17. Speciation of organotin compounds in environmental samples by GC–MS

ARTICLE *in* TECHNIQUES AND INSTRUMENTATION IN ANALYTICAL CHEMISTRY · DECEMBER 1995

DOI: 10.1016/S0167-9244(06)80018-1

3 AUTHORS, INCLUDING:

Salvatore Chiavarini
ENEA
59 PUBLICATIONS **1,021** CITATIONS
SEE PROFILE

Carlo Cremisini
ENEA
104 PUBLICATIONS **1,977** CITATIONS
SEE PROFILE

Quality Assurance for Environmental Analysis
Quevauviller/Maier/Griepink (editors)

17.

Speciation of organotin compounds in environmental samples by GC-MS

R. Morabito, S. Chiavarini and C. Cremisini
Environmental Chemistry Division, ENEA Casaccia, Via Anguillarese 301, 00060 Rome, Italy

The total worldwide consumption of organotin compounds has dramatically increased in the last thirty years from about 5,000 tons per year at the beginning of the '60s to over 60,000 tons per year in the midst of '80s. They are mainly used as stabilizers for rigid PVC (mono- and di-organotins) and as biocides (triorganotins). Even if the use as biocides accounts for only 30 % of the total world consumption, it contributes, due to the direct introduction, to the largest portion of organotins in the environment. Furthermore, the total production of organotins in the last thirty years increased by about 10-fold while the production of triorganotins for biocide uses has increased by about 20-fold in the same period. The environmental aspects of non-biocidal organotin compounds has been recently reviewed by Maguire [1]. In the conclusions it is stated that the most important non-pesticidal route of entry of mono- and dimethyltin, butyltin and octyltin to the environment is through leaching of PVC by water. Triorganotin biocides are used in pesticide formulations (mainly triphenyltin (TPhT)) [2] and, above all, in antifouling paints (mainly tributyltin (TBT) but also, increasingly, TPhT) [3]. Triphenyltin acetate (Brestan™) and triphenyltin hydroxide (Duter™) are used for the control of Phytophthora infestants, tricyclohexyltin hydroxide (Plictran™) for the control of Phytophagous; 1-tricyclohexylstannyl-1,2,4-triazole (Peropal™) and triphenylbutatin oxide (Vendex™) are both used as miticides [5]. These products are largely used in agricultural application and contamination could result from run-off water and overspray. Tributyltin-based antifouling paints were introduced at the beginning of the 60's but their widespread use started only in the 70's, replacing copper-based paints due to a superior performance: TBT paints are effective for about 5-7 years while copper paints are effective for no more than two years [4-5].

There are two types of organotin based antifouling paints: *(i)* conventional or "free association" paints in which the toxicant is loose in the paint and *(ii)* non conventional or polymeric paints in which the toxicant is chemically bound to a polymeric matrix. Conventional paints are more polluting and have a lower effect duration than polymeric paints, having a higher TBT release rate [6].

TBT is directly released into aquatic environment and its immission can be both continuous (release from the hulls of the boats) or intermittent (release from dockyard activities as paint removal, cleaning, painting, *etc.*). Environmental persistence and fate of TBT are strictly correlated to the specific characteristics of the aquatic ecosystem such as temperature, salinity, pH, suspended matter, microbial populations, flushing rates, etc. Distribution of TBT among the different environmental compartments is regulated by *(i)* physical mechanisms (including volatilization, adsorption, *etc.*), *(ii)* chemical mechanisms (including photochemical reactions) and *(iii)* biological mechanisms (including uptake and transformation) [7].

Both TBT and TPhT undergo degradation processes in marine environment, such as microbial and UV degradation, consisting in a progressive dealkylation down to inorganic tin [8]. Sufficient evidence exists of a faster rate for the DBT-->MBT degradation in some experimental and environmental conditions [9-11]. As the toxicity of the organotins is maximum for the trisubstituted compounds, the degradation can be considered as a mechanism of detoxification. In fact, elemental Sn and its inorganic compounds are practically non toxic for all living systems: due to their very low solubility in lipids, they are scarcely accumulated by the organisms [12-13]; furthermore, at physiological pH, the element is not reactive and its oxides are practically insoluble [14].

On the contrary, the progressive introduction of organic groups at the Sn atom exerts a profound influence on chemical-physical properties, biological activity, mobility and persistence. This leads to an increasing toxicity of the molecule, reaching a maximum for the trisubstituted compounds [15-16]. For marine organisms the highest toxicity is shown by tributyl, triphenyl and tricyclohexyltin compounds [17]. The inorganic substituents do not significantly affect the toxicity of the compounds, unless they are strongly coordinating groups [18-20]. The relative lipophilicity of triorganotin compounds as long as the tendency to bind with complex and simple lipids, make them able to cross biological membranes, producing toxic effects. Many reviews containing toxicological data on organotin compounds have been published [19-23].

The bioaccumulation process depends on the lipophilicity of the substance and on its resistance to metabolism and excretion processes [24]. Studies of kinetics and mechanism of accumulation showed that marine bivalves rapidly and effectively accumulate organotins even when exposed to low concentrations of dissolved material [25]. Bivalves accumulate dissolved TBT from sea water, presumably directly into exposed tissues such as gills, followed by migration to other tissues, or by ingesting tainted food. Very high concentrations can be reached in these organisms, because they are not capable, due to a low activity of the mixed function oxidase system, to metabolize a wide range of xenobiotics, including organotins [26-27].

Laboratory experiments on the accumulation of TBT demonstrated high bioconcentration factors for oysters [28] and for mussels [29-30]. Bioconcentration factors calculated in the field [11, 23, 31-32] resulted to be even higher than those predicted on the basis of octanol-water partition coefficient or calculated from laboratory experiments. The reasons for the discrepancies between laboratory experiments and field data are probably to be found in the difficulty of considering all the important parameters regulating the environmental behaviour in laboratory experiments.

The direct introduction in the marine environment and the successive accumulation together with the high toxicity of these compounds towards "non-target" organisms, such as oysters and mussels, can cause environmental and economic damages as observed in the past in the Arcachon Bay (France) [33].

France was the first country to restrict the use of the TBT based antifouling paint by a legislation that regulated the use of these paints on boats with hulls less than 25 m long [34]. Similar regulations were enacted by many other countries such as UK, USA, Canada, *etc.*

Recently, recommendations to extend the restriction to all organotin paints not only for boats, but also for industrial water cooling systems, mariculture structures, etc. were taken into account. Many references on organotin legislative information can be found in literature [6, 34-37].

17.1 Analytical methods for the environmental analysis of organotins

Many analytical methods have been developed to determine organotin compounds in environmental matrices and several monitoring programmes have been carried out in order to evaluate the effectiveness of legal provisions. As the environmental quality target (EQT) for TBT in water is generally put at the $ng.l^{-1}$ level, analytical methods should be sensitive enough to detect organotin compounds down to these very low concentrations. Furthermore, these methods should be able to discriminate among different chemical forms providing information on the speciation of organotin compounds. In fact, due to the wide differences in toxicity among tin species, speciation is an invaluable tool to understand the distribution and fate of these compounds in the environment and to assess the environmental risks in the studied areas.

Obviously, analytical methods should be characterized by good precision and accuracy, avoiding mistakes that could lead to wrong environmental considerations.

It is worth to point out that mistakes occur in the whole analytical procedure and not only during the instrumental measurement steps. Sampling, storage of samples, sample pre-treatment and analytical measures are all critical stages in speciation studies.

Various papers discuss errors occurring during sampling [38-39] and storage [10, 40].

Sample pre-treatment generally involves an extraction (in order to separate the analytes from the matrix and concentrate) and a derivatization/clean-up step (in order to improve analytes detection).

17.1.1 Extraction

A large number of extractants have been used for the extraction of organotins from water, sediment and biological samples: acids [41-42], organic solvents with [43-46] and without [47-49] complexing agents (*e.g.* tropolone) and a mixture of them [50-56].

Recently, a supercritical fluid extraction was developed for the determination of TBT in sediments [57]. This development is described in detail in Chapter 18 of this book.

Low extraction yields, losses of analytes and contamination directly affect the quality of the results. Moreover, change in the organotin speciation occurring during this step lead not only to wrong information about the contamination levels but also to wrong considerations about the extension of the ongoing degradation phenomena. Thus, recovery should be carefully tested for each organotin compound. In absence of reference materials, spiking experiments are generally used. However, the adequacy of such experiments to represent a realistic surrogate is still a matter of controversy [39] and further studies are needed.

17.1.2 Derivatization

As said in previous Chapters, the most used derivatization methods in organotin analysis are hydride generation and alkylation with Grignard reagents.

Hydride generation [41,49,55,58-62] is generally used when the final determination is performed by atomic absorption spectrophotometry (AAS) while using flame photometry detector (FPD) or mass spectrometry detector (MS) Grignard derivatization [31, 44-46, 63-68] is generally preferred.

Hydride generation seems very attractive for butyltins analyses, even if poor recoveries are obtained from sediments containing high sulphur, hydrocarbons and chlorophyll content due to an inhibition of the hydride generation [69,70]. Determination of phenyltin species is generally hindered due to the low yields and poor reproducibility of hydridization. Furthermore, losses of the highly volatile mono-alkyltin species could occur if the analytical method requires a concentration step by evaporation of the final solution. Problems concerning the hydride generation for the determination of butyltin compounds are treated in detail in Chapter 19 in this book [71].

Grignard derivatization is generally performed by methylation [63,64], ethylation [44], pentylation [55,66,68], hexylation [31,67], *etc.*. Pentylation seems to offer the best compromise to obtain non-volatile derivatives and good gaschromatographic properties both in terms of separation and detectability (see also section 17.1) [66]. Grignard derivatization needs more steps than hydride generation *i.e.* the reagent destruction (usually performed by careful addition of water and then of a strong acid) and the back-extraction of the alkylated organotins, increasing risks of contamination, decomposition, losses, *etc.*

Recently, a new derivatization method with sodium tetraethylborate was proposed for butyltin compounds [70]: its characteristics are situated somewhere in between those of hydridization and Grignard alkylation.

