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WORKING METHODS PAPER:

Critical Considerations with Respect to the Identification of Tin Species in the Environment

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Biogeochemical pathways of tin species in the environment are still controversial, e.g. with regard to methylation and transmethylation phenomena, owing to the fact that the identification of methylated tin-compounds is often difficult. The previous tentative identification of a mixed methylbutyltin compound in sediment and biological samples by GC/AAS after hydride generation gave an illustration of this problem. This compound was previously identified in sediments by other authors and also suspected to occur in a contaminated sediment sample from the Boyardville Marina, France. The retention time obtained by GC/AAS corresponded to the actual retention time of a mixed methylbutyltin calibrant. However, additional checks demonstrated that the compound detected was actually monophenyltin. This evidence was produced by a thorough analysis of a selected sediment sample by alternative techniques such as GC/AAS and GC/AES after pentylation, GC/FPD and GC/MS. The results presented highlight the need for a full identification of compounds to avoid mis-interpretation.

Keywords: Organotin, identification, mixed methylbutyl forms, monophenyltin, cross-comparison, mis-interpretation

Glossary: GC = gas chromatography, AAS = atomic absorption spectroscopy, AES = atomic emission spectroscopy, FPD = flame photometric detection, MS = mass spectroscopy, HG = hydride generation, QF = quartz furnace, MBT, DBT, TBT = mono-, di- and tributyltin respectively. TRIT = total recoverable inorganic tin, MPhT, DPhT, TPhT = mono-, di- and triphenyltin respectively, CyH = cyclohexyl group

INTRODUCTION

The determination of organometallic species is now recognized to be a necessary approach for the understanding of biogeochemical cycles of trace metals. This is particularly true in the case of tin which is used in a variety of biocides (e.g. TBT-based antifouling paints) and which usage has greatly increased in the last decade.[1] Superimposed upon inputs of anthropogenic compounds, natural alkylation/dealkylation processes (e.g. methylation, volatilisation) can occur which may take place mediated by the high biochemical activity developed, e.g. in estuaries. Methylation of tin has been extensively discussed over the past ten years and has been the subject of some controversy. It is now generally accepted that such phenomena may occur in various environmental compartments (water, sediments, biological tissues) due to biological (e.g. microbial) or physicochemical reactions (e.g. in the presence of methyl iodide) reactions.[2,3]

However, before developing theories on biogeochemical pathways for organotins in the environment, it is necessary to ensure that the analytical methods are well under control and that the identification of the compounds is

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demonstrated. If this identification is not clearly verified then the interpretation can be misleading and controversy is likely. The results presented in this paper are intended to illustrate this. A series of analytical experiments has been carried out in order to attempt the identification of an unknown compound detected almost systematically in biological (mussel and oyster) and sediment samples by GC/AAS after hydride generation in a tin species monitoring campaign of Dutch coastal environments[4] and in contaminated sediments from the Boyardville marina, SW France.[5,6] Based on previous findings,[7–9] it was suspected that this compound could correspond to a mixed methylbutyl form of tin which would in turn mean that a new biogeochemical pathway, e.g. the methylation of a TBT-degradation product, could be suspected to occur in the environment.

$MeBu_3Sn$ and Me_2Bu_2Sn had been previously detected in sediments[7,8] by GC/AAS and GC/FPD, respectively, and $MeBuSn^{2+}$ was tentatively identified in oyster samples[9] by GC/AAS after hydride generation. A compound with a similar retention time to a Me_2BuSn^+ calibrant was also detected using the same technique, suggesting the presence of mixed methylbutyltin forms in this medium. In order to firmly identify this organotin compound, several techniques based on different extraction, derivatization and detection methods were compared. This paper presents the results obtained on a selected sediment sample using techniques such as GC/AAS after pentylation, GC/FPD and GC/MS.

EXPERIMENTAL

Sample collection

A sample containing large amounts of organotins was used in this study; this was collected in the Boyardville Marina (Oléron Island, France) at low tide, wet-sieved at 60 μm with water from the area of collection, air-dried and homogenised prior to analysis. This sample was later dried at 40–50 °C and ground with an agate mortar and pestle before shipment to other laboratories. This procedure was shown to be adequate to preserve the stability of butyltins in sediment, at least over a period of four months.[10] After re-homogenisation, sub-samples were distributed to different laboratories for the formal identification of the organotins.

