples for up to two days, was a polystyrene transport box filled with crushed dry ice. This packing procedure is therefore recommended for transport of blood samples collected for evaluation of occupational exposure to alkyl-lead compounds.

Keywords: Blood, dimethyl-lead, occupational exposure, stability, storage, trimethyl-lead

INTRODUCTION

Biological monitoring is frequently used for the assessment of occupational exposure to some toxic metals and organic compounds.[1] Samples for biological monitoring are normally collected from exposed subjects at local health clinics or by a company nurse. In general, however, the analyses for biological monitoring are performed in a specialized laboratory. In reality, this means that it may take more than a week, of which one to two days are used for transportation, from sampling to analysis. Since biological samples are a living material, changes of the analyte composition may be caused both by microbial and by chemical reactions. Consequently, in order to preserve the sample composition, it is essential that the samples are properly transported and handled during the time between sampling and analysis.

For elements, such as metals, the total concentration of the element is of interest for biological monitoring and it is important that it can be reliably determined by standardized procedures.[1] Many organic compounds and organometallic compounds—such as alkyl-lead compounds—have different toxicities. Speciation of these compounds is therefore essential for assessment of toxic effects of exposure to such compounds. Besides contamination and analyte loss, various chemical reactions, such as dealkylation and hydroxylation, may also occur in biological samples and change the analyte composition during storage prior to analysis. If these reactions occur, the value of biological monitoring for determination of occupational exposure will decrease. It is therefore important to evaluate proper conditions for handling, transportation and storage of samples for biological monitoring.

Although new automobiles do not need it, leaded petrol can be expected to be used in older cars for several years to come. Occupational exposure to petrol and, consequently, to tetra-alkyl-lead compounds, will occur in many occupations, e.g. garage work, pump maintenance, tank cleaning and fuel-truck driving.

One way of assessing exposure to tetra-alkyl-lead compounds is to determine these compounds, and their dealkylated metabolites (trimethyl- and dialkyl-lead ions), in blood samples from exposed subjects. A method for determination of alkyl-lead compounds in blood has previously been described by us and is based on extraction and Grignard derivatization followed by gas chromatographic separation and metal specific detection by graphite furnace atomic absorption spectrometry.[2,3] Tetramethyl-lead as well as trimethyl- and dimethyl-lead ions have also been found in blood from workers with the occupations mentioned above.[2,4]

In this study, the aim has been to investigate

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the stability of some alkyl-lead compounds in blood during storage at different temperatures. In conjunction, the insulation capacity of commonly used transport package materials has been investigated. The purpose has been to find suitable procedure for packing blood samples for one to two days of transport and adequate storage conditions in order to preserve the samples for up to one month prior to analysis.

EXPERIMENTAL

Chemicals, materials and blood samples

All chemicals were of analytical grade and the water was purified in a Milli-Q Water Purifier (Millipore, Bedford, MA, USA). A buffer, consisting of 26 mg ml^{-1} sodium citrate, 7 mg ml^{-1} EDTA and ammonia to pH 9, was used as extraction buffer and sodium diethyldithiocarbamate (0.1 g ml^{-1}) in water was used as complexing agent. Butylmagnesium Grignard reagent (2.23 mol l^{-1} in THF, Alfa-Ventron, Karlsruhe, Germany) was used as derivatization reagent. Stock solutions (50 μg ml^{-1}) of trimethyl-lead chloride [$(CH_3)_3PbCl$] and dimethyl-lead chloride [$(CH_3)_2PbCl_2$] (both Alpha-Ventron) were prepared in ethanol. Both substances were used without any pre-treatment or further purification. All glassware was cleaned and thoroughly rinsed in deionized water in a washing machine.

Blood from five donors, supplied by the blood bank at Umeå University Hospital, Sweden, was pooled and sodium citrate was added to prevent rapid natural clotting. With a micropipette, 500 μl of the 50 μg ml^{-1} standards of trimethyl- and dimethyl-lead chloride were added to a 500 ml portion of the pooled blood, resulting in a concentration of 50 ng ml^{-1} each in the spiked blood.

Four blood samples were also withdrawn from two subjects, previously known to have measurable amounts of alkyl-lead compounds in their blood.

