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Analysis of butyltin compounds by gas chromatography–mass spectrometry: an application to the Antarctic bivalve *Adamussium colbecki*

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A gas chromatography–mass spectrometry method was developed for the determination of butyltin compounds in biota samples. Tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) were extracted in methanol-containing tropolone (0.05% w/v) and subjected to Grignard pentylation. A solid-phase extraction procedure on florisil was optimized in order to purify the extracts. Quantitative determinations were carried out in single ion monitoring mode (TBT *m/z* 305, DBT and MBT *m/z* 319) using tripropyltin as internal standard (*m/z* 277). The accuracy of the whole methodology was verified on a certified reference material (CRM 477 from BCR), obtaining a recovery of about 95% for TBT and DBT and 116% for MBT.

Detection limits (organotin cation per tissue dry weight) were 6.4 ng g^{-1} for TBT, 6.2 ng g^{-1} for DBT and 4.5 ng g^{-1} for MBT.

Butyltin compounds were determined in the marine bivalve *Adamussium colbecki*, collected near the Italian Antarctic Base of Terra Nova Bay, during the XIII Italian Antarctic Campaign. The presence of the analytes, although at low levels, was verified in the whole tissue, gills and digestive glands; gills showed the highest concentrations, ranging from 31 to 133 ng g^{-1}. The occurrence of butyltin compounds in the southern polar region studied suggests their ubiquitous distribution. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: butyltin compounds; marine bivalve; Antarctica

INTRODUCTION

Organotin compounds are widely used in many industrial, chemical and agricultural applications. Their environmental relevance and toxicity towards aquatic organisms are well documented.[1] In particular, tributyltin (TBT) is used in a large number of commercial applications, including biocide additives in antifouling ship-paint formulations. The International Maritime Organization has recently established[2] that, since 1 January 2003, TBT must no longer be used in antifouling paint formulations. In spite of this restriction, in several countries the levels in the marine environment are still high.

TBT released from the paints has been found to affect many non-target organisms, particularly molluscs and gastropods.[3,4] A few nanograms per litre in the water can produce chronic toxic effects on oysters (shell deformation), mussels (growth inhibition of larvae and veliger stages) and marine gastropods (sterilization of females).

In a marine environment, dibutyltin (DBT) and monobutyltin (MBT), which are less toxic than TBT to aquatic organisms, are also present as a result of TBT degradation processes, together with triphenyltin (TPhT), employed as a co-toxicant with TBT in some long-performance antifouling paints.[5,6] Several studies on TBT, TPhT and their degradation products are present in the literature, with butyltin compounds being the most frequently investigated.[7–9]

The fate of organotin compounds in the water column is a result of different processes: input rate, mixing and dilution, biodegradation, photodegradation, and sorption

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onto particulate suspended matter.[10] In order to provide a time-integrated estimation of the concentrations of these compounds, bioindicator organisms have been used in monitoring programmes performed all over the world.[11–13]

The presence of butyltin compounds was investigated in blue mussels in the Arctic and semi-Arctic environment in Greenland, Norway, Iceland and the Faeroe Islands.[14–16] The concentration levels in Iceland ranged from 1 to 65 μg organotin per kilogram wet weight, and in Greenland they ranged from 0.5 to 1 μg tin per kilogram wet weight, showing the occurrence of these compounds in the northern polar environment. However, no data were found in the literature for the southern polar region. Nevertheless, the presence of several persisting organic pollutants (POPs), such as polychlorinated biphenyls, has been assessed in the Antarctic environment, showing the ubiquitous distribution of xenobiotics.[17]

Recently, *Adamussium colbecki*, the most abundant bivalve of the Antarctic coastal marine ecosystem, has been proposed as a biomonitor for heavy metals such as lead, employing as check organs the digestive gland, responsible for accumulating and detoxifying mechanisms, and gills, which reflect levels of contamination in sea water.[18]

Different methods have been proposed and reviewed[19–21] for the analysis of organotin compounds in environmental matrices; these involve various analytical steps, such as extraction, clean up, derivatization if required, separation, and final detection. Most of the available methods for environmental samples are based on different separation techniques, e.g. gas chromatography (GC) or high-performance liquid chromatography (HPLC), coupled with highly sensitive detection methods like atomic absorption spectrometry, mass spectrometry (MS), flame photometry, atomic emission spectrometry (AES) and inductively coupled plasma (ICP) MS.

