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A simple method for synthesis of organotin species to investigate extraction procedures in sediments by isotope dilution-gas chromatography-inductively coupled plasma mass spectrometry Part 2.† Phenyltin species

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Received 30th October 2003, Accepted 14th January 2004
First published as an Advance Article on the web 17th February 2004

A rapid method for the synthesis of phenyltin species based on the phenylation of tin iodide was developed and a standard of ^{124}Sn, enriched monophenyltin (MPhT), diphenyltin (DPhT) and triphenyltin (TPhT) was produced. Isotope enriched species were added to and equilibrated with the certified reference material BCR 646 to evaluate different extraction procedures currently in use for the determination of organic tin species in sediments. Samples were measured by gas chromatography-inductively coupled plasma mass spectrometry (GC-ICP-MS) with species specific isotope dilution (SSID) calibration. For TPhT measurement results agreed with the certified values for extraction methods using tropolone in diethyl ether alone or in the presence of NaCl and HCl as well as with 50% HBr. However, with 50% HBr, concentrations obtained for DPhT and MPhT were above the upper limit (2σ) of the certification. The stability of phenyltin species was studied by comparing their signal magnitudes in spike solutions generated directly after derivatisation with those obtained after applying the extraction–derivatisation procedures. Degradation of phenyltin species was matrix dependent and appeared for most of the extraction methods investigated. For water standards and BCR 646, extraction with methanol combined with dichloromethane or methanol combined with acetic acid gave no degradation when applied with less than 20 min ultrasonication. Extraction efficiencies for these two methods were however low for the BCR 646 matrix, in particular for DPhT and MPhT.

Introduction

In the last four decades, organotin compounds were extensively used as additives in antifouling paints, wood preservatives, biocides, and plastics.[1] In countries such as The Netherlands, Germany and USA, triphenyltin (TPhT) was used as agricultural fungicide.[2,3] Studies on the toxicity of phenyltins have shown that it affects the early life stage of marine organisms.[4] While the effects of phenyltins on humans have rarely been studied, a recent report showed that these compounds are biologically active with the ability to penetrate lipid membranes.[5] Exposition to TPhT for 1 h is reported to suppress 50–60% of lymphocyte activity, which as the natural killer cells are essential for the human immune system.[6] As a consequence of the complex interactions of TPhT with living systems, phenyltin species are included in the European Union pollutant list.[7–9]

TPhT was frequently applied by spraying, which risked contaminating the surrounding soil. As the vapour pressure of TPhT hydroxide is relatively low at 1×10^{-7} mm Hg at 25 °C,[1] losses by volatilization will be insignificant. Therefore, TPhT and its degradation products are expected to be persistent pollutants of soil, increasing the risk for bioaccumulation of these species.

In view of the great impact of TPhT on living organisms it is important to develop reliable methods to monitor TPhT and its degradation products in the environment.[10] Methods currently used involve several analytical steps such as extraction, preconcentration, cleanup, derivatisation, separation, and finally detection by element or molecule specific techniques.[11–13] Incomplete extraction, transformation or losses of species in any one of these steps will deteriorate the accuracy of results and new approaches such as pressurised liquid extraction[14] and atmospheric pressure ionisation[15] have been applied.

A number of different, independent measurement techniques are now available for separation and detection of tin species. Normally species are separated by suitable chromatography (LC, GC, SFC) and detected by element specific (ICP-MS, MIP-AES, AAS) or species specific detectors (MS).[16]

For speciation analysis with SSID[17–20] it is necessary to use species prepared from isotopically enriched Sn, however to date, such species are not commercially available.

This paper reports the development of a fast and simple method to synthesize a mixed standard of phenyltin species (MPhT, DPhT and TPhT) using enriched ^{124}Sn metal. The isotope-enriched standard was prepared by the synthesis of tiniodide and dissolution in diethyl ether followed by reaction with phenylmagnesium bromide (a Grignard reagent). The standard was used to evaluate, using SSID, different extraction procedures currently in use for the determination of phenyltin species.

In separate experiments the stability of phenyltin species during sample preparation procedures has been investigated.