Insulation capacity of packing materials

The temperature changes of frozen blood samples packed in different materials were measured with time. Four rubber-capped 10 ml blood collection tubes were two-thirds filled with blood. A thermocouple was placed in the blood in one of the tubes and was fixed with the cap. The tubes were frozen at −20 °C in a standing position to avoid contamination of the thermocouple wire. The tubes, placed in transport cassettes, were packed according to the four procedures described below. The thermocouple wire was led out of each package and connected to a digital read-out instrument. One tube was placed directly in a tube-stand at room temperature without any insulation and the time for the tube to reach room temperature was measured as a reference value. The following packing procedures were used.

(1) Each cassette was wrapped in household paper and the cassettes were then packed together in a padded envelope.

(2) The cassettes were packed together with cellulose wadding in a cardboard box (300 mm × 200 mm × 100 mm).

(3) The cassettes were placed with a household freezing block frozen at −20 °C in a plastic bag and packed with cellulose wadding in a polystyrene transport box (300 mm × 300 mm × 300 mm; 50 mm wall).

(4) The cassettes were packed with crushed dry ice and cellulose wadding in a polystyrene transport box (300 mm × 300 mm × 300 mm; 50 mm wall).

Storage procedures

The spiked blood was transferred to 30 10-ml screw-capped test-tubes. Two tubes of spiked blood and one sample from each subject were analysed at once. Duplicate tubes of spiked blood were stored prior to analysis at room temperature for 1, 6, 24 and 48 h before analysis, at +4 °C (in a refrigerator) for one and three days, one and two weeks and finally at −20 °C (in a freezer) for one, two, four and eight weeks, respectively. One of the remaining three samples from each subject was stored in a refrigerator for one week. One was stored for two days with dry ice in a polystyrene transport box as described above and then at −20 °C during four weeks prior to analysis. The last sample from each exposed subject was stored at −70 °C for one year prior to analysis.

Analytical procedure

Trimethyl- and dimethyl-lead were analysed in the samples utilizing the previously described method for determination of trimethyl- and dimethyl-lead in blood.[3,4]

Standards of freshly spiked blood (5–100 ng

ml^{-1}) were prepared at the time for each analysis. A 50 ng ml^{-1} standard in water was prepared as a control for the determinations. Aliquots (5 ml) of the blood samples, blood and water standards were transferred to screw-capped 50-ml polyethylene test-tubes. To the tubes 15 ml of extraction buffer and 1 ml of complexing agent were added. The samples were extracted twice with 2 ml n-pentane on a vibrating shaker for 2 min. Phase separation was facilitated by centrifugation. The pentane phases were combined in a three-necked flask, which was left open in a fume cupboard overnight to allow evaporation of the pentane.

After evaporation of the pentane, a condenser, a thermometer and a dropping funnel were mounted on the three-necked flask and on an ice-bath; 1 ml of Grignard reagent and 500 μl of n-heptane were added. The sample was left for 10 min to complete the Grignard reaction and then 2.5 ml of 1 M hydrochloric acid was added slowly through the dropping funnel, not allowing the temperature to increase above 30 °C. After the excess of Grignard reagent had decomposed, the sample was transferred to an extraction funnel with a 50-mm capillary (1 mm i.d.) between the bulb and the drain valve. The acid–water phase was discarded, not allowing the heptane phase to enter the capillary. The heptane phase was washed with 5 ml of water, which was then discarded allowing the heptane phase to enter the capillary. The heptane extract was withdrawn using a glass syringe. The heptane extract was transferred to a screw-capped glass vial containing some crystals of sodium sulphate.

The alkyl-lead compounds were determined using a high-resolution gas chromatography–graphite furnace atomic absorption spectrometry (HRGC–GF AA) system. An aliquot (2 μL) of the extracts were injected at 30 °C on a 50 m OV-101 fused silica column (0.32 mm i.d.) using on-column injection. Hydrogen was employed as carrier gas (3.5 ml min^{-1}). The following GC oven temperature programme was used: 30 °C for 1 min, 16 °C min^{-1} to 220 °C, which was held for 5 min. The fused silica was led from the GC (Model 204, Pye–Unicam) to the graphite furnace (CRA-90, Varian) through copper tubing heated to 220 °C. The column entered the furnace through a 0.5 mm hold drilled in the tube wall and ended 1 mm inside the graphite tube. The ordinary injection port in the tube was covered by one of the electrodes.