Methods

GC/QFAAS after hydride generation

This method was used by two different laboratories using similar techniques (University of Bordeaux and Rijkswaterstaat). Organotin extraction was performed using analytical grade acetic acid and the samples were analysed by hydride generation/cryogenic trapping/GC separation, with detection in a quartz cell in an atomic absorption spectrometer (HG/GC/QFAAS). In this method, inorganic tin and alkyltin compounds react with a 5% sodium borohydride solution under acidic conditions to yield alkyltin hydrides. These hydrides are cryogenically trapped (in liquid nitrogen) and separated on a chromatographic column packed with Chromosorb GNAW 60/80 mesh, coated with 3% SP-2100. Hydride species are sequentially desorbed after heating of the column (−196 to 200 °C), in relation to their specific evaporation points. Detection is performed by AAS. Dimethylbutyltinbromide ($Me_2BuSnBr$) was used as internal standard; this compound was later used for the GC/MS verification. This method is described in detail elsewhere.[11,12]

GC/flame photometric detection after ethylation

This method was used by the Environmental Chemistry Department of the CSIC (Barcelona). Organotins were extracted with pesticide grade methanol by sonication. The extracts were concentrated to dryness and the residue were derivatized with EtMgCl in tetrahydrofuran (THF) (Aldrich). The derivatized organotins were recovered by liquid-liquid extraction from the aqueous solution with diethyl ether and organotin determination was performed by capillary GC (Carlo Erba) coupled to a tin selective flame photometric detector (FPD). This method has been described in detail elsewhere.[13]

GC/QFAAS and GC/AES after pentylation

Organotin determinations using this method were performed at the University of Antwerp. The sample was extracted with a mixture of doubly deionized water, acetic acid, and diethyldithiocarbamic acid solution in pentane/hexane also using sonication. After phase separation, the hexane was collected and back-extracted with a fresh hexane portion. The extract was then centrifuged and dried over sodium sulphate which was rinsed several times with hexane and the total hexane was evaporated to dryness by rotary evaporation at 40 °C. Finally, the sample was re-dissolved in n-octane containing Pr_3SnPe as internal standard after which pentylation with PeMgBr was carried out as described earlier.[14]

The mixture of pentylated tri-, di- and monobutyltin compounds together with Pr_3SnPe as internal standard were measured using two different hyphenated techniques, namely GC/QFAAS and GC/AES optimized operating parameters have been described elsewhere.[15] Recovery experiments were performed by spiking sediment samples with an aqueous solution of Bu_3SnCl, Bu_2SnCl_2 and $BuSnCl_3$ (ca. 4.5 $\mu g\, g^{-1}$ of each species).

GC/mass spectrometry

Organotin determinations by GC/MS were performed at the ENEA (Environmental chemistry Division, Rome). The sample was extracted with a methanol/tropolone solution (0.3 $g.l^{-1}$) with sonication. After solvent exchange, the extract was concentrated, derivatized with PeMgBr, purified by silica gel clean-up and injected for GC/MS determination. Tripropyltin was used as internal standard. The same calibrant of $Me_2BuSnBr$ as used in the HG/GC/AAS experiment was analysed by GC/MS.

RESULTS

HG/GC/QFAAS results

Within the framework of two butyltin monitoring campaigns carried out in The Netherlands in 1988/89, analyses of sediment and biological samples (mussel and oyster tissues) performed by HG/GC/QFAAS at the University of Bordeaux suggested the presence of an unknown compound eluting between MBT and DBT [Fig. 1(a)].[4] This unknown compound was also later observed in a contaminated sediment sample from the Boyardville Marina [Fig. 1(b)], which was taken as a reference sample for further investigations. The butyltin contents determined in the fresh sediment were the following (on a dry matter basis): 3.4 ± 0.3 $\mu g\, g^{-1}$ of MBT (as Sn), 4.5 ± 0.5 $\mu g\, g^{-1}$ of DBT (as Sn) and 8.2 ± 0.9 $\mu g\, g^{-1}$ of TBT (as Sn). At this point, it was not possible to formally identify the unknown compound; the only indication was the observation of a similar peak also eluting between MBT and DBT by Rapsomanikis and Harrison[9] using a technique involving hydride generation and AAS—this was assumed to be $MeBuSn^{2+}$. This indication encouraged us to perform analyses of mixed butylmethyl

