APPLIED ORGANOMETALLIC CHEMISTRY, VOL. 8, 639–644 (1994)

Interlaboratory Study for the Improvement of Tributyltin Determination in Harbour Sediment (RM 424)

Ph. Quevauviller,* M. Astruc,† L. Ebdon,‡ G. N. Kramer§ and B. Griepink¶
*European Commission, Measurements and Testing Programme (BCR), rue de la Loi 200, B-1049 Brussels, Belgium, †Université de Pau et des Pays de l'Adour, Laboratoire de Chimie Analytique, Avenue de l'Université, F-64000 Pau, France, ‡University of Plymouth, Department of Environmental Sciences, Drake Circus, Plymouth PL4 8AA, UK, and §European Commission Institute for Reference Materials and Measurements, Steenweg op Retie, B-2440 Geel, Belgium

Following two interlaboratory studies on tributyltin species (TBT) in water and TBT-spiked sediment, the EC Community Bureau of Reference (BCR) (Measurements and Testing Programme) organized in 1992 an interlaboratory study on TBT in a harbour sediment (RM 424) to investigate sources of error possibly occurring in the analysis of a complicated matrix and to attempt to certify this material. The sediment was collected in the Sado Estuary (P), then carefully prepared, and its homogeneity and stability were verified. The low TBT content encountered in this material, and the high level of interferences, created difficulties with the techniques using hydride generation, whereas better agreement was obtained for other derivatization techniques with GC/FPD and GC/MS. This paper presents the results of the interlaboratory study. The reference value for TBT in this material is 20 ± 5 ng g^{-1} (as TBT cation) and the indicative values for dibutyltin (DBT) and monobutyltin (MBT) are 53 ± 19 ng g^{-1} (as DBT cation) and 257 ± 54 ng g^{-1} (as MBT cation), respectively. Owing to the difficulties encountered, the reference material was not certified and is considered as a research material to be used for the evaluation of the performance of analytical techniques for the determination of low TBT levels in a difficult matrix.

Keywords: Interlaboratory study, harbour sediment, analytical quality control, reference material, tributyltin, dibutyltin, monobutyltin

INTRODUCTION

The toxicity of tributyltin (TBT) to marine organisms is now widely recognized.[1,2] This compound is released from the leaching of TBT-based antifouling paints used on boats and ships. Such paints have been controlled, e.g. in France, the UK and The Netherlands. In addition, organotin compounds (including TBT) have been included in the list of priority contaminants in some EC Directives on the protection of the aquatic environment. Consequently, this compound is routinely monitored in environmental matrices (mostly sediment and biological tissues) by a number of laboratories in Europe. Accurate measurements are a prerequisite to achieve a good assessment of contamination levels and allow for the comparability of data obtained by different laboratories using different techniques. In order to control the quality of environmental analyses, various measures are necessary. One of the most powerful tools in detecting and removing sources of error due to a particular technique or a lack of quality control (QC) within a laboratory is to participate in interlaboratory studies.[3,4] When laboratories participate in an interlaboratory study, different sample pre-treatment methods and different techniques of final determination may be compared and discussed. If the results of such an exercise agree, the value obtained is likely to be the best approximation of the truth.[4]

Interlaboratory studies on TBT were organized within the BCR programme for improving the quality of the determinations.[5] Other projects have also been carried out, e.g. to compare extraction procedures for TBT determination in sediment.[6] More recently, certification campaigns were performed which allowed groups of labora-

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¶ Present address: European Commission, PHARE Programme, rue de la Loi 200, B-1049 Brussels, Belgium.