Derivatization yields should be carefully evaluated but the absence of commercially available derivatized compounds of proven purity makes it a difficult task. The use of radiolabelled compounds and/or synthesized derivatized standards in one's own laboratory [72] should be considered.

17.1.3 Analytical techniques

Chromatography is the most cited analytical technique for the determination of organotins. Atomic absorption spectrometry (AAS), flame photometry (FPD) and mass spectrometry (MS) are mainly used as detection system. Chromatography interfaced with atomic absorption spectrophotometry has been reviewed by Donard and Pinel [73]. Several papers describing analytical procedures based on FPD determination are reported in literature [64, 68, 74-78]. Mass spectrometry detection is described in detail in the next paragraph including literature references. Electron capture (ECD) [79-81] and flame ionization (FID) [82] detectors have been also used. The application of gas chromatography atomic emission spectrophotometry (GC-AED) [83] and of high performance liquid chromatography interfaced with isotope dilution - inductively coupled plasma mass spectrometry (HPLC/ID-ICP/MS) for tin speciation is described in Chapter 16 of this book [84].

Nevertheless, a number of other techniques, such as graphite furnace atomic absorption spectrophotometry (GFAAS) [85-89], voltammetry [90-93], fluorimetry [94-95] and spectrophotometry [96-97] are present in literature.

17.2 GC/MS analysis of organotins

Mass spectrometric detection systems have distinct advantages over the other specific detectors that have been used for organometallic compounds analysis (like AAS, ICP, AED, *etc.*):

1) they provide both sensitivity and selectivity together with structural confirmation capabilities;
2) coupling to HRGC is straightforward and there is no need to "adapt" each other different instruments;
3) modern instrumentation is robust, easy to use, relatively inexpensive (benchtop instruments) and is now really at an industry-standard level of reliability;
4) there is no need to be a "MS specialist" to perform analyses.

Mass spectrometry is particularly well suited for the analysis of organometallic species (provided they are amenable to GC): the metal atom (particularly the heaviest ones) gives the compound an "extra-mass" with respect to the possible coeluting organic compounds, making detection and confirmation easier and less prone to interferences. This obvious advantage is not confined to GC/MS only, but is also the key feature of such powerful detection techniques as He-MIP/MS [98], ICP/MS [99-102], ionspray MS/MS [50], laser ionization/TOF-MS [103], *etc.*

17.2.1 Which MS detector ?

The correct choice of a MS detector is usually a complex task, involving both technical and budget aspects. Generally speaking, one should answer some questions:

- Do I need a mass spectrometer or a mass spectrometric detector ?
- Do I really need high mass resolution ?
- Do I need extra features (NICI, PICI, LC interfaces, solid probes, FAB, *etc.*) ?
- Is not productivity one of my major goals ?
- Do I have well trained personnel and strong technical assistance support ?

If the answers were mainly no, a benchtop quadrupole MS detector is the best choice, progressively upgrading to high-costs, complex and technically demanding magnetic sector HRMS spectrometers.

In the field of organotin compounds analysis the MS system of choice is by far the quadrupolar type: high mass resolution is not needed and a "workhorse" as the quadrupole is important when one is frequently facing relatively "dirt" samples as those from environmental matrices. The ion trap detector is also gaining relevance as an all-purpose mass detector, after some technical drawbacks that affected the oldest versions of the instrument were partially solved.

17.2.2 Gas chromatographic characteristic of organotins

The tremendous separating power of capillary gaschromatography is fully exploited if the analytes are in a suitable chemical form in order to match the technical characteristics of the injection system, the capillary column and the detection system. Various authors reported results of investigations on the chromatographic behaviour of organotins derivatives [76, 104-106] and even if the analysis of organotin chlorides and hydrides were found feasible, the range of applicability of Grignard derivatization is by far superior for a number of reasons: it gives extremely stable compounds and the derivatized samples can be easily stored for later analysis or other investigations;

- Grignard derivatives can be obtained also from extracts of extremely dirt samples;
- Grignard derivatives can be obtained from almost the whole range of organotin compounds, whereas useful chlorides and hydrides are limited;
- Grignard derivatives show excellent chromatographic properties on the most common capillary phases;
- samples after derivatization are easily subjected to cleanup to improve chromatography and detection.

The Grignard reagent of choice is, in our opinion, n-pentylmagnesium bromide for various practical reasons:

- it is less reactive than *e.g.* methylmagnesium bromide, but is less hazardous and still sufficiently strong to act on dirty extracts;

- it gives derivatives of optimal volatility to be analyzed on "all-purpose " columns; the derivatives show a wide chromatographic separation;
- it allows a good separation of butyltins from phenyltins to be achieved.

The fully derivatized organotins behave chromatographically like hydrocarbons and their separation is perfectly achieved on nonpolar phases of the SE-30 (methylsilicone) or SE-54 (methyl-5 % phenylsilicone) type. Capillary columns with phase ratios from 200 to 450 can be successfully used. Since separation of the compounds is easily achieved, column length and thermal program are not critical: relatively short columns (15-25m) and fast thermal programs (8-10°C/min increase) can significantly speed analysis and improve sensitivity. An example of a typical pentylated organotins chromatogram is shown in Figure 1.

The good thermal stability and absence of active groups in the Grignard derivatized compounds allow the use of rugged inlet systems like the split-splitless one, whereas chlorides or hydrides are amenable to cool on-column injection in deactivated precolumns only. The split-splitless injector is much more resistant to the buildup of involatiles and it is particularly suited for environmental samples, even if it has a lower reproducibility than the on-column one.

The use of an internal standard is always advisable in quantitative GC and is simply mandatory for GC/MS. Due to the complex manipulation of the samples, recovery standards (surrogate compounds) analysis is highly desirable. The most commonly used compound is TPrT, which is usually added to the sample at the beginning of the whole procedure and therefore acting as a surrogate compound. Because of the very good recoveries of trisubstituted compounds, TPrT is also considered as the internal standard for the quantitative calculations. A more rigorous approach would be to add a complete set of surrogate compounds (for mono- and disubstituted species too) to the sample and to add a true internal standard (e.g. tetrabutyltin) to the final solution. To our knowledge, this approach has never been fully addressed by the scientific community, probably because of the lack of pure reference compounds.

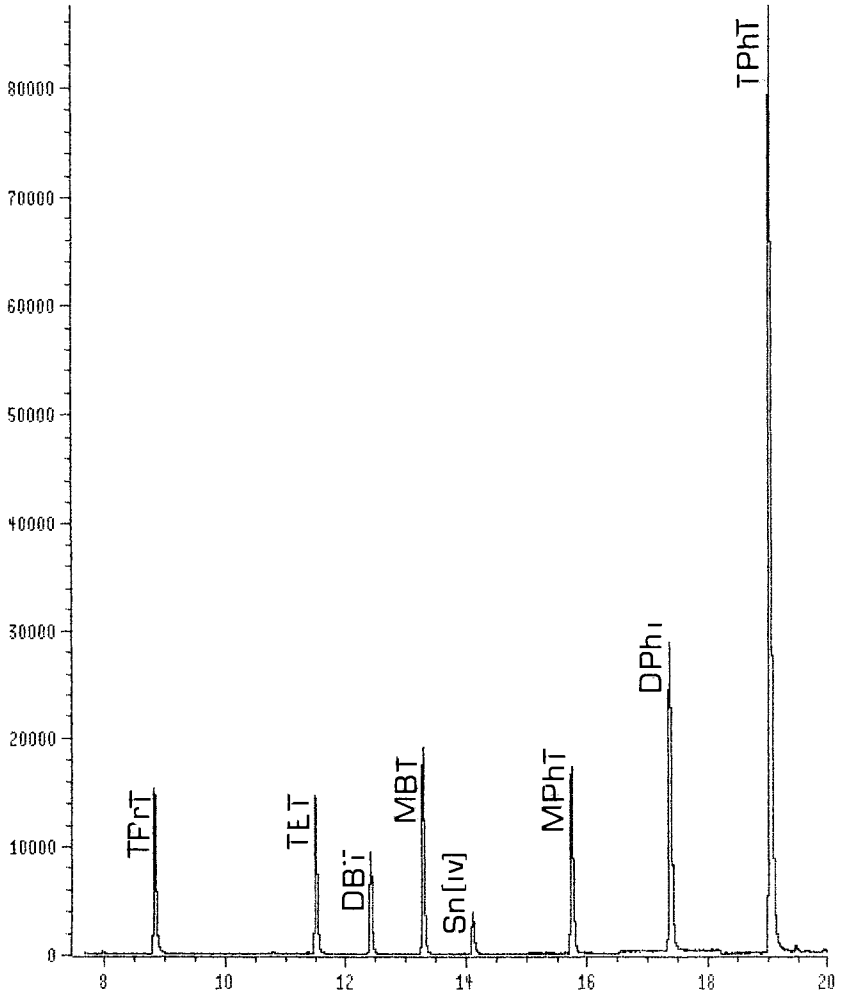

Figure 1: GC-MS of pentylated organotin compounds standard mixture. Amounts injected on column: TPrT, 0.20 ng; TBT, 0.17 ng; DBT, 0.12 ng; MBT, 0.20 ng; MPhT, 0.12 ng; DPhT, 0.11 ng; TPhT, 0.12 ng.

17.2.3 Mass spectral characteristics of organotins

Various papers report mass spectral data for organotin compounds [63,72,76,104,107-110], but it is always worth to remind their key MS characteristics.