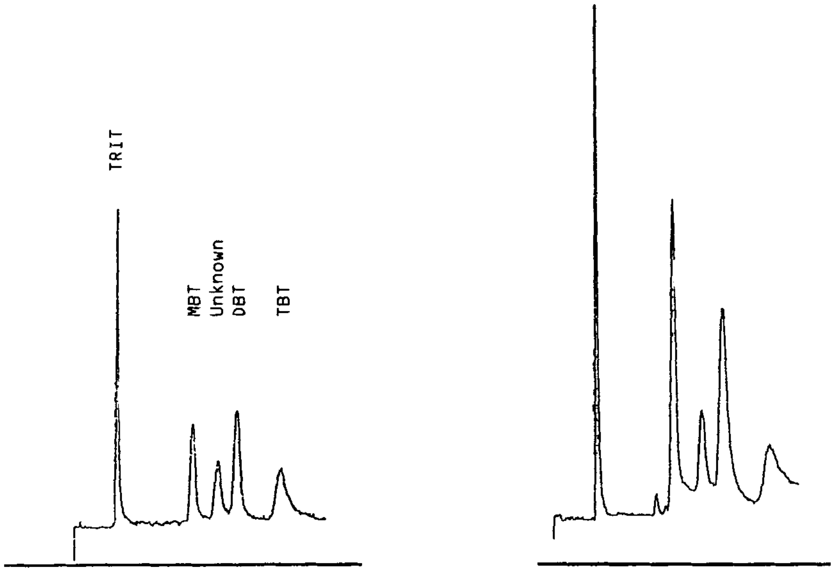

Figure 1 (a) HG/GC/QFAAS chromatogram of mussel tissue. The sample was collected in the Eastern Scheldt, The Netherlands. The chromatogram shows a succession of five peaks which are, from left to right: acetic acid extractable inorganic tin, MBT, unknown compound, DBT and TBT. TRIT = 0.56, MBT = 1.58, unknown = 1.70, DBT = 2.09 AND TBT = 2.65 min. (b) HG/GC/QFAAS chromatogram of a contaminated sediment analysed under the same conditions. The sample was collected in the Boyardville Marina, France. The distribution of organotin compounds observed is similar to that of the mussel sample, Fig. 1(a). Retention times are 0.58, 1.59, 1.95, 2.22 and 2.79 min.