Experimental

Instrumental

The same instrumentation as described in Part 1[20] of this series was used. Briefly, a Varian 3300 gas chromatograph (Varian, Palo Alto, CA, USA) fitted with an on-column injector liner and a 30 m SBP-1 capillary column (Supelco, Sweden) was used

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DOI: 10.1039/b313787b

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for separation of Sn species with an Agilent 7500 ICP-MS for detection. The instruments were connected through an in-house interface.[21] The operating parameters of the ICP-MS were optimised for maximum sensitivity by measuring ^{129}Xe, which was continuously added during optimisation at a flow rate of 0.5 ml min^{-1} to the ICP nebuliser gas flow. The optimised operating parameters of the GC and ICP-MS are given in Table 1 of Part 1.[20]

Determination of tin species by GC-quartz tube AAS. A Varian 3300 gas chromatograph was used as described above. A 0.4 m deactivated fused silica transfer line, heated to 280 °C interfaced the GC to an electrically heated quartz t-tube atomiser, length 80 mm and inner diameter 10 mm. This was positioned in the optical axis of a PerkinElmer Analyst 700 atomic absorption spectrophotometer provided with an EDL lamp for tin using the 224.6 nm line. Make up gases, hydrogen and synthetic air (AGA, Sweden), were added to the quartz tube atomiser heated to 750 °C. An in house constructed program was used to collect and evaluate data with Lab View. Sample pre-treatment and GC parameters followed the procedures used for ICP-MS measurements.

Reagents

Tin metal, 98.6% enriched in ^{124}Sn, was purchased from JSC JV ISOFLEX, Moscow, Russia. Iodine (99.8%, p.a. grade) and the solvents dichloromethane, diethyl ether, toluene, and acetic acid were obtained from Merck (Darmstadt, Germany). Methanol (HPLC gradient grade) and hydrobromic acid were obtained from J.T. Baker, Deventer, The Netherlands. Sodium tetraethylborate (98%) was obtained in sealed septum vials from Galab, Geestach, Germany. The solution for the organoborate derivatisation of samples was prepared as described earlier.[20] Water was purified by a Milli-Q system, Millipore, Bedford, MD, USA.

Phenyl tin stock standards in methanol (1000 μg g^{-1} as Sn) with natural isotope distribution were prepared from monophenyl- (98%), diphenyl- (96%) and triphenyl- (97%) tin chloride, which together with tropolone (98%) was obtained from Aldrich (Germany).

All organotin stock and diluted solutions were kept in 20 ml glass vials provided with Teflon faced silicone septum caps, Coricon, Knivsta, Sweden. Solutions were withdrawn using dedicated glass syringes, Hamilton, Australia. Solutions recovered after extraction and derivatisation were stored in 1.5 ml glass vials with rubber/TEF septum caps. The amount of Sn in standards with natural isotope distribution were based on gravimetric measurement of their preparation, corrected for reagent purity. PhT concentrations in the isotopically enriched standards are determined by reversed isotope dilution and the equation proposed by Fasset *et al.*[22] is used for the calculations. All gases used (O_2, He, Ar, N_2) were of at least 99.995% purity.

Procedures for synthesis of phenyltin compounds

The synthesis of phenyltin species involved two steps. Tin iodide was prepared from tin metal 98.6% enriched in ^{124}Sn, which was reacted with the Grignard reagent.

Preparation of tin iodide. A similar method as described in Part 1[20] was used. Briefly, 70.4 mg of iodine was added to 24.1 mg of ^{124}Sn metal with 2 ml of dichloromethane in a 10 ml glass tube. The tube was closed and placed in a sand bath kept at 100 °C. The reaction ceased after 60–90 min when the violet color of iodine changed to orange. The container was cooled and the dichloromethane evaporated leaving unreacted tin metal and solid tin iodide. The salt was dissolved in 4–5 ml of diethyl ether, which was then transferred to another 10 ml glass tube and the unreacted tin (12.6 mg) was collected.

