REGIONAL SEAS

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Determination of organotins in environmental samples

Reference Methods For Marine Pollution Studies No. 59

UNEP 1994

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PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes 12 regions and has some 140 coastal States participating in it (1), (2).

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment and of its resources and of the sources and trends of the pollution, and the impact of pollution on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies is being developed as part of a programme of comprehensive technical support which includes the provision of expert advice, reference methods and materials, training and data quality assurance (3). The methods are recommended to be adopted by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialized bodies of the United Nations systems as well as other organizations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines the style used by the International Organization for Standardization (ISO) is followed as closely as possible.

The methods and guidelines, as published in UNEP's series of Reference Methods for Marine Pollution Studies, are not considered as final. They are planned to be periodically revised taking into account the development of our understanding of the problems, of analytical instrumentation and the actual need of the users. In order to facilitate these revisions the users are invited to convey their comments and suggestions to:

Marine Environmental Studies Laboratory
IAEA Marine Environment Laboratory
B.P. No. 800
MC-98012 MONACO Cedex

which is responsible for the technical co-ordination of the development, testing and intercalibration of Reference Methods.

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(1) UNEP: Achievements and planned development of the UNEP's Regional Seas Programme and comparable programmes sponsored by other bodies. UNEP Regional Seas Reports and Studies No. 1. UNEP, 1982.

(2) P. HULM: A strategy for the Seas. The Regional Seas Programme: Past and Future. UNEP, 1983.

(3) UNEP/IAEA/IOC: Reference Methods and Materials: A Programme of comprehensive support for regional and global marine pollution assessments. UNEP, 1990.

The present document was prepared by Dr. M. Waldock of the Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, Burnham-on-Crouch, United Kingdom. The methods described within this Reference Method have proven to be robust and effective within monitoring programmes. Various choices are afforded within the methods described to allow for the availability of equipment, etc. and range from simple screening for organotin contamination using less specialist atomic absorption equipment, to high resolution separation and sensitive and selective quantification of individual species. The document does not attempt to provide details of all available techniques. Further information concerning other protocols can be found through a UNEP bibliography on marine pollution by organotin compounds [4], or alternatively through a review article by Müller et al on the analysis of organotins [5].

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(4) UNEP: Bibliography on marine pollution by organotin compounds. MAP Technical Reports Series No. 35. UNEP, Athens, 1989.

(5) D. Müller, L. Renberg and G. Rippen (1989): Tributyltin in the Environment - Sources, Fate and Determination. An assessment of Present Status and Research Needs. Chemosphere, 18 (9/10), 2015-2042.

CONTENTS

1. SCOPE AND FIELD OF APPLICATION

This publication describes reference methods for the determination of organotin compounds in environmental samples. Inputs of organotin compounds to the aquatic environment arise from the use of triorganotins as biocides and diorganotins as catalysts and stablisers in the plastics industry. Tributyltin (TBT) and triphenyltin (TPhT) compounds are widely used as the toxic component in boat antifouling preparations. Either compound may be simply mixed into the paint matrix, but tributyltin may be used in a copolymer formulation, chemically bonded to methyl methacrylate to provide a self polishing paint which chemically erodes by hydrolysis of TBT from the methacrylate chain. This controlled release of toxin confers a much longer active life to the paints, and hence TBT based formulations have become the most commonly used antifouling preparations in Europe. TBT and TPhT are also used in antifouling preparations to protect nets used to contain salmon in mariculture. Indirect inputs of TPhT to the aquatic environment may also occur as a result of the use of TPhT in agriculture as a fungicide, and from TBT used as a timber preservative.

TBT is commonly detected in environmental samples close to harbours, and popular pleasure-craft centres. In water, sediments and shellfish, the parent compound is most abundant, but the breakdown products dibutyltin and monobutyltin and inorganic tin may also be found. The breakdown route for TPhT is also by sequential de-arylation to inorganic tin. Typically, concentrations of TBT found in harbour waters may be in the upper nanogram to microgram per litre range, and more remote from harbours, but within the same estuaries, in the sub- to tens of nanograms per litre range.