In our laboratories, an HPLC–hydride generation-ICP-AES analytical procedure was developed[22] and used[23] for the certification of CRM 477 (a lyophilized marine mussel tissue produced by BCR of the European Community). The method was suitable for the identification and the determination of organotin species in real samples, such as tissues of marine organisms and sediments, combining the separation capability of HPLC with the specificity of ICP-AES detection.[24–26] Evidence of the presence of butyltin compounds in Antarctic samples had already been highlighted using this technique,[27] although the sensitivity was not good enough for their quantitation.

A GC–MS method was then developed and applied to the determination of TBT and its degradation products in the Antarctic bivalve *A. colbecki*.

EXPERIMENTAL

Instrumentation

A Hewlett Packard 5890 series II gas chromatograph was coupled to an HP mod. 5989A quadrupole mass spectrometer. The gas chromatograph was equipped with a Phenomenex ZB5 column (30 m × 0.25 mm ID × 0.25 μm) coated with 5% phenylpolysiloxane. Samples were injected by autosampler (HP 6890 series) using an on-column injector equipped with electronic pressure control, which enables a constant flow rate of 1.4 ml min^{-1} to be maintained during the entire separation. Helium was used as carrier gas. The oven temperature was programmed as follows: 90 °C for 1 min, from 90 to 140 °C at 30 °C min^{-1}, from 140 to 250 °C at 12 °C min^{-1} and then held at 250 °C for 1 min, from 250 to 290 °C at 30 °C min^{-1} and then held at 290 °C for 10 min. The injector temperature program was the same as that for the oven.

The capillary column was connected directly to the ion source of the mass spectrometer by means of a transfer line maintained at 280 °C. The electron ionization (EI) ion source conditions were: electron energy 70 eV, temperature 250 °C. The quadrupole temperature was set at 100 °C. Each instrumental analysis was performed in triplicate.

Reagents

Monobutyltin trichloride (95%), dibutyltin dichloride (96%), tributyltin chloride (96%), tropolone (2-hydroxy-2,4,6-cycloheptatrienone, 98%) and *n*-pentylmagnesium bromide were obtained from Aldrich (Milan, Italy).

Tripropyltin (TPrT) chloride, *n*-hexane, isooctane and dichloromethane were purchased from Merck (Darmstadt, Germany), and methanol and acetone from Riedel-de Haen (Seelze, Germany). All organic solvents were of analytical or chromatographic grade.

All standard solutions were prepared in isooctane unless stated otherwise.

Certified reference material CRM 477 was provided by the BCR of the Commission of the European Community.

Pentylated compounds (purity >98%) used in the preliminary part of this work were provided by BCR in the framework of the certification of CRM 477. Afterwards, pentylated organotin compounds were prepared in our laboratory, following the same procedure described below for the biota samples.

The water was obtained by a Milli-Q system (Millipore, Watford, Hertfordshire, UK). LiChrolut SPE columns (6 ml) containing 1000 mg of florisil (150–250 μm) were purchased from Merck.

All glassware was soaked overnight in 4 M HNO_3 to remove sorbed organotin compounds, rinsed with Milli-Q water and then with acetone. A blank was prepared for each set of samples and the relative measured analyte signals were subtracted from the sample values.

Sample collection and treatment

A. colbecki samples of 7–8 cm in length were collected during the austral summer 1997–98 Italian Antarctic Campaign, in the framework of 'Contaminazione Ambientale' project activities. Bivalves were sampled by scuba near the Terra Nova Bay Italian Base (Ross Sea) at about 20 m depth. A map of the sampling site is shown in Fig. 1.

