APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 10, 69-73 (1996)

WORKING METHODS PAPER

Preparation of Candidate Reference Materials for Trimethyl-lead Analysis and Assessment of their Homogeneity and Stability

Yun Wang,† Roy M. Harrison*† and Philippe Quevauviller‡
† Institute of Public and Environmental Health, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK, and ‡ European Commission, Measurements and Testing Programme, rue de la Loi 200, B-1049 Brussels, Belgium

This study was conducted to develop certified reference materials containing trimethyl-lead (TriML) in environmental matrices. A detailed description of the process to prepare candidate artificial rainwater and road dust reference materials, which includes pretreatment, homogenization and bottling procedures, is described. The homogeneity was assessed by comparing the variations within a bottle and between different bottles. The results demonstrated that the distribution of TriML in the same material is homogeneous. The stability of TriML in these materials was verified on the day of preparation and after 1, 3, 6 and 12 months. The results showed no significant difference between the initial TriML concentration and the concentration after 12 months' storage in candidate artificial rainwater kept at room temperature and in road dust stored at −20 °C and 4 °C, based on a statistical analysis of the results. At 37 °C, however, TriML was shown to be unstable: this highlighted the need to store the materials in the dark, preferably at 4 °C. TriML in both of the candidate reference materials was hence concluded to be stable for at least 12 months under the appropriate storage conditions.

Keywords: trimethyl-lead; reference material; homogeneity; stability; rainwater; road dust

INTRODUCTION

Environmental contamination by organolead compounds is widespread. The dominant source of organolead compounds in the environment is tetra-alkyl-lead, used in petrol as an antiknock additive. Although their use has been dramatically decreased in many countries by the recently increased market penetration of unleaded petrol, it is anticipated that the monitoring of organolead species in the environment will be continued over the next decade.[1] A number of laboratories are performing the analyses of organolead compounds in rainwater or snow to evaluate organic lead burden in the environment,[2-4] but the lack of reference materials for organolead analysis in environmental samples does not permit quality control of the measurements. Furthermore, no valid comparisons can be made on the basis of analytical results obtained by different laboratories, unless the same reference materials have all been measured.[5,6] There is thus an urgent need to develop certified reference materials for trimethyl-lead (TriML) analysis in environmental samples.

The aim of this study was to develop methodology to produce candidate reference materials with a homogeneous and stable distribution of TriML. This work involves specifically:

(1) the preparation of an artificial rainwater sample enriched with TriML;
(2) the preparation of an urban road dust sample contaminated with organolead compounds;
(3) the study of the homogeneity and long-term stability of these materials with respect to TriML.

TriML in candidate materials was determined by using a well-tested analytical procedure based on extraction, derivatization and gas chromatography–atomic absorption spectrometry.[2] Here we report the results of such a study, which was carried out at University of Birmingham under the auspices of the EC Measurement and Testing Programme.

---

* Author to whom correspondence should be addressed.

CCC 0268-2605/96/010069-05

Received 13 February 1995
Accepted 23 May 1995

EXPERIMENTAL

Reagents and apparatus

Trimethyl-lead chloride calibrant was obtained from Johnson Matthey Ltd, Royston, UK. Its purity has been determined at the University of Birmingham by a number of parallel techniques, and has shown to be greater than 98%.[7] Propylmagnesium chloride (2.0 mol l^{-1} solution in diethyl ether) and sodium diethyldithiocarbamate (NaDDTC) were obtained from Aldrich Chemicals. All other reagents employed in the study were obtained from BDH Chemicals. Distilled deionized (Milli-Q) water was used throughout the study.

A gas chromatograph (Perkin–Elmer F 17) was fitted with a glass column (1.2 m × 6 mm) packed with 10% OV-101 on Chromosorb W (80–100 mesh) and was interfaced to a Perkin–Elmer 2380 atomic absorption spectrometer using PTFE tubing (1.6 mm × 0.75 m). A detailed description of the installation and optimum operating conditions for this system have been described in a previous paper.[2]