Such concentrations are toxic to a variety of marine and freshwater species, and molluscs have been shown to be particularly sensitive to TBT. The lowest concentration of TBT known to elicit a biological effect is for the dogwhelk *Nucella lapillus* which exhibits a phenomenon termed imposex, whereby male sexual characteristics are imposed on the female of the species. Sterility of female dogwhelks occurs at approximately 5 ng TBT l^{-1} and the no-effect concentration for the response has been estimated to be less than one nanogram per litre (Gibbs *et al*, 1988).

Since organotins are highly toxic to aquatic organisms, methods of analysis must be adequate for the determination of trace quantities (nanograms per litre or gram) of analyte. The methods described here are designed to be rapid and robust and should allow the processing of large numbers of samples in support of monitoring programmes.

Methods fall into two categories, (a) those using Atomic Absorption Spectrophotometry (AAS), and (b) those using Gas Chromatography (GC). The former allow screening for organotin contamination of samples using less specialist equipment. The latter are recommended for sensitivity, and detailed speciation.

2. REFERENCES

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3. GENERAL CONSIDERATIONS AND SAMPLE HANDLING

Contamination and losses:

Contamination of laboratory apparatus with organotin compounds arises from the use of diorganotin stabilising compounds in many plastics, e.g. PVC, and use of such materials should be avoided. However, organotins are readily adsorbed onto glassware, and losses may occur during use of glass equipment. The methods described here use the minimum of glassware and wherever possible use the same vessel for sampling and extraction.

Preparation of glassware:

Before use, all glassware is soaked in a suitable non-contaminating laboratory detergent (e.g. 10% Pyroneg) for 8h, then rinsed with tap water and soaked for a further 8h in conc. HCl (Analar). Finally it is rinsed in double distilled water and dried in a glass oven at 50°C.

Sample collection and storage:

Water samples are collected in 2.7 l glass bottles with PTFE lined screw capped lids. Before use they are rinsed with conc. HCl, tap water, and finally solvent rinsed.

During use, water sampling bottles are either hand held (for surface and sub-surface samples) or mounted in a weighted stainless steel frame, which is deployed by means of a nylon rope (see fig. 1). Water samples are stored in a cool room (4 °C) and extracted within 48 h of collection. Extracts have been held at -20°C for several months with no measurable breakdown of TBT. TBT hydrides do, however, revert to TBT chlorides on storage and in the hydride derivative technique for organotin speciation, additional sodium borohydride is added to extracts if previously stored (see Section 5).

Shellfish are stored as whole animals in polyethylene bags, and held at -20°C. After dissection and homogenisation, samples may also be stored by freezing in glass jars. No measurable breakdown of TBT in stored samples has been recorded after holding at -20°C for six months.

Sediment samples are collected by scraping the surface layer (top 1 cm) into clean glass jars, using a PTFE coated spatula. Samples are stored at -20°C prior to analysis.