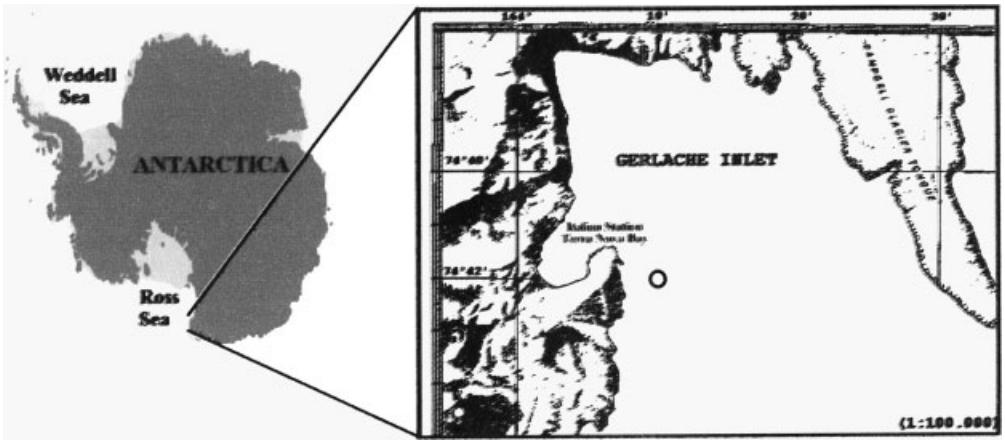

Figure 1. Map of the sampling site.

Samples were kept frozen at −150 °C at Italian Antarctic Environmental Specimen Bank (BCAA) until analysis.

The whole soft tissues of 10 individuals and the digestive glands and the gills of 10 animals were dissected, homogenized and lyophilized. The freeze-dried whole tissues represented 14% in weight of the whole wet tissues, and the lyophilized gills and digestive glands were respectively 7% and 20% of the wet organs.

Organotin compounds extraction and derivatization

The extraction procedure of organotin compounds from the tissues is based on the method employed by Caricchia *et al*.[28] and modified in our laboratories.[27]

A subsample of freeze-dried homogenized tissue (0.4 g), after the addition of TPrT chloride as internal standard, was subjected to extraction in methanol-containing tropolone (0.05% w/v). The organotin compounds were extracted with 30 ml dichloromethane by shaking vigorously for 5 min in a separating funnel. The dichloromethane phase, containing the organotins, was collected and evaporated to dryness by a vacuum rotary evaporator. The samples were redissolved in 3 ml of dichloromethane. Derivatization was carried out by the addition of 1.6 ml of *n*-$C_5H_{11}MgBr$ as Grignard reagent. The excess of Grignard reagent was destroyed by adding 2 ml of 2 M hydrochloric acid. The organic phase was collected and re-extracted by a liquid–liquid procedure. The dichloromethane phase, containing the organotins, was collected and evaporated to dryness by a vacuum rotary evaporator. The sample was redissolved in 1 ml of *n*-hexane. A clean-up step was performed by solid-phase extraction (SPE), using florisil as adsorbent. After a preliminary washing with dichloromethane, the column was conditioned with *n*-hexane (3 ml) and the elution was performed with a 1 : 1 *n*-hexane : dichloromethane mixture (2 ml). The eluted sample was evaporated under a gentle stream of nitrogen and redissolved in 1 ml isooctane. The analysis was carried out injecting 0.5 µl of this solution in the GC–MS system.

The accuracy of the method was tested by using CRM 477, a freeze-dried and homogenized mussel tissue, produced by BCR of the Commission of the European Community.

RESULTS AND DISCUSSION

GC–MS quantitative analysis

Standard mixtures of pentylated analytes were prepared with the calibrants provided by BCR in the framework of the certification[23] of CRM 477 and were used to obtain the optimum chromatographic conditions described in the Instrumentation section. The following retention times were observed: 9.34 min for TBT, 10.05 min for DBT and 10.74 min for MBT.