Tin has a "rich" isotopic composition as shown below:

Mass number	Abundance (%)
116	14.30
117	7.61
118	24.03
119	8.58
120	32.85
122	4.72
124	5.94

Natural abundances of main isotopes of Sn

This isotopic distribution gives a peculiar aspect to the MS spectra of tin compounds: each fragment ion appears as a cluster of m/z values with the above mentioned ratios. From the analytical point of vue, this improves identification of the analyte but decreases GC/MS sensitivity because the ions produced by a single fragmentation are spread over a range of m/z.

The fragmentation pattern under classical EI conditions of fully alkylated organotins (which are the most frequently analyzed derivatives: hydrides are rarely used [105] unless for methyltins [111]) is quite simple, consisting in a stepwise loss of the alkyl (aryl) groups. Examples for the pentylated organotin compounds considered in this chapter are shown in Figures 2-8. The molecular ion is virtually absent. Losses of alkyl groups seem to be almost equiprobable (at least in the C3-C5 range) and the structure of the compound can be deduced by mass losses and by ratios of the relative isotope clusters. The presence of aryl groups gives additional stability to fragment ions. The case of TPhT is particularly significant,

where the cluster originating from the loss of the pentyl group is the only present, despite the 3:1 statistical favour to the loss of a phenyl. Different ionization modes as PICI [112] give a predominant molecular cluster ion, useful for identification and confirmation purposes, but when coupled to GC, this approach gave higher detection limits than the usual EI-SIM.

The scan mode detection of quadrupolar detectors cannot cope with the low environmental concentrations of organotins (the ion trap detector could be a valuable alternative for high sensitivity scan) and diagnostic ions have to be chosen for SIM.

As a general rule, diagnostic ions for SIM have to fulfill some basic requirements:
they should be among the most abundant ions of the spectrum in order to increase sensitivity;

- they should be unique and characteristic of the compound in the matrix under analysis;
- the selected m/z should have the lowest possible instrumental noise in the experimental setup;
- m/z values whose ratios are checked for confirmation should be isotopic peaks rather than different fragment ions.

In the case of pentylated organotins these requirements are easily met thanks to the simple fragmentation and the peculiar isotopic composition.
The GC separation of pentylated organotins is very good, as shown before, and the number of ions to be monitored in each time window can therefore be kept to a minimum, again increasing sensitivity.

In our experience, organotin analyses by GC/MS rarely suffer from serious interferences: the only problem could be an overload of the ion source from high amounts of coeluting compounds in case of extremely polluted samples (petroleum hydrocarbons in harbour sediments or lipids from non perfectly cleaned-up biological extracts). In those cases a retention times shift is usually evident, due to the overloading of the capillary column.

GC/MS data are ideal for the implementation of automatic data reduction and interpretation routines: integration of ion chromatograms, peaks detection and identification, identity confirmation and quantitation can be performed in an automatic way, using the programming capabilities of modern GC/MS workstations. Care should be taken in revising the results so generated, as the integration of different ion traces is the weak link in the automation: widely different noise or interference level are not uncommon even for $Dm/z=2$, which is the case of organotins, seriously affecting data quality. Current integration software is not sufficiently intelligent to cope with these cases and nothing can still replace the expert's eye evaluation capabilities.

The other great advantage of the MS detector (and particularly the quadrupolar one) is its wide linear range (up to 7 decades) which makes calibration with the internal standard method an easy task: the injection of two standard solutions (bracketing the expected amounts to be analyzed) is sufficient to verify the constancy of the response factors. The repetition of the standards injection between samples ensures that the whole system is working properly and results are trustable.