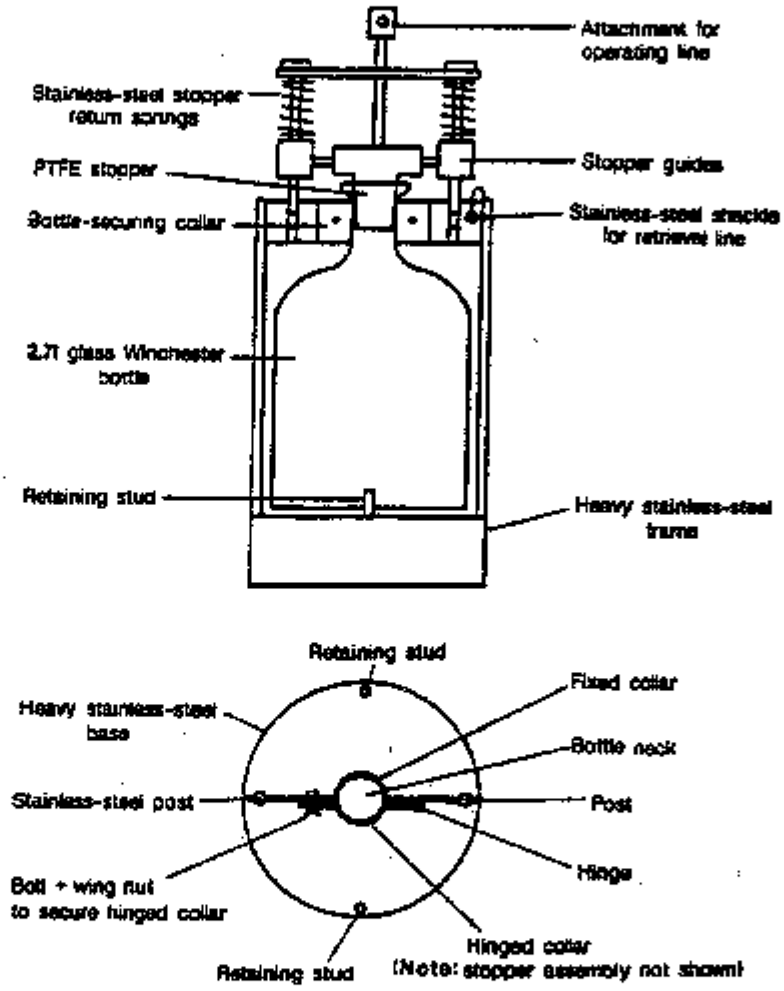

Fig. 1. Stainless steel and PTFE water sampler.

4. ATOMIC ABSORPTION SPECTROPHOTOMETRIC METHODS

4.1. DETERMINATION OF TOTAL TIN CONCENTRATIONS IN WATER

4.1.1. PRINCIPLE

Water samples are acidified, tin containing compounds are bound to a complexing agent (tropolone), and extracted into toluene. The extract is analysed by electrothermal atomic absorption spectrophotometery (EAAS). The detection limit is approx. 0.1 $\mu g\ l^{-1}$ Sn in the original sample.

4.1.2. REAGENTS

Hydrobromic acid, 47%, s.g. 1.46-1.49 (e.g. AristaR).

Toluene/tropolone reagent: 1.25g tropolone (pure; m.p. 49-51°C) dissolved in 2.5l toluene (99.5% or better e.g. AnalaR).

Bis (tributyltin) oxide [hexabutyldistannoxane] (TBTO) standards (97.6% pure or better). Stock solutions are prepared from 0.0512 g (allowing for impurity) TBTO dissolved in glacial acetic acid and made up to 50 ml. Intermediate stock solutions are prepared by serial dilution into glacial acetic acid to 10 mg l^{-1} (w.s.1) and 1 mg l^{-1} (w.s.2). These are stable for at least three months. Instrument calibration solutions (in toluene) in the range 0.005 to 0.2 mg l^{-1} are prepared by adding suitable aliquots of w.s. 1 or 2 to 10 ml toluene.

4.1.3. APPARATUS

1 l glass sampling bottles (calibrated at 750 ml), with PTFE lined screw-capped lids.

Polyethylene capped centrifuge tubes, 1.9 ml capacity.

A range of volumetric flasks, graduated measuring cylinders and pipettes.

An orbital shaker capable of holding the sample bottles.

Centrifuge with rotor to accept the centrifuge tubes described above.

An atomic absorption spectrophotometer adapted for electrothermal operation to be operated in accordance with the manufacturers instructions. The use of an autosampler is beneficial.