CCC 0268-2605/94/070639-06

Received 22 February 1994
Accepted 8 August 1994

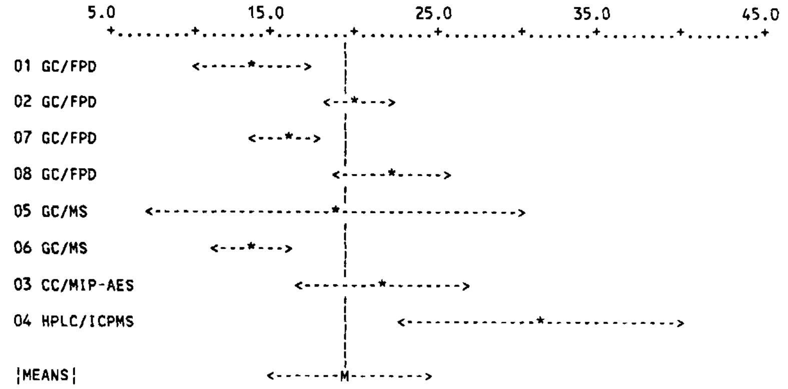

Figure 1 Bar-graph for TBT results, with 95% confidence interval. The laboratory codes are indicated along with the methods used (abbreviations defined in Table 2). The results plotted correspond to five replicate determinations. 'MEANS' is the mean of laboratory means with its standard deviation.

tories to achieve the best obtainable agreement between different techniques: a Certified Reference Material (CRM) was produced by the National Research Council Canada (PACS-1)[7] with a TBT content of $1.27 \pm 0.22\,\mu g\,g^{-1}$. Considering that materials with lower TBT contents were necessary (typical environmental TBT sediment levels range from 50 to $200\,ng\,g^{-1}$ of TBT), the Measurements and Testing Programme (BCR) has produced a Certified Reference Material (CRM 462) with a TBT content of $70 \pm 14\,ng\,g^{-1}$ as TBT.[8] However, in some cases the butyltin levels are even lower and there is presently no means of controlling the quality of the determination of TBT content below $50\,ng\,g^{-1}$; the problems are even more acute when complicated (organic) matrices are to be analysed. Consequently, the BCR decided to produce a sediment rich in organic matter containing low levels of TBT in order to test the performance of existing analytical techniques under extreme conditions.

ORGANIZATION OF THE INTERLABORATORY STUDY

An interlaboratory study can be organized (i) to detect the pitfalls of a commonly applied method and to ascertain its performance in practice, (ii) to assess the quality of a laboratory or a part of a laboratory (e.g. audits for accreditation of laboratories), (iii) to improve the quality of a laboratory in a collaborative and mutual learning process, and (iv) to certify the contents of a reference material.[9] The work described here is of type (iv). Each of the laboratories which participated in the exercise (see Acknowledgements) received two bottles of RM 424 and was requested to make a minimum of five independent replicate analyses on different days. Recommendations were made to verify the calibrants used with a pure tributyltin acetate calibrant prepared for this interlaboratory programme.[5] The participants were also requested to report their method and extraction recovery. The results submitted by the laboratories were discussed amongst all participants in a technical meeting; they were presented in the form of a bar-graph showing the laboratory codes and the methods used, the individual means and standard deviations and the mean of laboratory means with its standard deviations (see Fig. 1).

PREPARATION OF THE REFERENCE MATERIAL

Collection and preparation of the reference material for the interlaboratory study

The harbour sediment to be used in the interlaboratory study (RM 424) was collected in the Sado Estuary in the vicinity of the harbour of Setúbal. The first 10-cm sediment layer was collected with a shovel and stored at ambient temperature in plastic containers. After decantation, the sediment sample was air-dried for seven days at ambient temperature on a cotton sheet in a well-ventilated room, and stored at −20 °C in polythene bags. The material (about 180 kg dry mass) was then stored in ice boxes for transportation to the Institute for Reference Materials and Measurements, IRMM (Geel, Belgium). The material was dried at 80 °C in air for 100 h; it was then ground and sieved through a sieve of 75 μm mesh size. The powder obtained was sterilized by heating at 120 °C for 1 h, then homogenized in a mixer for 1 h. The material was finally stored in 60-ml well-cleaned brown glass bottles with polyethylene inserts and plastic screw caps, each containing *ca* 25 g of powder.

The moisture content of the material was determined by Karl Fischer titration on ten samples selected during the bottling procedure.[10] The mean moisture mass fraction measured was 2.24±0.36%.

The between-bottle homogeneity was verified by the determination of TBT on intakes of 2 g taken from 20 bottles which were set aside at regular intervals during the whole period of bottling. The within-bottle homogeneity was assessed by ten replicate determinations on the well-mixed contents of one bottle. The technique used for this study was hydride generation/cold trapping–gas chromatography/atomic absorption spectrometry.[11] No significant differences were observed between the coefficient of variation (CV) of the method 8.7±2.8% and the CV within-bottles (8.2±1.8%). The CV between-bottles appeared to be significantly higher than the CV within-bottles (18.3±2.9%); however, the value found corresponded to determinations performed over a period six months and therefore included the long-term reproducibility of the analytical method used, which was realistic at this mass fraction level. Any inhomogeneity of the material would have been detected in the stability study.