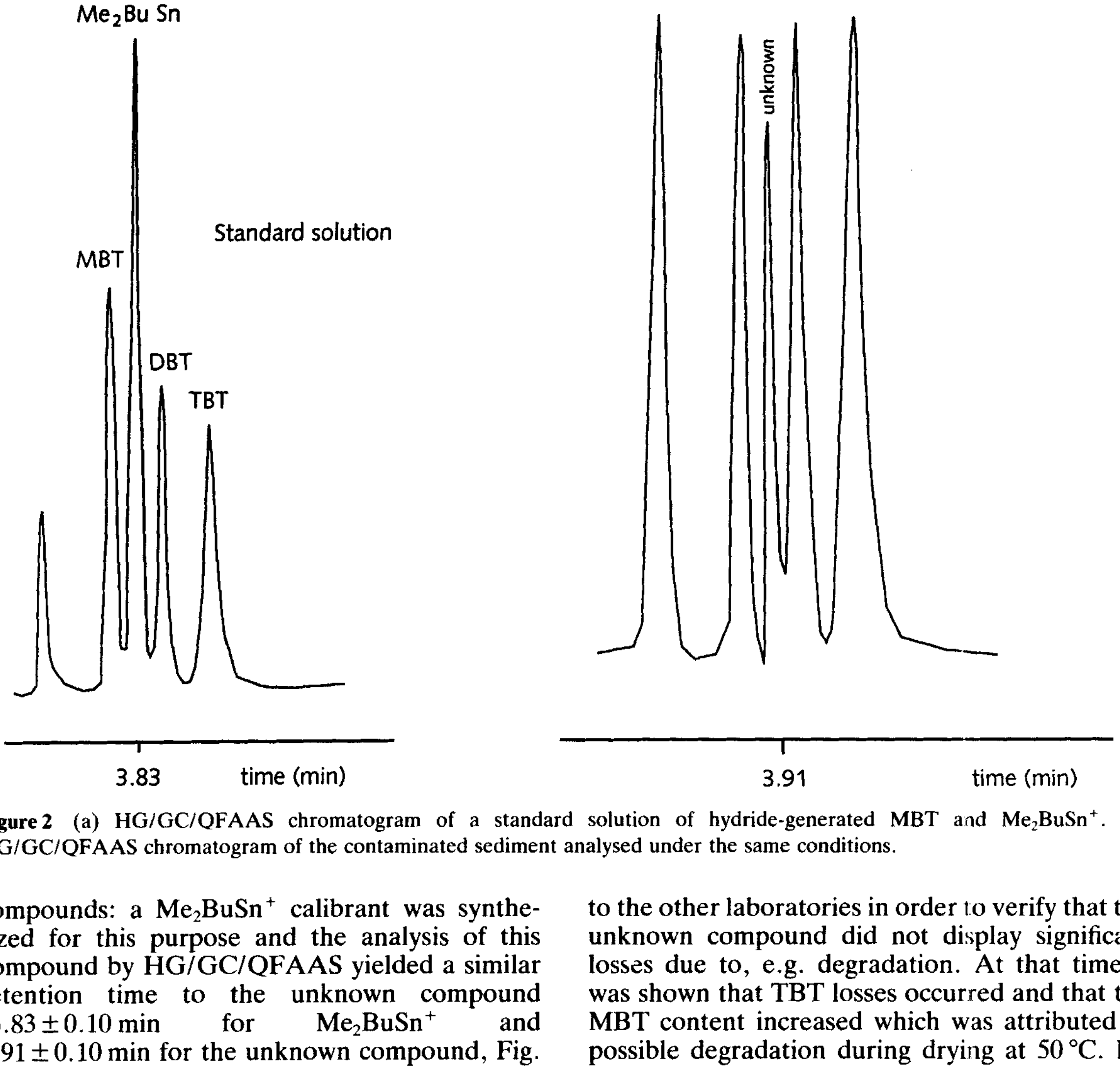

Figure 2 (a) HG/GC/QFAAS chromatogram of a standard solution of hydride-generated MBT and Me_2BuSn^+. (b) HG/GC/QFAAS chromatogram of the contaminated sediment analysed under the same conditions.

compounds: a Me_2BuSn^+ calibrant was synthesized for this purpose and the analysis of this compound by HG/GC/QFAAS yielded a similar retention time to the unknown compound (3.83 ± 0.10 min for Me_2BuSn^+ and 3.91 ± 0.10 min for the unknown compound, Fig. 2). These analyses were performed at the Rijkswaterstaat under analytical conditions slightly different from those used by the University of Bordeaux, which explains the different retention times (the unknown eluted at 1.90 ± 0.10 min in the latter case).

At a later stage, further verification was attempted using other tin compounds; the last HG/GC/QFAAS experiment concerned the analysis of a monophenyltin calibrant which was shown to elute at approximately the same retention time as the unknown and Me_2BuSn compounds (3.88 ± 0.10 min). At this stage it was therefore impossible to formally identify the unknown compound with this technique.

The analysis of the sediment sample by HG/GC/QFAAS was repeated prior to shipment to the other laboratories in order to verify that the unknown compound did not display significant losses due to, e.g. degradation. At that time it was shown that TBT losses occurred and that the MBT content increased which was attributed to possible degradation during drying at 50 °C. No quantification of the new butyltin pattern was carried out as the purpose was the identification of the unknown compound.

GC/FPD results

Chromatograms obtained by GC/FPD are shown in Figs 3(a) and 3(b). TBT, DBT and MBT were formally identified with this technique. However, the compound eluting at 17.25 min in Fig. 3 could not be identified. Furthermore, no mixed butylmethyl and phenyltin compounds could be identified; it was assumed at that stage that the technique was not optimised for the determination of phenyltin compounds which would explain their absence in the sample.