4.1.4. METHOD

750 ml of water is collected in a calibrated 1 l glass sampling bottle, 20 ml of hydrobromic acid are added, mixed and allowed to stand for 15 min. 25 ml of the toluene reagent is added and the bottle capped. The bottle is then shaken on an orbital shaker for 10 min. After standing to allow phase separation, the toluene extract is transferred to a polyethylene centrifuge tube and centrifuged at 2500 rpm for 5 min to separate traces of water from the solvent. The toluene extract is then analysed by electrothermal atomic absorption spectrophotometry (EAAS).

Calibration standards in the range 0.005 mg l^{-1} to 0.2 mg l^{-1} Sn in toluene are prepared from the intermediate stock solutions(see Section 4.1.2.). Duplicate reagent blanks are prepared using tin-free distilled water and analysed with each batch of samples. Spiked samples are prepared to monitor extraction efficiency. The sample extracts are reasonably stable, but if not analysed immediately after preparation, should be stored in the dark and cool (4°C) to prevent photolytic decay and evaporative loss, and analysed within 24 hours.

AAS operating conditions:

The following conditions have been found to give optimum performance on a PyeUnicam SP 9:

Wavelength 286.3 nm

Deuterium background correction

Uncoated graphite tubes

Dry	120°C	35 sec
Ash	600°C	30 sec
Atomise	2800°C	3 sec
Clean up	2900°C	3 sec

4.1.5. SUMMARY OF METHOD

Total tin in water:

Sample, 750 ml water/blank/recovery standard

add 20 ml HBr

Stand 15 min

add 25 ml toluene/tropolone

shake

stand

toluene extract to centrifuge tube

centrifuge

analyse by EAAS

4.1.6. CALCULATION OF RESULTS

The concentration of tin (C) in the sample is given by:

$$C = \frac{T \times v}{V \times Rf} \quad \mu g\ l^{-1}\ Sn$$

where:
T = concentration of tin measured in the toluene extract in $\mu g\ ml^{-1}$
v = volume of extract in ml
V = volume of sample taken in litres
Rf = recovery factor for the determination of a known amount of stannous chloride spiked into a tin free sample (see 4.1.7.).

4.1.7. PRECISION AND ACCURACY

The recovery of stannous chloride spiked into a tin free sample at a concentration of 1 $\mu g\ l^{-1}$ was found to be 81% (SD=7, n=8)(Rf=0.81). The technique of standard additions to samples has generally shown that the method is generally free from matrix interferences. Parks *et al.* (1985) have listed some possible matrix interference problems, and where matrix problems are suspected, standard additions should be used to verify results.

4.2. DETERMINATION OF ORGANOTIN CONCENTRATIONS IN WATER

4.2.1. PRINCIPLE

By omission of the tropolone complexing agent in the previous method (Section 4.1), organotin compounds are extracted, but not inorganic tin compounds. In this procedure glacial acetic acid has been demonstrated to give better extraction efficiency than HBr for organotins. The detection limit of the method is approx 0.04 $\mu g\ l^{-1}$ Sn in the original sample.

4.2.2. REAGENTS

Toluene, 99.5% purity or better (e.g. AnalaR).

Glacial acetic acid, s.g. 1.049, 99.8% (e.g. AnalaR).

TBTO standards (97.6% pure or better). Stock solutions are prepared from 0.0512 g (allowing for impurity) TBTO dissolved in glacial acetic acid and made up to 50 ml. Intermediate stock solutions are prepared by serial dilution into glacial acetic acid to 10 mg l^{-1} (w.s.1) and 1 mg l^{-1} (w.s.2). These are stable for at least three months. Instrument calibration solutions (in toluene) ranging from 0.005 to 0.2 mg l^{-1} are prepared by adding suitable aliquots of w.s. 1 or 2 to 10 ml toluene.

4.2.3. APPARATUS

2.7 l glass sampling bottles (calibrated at 2 l) with PTFE lined screw-capped lids.

Polyethylene capped centrifuge tubes, 1.9 ml capacity.

A range of volumetric flasks, graduated measuring cylinders and pipettes.