The stability of the TBT content was tested to determine the suitability of this material as a reference material. Bottles were kept at −20 °C, +20 °C and +40 °C respectively over a period of 12 months and TBT was determined at regular intervals during the storage period. Tests were made at the beginning of the storage period and after one, three, six and 12 months. TBT was determined ten times on each occasion of analysis. The samples stored at −20 °C were used as the reference for the samples stored at +40 °C and at +20 °C respectively. Table 1 gives the ratios (R_T) of the mean values ($\bar{X}_T$) of five measurements made at +20 °C and +40 °C, respectively, and the mean value ($\bar{X}_{-20\,°C}$), from five determinations made on the same occasion of analysis on samples stored at a temperature of −20 °C:

Table 1 Stability tests of TBT in coastal sediment RM 424

Time (months)	Temperature (°C)	R_T[a]	U_T[b]
1	+20	0.984	0.141
3	+20	1.036	0.227
6	+20	1.059	0.293
12	+20	0.967	0.139
1	+40	0.938	0.160
3	+40	1.000	0.227
6	+40	0.922	0.138
12	+40	0.883	0.123

[a] $R_T = X_T/X_{-20\,°C}$, where X_T mean of 10 replicates at temperature T (+20 °C or +40 °C).

[b] $U_T = (CV_{T^2} + CV_{-20\,°C^2})^{1/2} . R_T$, where CV_T = coefficient of variation of 10 replicates at temperature T.

$$R_T = \bar{X}_T / \bar{X}_{-20\,°C}$$

The uncertainty U_T has been obtained from the coefficient of variation (CV) of five measurements obtained at each temperature:

$$-U_T = (CV_T^2 + CV_{-20\,°C}^2)^{1/2} . R_T$$

In the case of ideal stability, the ratio R_T should be 1. In practice, however, there are some random variations due to the error on the measurement. In most cases, the value 1 existed between $R_T - U_T$ and $R_T + U_T$. The uncertainty on the coefficient of variations (CV) can account for the deviations observed. In the case of storage at +40 °C, slight TBT losses were, however, suspected to occur after six-months of storage. Although the CRM 462 was shown to be stable at +20 °C, a recommendation was given to keep this material at +4 °C for long-term storage.

TECHNIQUES USED IN THE INTERLABORATORY STUDY

The techniques used in the interlaboratory exercise involved different types of extraction (solvent or acid), derivatization, separation and final

Table 2 Summary of the techniques used in the interlaboratory study

Lab. code	Extraction, derivatization and separation[a]	Final determination[a]
01	NaOH/hexane extraction–hydride generation–CGC separation	FPD at 610 nm
02	Toluene/tropolone extraction–ethylation–CGC separation	FPD at 610 nm
03	Acetic acid/hexane/DDTC extraction in pentane–pentylation–CGC separation	QFAAS at 286.4 nm
03	HCl/HAc/hexane/tropolone extraction–pentylation–CGC separation	MIP–AES at 303.4 nm
04	Acetic acid extraction–back-extraction with toluene–HPLC separation	ICP–MS of mass 120
05	Soxhlet extraction with hexane/acetone–pentylation–CGC separation	MS of masses 305 and 319
06	HCl/methanol/tropolone extraction–pentylation–CGC separation	MS of masses 301, 305 and 305
07	HBr/tropolone extraction–pentylation–CGC separation	FPD at 610 nm
08	SFE (CO_2/HCl/MeOH)–ethylation–CGC separation	FPD at 610 nm
09	Acetic acid extraction–hydride generation–cold trapping/GC separation with packed column	QFAAS at 286.4 nm
10	Dichloromethane extraction–ethylation–GC separation with packed column	QFAAS
11	Acetic acid extraction–hydride generation–cold trapping/GC separation with packed column	QFAAS at 224.6 nm
12	Dichloromethane extraction	ETAAS
13	Diethylether/HCl/tropolone extraction–pentylation–CGC separation	MS of masses 247, 303 and 305
14	Leaching with HCl–dichloromethane extraction	ACP (Ag/AgCl electr.)
15	Methanol/HCl extraction–hydride generation–cold trapping/GC separation with packed column	QFAAS at 224.6 nm
16	Acetic acid extraction–hydride generation–cold trapping/GC separation with packed column	QFAAS at 224.6 nm
17	Tropolone/toluene extraction–back-extraction with acetone–pentylation–CGC separation	MS of masses 305 and 319
18	Methanol/HCl extraction–back-extraction with NaOH	ETAAS at 235.4 nm