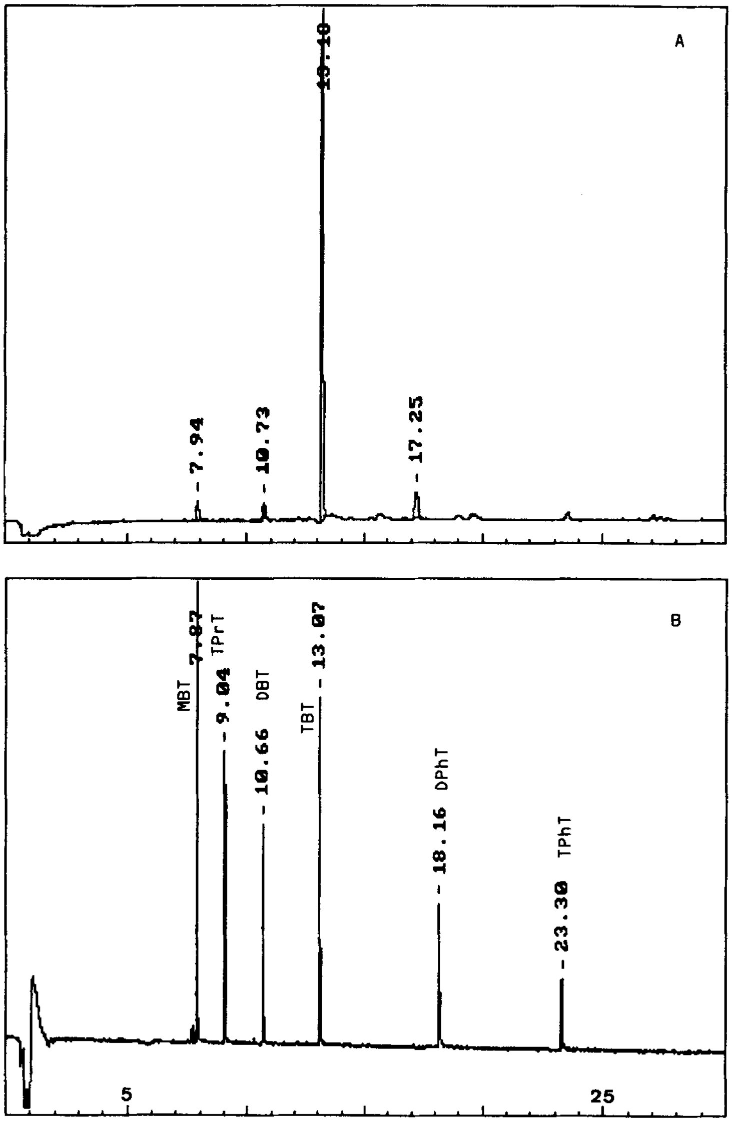

Figure 3 (a) GC/FPD chromatogram of the contaminated sediment sample. The three peaks on the left side correspond to ethylated MBT, DBT and TBT respectively. The compound eluting at 17.25 min could not be identified. (b) GC/FPD chromatogram of an ethylated mixture of MBT, tripropyltin, DBT, TBT, DPhT and TPhT.

GC/QFAAS after pentylation results

Butyltin compounds (MBT, DBT and TBT) were formally identified with this technique as well as mono-, di- and trimethyltin. In addition, traces of monophenyltin (MPhT) were also detected as shown in Fig. 4(a). The respective contents of TBT and DBT were 3.94 ± 0.16 μg g^{-1} (as Sn) and 2.92 ± 0.02 μg g^{-1} (as Sn). No attempts were made to quantify MBT because spiking experiments showed low recovery for this species. It was hardly possible to draw any conclusions as to the occurrence of mixed MeBuSn compounds in the sample. Further confirmation of the presence of MPhT was obtained by analysing a mixed calibrant solution obtained from the University of Amsterdam (J. Stäb, with courtesy) containing pentylated butyl-, phenyl- and cyclohexyltin compounds.