The graphite furnace was mounted in an AAS instrument (SP 192, Pye–Unicam) and operated at 1100 °C during the whole run with nitrogen (5 l min^{-1}), as shield gas. An electrodeless discharge lamp for lead was operated at 217 nm and a deuterium lamp was used for background correction. The background-corrected absorbance signal was sampled in a personal computer (ABC 806, Luxor, Sweden) and evaluated using chromatography software (Chromatic, Kebo Computer, Sweden).

The trimethyl- and dimethyl-lead concentrations in the samples were determined from calibration curves calculated after analysis of the freshly spiked blood. The water standard was used as a control of the determination.

RESULTS AND DISCUSSION

Table 1 shows the results of the storage experiments. For all samples, the difference between the duplicates was below 10%. At room temperature, the recovery of trimethyl-lead had decreased to less than 90% in 6 h and was only 72% after 24 h. Clotting had already begun to occur after 6 h but did not seem to affect the recovery as long as enough fluid blood could be withdrawn. However, after two days it was impossible to analyse the samples because the clotting was too heavy. The dimethyl-lead recovery increased with time, indicating that trimethyl-lead was dealkylated to dialkyl-lead. For the samples stored at +4 °C the same tendency was seen, but the recovery of trimethyl-lead was above 90% for one week. The samples stored at −20 °C maintained a recovery of trimethyl-lead above 90% for at least eight weeks. The overall recovery of organic lead was, however, higher than 93% for all stored samples. This show that further dealkylation of dimethyl-lead occurs at a significantly slower rate than dealkylation from trimethyl- to dimethyl-lead. In the environment, trialkyl-lead species are considered to be the most stable ionic alkyl-lead compounds.[5] One possible explanation of the more rapid dealkylation of trimethyl-lead in blood can be that the elimination of tetra-alkyl-lead in man occurs by enzymatic dealkylation in order to increase solubility in water.

Table 2 shows the recovery of trimethyl-lead in the blood samples from the exposed subjects after different storage conditions and times. No dimethyl-lead above the detection limit (5 ng ml^{-1}) was found in any sample. All stored samples showed recoveries above 95% for trimethyl-

Table 1 Recovery from blood spiked with 50 ng ml^{-1} (as lead) of trimethyl-lead (3ML) and dimethyl-lead (2ML), respectively, after different storage times and conditions[a]

Time	Storage place	Recovery 3ML (ng Pb ml^{-1})	3ML (%)	2ML (ng Pb ml^{-1})	2ML (%)	Total (%)	Comments
0	Direct	49	98	48	96	97	
1 h	Room	47	94	48	96	95	
6 h	Room	44	88	51	102	95	Slightly clotted
24 h	Room	36	72	57	114	93	Partly clotted
48 h	Room	—	—	—	—	—	Totally clotted
1 d	Refrigerator	49	98	48	96	97	
3 d	Refrigerator	47	94	48	96	95	
1 week	Refrigerator	45	90	48	96	94	Slightly clotted
2 weeks	Refrigerator	38	76	55	110	93	Partly clotted
1 week	Freezer	50	100	48	96	98	
2 weeks	Freezer	48	96	48	96	96	
4 weeks	Freezer	47	94	49	98	96	
8 weeks	Freezer	46	92	50	100	96	

[a] Each value is a mean of two replicates.

lead, compared with the directly analysed samples. Although there were only two subjects and only one sample from each subject was stored under the specified conditions, the results indicate that it would be possible to store blood samples at +4 °C for one week, at −20 °C for two months and at −70 °C for at least one year for assessment of exposure to tetra-alkyl-lead compounds.