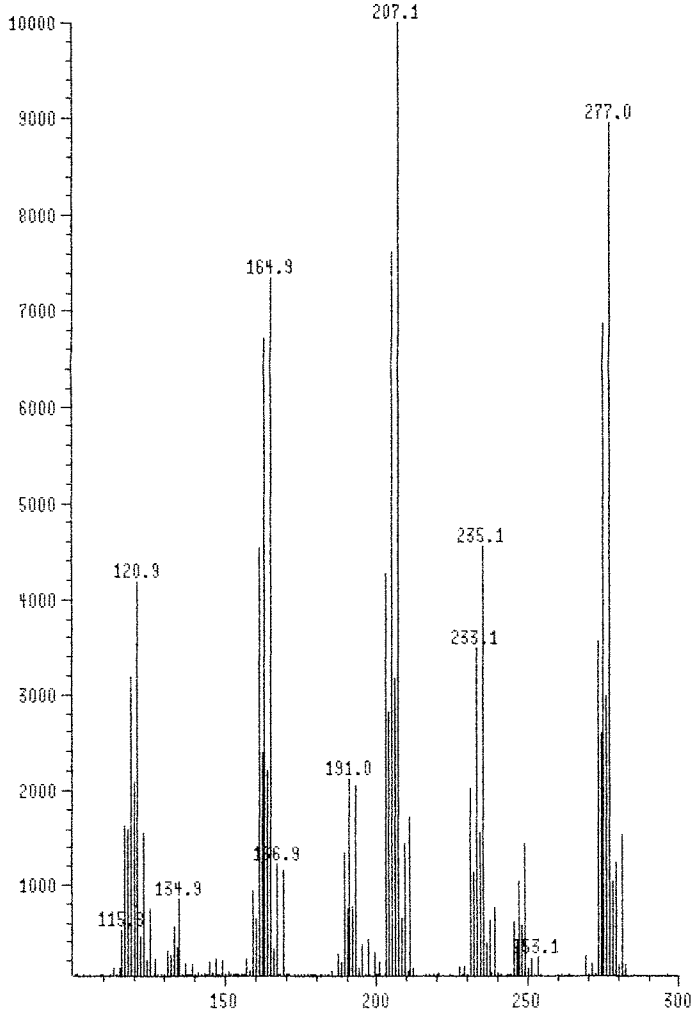

Figure 2: Mass spectrum of TPrT

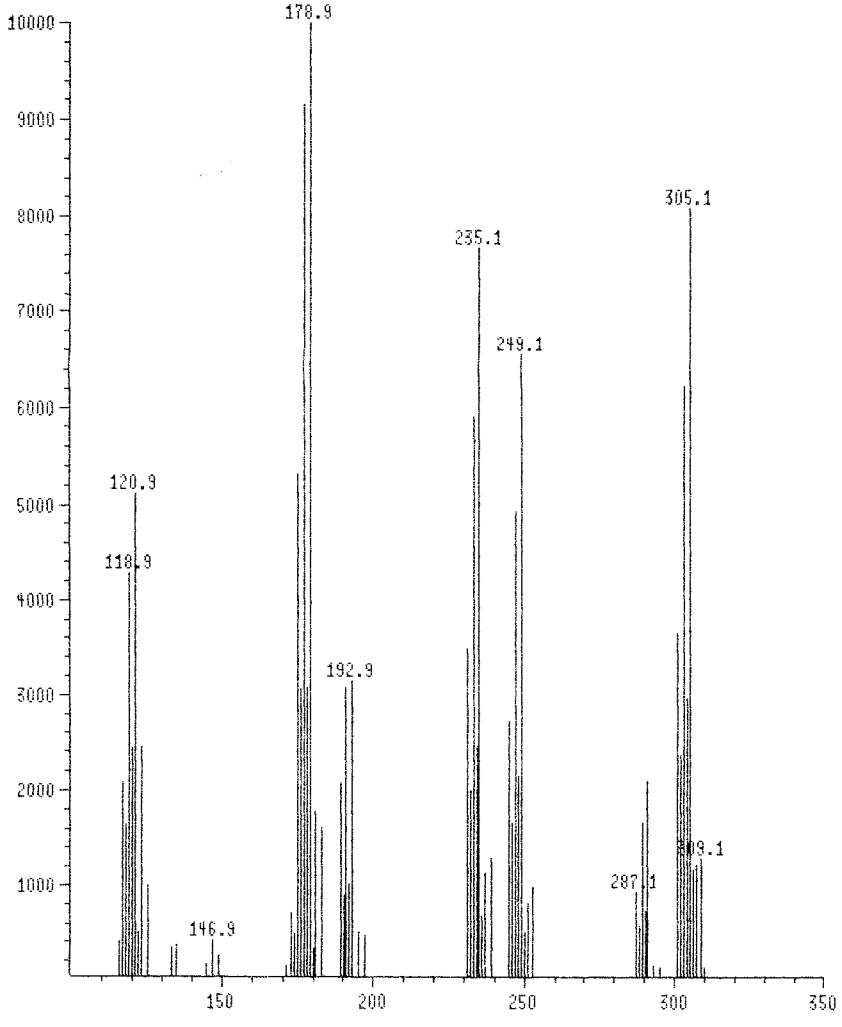

Figure 3: Mass spectrum of TBT

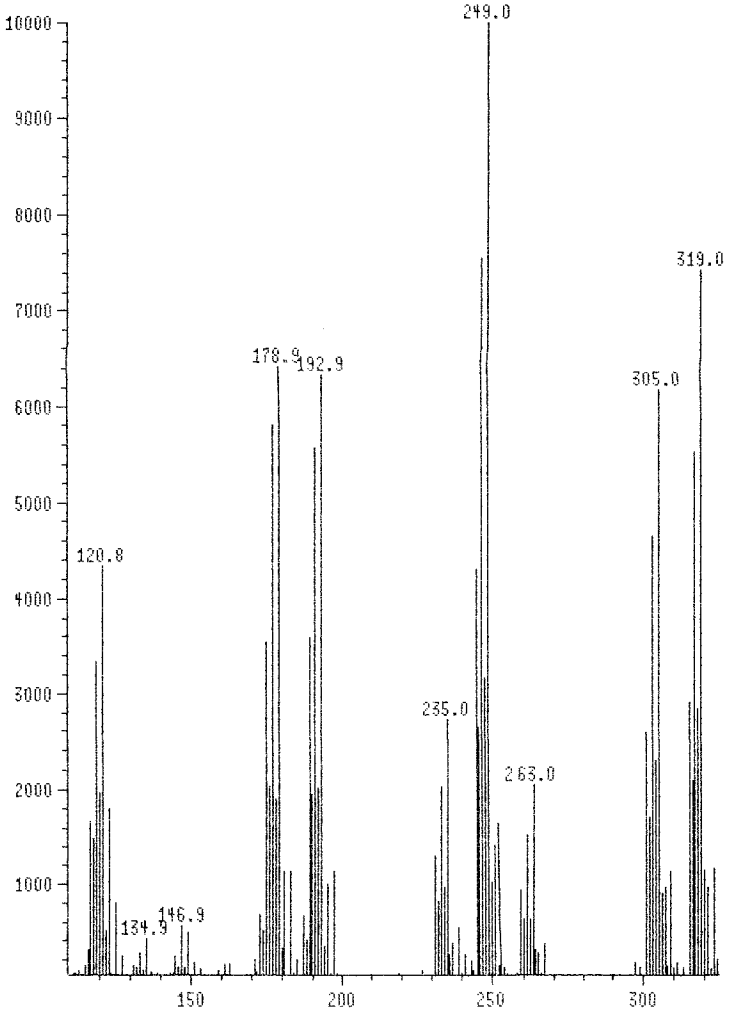

Figure 4: Mass spectrum of DBT

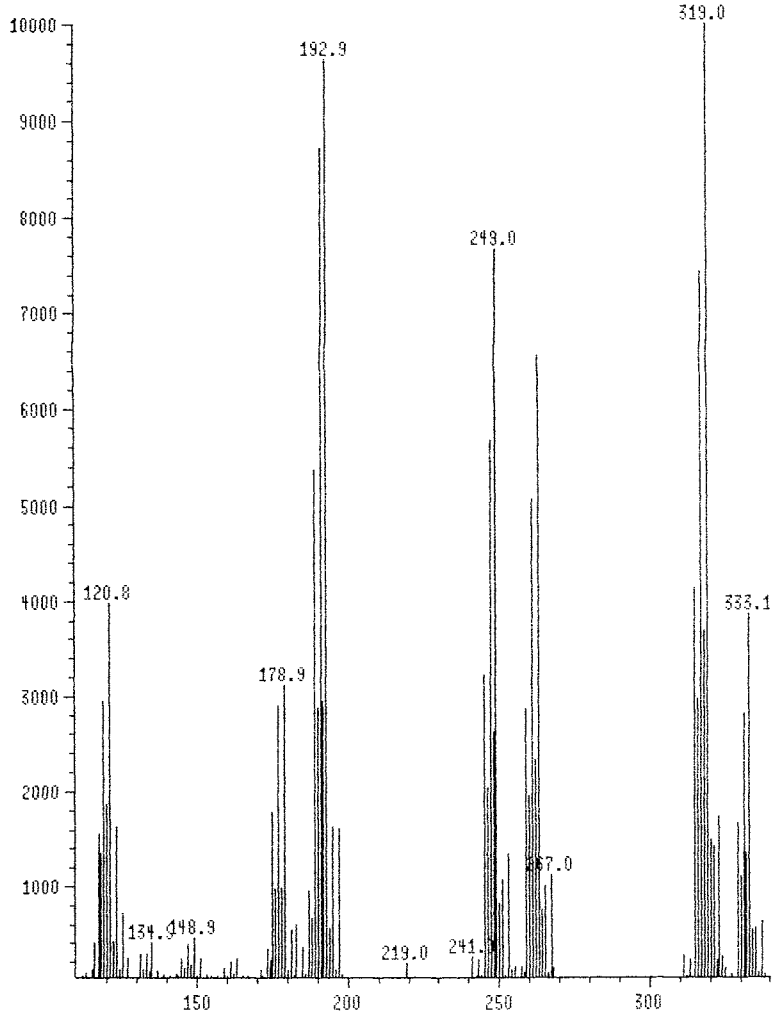

Figure 5: Mass spectrum of MBT

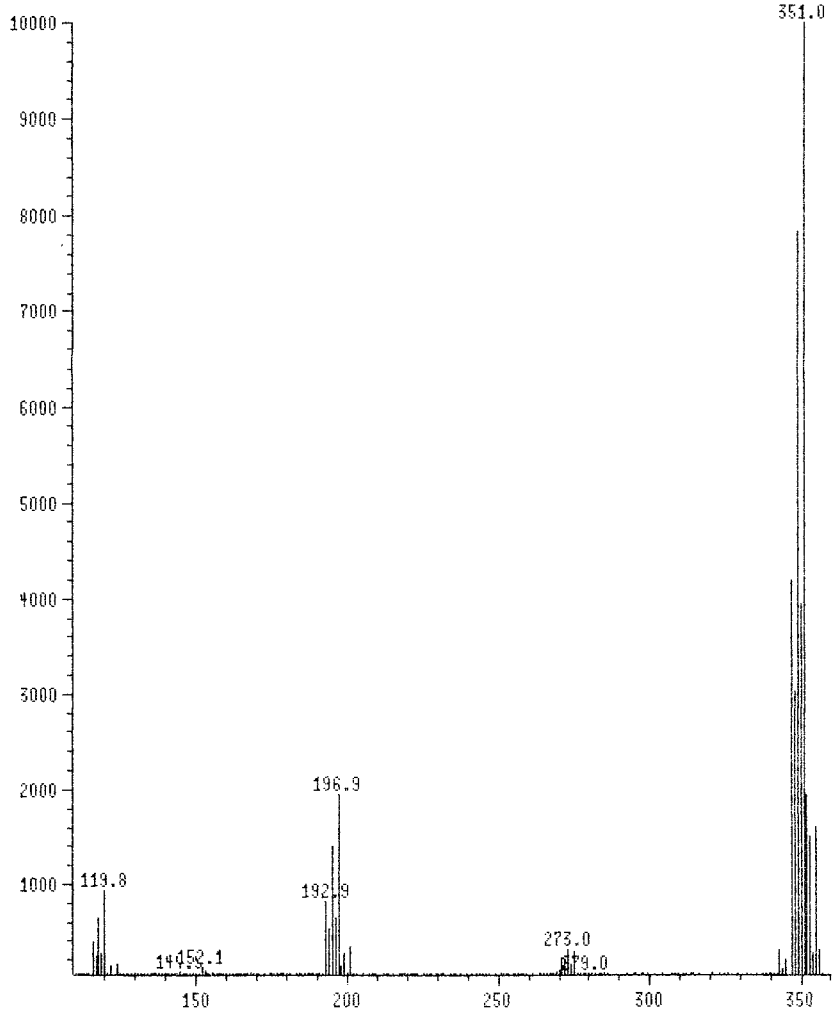

Figure 6: Mass spectrum of TPhT

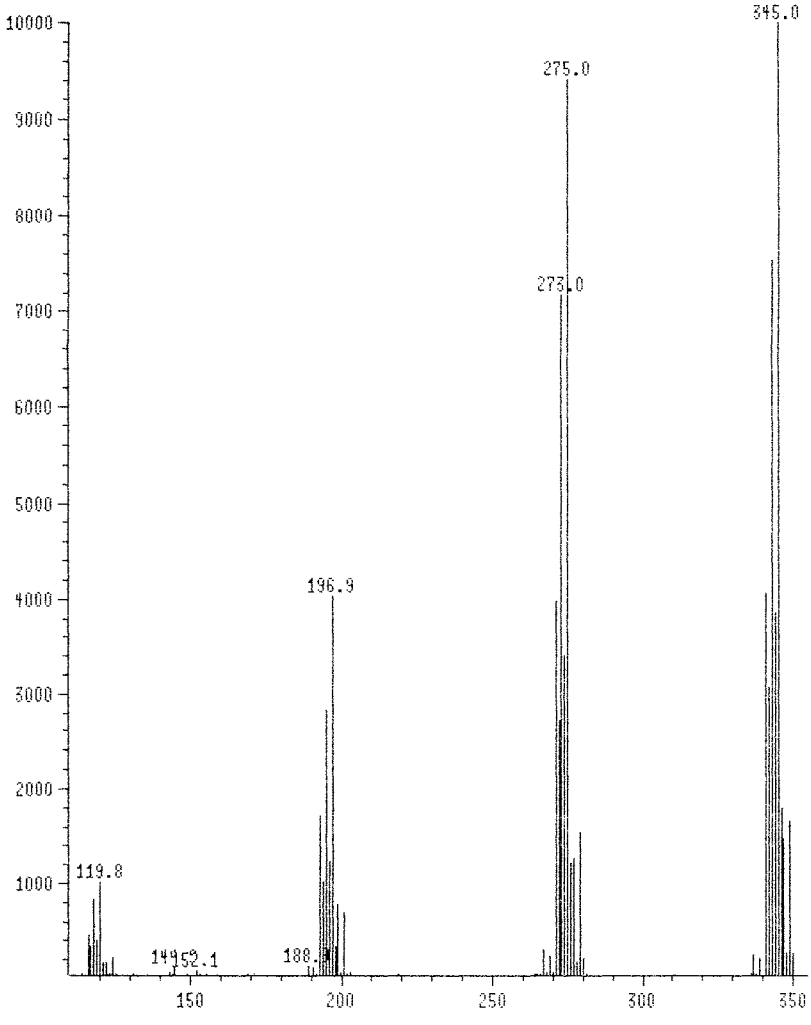

Figure 7: Mass spectrum of DPhT

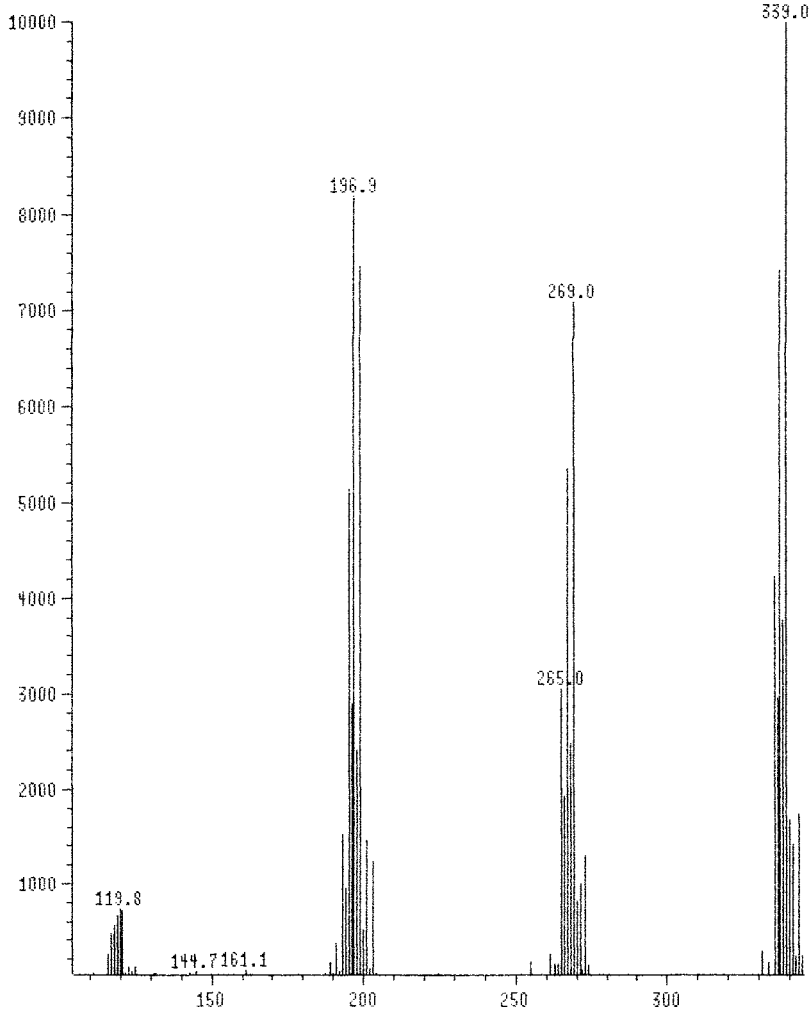

Figure 8: Mass spectrum of MPhT

17.3 Analytical methods

In recent years procedures for butyltins and phenyltins analyses in water, sediments and biological samples were studied on the basis of the literature and optimized in our laboratory. These procedures were frequently tested by using of reference materials, when available, by spiking experiments on real samples and participating to european intercomparison and certification exercises. In this paragraph these procedures are described in detail together with comments on specific topics or hints and recommendations for critical steps in the determinations.

17.3.1 Reagents and materials

- Organic solvents: pesticide analysis grade;
- Tropolone (2-hydroxy-cycloheptatrienone);
- n-Pentyl magnesium bromide 2 mol.l^{-1} in diethylether;
- Nitric acid;
- Sulphuric acid;
- Potassium dichromate;
- Silica gel: Davison 923 type (100-200 mesh) activated at 180°C overnight.
- Anhydrous sodium sulphate treated at 550°C for 6h before use;
- Tributyltin chloride (TBT); Dibutyltin chloride (DBT), Monobutyltin chloride (MBT); Triphenyltin chloride (TPhT); Diphenyltin chloride (DPhT); Monophenyltin chloride (MPhT);
- Tripropyltin chloride (TPrT);
- SPE LC18 extraction tubes;
- Carbopack B 80-120 mesh;
- Sn(IV) calibrant solution for GFAAS
- Common laboratory glassware
- Glass reaction vials (8 ml and 15 ml volume)
- Rotary evaporator with vacuum and thermostated bath
- Nitrogen gas blowdown apparatus
- Efficient fume hoods

The purity of commercially available tin compounds is usually based on total tin content and they should be checked for the presence of degradation products with GC-FPD and/or GC/MS after Grignard derivatization. The preparation of calibrant solutions of pentylated organotins following the guidelines given by Stäb *et al.* [72] are strongly recommended, due to the absence of pure standard compounds.

Organotin chlorides stock solutions are prepared gravimetrically in methanol at about 1 mg. ml^{-1} (as Sn). All data in the text are expressed on the basis of tin) and diluted 1000-fold to give the working standard solutions. When stored refrigerated in the dark, stock solutions are stable for at least 3 months and the working solutions at least for 1 month, but the latter are renewed weekly.

17.3.2 Instrumentation

GC/MS analyses were performed on a Hewlett-Packard HP 5890 GC/ HP 5970B MSD system with the following conditions:

- electron impact ionization mode (70 eV);
- carrier gas: helium, 120 kPa head pressure;
- column: HP-5 (methyl-5 % phenylsilicone, 0.20 mm i.d.,0.11 μm film thickness, 25 m length; Hewlett-Packard);
- temperature program: 80 °C x 2 min, then 10 °C min^{-1} to 280 °C; injector: splitless, 240 °C;
- transfer line temperature: 280 °C;

SIM (selected ions monitoring) operation with the following program (dwell time was 100 ms for all ions):

Compounds	Starting time (min)	m/z
TPrT	8	277, 275, 273
TBT	10	305, 303, 301
DBT	12	319, 317, 315
MBT	12.9	319, 317, 315
Sn(IV)	13.8	333, 331, 329
MPhT	15	339, 337, 335
DPhT	16.5	345, 343, 341
TPhT	18.2	351, 349, 347

Table 1

The timings reported above are only indicative and should be adapted to the particular instrumental conditions in use.

Peak identification was based on the matching of retention times (± 0.5 %) and isotopic mass ratios (± 20 %) for the diagnostic ions. The relative response factors were controlled by injecting standard mixtures on a regular basis (one injection every 3-4 samples) to follow the tuning conditions of the MS system.