GC/AES after pentylation results

Injections on the GC/AES were performed both in split and splitless mode. Similar results were obtained as with the other techniques; no mixed

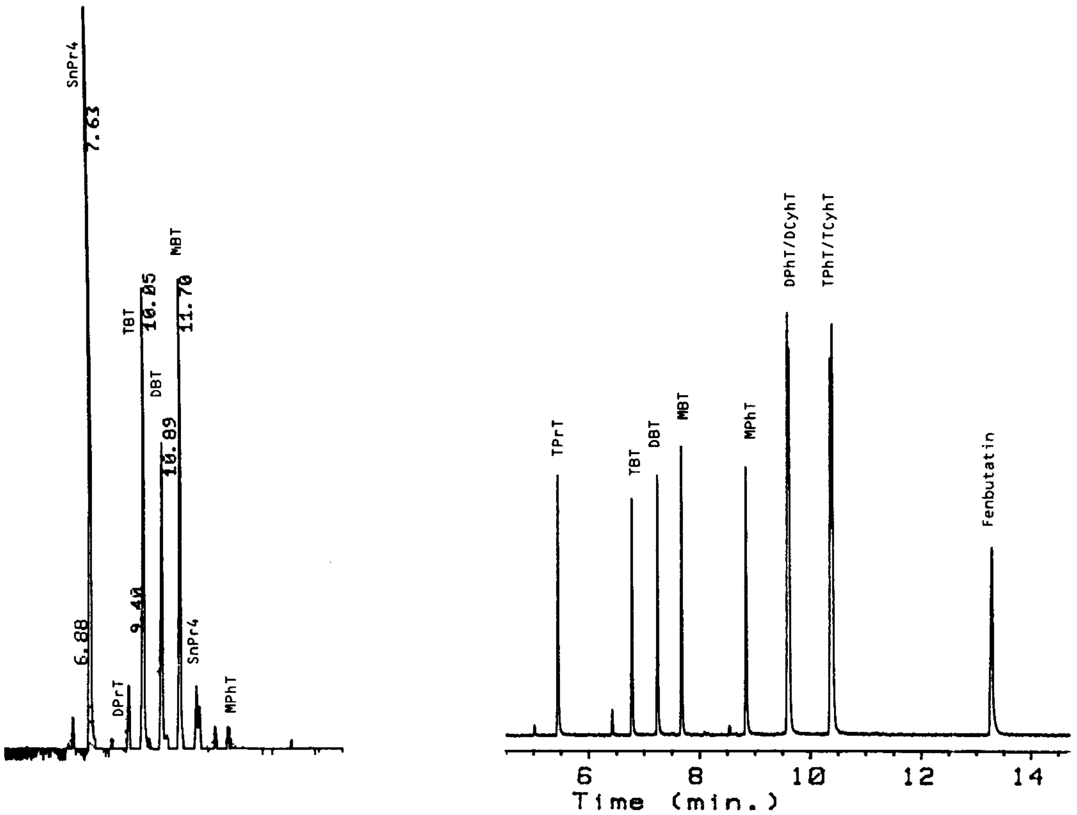

Figure 4 (a) GC/QFAAS chromatogram of the contaminated sediment sample after pentylation. The compounds are indicated in the figure. (b) GC/AES chromatogram of a calibrant mixture of organotin compounds after pentylation. The compounds quoted in the paper are indicated in the Figure.

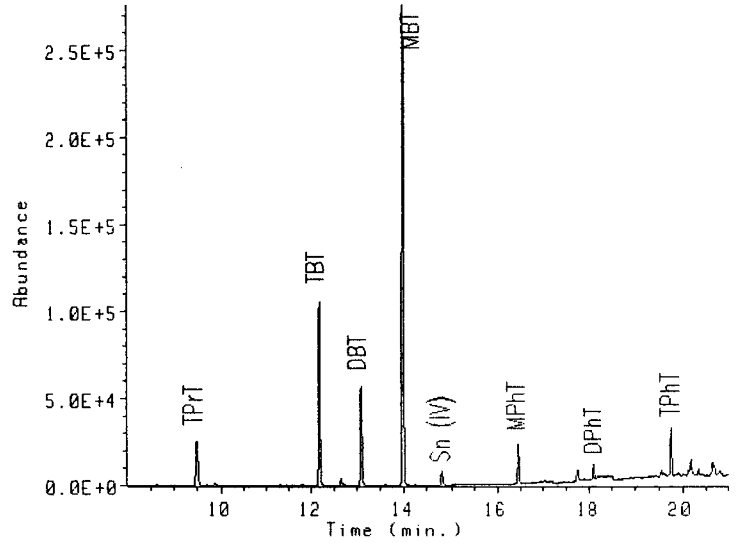

Figure 5 Ion chromatogram of the contaminated sediment sample analysed by GC/MS after pentylation.