Sample preparation

Candidate artificial rainwater reference material

In most laboratories, 1 litre of rainwater sample is required to quantify alkyl-lead species. Considering the difficulty of storage and transportation for such a large volume, the design concentration for all components in the artificial rainwater reference material (stored solution) was ten times higher than that in simulated rainwater. Prior to analysis, 100 ml of this solution was to be diluted with 900 ml Milli-Q water to correspond to a simulated rainwater composition. The artificial rainwater composition was chosen as being broadly similar to the composition of rainwater over continental land masses. Candidate artificial rainwater contains 500 ng l^{-1} (as Pb) of trimethyllead in an artificial rainwater matrix. The composition of artificial rainwater is presented in Table 1. A large batch of artificial rainwater (100 litres) was prepared in a 0.14 m^3 bin with a lid, made from rigid high-density polythene. The bin was wrapped with aluminium foil to protect the solution from daylight. The solution was thoroughly homogenized by stirring over an extended period with a glass rod which was passed through the hole in the lid. Teflon tubing ($\frac{1}{4}$ in; 6.4 mm) was passed through the hole in the lid to reach the bottom of the bin. The liquid was transferred with a siphon into 125 ml Nalgene bottles. An accurate amount of 100 ml of the solution was measured into each of the 800 bottles by using a balance. Prior to filling, bottles were rinsed twice with a small volume of the solution. Full bottles were wrapped with aluminium foil, sealed in plastic bags and stored in the cold room at 4 °C.

Table 1 Composition of candidate artificial rainwater

Components	Concentration for stock (μmol l^{-1})	Concentration for analysis (μmol l^{-1})
NH_4^+	600	60
K^+	50	5
Ca^{2+}	120	12
Mg^{2+}	100	10
Na^+	600	60
Cl^-	900	90
SO_4^{2-}	300	30
NO_3^-	400	40
Me_3Pb^+ as Pb (ng l^{-1})	500	50

Candidate road dust reference material

About 15 kg of surface road dust was collected from sweeping a lay-by in the central section of the Queensway tunnel (Birmingham, UK). The tunnel is approximately 850 m in length, and is a major traffic route through Birmingham from the motorway system. The dust sample was first passed through a 500 μm sieve to remove large debris particles. It was then air-dried at room temperature for five days in a flat tray covered with clean paper in a well-ventilated dark place. Finally, the dust was ground in a ballmill (Tema Swing) for 3 min and sieved through a 125 μm sieve. About 10 kg of the pre-treated dust sample was homogenized by the following procedure: the whole amount of dust was divided in four subsamples, each of about 2.5 kg. Each subsample was stored in a 5-litre dark glass bottle and shaken on the mechanical shaker for a few hours (using different shaking directions). When all four subsamples had been homogenized, they were redistributed again into a second four subsamples in such a way that each of the first four subsamples contributed equally to each of the second four subsamples. The procedure was repeated three times, and after this the final four subsamples were again combined in one sample. The homogenized dust sample was dried using freeze-drying at −50 °C and 8 mbar pressure for 24 h, as it was demonstrated that TriML remained stable under these conditions. The bottling procedure followed immediately. Each of 600 30 ml amber glass bottles provided with screwcaps was filled with 15 g of the dust sample, sealed into a plastic bag and stored in the cold room at 4 °C.

Analysis

Artificial rainwater

Prior to analysis, a bottle of artificial rainwater (100 ml) was diluted with 900 ml of Milli-Q water. To this NaCl (20 g), NaDDTC (5 ml, 0.25 mol l^{-1}) and hexane (5 ml) were added, and the solution was shaken mechanically for 30 min. The hexane was transferred to a 25 ml conical flask. Propylmagnesium chloride (0.5 ml) was added to the extract, and the flask was gently shaken manually for 8 min. The extract was washed with sulphuric acid (H_2SO_4) (5 ml, 0.5 mol l^{-1}) to destroy any excess Grignard reagent (propylmagnesium chloride) present. The organic phase was dried over anhydrous sodium sulphate (Na_2SO_4) (approx. 100 mg) and then transferred to a 4 ml vial. This extract (50 μl) was injected into the GC–AAS system and quantified by means of a Hewlett–Packard 3396A integrator.

Road dust

Samples (10 g) were transferred to 250 ml screw-cap glass bottles with Milli-Q water (100 ml) and sodium chloride (10 g). The bottles were shaken on a mechanical shaker for 30 min. The slurry was filtered through two pieces of glass microfibre filter (Whatman GF/C) and rinsed with 50 ml of Milli-Q water. The combined filtrates were then transferred to a second clean 250 ml glass bottle. After the pH was adjusted to 9.0 with ammonia solution, ethylenediaminetetra-acetic acid (EDTA; 3 g), NaDDTC (5 ml, 0.5 mol l^{-1}) and hexane (15 ml) were added. After 30 min of agitation, the organic phase was removed and extraction was repeated with a second aliquot of hexane. The combined hexane extracts were then passed through anhydrous Na_2SO_4 with hexane rinses. The hexane extracts were transferred to a 25 ml conical flask and evaporated by purging with a stream of nitrogen in a waterbath set at 35 °C until approx. 5 ml of the hexane extract remained. This was transferred into a 10 ml concentrator receiver tube with a hexane rinse and further evaporated by purging with a nitrogen stream until 0.5 ml of the extract remained. Grignard reagent (0.3 ml propylmagnesium chloride) was added and the concentrator tube was shaken in an ultrasonic bath for 5 min. H_2SO_4 (5 ml, 0.5 mol l^{-1}) was added and the mixture was shaken in the ultrasonic bath for a further 5 min. The hexane layer was removed from above by means of a 200 μl pipette. A small amount of anhydrous Na_2SO_4 was placed within the pipette tip to dry the extract. Then 25 μl of the final extract was injected into the GC–AAS system. Moisture content was determined by drying a separate aliquot at 105 °C in an oven to constant mass. The dust results have been corrected for the moisture content in each sample.