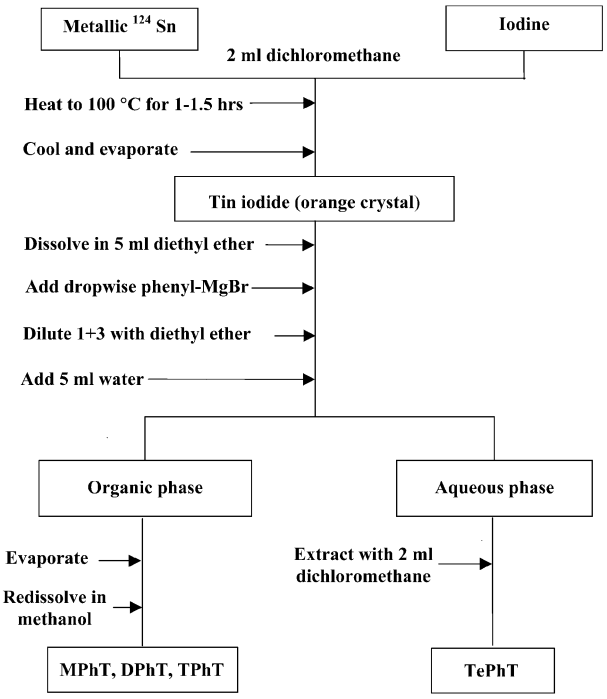

Fig. 1 Schematic presentation for the synthesis of phenyltins.

Preparation of phenyltin compounds. Fig. 1 shows the scheme for the preparation of phenyltin compounds. A Teflon coated magnetic stirrer was placed in the container containing ^{124}Sn iodide solution to allow continuous stirring.

To a 5 ml glass container, 250 μl of 3 M phenylmagnesium bromide in diethyl ether and 2–3 ml of diethyl ether were added, after which the vessel was closed with a screw cap. With a glass Pasteur pipette, the diluted reagent was added to the tin iodide solution until the yellowish colour had just disappeared. This occurred after about 2 min, on consumption of about 70% of the reagent. The reaction was then immediately quenched by the addition of 5 ml water. The diethyl ether layer was transferred to another glass tube by a Pasteur pipette, evaporated under a stream of nitrogen and the residue was re-dissolved in 7 g of methanol. The concentration of the species prepared was determined by reverse isotope dilution-GC-ICP-MS, spiking with one species at a time, immediately after production as well as immediately before use. The synthesized species were stored at −18 °C.

Extraction procedures

The same extraction procedures were used as in Part 1[20] and therefore only an abbreviated description is given below.

Spiking. If not otherwise stated, samples were equilibrated with the spike for 14 h for Method 1 and for about 1 h for the other methods. Samples were stored at 4 °C in the dark.

Method 1, extraction with 0.02% (w/v) tropolone in diethyl ether. To 2 g of sample, 10 ml of tropolone in diethyl ether was added. The mixture was sonicated in an ultrasound bath for 15 min, mechanically shaken for 10 min and centrifuged to separate diethyl ether. The process was repeated with another 10 ml diethyl ether and was combined with the ether from the first extraction, evaporated on anhydrous sodium sulfate to about 5 ml, transferred into another container and evaporated to dryness. To the residue, 1 ml of toluene was added followed by the derivatisation procedure in Method A, see below.

Method 2, extraction with methanol and dichloromethane. To 0.25 g of sample, 10 ml of 1 + 1 methanol and dichloromethane

were added and sonicated for 15 min. The mixture was centrifuged and the organic layer was separated and evaporated. The residue was dissolved in 1 ml of toluene and derivatised by Method A.

Method 3, extraction with NaCl and HCl. To 2 g of the sample, 5 ml of 0.2 M HCl in 10% (w/v) NaCl was added. The mixture was sonicated for 15 min and shaken with 12 ml tropolone in diethyl ether followed by centrifugation. The organic layer was separated and evaporated. The residue was dissolved in 1 ml of toluene and derivatised by Method A.

Method 4, extraction with methanol and acetic acid. To 0.25 g of the sample, 1 ml of methanol and 3 ml of acetic acid was added. The sample was sonicated for 15 min and centrifuged as above. 0.2 ml of the solution was derivatised by Method B see below.

Method 5, extraction with diluted HBr. To 0.5–0.7 g of the samples 10 ml of 50% (v/v) HBr was added and sonicated for 15 min. The samples were extracted with 20 ml of tropolone (0.04%) in dichloromethane for 1, 2 or 14 h. The mixture was centrifuged as before and the dichloromethane layer was removed with a Pasteur pipette, evaporated, and treated in the same manner as in Method 1.