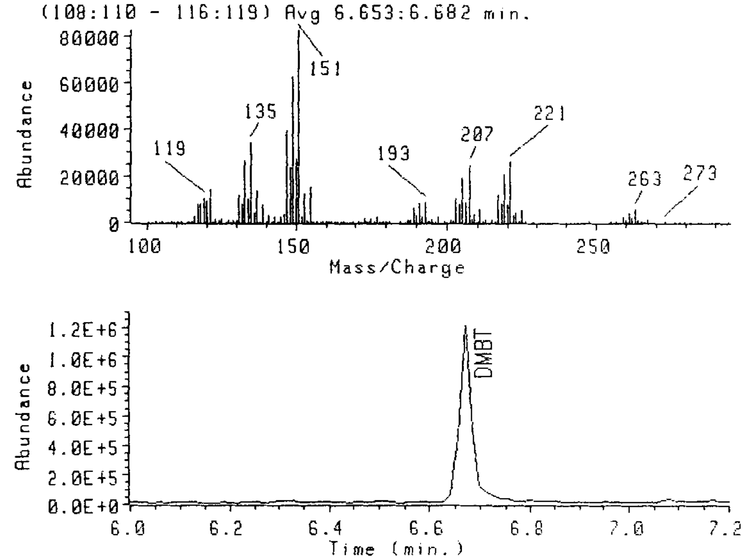

Figure 6 Total ion chromatogram (bottom) and mass spectrum (top) of the pentylated $BuMe_2Sn^+$ calibrant analysed by GC/MS.

methylbutyltin compounds could be detected. However, this technique allowed us to identify butyltins as well as phenyl- and cyclohexyltin compounds, Fig. 4(b). MPhT was clearly identified along with traces of DPhT, TPhT and tricyclohexyltin. No attempts were made to quantify these compounds.

GC/MS results

Although the purpose of the experiments was the identification and not the quantification of the compounds present in the sample, a tentative quantitation could be done by GC/MS by duplicating the sediment analysis. The butyltin contents were as follows: 3.9 ± 0.3 $\mu g\,g^{-1}$ (as Sn) for TBT, 1.7 ± 0.3 $\mu g\,g^{-1}$ (as Sn) for DBT and 9.4 ± 0.5 $\mu g\,g^{-1}$ (as Sn) for MBT. These results are in good agreement with those obtained for TBT by GC/QFAAS; the agreement was poorer in the case of DBT. Traces of other tin compounds were also detected, e.g. DPhT (ca. 0.2 $\mu g\,g^{-1}$ as Sn) and TPhT (ca. 0.2 $\mu g\,g^{-1}$ as Sn) and a high content of MPhT (1.5 ± 0.4 $\mu g\,g^{-1}$ as Sn) was observed (Fig. 5). Tests with the dimethylbutyltin calibrant showed that this compound is easily recovered and determined with the technique used (Fig. 6). However, as for the other techniques, no mixed methylbutyl forms could be identified in the sediment sample.

DISCUSSION

Formal identification of the unknown compound

The detection of a compound by HG/GC/QFAAS eluting between MBT and DBT, referred to as the unknown species in this paper, was first made by Rapsomanikis and Harrison with a similar technique[9] in a comparative study on extraction procedures for the determination of butyltins in different oyster samples. In the present study, two other laboratories have confirmed the occurrence of such a compound by using HG/GC/QFAAS. A third laboratory (also using HG/GC/QFAAS) reported the detection of a similar compound.[6] Although it could not be formally identified, nine different compounds on the basis of their similar molecular masses (and similar evaporation points and retention time) were proposed and the following compounds: Et_3SnH, Me_2BuSnH and Me_2Et_2Sn, were assumed to be the most likely composition of the compound of concern. The author[6] concluded that the mixed methylbutyl form would be the best approximation as this compound had previously been observed in the environment.[8] The results presented above have shown that Me_2BuSn^+ calibrant used as the internal standard eluted at the same retention time as the unknown compound, which was a further indication that this compound in fact be a mixed methylbutyl form as previously suspected. Hydride generation systems coupled to cryogenic trapping and AAS detection are highly sensitive and selective. However, the technique only provides relatively poor chromatographic resolution. Consequently, a large variety of alkyltin compounds with evaporation points ranging from 90–100 °C may coelute and would not be sufficiently separated to allow their identification.