[a] Abbreviations: DDTC, diethyldithiocarbamate; ETAAS, electrothermal atomic absorption spectrometry; FPD, flame photometric detection; CGC, capillary gas chromatography; HPLC, high-performance liquid chromatography; ICPMS, inductively coupled plasma mass spectrometry; MIP–AES, microwave-induced plasma atomic emission spectrometry; MS, mass spectrometry; QFAAS, quartz furnace atomic absorption spectrometry; SFE, supercritical fluid extraction.

determination. Table 2 gives an account of these techniques. Calibration was performed by calibration graph, except for Laboratories 04, 05, 09, 14, 15, 16 and 18, which carried out standard additions. Calibrants used by the participants (either TBTCl or TBTO) were checked against a TBTAc calibrant of verified purity (>99%). Tripropyltin chloride was often used as internal standard.

TECHNICAL DISCUSSION

The original bar-graph presentation showed a very large scatter of data which ranged from less than 10 ng g^{-1} to more than 150 ng g^{-1} of TBT. Detailed discussions were necessary to explain the sources of discrepancies.

Some laboratories reported not-detected values which corresponded to, respectively, less than 15 ng g^{-1} (Laboratory 03), less than 20 ng g^{-1} (Laboratory 11), less than 49 ng g^{-1} (Laboratory 12), less than 60 ng g^{-1} (Laboratory 13), less than 146 ng g^{-1} (Laboratory 15) and less than 20 ng g^{-1} (Laboratory 16); Laboratory 03 submitted two results which were below their limit of determination and could not be accepted for certification: 15.6 ng g^{-1} and 22.4 ng g^{-1}. Later (after optimization of the method), this laboratory carried out five other replicate determinations, of the results of which were slightly above the limit of determination of their method (mean of 12.0 ± 3.9 ng g^{-1}), which confirmed the values found; these values were, however, not used for the calculation of the assigned value.

The laboratories reported their results of extraction recovery, which were generally acceptable (from 80 to 100%). It was assumed that the main problems were not due to extraction but to possible interferences in the derivatization and/or in the detection step. Laboratory 10 noted that aromatic compounds could have inhibited hydride generation but not the ethylation reaction. Laboratory 15 used methanol–HCl for extraction and found they did not extract the oil present in the sample but still observed a 35% suppression of hydride generation. Laboratory 11 felt, however, that the interferences were occurring at the atomization stage rather than in the hydride generation step; high content of iron and chromium could have led to a suppression of the TBT signal, as demonstrated with spiking experiments. An extensive study was carried out by the University of Bordeaux (France) on interference effects from inorganic (metals) and organic substances (organic solvent, PCB, pesticides, n-alkane, humic substances) on the yield of hydride generation. The major effects observed were mostly linked to high levels of trace metals; organic compounds had generally negligible effects on the signal suppression.[12]

The methods using hydride generation and AAS as final determination were, however, in considerable difficulty with this complicated matrix, due to unknown interferences, either at the derivatization step or in AAS detection, or with determination limits which were too low. As observed by the participants, the laboratories using other derivatization techniques and gas-chromatographic separation and detection either by FPD or MS tended to agree, which would confirm that these methods would be more suited to the determination of TBT in this particular material.

It was agreed that analyses would be repeated by either independent laboratories or alternative techniques. Laboratory 03 submitted new results obtained by GC/MIP–AES (mean of 21.5 ± 4.2 ng g^{-1}) and Laboratory 08 obtained a new set of results using a newly developed supercritical fluid extraction method (mean of 22.2 ± 3.2 ng g^{-1}). A new laboratory (Laboratory 07) submitted an additional set of GC/FPD results (15.6 ± 1.6 ng g^{-1}). Finally, Laboratory 16 repeated the analysis and found two values of 20 and 23 ng g^{-1}, which confirmed the data found by the other laboratories; this laboratory confirmed, however, the extreme difficulty of obtaining reliable results with the hydride generation/AAS technique with this material.