Derivatisation

Method A. Portions of 40 μl of 25% (w/v) sodium tetraethyl borate in THF were added to samples. Mixtures were left at room temperature for 1 h and centrifuged at 5000 rpm for 2 min. Part of the organic layer was transferred to 1 ml glass tubes equipped with septa.

Method B

Samples of 200 μl were buffered with 3 ml of sodium acetate/acetic acid buffer. They were derivatised with 40 μl of 25% (w/v) sodium tetraethyl borate in THF. The mixture was manually shaken for 5 min with 1 ml of hexane. The hexane layer was then treated as with Method A.

Results and discussion

Optimisation of GC and ICP-MS operating parameters

As phenyltins are less volatile than butyltins, optimisation of the GC temperature program and the GC-ICP-MS interface heating was even more critical for separation and reproducible peak shapes. TPhT was the most difficult species to elute. Careful optimisation of the voltage at the interface was necessary as high temperatures resulted in breakage of the interface quartz capillary, while low temperatures gave broad TPhT peaks. The optimised GC operating parameters as well as those of the ICP-MS are given in Table 1 of Part 1.[20]

Mass bias correction

The method used to correct for mass bias is described elsewhere.[20] Briefly, on each measurement occasion, the ratio $^{118}Sn/^{120}Sn$ for natural tin was measured under the same instrumental conditions as the sample. This ratio was compared with ratios calculated from agreed natural abundances for natural isotopes and used for correction.

Synthesis of phenyltins

Attempts to directly synthesize phenyltins from tin metal by using chloro- or dichloro-benzene, triethyl amine and iodine were not successful. Therefore a similar method as presented in Part 1[20] based on derivatisation of tin iodide with Grignard reagent (phenylmagnesium bromide) was used, see Fig. 1. The scheme is similar to the preparation of butyltins except that the Grignard reagent was added drop wise until the yellow colour of tin iodide started to disappear. Any excess of the reagent resulted in a white precipitate of tetraphenyltin. After quenching the reaction with water, the aqueous phase was turbid. It contained decomposed ingredients such as magnesium and some tetraphenyltin, which could also be recovered by extracting the aqueous phase with 3–4 ml of dichloromethane. The final methanol standard contained typically 1.1 mg MPhT, 2.4 mg DPhT, 1.6 mg TPhT and 2.5 mg precipitated TePhT all as tin. The total yield of the mixed species including TePhT was 66%. To establish whether any isotopic fractionation, change in the isotopic composition, had occurred during preparation, the isotopic composition of the species was determined by GC-ICP-MS. As the enriched isotope contained 98.6% ^{124}Sn, most of the remaining isotopes were below the limit of detection except ^{122}Sn, however, the agreement of isotope content between the different species indicated that isotope fractionation was negligible (Table 1).

Evaluation of extraction methods

Analogous to the approach adopted by us for BT species, SSID was applied to the certified fresh water sediment (BCR 646). Tests were made with extraction reagents of increasing ionic strength, from pure organic solvents in the presence of tropolone to 50% hydrobromic acid. Compared to the certified values, analytical results too low or too high would indicate differences in the extraction efficiency between added and incipient tin species or/and changes in the recovery as well as inter conversion between the tin species investigated.

As will be discussed below, the binding strength of butyl substitutes to tin is larger than that for phenyl substitutes and treatment procedures involving phenyl species might therefore more easily lead to transformations of species. The binding strengths of phenyltin species to active groups of matrix constituents can also be expected to be smaller, which could increase their extraction efficiencies. Fig. 2 shows the results of our investigation on different extraction procedures. We found almost 100% recovery for extraction with tropolone in diethyl ether or for NaCl/HCl extraction while erroneously high results were obtained for MPhT when using 50% HBr for extraction. Overnight extraction with HBr resulted in even higher concentrations of MPhT (Fig. 2). When using HBr other workers have also obtained too high extraction efficiency, 166% for MPhT in mussel tissue.[23]