An orbital shaker capable of holding the sample bottles.

Centrifuge with rotor to accept the centrifuge tubes described above.

An atomic absorption spectrophotometer adapted for electrothermal operation to be operated in accordance with the manufacturers instructions. The use of an autosampler is beneficial.

4.2.4. METHOD

The procedure is essentially the same as described for total tin, but 25 ml of toluene is substituted for the toluene/tropolone reagent used in method 4.1.

A 2 l sample of water is collected in a calibrated 2.7 l bottle. 50 ml of glacial acetic acid are added, mixed and allowed to stand for 15 min. 25 ml of toluene is added and the bottle capped. The bottle is then shaken on an orbital shaker for 10 min. After standing to allow phase separation, the toluene extract is transferred to a polyethylene centrifuge tube and centrifuged at 2500 rpm for 5 min to separate traces of water from the solvent. The toluene extract is then analysed by electrothermal atomic absorption spectrophotometry (EAAS).

Calibration standards in the range 0.005 mg l^{-1} to 0.2 mg l^{-1} Sn in toluene are prepared from the intermediate stock solutions (see section 4.2.2.). Duplicate reagent blanks and spiked samples are prepared using tin-free distilled water and analysed with each batch of samples. Spiked samples are prepared to monitor extraction efficiency.

The AAS operating conditions are described in Section 4.1.4

4.2.5. SUMMARY OF METHOD

Organotins in Water:

Sample 2l/blank/recovery standard

add 50 ml acetic acid

stand 15 min.

add 25 ml toluene

shake

stand

decant toluene extract to centrifuge tube

centrifuge

analyse by EAAS

4.2.6. CALCULATION OF RESULTS

The concentration of organotin (as TBT equivalents) (C) in the sample is given by:

$$C = \frac{T \times v \times 2.5}{V \times Rf} \quad \mu g\ l^{-1}\ TBT$$

where:
T = concentration of tin measured in the toluene extract in $\mu g\ ml^{-1}$
v = volume of extract in ml
V = volume of sample taken in litres
Rf = recovery factor for the determination of a known amount of TBT spiked into a tin free sample (see 4.2.7).

4.2.7. PRECISION AND ACCURACY

Typical recoveries of TBT by this method are 96 $\pm$ 15% (Rf=0,96), but in the hands of a skilled analyst can be better. In an intercalibration exercise organised by the US National Bureau of Standards in 1986, a reference water was supplied containing an undisclosed concentration of TBT. After 1000 fold dilution, the above method produced a result of 1.06 $\mu g\ l^{-1}$ (SD= 0.05, n= 5). The standard was later certified to contain 1.06 $\pm$ 0.05 mg l^{-1} TBT (Blair *et al*, 1986).

Recovery of dibutyltin by this method is low (approximately 16%) because DBT is more water soluble than TBT and does not readily partition into the solvent phase.

The AAS methods described in Sections 4.1 and 4.2 above were developed by M&T Chemicals Ltd. (see Trachman *et al*, 1977 for background), and use of the organotin method has been described for seawater (Cleary and Stebbing 1985). Cleary has since increased the sensitivity of the method approximately 10 fold by introducing an evaporation step at the end of the procedure (Cleary pers.comm., Anon 1992). See also Parks *et al* (1985) for descriptions of possible matrix interferences. The United Kingdom Standing Committee of Analysts also recommend lanthanum coated furnace tubes to increase sensitivity (Anon 1992).

4.3. THE DETERMINATION OF TOTAL TIN CONCENTRATIONS IN SHELLFISH TISSUE

4.3.1. PRINCIPLE

Shellfish tissue is dispersed by mixed acid digestion, total tin is extracted by toluene and tropolone, and analysed by EAAS. The limit of detection is 0.05 μg Sn g^{-1} wet weight in the original sample.