With these chromatographic settings, the limit of detection for TBT, DBT and MBT at a signal-to-noise ratio of 3 is around 8 pg injected. Phenyltins detection is somewhat more sensitive, particularly for TPhT (LOD=1.5 pg), owing to their peculiar fragmentation pattern.

17.3.3 Procedures

17.3.3.1 Sediments and mussels samples

The sample is homogenized and freeze-dried before extraction. 50-500 ng of TPrT, as internal standard (see 17.3.3.3a), are added to 100-500 mg of sample as methanolic solution before extraction, allowing 30 minutes for equilibration. Longer equilibration times, up to 16 hours, do not affect absolute recovery of TPrT. The sample is placed in a Pyrex vial and 15 ml of tropolone 0.05 % in methanol and 1 ml of concentrated HCl are sequentially added (see 17.3.3.3.b).The vial is put in an ultrasonic bath at a water temperature lower than 40 °C and left under sonication for 15 minutes (see 17.3.3.3.c).The vial is then transferred for centrifugation at 3000 rpm for 10 minutes. The surnatant is transferred in a 250 ml separatory funnel filled with 150 ml of a 10 % NaCl solution (see 17.3.3.3.d) and the extraction procedure is repeated (see 17.3.3.3.e).After the second surnatant is transferred in the separatory funnel, liquid-liquid partitioning is performed twice with 15-20 ml of CH_2Cl_2. The methylene chloride phases are collected through anhydrous sodium sulphate. After washing the sulphate with 1-2 ml of CH_2Cl_2, the extract is added of 1 mL of isooctane and the volume is approximately reduced to 5 ml in a rotary evaporator at a bath temperature lower than 40 °C and under moderate vacuum. The concentrated extract is transferred to a 15 ml vial and lead to almost dryness under moderate flow of nitrogen, operating solvent exchange (methylene chloride to isooctane) (see 17.3.3.3f). 1 ml of 2 $mol.l^{-1}$ ethereal solution of pentylmagnesium bromide (see 17.3.3.3g) is added and the vial is put under sonication for 1 minute and then in a 50 °C water bath under mechanical agitation; the reaction is allowed to proceed at least for 30 minutes (see 17.3.3.3h). 1 ml of hexane is added and the vial is put in a beaker half-filled with cold water. 2 ml of distilled water are carefully added drop by drop and then 6-7 ml of H_2SO_4 1 $mol.l^{-1}$ are added too (see 17.3.3.3.i).Derivatized organotins are extracted with 2-3 ml of hexane; the extraction is repeated twice. The organic phase is put in a vial and concentrated under moderate flow of nitrogen to *ca.* 0.5 ml. The extract is transferred on top of a silica-gel column (3 g in a glass column 30 cm length and 8 mm as internal diameter) previously wet with 0.5 ml of hexane/benzene 1:1. Hexane/benzene 1:1 mixture is passed through the column until 5 mL are collected in a vial (see 17.3.3.3l). Finally, the solution is concentrated under moderate flow of nitrogen to *ca.* 0.5 ml. 1 ml is injected for GC-MS determination.

17.3.3.1.1 Recovery tests from sediment samples

Recovery tests from sediment samples were carried out by spiking freeze-dried uncontaminated sediments collected in the open Adriatic Sea. Organotins were added as solutions in methanol to the sediments previously wetted with distilled water. After the addition, sediments were shaken at least for 30 minutes and allowed to equilibrate overnight. Sediments were spiked at two different concentration level in the ranges 140-160 $ng.g^{-1}$ and 880-1000 $ng.g^{-1}$. Results, as average of five independent experiments for both the concentration level, are shown in Table 2. As it can be seen recoveries were always satisfactory even if for monosobstituted compounds they were slightly lower than for di- and trisubstituted ones.