Calibrant

Propylated trimethyl-lead chloride standards were used as calibrants for determination of TriML by GC–AAS. The stock calibrants of Me_3PrPb in hexane were prepared by reacting trimethyl-lead chloride, extracted into hexane first, with propylmagnesium chloride. After removal of the excess Grignard reagent, the stock calibrants were stored in the dark at −20 °C. Fresh working calibrants were prepared by dilution for each individual set of analyses. The stability of this material was established by comparing with a freshly propylated calibrant at each calibration to achieve a long-term reproducibility for the stability study. The concentration of TriML in road dust was determined by using a method of standard additions. A series of aqueous trimethyl-lead chloride standards were spiked into several subsamples from the same road dust sample, and then these were extracted with hexane, concentrated, derivatized and analysed.

RESULTS AND DISCUSSION

Homogeneity study

Eight bottles (between-bottle homogeneity), selected randomly as one bottle out of each set of 100 from the total set of 800 bottles, were analysed to verify the homogeneity of TriML in the whole batch of artificial rainwater. The methodological uncertainty of the determination, regarded as within-bottle homogeneity, was determined by five replicate analyses of TriML from one bottle.

The homogeneity of TriML in road dust was determined by 10 replicate analyses from the well-mixed contents of one large bottle containing

Table 2 Statistical results of homogeneity study

Material	X_w[a]	S_w[b]	X_b[c]	S_b[d]	$F=S_b^2/S_w^2$	Critical F for a two-tailed test	Significance
Rainwater	613.2	26.3	622.9	42.4	2.59	9.07	N.S.[e]
Road dust	8.51	0.69	8.12	0.70	1.03	3.77	N.S.

[a] X_w = the mean within the bottle; [b] S_w = the standard deviation within the bottle. [c] X_b = the mean between bottles; [d] S_b = the standard deviation between bottles; [e] N.S., no significant difference at 95% confidence level.

400 g of dust (within-bottle homogeneity), and by 15 determinations from different bottles (between-bottle homogeneity) selected as five out of each set of 200 from the total set of 600 bottles.

The analytical results obtained from the homogeneity studies are presented in Table 2. The F-test was used to assess the homogeneity of TriML in both artificial rainwater and road dust samples. The statistical results are also presented in Table 2. If the variations within a bottle and between bottles have no significant difference at the 95% confidence level based on the results of the F-test, the distribution of TriML in candidate reference materials can be considered to be homogeneous. As shown in Table 2, there is no significant difference between bottles of the same reference material at 95% confidence levels.

Stability study

The stability of candidate reference materials was tested at beginning of the storage periods and after 1, 3, 6 and 12 months. Twelve bottles of candidate artificial rainwater were stored at room temperature in the dark. Twelve bottles of candidate road dust were stored at −20 °C, 12 at 4 °C and 12 at 37 °C, respectively, to test the stability of road dust under three different storage conditions. Three of these samples were analysed on the day of preparation, three after 1 month, three after 3 months and three after 12 months. Instability could be determined by comparing the concentration of TriML on the day of preparation with those obtained later. In Table 3, R_T expresses the ratio of the mean (X_t) of three replicates analysed at $t=1$, 3, 6 or 12 months to the mean ($X_{t=0}$) from three measurements made at $t=0$, and U_T describes the uncertainty obtained from the relative standard deviations of three replicates at $t=0$ and at different intervals of analysis ($t=1$, 3, 6 or 12 months), as proposed by Fiedler *et al.*:[8]

$$U_T = \frac{R_T\sqrt{Sr_t^2 + Sr_{t=0}^2}}{100} \qquad [1]$$

where Sr_t^2 = the square of the relative standard deviation from three replicates measured after 1, 3, 6 or 12 months of storage;

$Sr_{t=0}^2$ = the square of the relative standard deviation from three replicates analysed on the day of preparation;

$R_T = \frac{X_t}{X_{t=0}}$;

X_t = mean value at $t=1$, 3, 6 or 12 months;

$X_{t=0}$ = mean value at $t=0$.