The results obtained by GC/MS did not allow us to detect any mixed butylmethyl forms in the sediment sample; however this compound was easily determined in a Me_2BuSn^+ calibrant solution by this technique and hence should have been detected if present in the sediment sample.

Besides the butyltins, high contents of MPhT were found. It was therefore assumed that the unknown compound was MPhT and not Me_2BuSn. Further, the analysis of MPhT by HG/GC/QFAAS, as indicated in the results, confirmed that this compound eluted at the same retention time as both Me_2BuSn and the unknown. MPhT has also been detected by GC/QFAAS and GC/AES after pentylation, techniques which did not allow the detection of mixed butylmethyl forms.

Likelihood of the presence of MPhT in the samples

Additional evidence could be given to confirm the formal identification of MPhT as the unknown. First, the occurrence of mixed methylbutyl forms of tin has been assumed to be a minor phenomenon.[7,8] However, the compound investigated in this study was widely distributed in water, sediment and biological samples collected in Dutch coastal environments[4] including freshwater areas (this compound was present in more than 70% of the samples analysed); the presence of MPhT is not at all surprising as TPhT is widely used as a pesticide in these areas and it may be directly degraded to MPhT as described below. TPhT is also increasingly used as an antifouling agent (at the same percentage as TBT) and hence MPhT could originate from degradation of TPhT from this contamination source. A second confirmation is that methylation phenomena have never been shown to occur to a great extent in water. Indeed, methyltin species have very rarely been reported in water at ultratrace levels. High contents of methyltin species were reported in sewage waters[17] and at a low depth in water from the Venice Lagoon[18] but, although MBT and DBT were present, no methylation of butyl forms was observed. It is therefore not likely that this pathway could occur in natural water to a great extent. Hence, the systematic detection of MPhT in environmental matrices from Dutch coastal environments is a more logical explanation than the hypothetical occurrence of Me_2BuSn. A further argument is that no unidentified tin compounds were detected during several monitoring campaigns carried out along Italian coasts on water, sediment and mussel samples.[19,20] However, phenyltin compounds (including MPhT) were detected in nearly all the sediments and mussels and in a few water samples, and their occurrence in sediment was reported by other authors.[21] In the case of the sediment sample selected, the presence of MPhT could be explained both by the vicinity of agricultural areas in Oléron Island and the release of TPhT from antifouling paints in the marina.

Degradation pathways

A further argument to justify the presence of MPhT in the sediment sample used in this study was the observation of a facile degradation pathway for triphenyltin. Triphenyltin was shown to degrade directly to MPhT in samples dried at more than 50 °C which was the case for the sample selected (dried at 60 °C).

The direct degradation of trialkylforms to monoalkyl forms is not an unknown pattern, e.g. TBT has been shown to degrade directly to MBT both in water[10] and sediment,[22] which is confirmed by the actual change of butyltin contents observed from 1989 (HG/GC/AAS results obtained by analysing the fresh sediment) to 1991 (GC/MS and GC/QFAAS after pentylation results obtained with the analysis of the dried sediment) in the sediment used in this study; indeed a decrease in the TBT content was observed, compensated by a corresponding increase in MBT content.

CONCLUSIONS

This paper presents the results of investigations carried out to formally identify a compound occurring almost systematically in samples collected in Dutch coastal environments. Based on previous studies, this compound was assumed to be a mixed methylbutyltin form which in turn would have meant that an important transformation pathway (degradation/methylation) would systematically occur in the matrices considered. However, through analyses of a selected sediment sample have shown that this compound was actually a monophenyltin form, probably issued from the degradation of triphenyltin used in agriculture. This example clearly demonstrates the problem of the likelihood of misinterpretation of possible environmental pathways in cases in which the identification of a compound is not formally verified and the preparation of samples (e.g. degradation of compounds upon drying) is not sufficiently investigated.

It gives also a clear warning to environmental chemists that the adoption and application of good quality control principles are vital in order to ensure the reliability of analytical data prior to the establishment of new theories, particularly in the field of speciation analyses.

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