Compound	Spiked amount (ng Sn)	Recovery (%)	Spiked amount (ng Sn)	Recovery (%)
TBT	80	91 ± 10	480	94 ± 7
DBT	83	89 ± 12	496	88 ± 11
MBT	75	80 ± 13	450	85 ± 11
TPhT	75	92 ± 11	451	90 ± 9
DPhT	79	87 ± 15	472	91 ± 12
MPhT	74	78 ± 16	442	81 ± 13

Table 2: Recoveries of organotin compounds from 500 mg of spiked sediments. Results are the average of five different experiments. (Ref. [68])

17.3.3.1.2 Recovery tests from mussel samples

Recovery tests on biological materials are difficult to be performed because reference material are not still commercially available (except the NIES - Japanese National Institute for Environmental Sciences - fish tissue certified for TBT). Moreover, uncontaminated samples are rarely collected. Freeze dried mussel samples were analyzed before and after spiking and the recoveries were calculated with respect to the sum of the concentrations of the incurred compounds and the spikes. Spiking procedure was the same as for sediments. Results, as average of five independent experiments, are shown in Table 3. As for sediments, recoveries of monosubstituted compounds were lower than for the other ones. Anyway, recoveries were always higher than 80 % for each organotin compound tested.

Compound	non-spiked (ng Sn)	spiked amount (ng Sn)	found (ng Sn)	recovery (%)
TBT	185 ± 24	160	314 ± 27	91 ± 9
DBT	61 ± 10	165	201 ± 23	89 ± 11
MBT	80 ± 13	150	195 ± 29	85 ± 15
TPhT	nd	150	138 ± 13	92 ± 9
DPhT	nd	157	133 ± 18	85 ± 14
MPhT	nd	147	120 ± 20	82 ± 17

Table 3: Recoveries of organotin compounds from 500 mg of non-spiked and spiked mussels. Results are the average of five different experiments. (Ref. [68])

17.3.3.2 Water samples

Filtration of samples is usually performed to discriminate between dissolved and adsorbed fractions. As the toxicological data of organotin compounds and the Environmental Quality Target (EQT) are generally referred to the concentration in the dissolved phase, such a procedure is widely accepted to provide information about the contamination levels. Furthermore, analytical results on non-filtered samples are not easily comparable due to the variety of extraction techniques used for organotin analysis. However, the strong tendency of TBT to accumulate onto suspended matter can lead to underestimate the contamination of the site [38]. In fact, depending on the particular site, the concentration of adsorbed TBT can be equal and in some cases higher than in dissolved phase. Moreover, the contribution of adsorbed TBT in bioaccumulation and toxic effects towards marine organisms cannot be neglected. It is therefore recommended to determine both the concentration of dissolved TBT, after filtration of the samples, and of adsorbed TBT, by analyzing the filters.

The procedure for the determination of organotins adsorbed onto particulate matter is the same as for sediments and mussels.

Usually, a liquid-liquid extraction is employed for water samples due to high performances such as very good recoveries and high enrichment factors. However, it is difficult to be performed in the field and on oceanographic ships during sampling campaigns. In these cases, samples are usually stored and the extraction is performed later. Nevertheless, during sample storage, analytes losses and degradation processes could occur altering butyltins speciation in the samples. Recently, liquid solid extraction (LSE) of TBT [81,113,114]and butyltins [88] from water samples has been successfully applied.

17.3.3.2.1 Liquid/liquid extraction

The sample (1 l) is filtered though a 0.45 mm glass fiber filter in order to evaluate the dissolved organotins concentrations.

10-100 ng of TPrT standard solution in methanol are added, depending on the expected contamination level of the sample, and allowed to equilibrate for 15 min with occasional agitation. The sample pH is adjusted to 2 in order to improve the extraction efficiency of the monosubstituted species (see 17.3.3.3b) and the extraction is performed in a separatory funnel with at least 2 aliquots (30 ml) (see 17.3.3.3d) of a 0.03 % tropolone solution in dichloromethane, collecting the organic phases through anhydrous sodium sulphate. The procedure is then the same as in 17.3.3.1.

17.3.3.2.1.1 Recovery tests

Certified Reference Materials are not so far available for organotins in water samples; so recoveries from water samples have to be calculated by carrying out spiking experiments. The recovery tests for water samples were performed on uncontaminated filtered sea water. The sample was divided into 3 aliquots. The first aliquot was analyzed and the concentrations of butyltins (TBT, DBT and MBT) and phenyltins (TPhT, DPhT and MPhT) resulted to be below 2 $ng.l^{-1}$. The other subsamples were spiked with 20 $ng.l^{-1}$ and 200 $ng.l^{-1}$ of each organotin compound, respectively. After spiking, the samples were allowed to equilibrate under mechanical agitation for 2 hours. Recoveries were always higher than 90% for both the tested concentrations and for the six compounds (Table 4).

Compound	Recovery (%) at 20 ng.l^{-1}	Recovery (%) at 200 ng.l^{-1}
TBT	102 ± 6	102 ± 4
DBT	104 ± 6	100 ± 4
MBT	97 ± 8	95 ± 10
TPhT	93 ± 9	91 ± 9
DPhT	94 ± 8	91 ± 8
MPhT	91 ± 10	92 ± 13

Table 4: Recoveries of organotin compounds from spiked sea water samples by liquid-liquid extraction. Results are the average of six different experiments. (Ref. [68])

17.3.3.2.2 Solid phase extraction

The complete study is reported elsewhere [88]. The adsorption capacity of solid phases, the recovery and the enrichment factors obtainable with this kind of extraction were carefully evaluated. Adsorption tests were carried out with TBT, DBT, MBT and inorganic tin (Sn IV) respectively. Phenyltins were not systematically tested. On each solid phase (Carbopack, LC 8 and LC 18), inorganic tin was not retained at all; while the retention of organic tin was excellent. In fact, butyltins were completely retained on Carbopack up to 1 mg and on LC 8 and LC 18 up to 6 mg of butyltin compound per gram of solid phase.

Elution tests were carried out for TBT, DBT and MBT with five eluting agents (methanol/tropolone, methanol, dichloromethane, hexane and diethylether). The results obtained on Carbopack and on LC 18 are shown in Table 5; the results are the average of five different replicates and the final measurements was performed by GFAAS. As it can be seen, both for Carbopack and LC 18, methanol/tropolone was able to quantitatively elute all the butyltin compounds at the same time, with high efficiency. Similar results were obtained on LC 8 but, in this case, larger volume of eluants (about 10 ml) were needed.

	methanol/ tropolone	methanol	dichloro- methane	hexane	diethylether
TBT	105±7 94±7	105±4 96±8	27±8 <10	<10 0	0 0
DBT	102±9 101±5	0 0	0 0	0 0	0 0
MBT	107±5 89±4	0 0	0 0	0 0	0 0

Table 5: Butyltins elution test from Carbopack and LC 18. Results, expressed as percentage, are the average of five different experiments. The first column in each row indicates the results obtained with carbopack, whereas the second column gives the results obtained with LC 18 (Ref. [88])

17.3.3.2.2.1 Recovery tests

Recovery tests were carried out on deionized water and sea water.

LC18 pre-packed tubes (500 mg) and Carbopack B (100 mg) were tested. Both tube types were pre-treated sequentially with 10 ml of methanol and 10 ml of distilled water, avoiding the drying of the adsorbent bed. The sample volumes to be extracted were selected on the basis of acceptable sample flowrates (for LC 18 the manufacturer recommended flowrate of 5 - 10 $ml.min^{-1}$ was found to be satisfactory; for Carbopack B 10 $ml.min^{-1}$ was used, even if higher flowrates can be accommodated by this solid phase). TPrT as internal standard is added to the sample as in the liquid/liquid extraction procedure. Adsorbent bed must be vacuum dried before solvent elution.

Different aliquots of both deionized and sea water were spiked with 40 $ng.l^{-1}$ and 200 $ng.l^{-1}$ of each butyltin species, respectively. After spiking the samples were allowed to equilibrate under mechanical agitation for 2 hours. 1 l samples can be extracted without losses using Carbopack B, whereas volumes larger than 250 ml cannot be extracted by LC18 columns without significant losses (*e.g.* recovery from 500 ml sea water sample spiked at 200 $ng.l^{-1}$ was 78 %). Differences between recoveries from sea water and deionized water are not statistically significant. Results obtained from spiked sea water samples extracted with LC 18 and Carbopack are shown in Table 6.

Compound	LC 18 (250 ml) 40 $ng.l^{-1}$	LC 18 (250 ml) 200 $ng.l^{-1}$	Carbopack (250 ml) 40 $ng.l^{-1}$	Carbopack 250 ml) 200 $ng.l^{-1}$	Carbopack (1 l) 40 $ng.l^{-1}$	Carbopack (1 l) 200 $ng.l^{-1}$
TBT	96	96	95	103	99	102
DBT	90	90	88	99	96	98
MBT	88	90	87	94	90	94

Table 6: Recoveries of butyltin compounds from spiked sea water samples by liquid-solid extraction. Results are the average of two different experiments. (Ref. [68])

17.3.3.3 Remarks on the procedures.

As a general rule for trace analyses, chemical reagents should be of the highest purity available and should be individually tested for interferences and quality variations from batch to batch. Further purification steps are sometimes necessary in organotins analyses: in particular, dichloromethane pre-extraction of chloridric acid (often contaminated by MBT) was needed. Thermal treatment of other reagents (sodium sulphate, silica gel) is highly recommended, particularly if purchased in plastic containers.