The ratio (R_T) should equal 1.0, if the stored sample is entirely stable. However, as there are random errors in the analytical process, these are represented by $\pm U_T$ in Table 3. The results ($R_T \pm U_T$) from the stability studies for candidate artificial rainwater and road dust after 12 months'

Table 3 Stability study during 12 months[a]

Material	Interval (months)	$R_T \pm U_T$
Rainwater	1	1.00 ± 0.044
	3	0.98 ± 0.058
	6	0.98 ± 0.054
	12	0.96 ± 0.060
Road dust stored at −20 °C	1	1.02 ± 0.15
	3	1.06 ± 0.16
	6	1.04 ± 0.14
	12	0.98 ± 0.15
Road dust stored at 4 °C	1	1.08 ± 0.13
	3	1.04 ± 0.16
	6	1.05 ± 0.15
	12	0.99 ± 0.15
Road dust stored at 37 °C	1	0.87 ± 0.14
	3	0.90 ± 0.13
	6	0.84 ± 0.16
	12	0.78 ± 0.11

[a] The values analysed at $t=0$ were taken as a reference.

Table 4 Statistical results for stability study of artificial rainwater and road dust in different storage conditions

Materials	Source of variation	F	P-value	Critical F for a one-tail test	Significance at 0.05 level
Rainwater	Between intervals	0.47	0.71	4.07	N.S.[a]
Road dust	Between storage conditions	7.84	0.02	5.14	S.[b]
	Between intervals	0.08	0.97	4.76	N.S.

[a] N.S., no significant difference. [b] S., significant difference.

storage are shown in Table 3, from which it can be seen that no instability of TriML in either candidate artificial rainwater or road dust stored at −20 °C and 4 °C was demonstrated after 12 months' storage. However, Table 3 also shows some initial loss of TriML in the candidate road dust when stored at 37 °C, when compared with other storage conditions.

In order to assess further the stability of candidate artificial rainwater, a single-factor ANOVA was used to determine whether or not a significant difference exists among the TriML concentration results obtained at 0, 1, 3, 6 and 12 months. The statistical results are presented in Table 4. Since the calculated P-value is greater than the critical P-value in Table 4, the null hypothesis is accepted. Therefore, the TriML concentrations in candidate artificial rainwater, analysed at different intervals, did not differ significantly.

A two-factor ANOVA was applied (1) to test whether the different storage conditions have significantly influenced the concentration of TriML in candidate road dust, and (2) to test whether the temporal variation is significantly greater than the variation due to the random error of measurement. The variation caused by different storage conditions was regarded as an effect source, and the variation due to month-to-month variation as another source. The statistical results are shown in Table 4, from which it can be seen that there are no significant differences between different intervals. However, significant differences between three different storage conditions exist. Due to the risk of instability, it is therefore recommended that the candidate reference materials should be stored at 4 °C in the dark.

CONCLUSIONS

The results based on the statistical analysis show that the distribution of TriML in both candidate reference materials is homogeneous and stable for at least 12 months under the appropriate storage conditions (4 °C in the dark). However, the results also indicate that TriML may be unstable, if the candidate road dust is stored at a temperature as high as 37 °C. In summary, the feasibility of preparing homogeneous and stable candidate artificial rainwater and road dust reference materials for the certification of TriML concentration in environmental samples has been demonstrated, and the certification campaign can now be contemplated. It is expected that the certification analyses will be performed in 1996 by a group of European laboratories.

REFERENCES

1. Ph. Quevauviller, Y. Wang and R. M. Harrison, *Appl. Organomet. Chem.* **8**, 703 (1995).
2. A. B. Turnbull, Y. Wang and R. M. Harrison, *Appl. Organomet. Chem.* **7**, 567 (1993).
3. R. J. A. Van Cleuvenbergen and F. C. Adams, *Environ. Sci. Technol.* **26**, 1354 (1992).
4. R. Lobinski, C. F. Boutron, J. P. Candelone, S. Hong, J. Szpunar-Lobinska and F. C. Adams, *Environ. Sci. Technol.* **28**, 1467 (1994).
5. S. Dyg, T. Anglov and J. M. Christensen, *Anal. Chim. Acta* **286**, 273 (1994).
6. B. Griepink, *Fresnius' J. Anal. Chem.* **338**, 360 (1990).
7. Ph. Quevauviller, Y. Wang, A. B. Turnbull, W. M. R. Dirkx, R. M. Harrison and F. C. Adams, *Appl. Organomet. Chem.* **9**, 89–94 (1995).
8. H. D. Fiedler, J. F. Lopez-Sanchez, R. Rubio, G. Rauret, Ph. Quevauviller, A. M. Ure and H. Muntau, *Analyst (London)* **119**, 1109 (1994).