Although the analytical methods involving hydride generation were successfully applied in an interlaboratory study on TBT in spiked sediment[5] as well as in the certification of TBT and DBT in CRM 462 (coastal sediment),[8] it was concluded that the low TBT mass fractions and the complicated matrix did not allow the use of these methods for an accurate TBT determination in this material. It was stressed that, although certification was contemplated at the start of the interlaboratory study, this material would probably not be suited as a CRM for the following reasons:

(1) the low content of TBT is very close to the limits of determinations of most of the techniques used in this exercise;
(2) the high level of interferences makes this material difficult to analyse, which places doubt on its use as a CRM;
(3) both the low TBT content and the complicated matrix do not resemble sediments usually analysed for TBT monitoring. This unrepresentativeness is another reason for not proposing this material as a CRM.

Consequently, RM 424 was not certified and will be considered as a research material for laboratories willing to evaluate techniques such as GC/FPD, GC/MS or HPLC/ICPMS (see Table 2 for glossary). Figure 1 presents the results obtained by GC/FPD, GC/MS, GC/MIP–AES and HPLC/ICPMS. The good agreement found between these methods gave confidence that no systematic error was left undetected. Due to the potential risk of interferences, calibration by standard additions is a prerequisite.

The reference value (unweighted mean of eight accepted sets of results) and its standard deviation is 20 ± 5 ng g^{-1} as mass fraction (based on dry mass) of TBT cation. Indicative values were given for DBT and MBT, respectively: 53 ± 19 ng g^{-1} as DBT cation (six set of results—GC/FPD, GC/MS and GC/AAS) and 257 ± 54 ng g^{-1} as MBT cation (three sets of results—GC/FPD, GC/MS).

AVAILABILITY

RM 424 is available from the Measurements and Testing Programme (European Commission, rue de la Loi 200, B-1049 Brussels, Belgium; Fax +32-2.2958072).

Acknowledgements The sediment was collectd by the University of Pau (France) and prepared at the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). The verification of the homogeneity and stability was carried out by the University of Pau. The following laboratories participated in the interlaboratory study and the participants are gratefully acknowledged for their valuable contribution to this programme: CIBA–Geigy, Bensheim, Germany (H. Müller); CID–CSIC, Department of Environmental Chemistry, Barcelona, Spain (J. M. Bayona); De Monfort University, Department of Chemistry, School of Applied Sciences, Leicester, UK (P. J. Craig); ENEA, Casaccia Research Centre, Rome, Italy (R. Morabito and S. Chiavarini); Free University of Amsterdam, Institute for Environmental Studies, Amsterdam, The Netherlands (J. Stäb); IFREMER, Nantes, France (P. Michel); Institute for Spectrochemistry, Dortmund, Germany (G. Weber); Max-Planck Institute for Chemistry, Biogeochemistry Department, Mainz, Germany (S. Rapsomanikis); Ministry for Agriculture, Fisheries and Food, Burnham-on-Crouch, UK (M. Waldock); Ministry for Transport and Public Works, Tidal Water Division, Haren, The Netherlands (R. Ritsema); Schering AG, Bergkamen, Germany (P. Schultze); Technical University of Athens, Department of Chemical Engineering, Athens, Greece (M. Ochsenkühn-Petropulu); TNO–CIVO Institutes, Ijmuiden, The Netherlands (H. Luten); University of Antwerp, Department of Chemistry, Antwerp, Belgium (F. Adams and W. Dirkx); University of Bordeaux I, Laboratory of Molecular Photophysics and Photochemistry, Talence, France (O. Donard and F. Martin); University of Pau, Laboratory of Analytical Chemistry, Pau, France (A. Astruc, M. Astruc, V. Desauziers and R. Pinel); University of Plymouth, Department of Environmental Sciences, Plymouth, UK (L. Ebdon and S. Sparkes); University of Seville, Department of Chemistry, Seville, Spain (J. L. Gomez-Ariza).

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