Grignard reagents, as previously mentioned, are often contaminated by butyltins, the contamination varying among different batches [115]. Values of *e.g.* TBT contamination ranged from 4 to 15 $\mu g.l^{-1}$.Custom synthesis of Grignard reagents is therefore recommended if very low contaminated samples are to be analyzed.

a) Tripropyltin was used as internal standard for quantitative analysis, being a compound that closely matches the most environmentally relevant compounds (TBT and TPhT) to be determined and because it was neither detected in natural samples nor its presence is expected, lacking a widespread use.

b) The extraction of organotin compounds is to be performed in acidified medium to enhance the recovery of di- and particularly monosubstituted species [63,66]. Acidity acts therefore synergistically with the complexing agent, tropolone. In water samples, acidification is needed to break the organotin aquocomplexes, to avoid possible binding to container walls or dissolved matter (*e.g.* humic substances), *etc.*. It also helps to reduce emulsions. In solid samples and particularly in sediments, acidification destroys the carbonates matrix, helps the release of mono- and disubstituted organotins from complexes with sulphur compounds, humics, etc..The use of a low concentration of strong acid is a compromise between effective matrix destruction and the need to avoid speciation changes: phenyltins are very sensitive to pH extremes. Tropolone is a well suited ligand for tin. It is effective also in acidic solutions unlike *e.g.* 8-hydroxy-quinoline and does not give side effects (obnoxious smell) as APDC. It is therefore the ligand of our choice in the analysis of organotins in environmental matrices. Tropolone forms well stable complexes with mono- and diorganotins while triorganotins are not complexed at all at concentrations of environmental concern [116].

c) Ultrasonic bath and rotary evaporator bath temperatures are kept below 40°C to avoid risks of organotins degradation (and consequently speciation changes) or losses by volatilization (care must be taken to avoid the complete drying of the extracts under vacuum).

d) The addition of sodium chloride is necessary to reduce emulsion problems and to enhance back-extraction efficiency from the aqueous solution to methylene chloride.

e) Sequential extraction experiments on sediments and mussels showed that more than 90 % of extracted organotins were in the first extracting aliquot. Two extraction steps are a compromise between speed of analysis and efficiency.

f) Isooctane is added to the extract as a keeper to reduce the risks of evaporative losses.
Solvent exchange is needed because dichloromethane is not compatible with Grignard reagents.

g) CAUTION: Grignard reagents are hazardous chemicals. They violently react with acids, water, alcohols, ketones, *etc.* and should be handled with extreme care by well trained personnel and using opportune safety precautions (gloves, glasses, *etc.*).

h) Sample extracts from sediments or mussels contain high amounts of coextractants and after addition of the Grignard reagent a precipitate is often found. Sonication of the solution is performed to improve sample-to-reagent contact by partial resolubilization of the precipitate.
Although Grignard derivatization is rapid and effective also at room temperature on standard solutions, higher temperatures and time of reaction are necessary to improve recovery from dirty samples. The reaction conditions used do not affect speciation, as experimentally observed.

i) Excess Grignard reagent must be destroyed before cleanup and analysis. Extreme care must be paid by the operators in this step. The drop by drop careful addition of water, keeping the vial in a cold water bath, is necessary to reduce the risks of a too violent reaction. The addition of hexane (or isooctane) before this step reduces the risks of evaporation losses.

l) Recovery tests from cleanup were performed using derivatized standard of butyltins, phenyltins and tripropyltin. Recoveries higher than 90 % were found for all organotins using 5 ml of hexane/benzene.

Derivatized organotins are very stable compounds. Non derivatized organotins are subject to easy decomposition, particularly when they are in solution at room (or higher) temperature as usually happens during the procedure above described. Thus, it is necessary to ensure the shortest time between extraction and derivatization to minimize the risk of decomposition. This is particularly true for phenyltins: a degradation of TPhT directly to MPhT has been sometimes observed as a consequence of a delay in the derivatization.

REFERENCES

[1] R.J. Maguire,*Water Poll. Res. J. Canada*, **26**(3), 243 (1991)
[2] B. Sugavanam, *I.T.R.I. Publication,* **No. 607**, Greenford (1981)
[3] C.J. Evans and R. Hill, *Rev. on Si, Ge, Sn and Pb compds.*, 7, 57 (1983)
[4] A.R.D. Stebbing, *Mar. Poll. Bull.*, **16**, 383 (1985)
[5] M.A. Champ and F.L. Lowenstein, *Oceanus*, **30**, 69 (1987)
[6] M.A. Champ, *Proceedings of the Oceans Organotin Symposium*, vol. 4, Washigton DC, IEEE Piscataway N.J., pp. 1-8 (1986)
[7] P.F. Seligman, R.F. Lee, A.O. Valkirs and P.M. Stang, *Proceedings of the 3rd International Organotin Symposium - Monaco*, pp. 30-38 (1990)
[8] S.J. Blunden and A.H. Chapman, *Environ. Tech. Lett.*, **3**, 267 (1982)
[9] P.M. Stang and P.F. Seligman, *Proceedings of the Oceans - An International Workplace*, Halifax, pp. 1386-1391 (1987)
[10] Ph. Quevauviller and O.F.X. Donard, *Fresenius J. Anal. Chem.*, **339**, 6 (1991)
[11] S. Chiavarini, C. Cremisini and R. Morabito, *MAP Technical Reports Series* , **No. 59**, UNEP/FAO/IAEA, Athens, pp. 179-187 (1991)

[12] D.H. Calloway and J.J McCullen, *Am. J. Chem. Nutr.*, **18**, 1 (1966)
[13] H.A. Schroeder, J.J. Balassa and I.H. Tipton, *J. Chronic. Dis.*, **17**, 483 (1964)
[14] P.J. Smith and L. Smith, *Chem. Brit.*, **11**, 208 (1975)
[15] C.J. Evans and P.J. Smith, *J. Oil Col. Chem. Assoc.*, **58**, 160 (1975)
[16] R.B. Laughlin and O. Linden, *Ambio*, **14**, 88 (1985)
[17] A. Sylph, *I.T.R.I. Publication,* **No. LB 11**, Greenford (1984)
[18] P.J. Smith, *I.T.R.I. Publication,* **No. 538**, Greenford (1978)
[19] P.J. Smith, *I.T.R.I. Publication,* **No. 569**, Greenford (1978)
[20] S.J. Blunden, P.J. Smith and B. Sugavanam, *Pest. Sci.*, **15**, 253 (1984)
[21] S.J. Blunden and A.H. Chapman, in: P.J. Craig Ed., *Organometallic Compounds in the Environment*, Longman, Leichester, pp. 111-139 (1986)
[22] U.S. Environmental Protection Agency, Tributyltin Technical Support Document, *U.S. EPA Position Document* 2/3, Office of Pesticides Programs, Washington, D.C (1987)
[23] S. Chiavarini, C. Cremisini and R. Morabito, in: S. Caroli Ed., *Element speciation in bioinorganic chemistry*, J. Wiley & Sons, Chap. 10 (in press)
[24] R.F. Lee, *Proceedings of the 3rd International Organotin Symposium*, Monaco, pp.70-76 (1990)
[25] C. Zuolian and A. Jensen, *Mar. Pollut. Bull.*, **20**, 281 (1989)
[26] R.E. Anderson, *Mar. Environ. Res.*, **17**, 137 (1985)
[27] R.F. Lee, *Mar. Biol. Lett.*, **2**, 87 (1981)
[28] M.J. Waldock and J.E. Thain, *Mar. Pollut. Bull.,* **14**, 411 (1983)
[29] R.B. Laughlin, W. French and H.E. Guard, *Environ. Sci. Technol.*, **20**, 884 (1986)
[30] J.W. Short and F.P. Thrower, *Proceedings of the Oceans Organotin Symposium*, vol. 4, Washington DC, IEEE Piscataway N.J., pp. 1202-1205 (1986)
[31] T.L. Wade, B. Garcia-Romero and J.M. Brooks, *Environ. Sci. Technol.*, **22**, 1488 (1988)
[32] A. Caricchia, S. Chiavarini, C. Cremisini, M. Fantini, R. Morabito, R. Scerbo and M. Vitali, *Proceedings of Heavy Metals in the Environment*, Toronto, September 1993, pp. 52-55 (1993)
[33] C. Alzieu, J. Sanjuan, J.P. Deltreil and M. Borel, *Mar. Poll. Bull.*, **17**, 494 (1986)
[34] C. Alzieu, *Proceedings of the 3rd International Organotin Symposium*, Monaco, pp. 1-2, (1990)
[35] UNEP/FAO/WHO/IAEA, *MAP Technical Reports Series,* **no. 33**, Athens, 185 pp (1989)
[36] H. Vrijhof, *Sci. Tot. Environ.*, **43**, 221 (1985)
[37] R.J. Huggett, M.A. Unger, P.F. Seligman and A.O. Valkirs, *Environ. Sci. Technol.*, **26**, 232(1992)
[38] Ph. Quevauviller and O.F.X. Donard, in: S. Caroli Ed., *Element speciation in bioinorganic chemistry*, J. Wiley & Sons, Chap. 11 (in press)
[39] R. Morabito, *Microchem. J.*, submitted
[40] A.M. Caricchia, S. Chiavarini, C. Cremisini, R. Morabito and R. Scerbo, *Anal. Chim. Acta* (in press)
[41] O.F.X. Donard, S. Rapsomanikis and J. Weber, *Anal. Chem.*, **58**, 772 (1986)