Species transformation during extraction

A mixture of phenyltin species prepared from a single enriched isotope (^{124}Sn) was used for SSID calibration. In contrast to investigations on butyl tin species, only a few publications deal with the degradation of PhT species during the analytical process.[8,10] For a preliminary investigation, the CAChe 3.1 Oxford Molecular work system was used to calculate the heat of formation for organic tin chlorides substituted with one to three phenyl or butyl groups. The heat of formation (KJ mol^{-1}) was −188.76 for MPhT, 414.92 for DPhT and 283.3 for TPhT. Corresponding values for the butyl tin species (MBT, DBT and TBT) were −407.5; −379.5 and −344.8, respectively. This indicated that PhT species are much more susceptible to

Table 1 Measured isotope composition of the tin species with the supplier's declaration for the bulk metallic tin

Isotope	MPhT	DPhT	TPhT	Average	Supplied
116–120	<DL	<DL	<DL	<DL	0
122	1.217	1.214	1.210	1.213	1.1
124	98.35	98.44	98.46	98.42	98.9

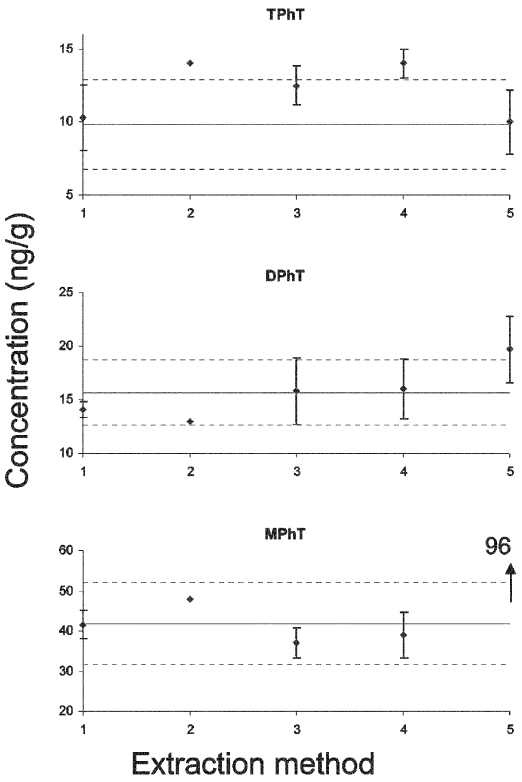

Fig. 2 Comparison of extraction methods for the determination of phenyltin species in BCR 646. 1: With tropolone in diethyl ether; 2: With methanol and dichloromethane in tropolone; 3: With tropolone in diethyl ether in the presence of NaCl and HCl; 4: With methanol and acetic acid; 5: With 50% HBr. The certified concentrations for BCR 646 are given as atomic tin whereas they are originally reported as cationic compounds. The error bars represent one standard deviation of the mean where the number of extraction replicates for methods 1–5 are 6, 1, 6, 2 and 6, respectively.