Since pentylated standards of a suitable purity are not easily available, in the last part of this study the pentylated analytes were prepared in our laboratories, according to the procedure described in the Experimental section. The completeness of the pentylation was verified by comparing the chromatographic areas of our derivatives with those obtained with the BCR calibrants, and the reaction yields were in the range 97–104%.

EI spectra of the three pentylated compounds were collected injecting 0.5 µl of a standard solution containing 50 µg ml^{-1} of each analyte; results are reported in Fig. 2.

Typical fragmentation patterns of organotin compounds, due to the isotopic distribution of tin, can be recognized. Although spectra of methylated and ethylated compounds are more frequently interpreted in the literature,[29,30] the fragmentation pattern of pentylated butyltins is quite similar. Major fragment ions of pentylated organotin compounds are reported in Table 1.

In order to choose the most suitable fragment for the quantitative analysis in single ion monitoring (SIM) mode, different ions were considered. Three calibration curves were drawn for each pentylated compound using m/z 305, 249, 179 for TBT and m/z 319, 249, 193 for DBT and MBT. All curves

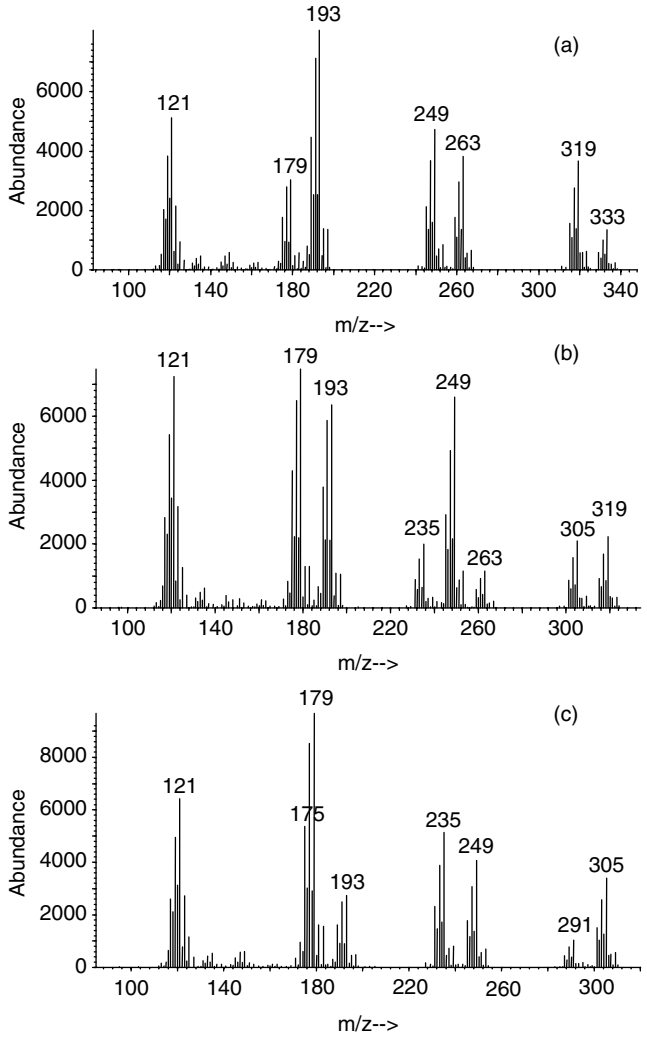

Figure 2. EI mass spectra of (a) $BuSnPe_3$, (b) Bu_2SnPe_2 and (c) Bu_3SnPe.

in the last part of this study the pentylated analytes were prepared in our laboratories as predicted by the spectra, the highest slope (i.e. sensitivity) was obtained using ions 179 and 193.

Preliminary experiments on biota samples were performed by monitoring each one of the above-mentioned fragments together with a qualifier ion (i.e. 177–179, 191–193, 247–249, 303–305 and 317–319).