[42] M. Astruc, R. Pinel and A. Astruc, *Mikrochim. Acta*, **109**(1-4), 73 (1992)
[43] R.J. Maguire, *Environ Sci. Technol.*, **18**, 291 (1984)
[44] M.D. Mueller, *Anal. Chem.*, **59**, 617 (1987)
[45] A.M. Caricchia, S. Chiavarini, C. Cremisini, M. Fantini, and R. Morabito, *Sci. Tot. Environ.*, **121**, 133 (1992)
[46] A.M. Caricchia, S. Chiavarini, C. Cremisini, R. Morabito and R. Scerbo, *Anal. Sci.*, **7**, 1193 (1991)
[47] J.W. Short and F.P. Thrower, *Mar. Pollut. Bull.*, **17**, 542 (1986)
[48] C.D. Rice, F.A. Espourteille and R.J. Huggett, *Appl. Organomet. Chem.*, **1**, 541 (1987)
[49] S. Chiavarini, C. Cremisini, T. Ferri, R. Morabito and A. Perini, *Sci. Tot. Environ.*, **101**, 217 (1991)
[50] K.W.M. Siu, G.J. Gardner and S.S. Berman, *Anal. Chem.*, **61**, 2320 (1989)
[51] C.A. Dooley and G. Vafa, *Proceedings of the Oceans Organotin Symposium*, vol. 4, Washington DC, IEEE Piscataway N.J., pp. 1202-1205 (1986)
[52] V. Desauziers, F. Leguille, R. Lavigne, M. Astruc and R. Pinel, *Appl. Organomet Chem.*, **3**, 469 (1989)
[53] T. Tsuda, M. Wada, S. Aoki and Y. Matsui, *J. Assoc. Off. Anal. Chem.*, **71**, 373 (1988)
[54] J.H. Weber, J.S. Han and R. Francois, in: M. Astruc and J.N. Lester Eds. *Heavy metals in the hydrological cycle*, London, pp. 395-400 (1988)
[55] R.J. Maguire, R.J. Tkacz, Y.K. Chau, G.A. Bengart and P.T. Wong, *Chemosphere*, **3**, 253 (1986)
[56] D.J. Hannah, T.L. Page, L. Pickstone and J.A. Taucher, *Bull. Environ. Contam. Toxicol.*, **43**, 22 (1989)
[57] J. Bayona and J. Dachs, this book.
[58] J.S. Han and J.H. Weber, *Anal. Chem.*, **60**, 316 (1988)
[59] C.L. Matthias, J.M. Bellama, G.J. Olson and F.E. Brinckman, *Int. Environ. Anal. Chem.*, **35**, 61 (1989)
[60] L. Randall, O.F.X. Donard and J.H. Weber, *Anal. Chim. Acta,* **184**, 197 (1986)
[61] C.L. Matthias, J.M. Bellama, G.J. Olson and F.E. Brinckman, *Environ. Sci. Technol.*, **20**, 609 (1986)
[62] A.O. Valkirs, P.F. Seligman, P.M. Stang, V. Homer, S.H. Lieberman, G. Vafa and C.A. Dooley, *Mar. Pollut. Bull.*, **17**, 319 (1986)
[63] H.A. Meinema, T. Burger-Wiersma, G. Versluis-de Haan and E. Gevers, *Environ. Sci. Technol.*, **12**, 288 (1978)
[64] M.D. Mueller, *Fresenius Z. Anal. Chem.,* **317**, 32 (1984)
[65] Y.K. Chau, P.T.S. Wong and G.A. Bengert, *Anal. Chem.*, **54**, 246 (1982)
[66] R.J. Maguire and H. Huneault, *J. Chromatog.*, **209**, 458 (1981)
[67] M.A. Unger, W.G. McIntytre, J. Greaves, and R.J. Hugget, *Chemosphere*, **15**, 461 (1986)
[68] A.M. Caricchia, S. Chiavarini, C. Cremisini, R. Morabito and C. Ubaldi, *Int. J. Environ. Anal. Chem.*, **53**, 37 (1993)
[69] M.P. Stang and P.F. Seligman, *Chemosphere*, **15**, 1256 (1986)

[70] J.R. Ashby and P.J. Craig, *Sci. Total Environ.*, **78**, 219 (1989)
[71] F. Martin, R. Ritsema and Ph. Quevauviller, this book.
[72] J.A. Stab, B. van Hattum, P. de Voogt, and U.A.Th. Brinkman, *Mikrochim. Acta*, **109**, 101 (1992)
[73] O.F.X. Donard and R. Pinel, in: R.M. Harrison and S. Rapsomanikis Eds. *Environmental analysis using chromatography interfaced with atomic spectroscopy* , Chap. 7, pp. 189-222. Hellis Horwood Limited, (1989)
[74] W.A. Aue and Ch.G Flinn, *J. Chromatogr.*, **142**, 145 (1977)
[75] W.A. Aue and Ch.G Flinn, *Anal. Chem.*, **52**, 1537 (1980)
[76] I. Tolosa, J.M. Bayona, J. Albaigés, L.F. Alencastro and J. Tarradellas, *Fresenius J. Anal. Chem.*, **339**, 646 (1991)
[77] S. Kapila and C.R. Vogt, *J. Chromatogr. Sci.*, **18**, 144 (1980)
[78] B.W. Wright, M.L. Lee, G.M. Booth, *J. High Resolut. Chromatr. Chromatogr. Commun.*, **1**, 189 (1979)
[79] Y. Hattori, A. Kobayashi, S. Takemoto, K. Takami, Y. Kuge, A. Sugimae and M. Nakamoto, *J. Chromatogr.*, **315**, 341 (1984)
[80] T. Tsuda, H. Nakanishi,S. Aoki and J. Takebayashi, *J. Chromatogr.*, **387**, 361 (1987)
[81] O. Evans, B.J. Jacobs and A.L. Cohen, *Analyst*, **116**, 15 (1991)
[82] C.J. Soderquist and D.G. Crosby, *Anal Chem.*, **50**(11), 1435 (1978)
[83] W. Dirkx, R. Lobinski and F.C. Adams, this book.
[84] S. Hill, A. Brown, C. Rivas, S. Sparkes and L. Ebdon, this book.
[85] E.J. Parks, W.R. Blair and F.E. Brinckman, *Talanta*, **32**, 633 (1985)
[86] T.M. Vickrey T.M. and G.V. Harrison G.V., *Anal. Chem.*, **53**, 1573 (1981)
[87] T. Ferri, R. Morabito and A. Perini, in: M. Astruc and J.N. Lester Eds., *Heavy Metals in the Hydrological Cycle*, London, pp. 413-418 (1988)
[88] S. Chiavarini, C. Cremisini, T. Ferri, R. Morabito and C. Ubaldi, *Appl. Organomet. Chem.*, **6**, 147 (1992)
[89] D.T. Burns, M. Harriott and F. Glockling, *Fresenius Z. Anal. Chem.*, **327**, 701 (1987)
[90] K. Hasebe, Y. Yamamoto and T. Kambara, *Fr. Z. Anal. Chem.*, **310**, 234 (1982)
[91] P. Kenis and A. Zirino, *Anal. Chim. Acta*, **149**, 157 (1983)
[92] S. Chiavarini, C. Cremisini, T. Ferri, R. Morabito and F. Roberti, Euroanalysis VIII, Edimburgh, September 5-11 (1993)
[93] M. Ochsenkuhn-Petropoulou, G. Poulea and G. Parissakis, *Mikrochim. Acta*, **109**(1-4), 93 (1992)
[94] S.J. Blunden and A.H. Chapman, *Analyst,* **103**, 1266 (1978)
[95] Y. Arakawa, O. Wada and M. Manabe, *Anal. Chem.*, **55**, 1901 (1983)
[96] L.R. Sherman and T.L. Carlson, *J. Anal. Toxicol.*, **4**, 31 (1980)
[97] M. Omar and H.J.M. Bowen, *Analyst*, **107**, 654 (1982)
[98] H. Suyani, J. Creed, J. Caruso and R. Duane Satzger, *J. Anal. At. Spectrom.*, **4**, 777 (1989)
[99] S. Branch, L. Ebdon, S. Hill and P. O'Neill, *Anal. Proc. (London)*, **26**, 401 (1989)
[100] H. Suyani, J. Creed, T. Davidson and J. Caruso, *J. Chromatogr. Sci.*, **27**, 139, (1989)
[101] W.L. Shen, N.P. Vela, B.S. Sheppard and J.A. Caruso, *Anal. Chem.*, **63**, 1491 (1991)

[102] N.P. Vela and J.A. Caruso, *J. Anal. At. Spectrom.*, **7**, 971 (1992)
[103] S.M. Colby, M. Stewart and J.P. Reilly, *Anal. Chem.*, **62**, 2400 (1990)
[104] C.A. Dooley, *NOSC Technical Report 1090*, Jan. 1986, San Diego CA, USA.
[105] D.J. Hannah, T.L. Page, L. Pickston and J.A. Taucher, *Bull. Environ. Contam. Toxicol.*, **43**, 22 (1989)
[106] K.W.M. Siu, P.S. Maxwell, S.S. Berman, *J. Chromatogr.*, **475**, 373 (1989)
[107] R. Weber, F. Visel and K. Levsen, *Anal. Chem.*, **52**, 2299 (1980)
[108] T.E. Stewart and R.D. Cannizzaro, in "*Pesticides Analytical Methodology*", ACS Symposium Series No. 136, American Chemical Society, pp 367-388 (1980)
[109] B. Humphrey and D. Hope, in *Proceedings Oceans '87 Conference*, Ed. W. MacNab, Halifax, Canada, p 1348 (1987)
[110] N.V. Fehringer and S.W. Walters, *J. High Res. Chromatogr.*, **13**, 575 (1990)
[111] C.C. Gilmour, J.H. Tuttle and J.C. Means, *Anal. Chem.*, **58**, 1848 (1986)
[112] J. Greaves and M.A. Unger, *Biomed. Mass Spectrom.*, **15**, 565 (1988)
[113] G.A. Junk and J.J. Richard, *Chemosphere*, **16**, 61 (1987)
[114] G.A. Junk and J.J. Richard, *J. Res. Natl. Bur. Stand.*, **93**, 274 (1988)
[115] R.G. Huggett, M.A. Hunger, F.A. Espourteille and C.D. Rice, *J. Res. Natl. Bur. Stand.*, **93**, 277 (1988)
[116] A. Astruc, M. Astruc, R. Pinel and M. Potin-Gautier, *Appl. Organomet. Chem*, **6**, 39 (1992)