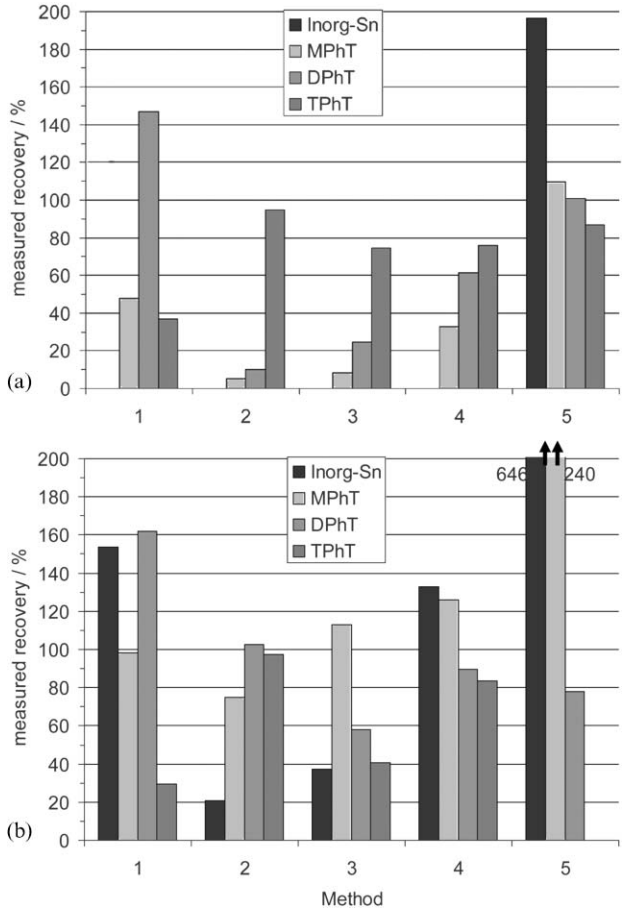

Fig. 3 Recovery (%) of phenyltin species and inorganic tin from BCR 646 (0.5 g) and water after applying various extraction methods. 1: Tropolone in diethyl ether; 2: Methanol and dichloromethane in tropolone; 3: Tropolone in ditheyl ether in the presence of NaCl and HCl; 4: Methanol and acetic acid; 5: 50% HBr. The following amounts, as μg tin, were added: MPhT, 0.496; DPhT, 0.544; TPhT, 0.506; inorganic tin, 0.077. All signals are normalised with the signals obtained for tin species in the standard solution used to spike the BCR 646. a. Measured recoveries of PhT species from BCR-646 sediment b. Measured recoveries of PhT species from water.

decomposition than butylated tin species. Therefore a separate series of experiments were performed, using quartz furnace atomic absorption spectrometry (QFAAS) for sensitive, species specific detection, to assess the effect of PhT species stability on their recovery when applying the different extraction procedures to mixtures of the mixed standard added to BCR-646 or to water.

Fig. 3a and b shows the recovery for the inorganic- and phenyl-tin species after extraction of tin species from water and BCR 646 following Methods 1–5. As a reference an aqueous standard was directly derivatised without any extraction. For both sediment and water matrices extracted with Method 1 (tropolone in diethyl ether) most of the TPhT had decomposed to DPhT. The lower recoveries for MPhT and inorganic tin with this method are due to their incomplete extraction in the presence of the sediment matrix. For Methods 2 and 4, involving extraction with (2) methanol and dichloromethane and (4) methanol and acetic acid, no significant degradation is visible, but extraction in BCR 646 is incomplete for the chosen conditions. Method 5 showed a clear difference in the degree of TPhT degradation with and without a sediment matrix. In the aqueous standard both TPhT and DPhT degraded substantially to be recovered as MPhT and inorganic tin. With the matrix present, these reactions are largely suppressed, however the measured concentration of MPhT is biased high, as is also evident in Fig. 2. Results for BCR-646 show an otherwise reasonable agreement with certified values.

In a separate experiment the extraction time for Method 4 was extended from 20 to 700 min. This eliminated the signal for TPhT and gave rise to results for MPhT and DPhT that were biased high. Uncontrolled extraction times with this method will therefore lead to substantial degradation of species and give unpredictable results.

It should be observed that incomplete extraction would be taken into account by SSID calibration, provided incipient and added species are extracted to the same degree. Even the decomposition of TPhT will not give rise to errors for this species provided incipient and added species are decomposed to the same degree. However, the decomposition product, DPhT, will change the isotope ratio between added and incipient DPhT and hence the measured result, unless the spiked and incipient ratios for TPhT/DPhT happen to be the same. Fig. 2 gives an example of such an occurrence in that the results for DPhT in BCR 646 obtained with Method 1 are in agreement with the certified value, because of the incidentally close agreement between the isotopically enriched spike and the certification ratio of TPhT/DPhT amount contents.

Conclusions

The fact that phenyl tin species are relatively unstable calls for enriched isotope standards prepared for single species. The mixed standard should only be used after careful optimisation of the extraction conditions. The results demonstrate that the matrix has a complex role in stabilization of the species as well as their extraction efficiency.

Acknowledgements

The authors thank MCN: North Sweden Soil Remediation Center (Funding: EU Structural Funds and New Objective 1, contract no 113-12534-00), The Swedish Environmental Protection Agency (SNV contract 2190102), and the Knut and Alice Wallenberg Foundation for financial support. Sunil Jai Kumar thanks the Royal Society of Chemistry, Cambridge, UK, for financial support in the form of a Journals Grant for International Authors.

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