The results highlighted that ions 177–179, 191–193 and 247–249 suffered from strong matrix interferences. In fact, their relative abundances showed altered isotopic patterns, e.g. the signal of 179 was lower than 177. Furthermore, the fragment 193, $[PeSnH_2]^+$, does not provide the necessary specificity for the analysis because it does not contain the butyl group. Therefore, m/z 305 for TBT and 319 for DBT and MBT were chosen for successive determinations.

As the sample preparation procedure consists of various steps, which can cause analyte loss, the use of an internal

Table 1. Major fragment ions of pentylated organotin compounds

Analyte	Fragment ions[a]	Ion formula[b]
Bu_3SnPe	305	$[Bu_2SnPe]^+$
	249	$[BuSnPeH]^+$
	179	$[BuSnH_2]^+$
	121	$[Sn(II)H]^+$
Bu_2SnPe_2	319	$[BuSnPe_2]^+$
	249	$[BuSnPeH]^+$
	193	$[PeSnH_2]^+$
	121	$[Sn(II)H]^+$
$BuSnPe_3$	319	$[BuSnPe_2]^+$
	249	$[BuSnPeH]^+$
	193	$[PeSnH_2]^+$
	121	$[Sn(II)H]^+$

[a] Fragment ion masses are reported for the ^{120}Sn isotope.
[b] All ions contain tin(IV) unless stated otherwise.

standard is thus necessary. TPrT is the most frequently used compound,[28,31–33] since it is chemically similar to butyltins and is not present in the marine environment. In the experimental conditions described, the retention time of pentylated TPrT was 7.37 min and, therefore, this compound was also suitable from the chromatographic point of view. Figure 3 is an SIM-mode chromatogram of a mixture of the pentylated analytes and internal standard.

Regression curves were drawn by plotting the ratio between the area of each analyte and the area of the internal standard against the ratio between their concentrations; the internal standard concentration was maintained constant at 500 ng ml^{-1}, while the analyte concentrations were 50, 100, 500 and 1000 ng ml^{-1}. Each point of these curves was the mean of three replicates (residual standard deviation ranged from 2 to 6%).

Table 2 shows the fragments used for the acquisition in SIM mode. For each compound, two ions were monitored

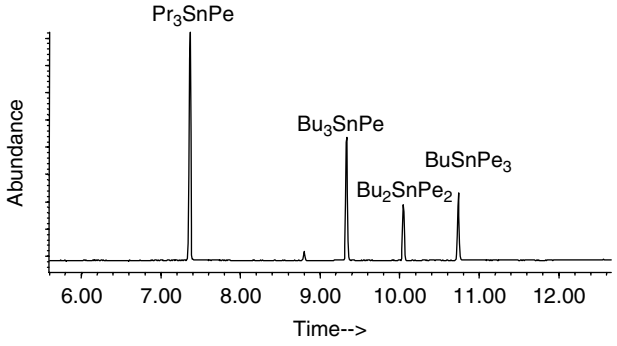

Figure 3. SIM chromatogram of a mixture of pentylated butyltin compounds (0.5 ug ml) containing the pentylated internal standard (1 ug ml^{-1}). Time expressed in minutes.

Table 2. Mass spectrometer programme for SIM-mode analysis

Pentylated organotin compound	Start time (min)	Monitored ions (m/z)
Pr_3SnPe	4.00	275–277
Bu_3SnPe	8.30	303–305
Bu_2SnPe_2	9.85	317–319
$BuSnPe_3$	9.85	317–319

to provide good specificity, using the most abundant for the quantitation. The equations obtained for the three regression curves are $y = 1.0779x - 0.0568$ ($R^2 = 0.9995$) for TBT, $y = 0.4876x - 0.0324$ ($R^2 = 0.9982$) for DBT, and $y = 0.5732x - 0.0394$ ($R^2 = 0.9990$) for MBT.