Figure 1 shows the insulation capacity of the packing materials investigated. The padded envelope and cardboard box maintained a temperature below 0 °C for less than 1 h. By packing

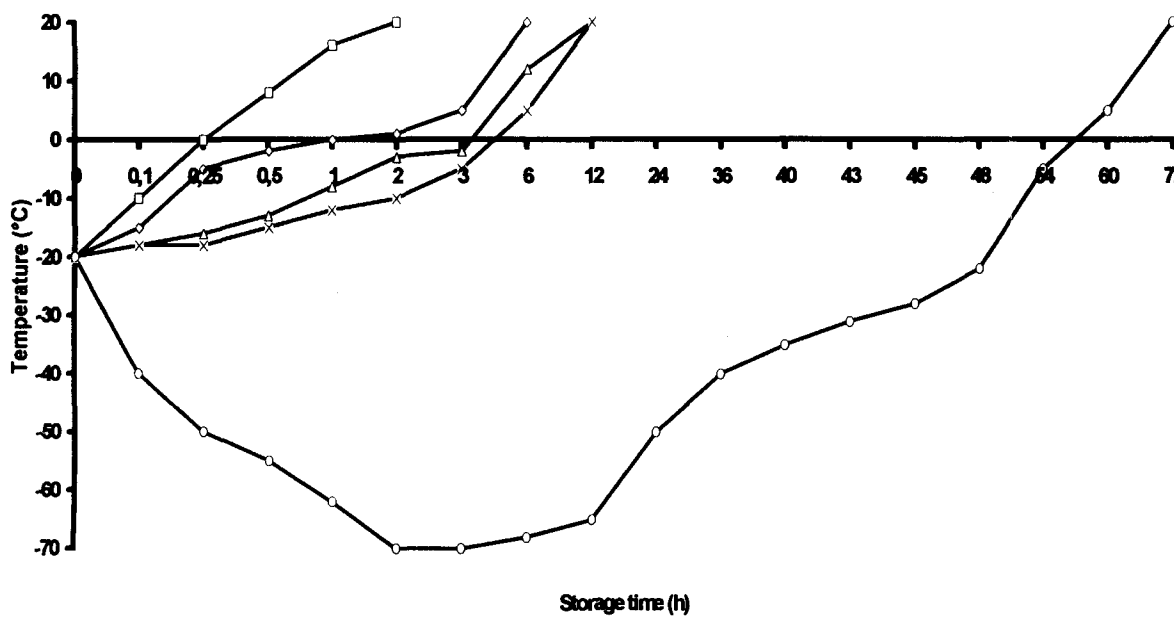

Figure 1 Changes of the temperature in blood samples during storage at room temperature in diffeent transport packages: □, no packaging; ◊, padded envelope; △, cardboard box; ×, polystyrene box with household freezing block; ○, polystyrene box with crushed dry ice.

Table 2 Concentration and recovery of trimethyl-lead (3ML) in blood samples from exposed subjects stored under various conditions.[a]

Sample	Concentration when directly analysed (ng Pb ml^{-1})	Recovery after storage (%)		
		A	B	C
Tank cleaner	22	96	98	97
Pump service man	12	95	97	96

[a] The trimthyl-lead concentration was determined directly after sampling. One sample from each subject was then (A) stored at +4 °C for one week; B, stored in a polystyrene box with dry ice for 2 days and then at −20 °C for eight weeks; C, stored at −70 °C for one year.

in a polystyrene box with a household freezing block a temperature below 0 °C could be maintained for 6 h, and by packing in the same box with dry ice a temperature below −20 °C could be maintained for two days. This result shows that blood samples for determination of alkyl-lead compounds should be transported in a polystyrene box with dry ice, in order to preserve the samples properly to avoid dealkylation of trimethyl-lead. If the transportation takes more than two days, a refill of dry-ice will be necessary to maintain a temperature below −20 °C throughout the transport process.

CONCLUSIONS

These investigations on spiked blood samples have shown that blood samples should be stored cold to prevent dealkylation of trimethyl-lead to dimethyl-lead. The samples can be stored in a refrigerator for one week, in a freezer for two months and in a deep freezer for at least one year. The results also indicate that samples from exposed subjects can be stored safely for the same time under the same conditions. To secure this temperature during a two-day transportation process the samples should be packed in a polystyrene transport box with dry ice. This packing procedure is therefore recommended for transport of blood samples collected for evaluation of occupational exposure to alkyl-lead compounds.

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