SPE

The original method[28] was improved by performing the clean-up of the extract by SPE instead of self-made columns and using a less toxic solvent mixture as eluent. Florisil is the most suitable adsorbent for biotic matrices with a high lipidic content, as reported by many workers[34,35] and had been previously used in our laboratories for the determination of butyltins by liquid chromatography–MS with a particle beam interface,[36] although in that study the analytes were not in the pentylated form.

In order to optimize the SPE procedure for the clean-up of the sample extract, elution curves on the florisil column were examined. A 500 µl aliquot of a mixture of the three pentylated analytes (1 µg ml^{-1} each in *n*-hexane) was loaded onto a 1000 mg florisil column. The elution was performed with a 1 : 1 *n*-hexane : dichloromethane mixture and the eluate was fractionated every 500 µl. The assay was repeated twice. All the analytes showed practically the same behaviour: about 80% of each compound was recovered after the collection of the first two aliquots, whereas no analytical signal was observed after the elution of 1.5–2 ml of solvent mixture. Therefore, 2 ml of eluate were collected in the final procedure. Mean recoveries were in the range 95–102%.

Detection limits and validation with certified reference material

Rajendran *et al*.[37] recently compared some of the analytical techniques reported in the literature for the determination of organotin compounds in sediments. They found a wide range of detection limits: from 0.019 to 10 ng tin per gram dry weight, depending on both the detection technique used and the method considered. Usually, methods using GC–MS have slightly higher detection limits (from 0.8 ng tin per gram dry weight[38] to 48 ng tin per gram wet weight[39]) than procedures using other detectors, such as flame photometric detection or ICP-MS,[37] although GC–MS provides a better specificity, together with structural information.

The detection limits of the present work, defined as the concentration which would give three times the standard deviation of the blank of the whole procedure, were 2.6 ng tin per gram dry weight (corresponding to 6.4 ng cation per gram dry weight) for TBT, 3.2 ng tin per gram dry weight (6.2 ng cation per gram dry weight) for DBT, and 3.0 ng tin per gram dry weight (4.5 ng cation per gram dry weight) for MBT, when 0.4 g of dry sample was used and the volume of the final extract was 1 ml. These values are very close to the lowest detection limits reported above for GC–MS.

The whole methodology was verified using the certified marine mussel CRM 477; the lyophilized tissue, after the addition of TPrT chloride, was submitted to extraction, derivatization and purification as described in the Experimental section. Finally, 0.5 µl of the resulting solution were injected for GC–MS analysis and the analytes quantified using the calibration curves discussed above.

The results, obtained as a mean value from three replicates of the whole procedure, are reported in Table 3. Data are in good agreement with the certified values, considering the uncertainty provided with the CRM 477.

The reproducibility of the analytical procedure is satisfactory. In fact, considering the relative standard deviation of the three analytes, DBT shows the highest value (13%), and both MBT and TBT are close to 7%.

Application to Antarctic bivalves

The organotin concentrations measured in the *A. colbecki* tissues analysed are shown in Table 4. The data represent the mean plus/minus the standard deviation of three replicates of the whole procedure. The precision of the data obtained is

Table 3. Comparison between CRM 477 certified values and the mean of three replicates with the standard deviation of the whole procedure

	Certified value (µg g^{-1})[a]	Value obtained (µg g^{-1})[a]
TBT	2.20 ± 0.19	2.06 ± 0.15
DBT	1.54 ± 0.12	1.47 ± 0.19
MBT	1.50 ± 0.27	1.74 ± 0.13

[a] Expressed as micrograms of organotin cation per gram dry weight.

Table 4. Concentration of organotin compounds in tissues of *A. colbecki* from Terra Nova Bay (Antarctica). Values represent the mean of three replicates with the standard deviation of the whole procedure

	Whole tissue (µg g^{-1})	Gills[a] (µg g^{-1})	Digestive glands[a] (µg g^{-1})
TBT	0.058 ± 0.034	0.133 ± 0.003	0.010 ± 0.004
DBT	0.035 ± 0.017	0.097 ± 0.002	0.022 ± 0.002
MBT	<d.l.	0.031 ± 0.007	<d.l.

[a] d.l. = detection limit. Values expressed as micrograms of organotin cation per gram of dry tissue considered.

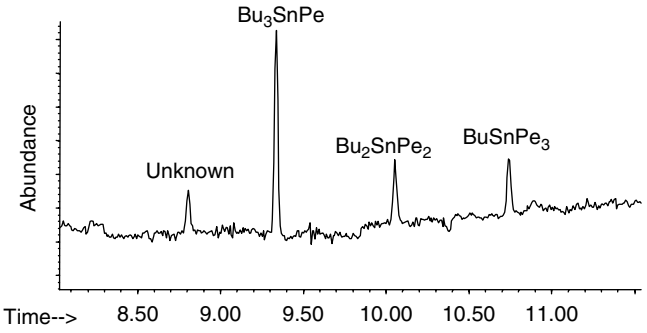

Figure 4. SIM-mode chromatogram of branchial tissue of *Adamussium colbecki*. Time expressed in minutes.

different for the whole tissue and the organ tissues. In fact, the standard deviation is satisfactory for the organ samples, with the exception of the TBT in digestive glands, but the measured value (0.010 μg g^{-1}) is very close to the detection limit. On the contrary, the standard deviation is rather high for the whole tissue. This is probably due to the different nature of the various tissues, as, despite a careful preparation of the sample, the whole tissue maintains a lower homogeneity compared with the organs. Figure 4 shows an SIM-mode chromatogram of a branchial tissue of *A. colbecki*.

In order to test the performances of the analytical method at these concentration levels, spiking experiments were conducted on the whole tissue: recoveries ranging from 89 to 96% were observed for the three analytes.

TBT and its degradation products were revealed in all the samples analysed, pointing to the occurrence of butyltin compounds in the Southern Ocean, as well as already observed in the northern polar region. Therefore, the presence of butyltins in the tissues indicates that these compounds are present in coastal waters of the Terra Nova Bay area, but physiological tests are needed to verify whether such low concentrations are exerting toxic effects on the animals.

Levels of TBT in organisms from the Ross Sea were low, compared with those reported for other marine bivalves of lower latitudes. The butyltin values were in the same ranges as those reported for the Faeroe Islands, Iceland and Greenland.[14–16]

The gills showed the highest butyltin total content. As mentioned previously, gills reflect levels of contamination in sea water. In a past study,[24] comparing the TBT and DBT trends in sea water and in mussel tissues, the best correlation was found in gills, so that it was considered as the most suitable tissue as a bioindicator. Moreover, MBT was detected only in this tissue, whereas it was below the detection limits both in the whole soft tissues and in the digestive glands.

The presence of degradation products in these tissues is due not only to metabolic processes, but also to direct take up from sea water. This hypothesis can be supported by considering that *A. colbecki* is a filter feeding and suspensivorous bivalve.

The contribution of the butyltin compounds deriving from the resuspended sediments, which can be ingested during feeding activity, seems to be very low, as the concentrations found in the digestive glands show.

CONCLUSIONS

The proposed analytical methodology proved to be effective for the present study. In particular, in the quantitative determination of the analytes, GC–MS provided good sensitivity and accuracy together with the peculiar specificity of MS. A further improvement in sensitivity could probably be reached using a new-generation GC–MS instrument.

TBT and its degradation products were detected in bivalve tissues collected in the Antarctic coastal marine environment. Butyltin compound concentrations were low and comparable to data referring to northern polar areas. The occurrence of these compounds emphasizes the ubiquitous distribution of this class of pollutants, and is similar to that of other xenobiotics, such as polychlorinated biphenyls.

Further studies will investigate the tissues of *A. colbecki* collected in different years to evaluate annual variability, as well as examine other biological samples in the Antarctic food web to compare butyltin contents and speciation with those observed in bivalves.

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