4.3.2. REAGENTS

Nitric acid, 70%, s.g. 1.42 (e.g. AnalaR)

Sulphuric acid, 98%, s.g 1.84 (e.g. AnalaR)

Perchloric acid 70%, s.g. 1.70 (e.g. AnalaR).

Hydrobromic acid, 47%, s.g.1.46-1.49 (e.g. AristaR).

Toluene/tropolone reagent: 1.25g tropolone (pure m.p. 49-51°C) dissolved in 2.5l toluene (99.5% or better e.g. AnalaR).

TBTO standards (97.6% pure or better). Stock solutions are prepared from 0.0512 g (allowing for impurity) TBTO dissolved in glacial acetic acid and made up to 50 ml. Intermediate stock solutions are prepared by serial dilution into glacial acetic acid to 10 mg l^{-1} (w.s.1) and 1 mg l^{-1} (w.s.2). These are stable for at least three months. Instrument calibration solutions (in toluene) ranging from 0.005 to 0.2 mg l^{-1} are prepared by adding suitable aliquots of w.s. 1 or 2 to 10 ml toluene.

4.3.3. APPARATUS

Top drive all stainless steel homogeniser (e.g. Ultraturrax).

30 ml capacity glass centrifuge tubes with PTFE lined screw-capped lids.

A range of volumetric flasks, graduated measuring cylinders and pipettes.

A heating block capable of heating the centrifuge tubes to 100°C.

A wrist action shaker capable of holding the centrifuge tubes described above.

Centrifuge with rotor to accept the centrifuge tubes described above.

An atomic absorption spectrophotometer adapted for electrothermal operation to be operated in accordance with the manufacturers instructions. The use of an autosampler is beneficial.

4.3.4. METHOD

1g of wet tissue homogenate is digested with 5 ml 1:1 nitric/sulphuric acid in a heated open centrifuge tube (100°C for 4 h.). 0.5 ml of perchloric acid is then added and the digest heated for another hour. After cooling, 23 ml of distilled water is added, followed by 2 ml of hydrobromic acid. The aqueous extract is then extracted by 5 ml of toluene reagent by shaking for 20 min. The toluene extract is then analysed by EAAS (see section 3.1.4.). Duplicate procedural blanks and quality control tissues are analysed with each batch of samples. Tissues spiked with TBTO are used to monitor extraction efficiency.

The sample extracts are stable, but if not analysed immediately after preparation should be stored cool (4°C) to prevent evaporative loss, and analysed within 24 hours.

4.3.5. SUMMARY OF METHOD

Total tin in Shellfish:

1 g sample/AQC material/blank/spiked sample

add nitric/sulphuric acid

heat 100°C 4 h.

add 0.5 ml perchloric acid

heat 100°C;1 h

cool, add 23 ml water

add 2ml HBr

extract into 5ml toluene/tropolone

analyse by EAAS

4.3.6. CALCULATION OF RESULTS

The concentration of organotin (as Sn equivalents) (C) in the sample is given by:

$$C = \frac{T \times v}{W \times Rf} \quad \mu g\ g^{-1}\ Sn$$

where:
T = concentration of tin measured in the toluene extract in $\mu g\ ml^{-1}$
v = volume of extract in ml
W = weight of sample in g.
Rf = recovery factor for the determination of a known amount of stannous chloride spiked into a tin free sample (see 4.3.7).

4.3.7. PRECISION AND ACCURACY

The mean recovery of stannous chloride spiked into oyster tissue at a concentration of 1 $\mu g\ g^{-1}$ was found to be 95% (SD=16; n=8)(Rf=0.95). The technique of standard additions to samples has shown that the method is generally free from matrix interferences. Parks *et al* (1985) have listed some possible matrix interference problems, and where matrix problems are suspected standard additions should be used to verify results.

4.4. THE DETERMINATION OF TRIORGANOTIN CONCENTRATIONS IN SHELLFISH TISSUE

4.4.1. PRINCIPLE

As in the method above, the tissue is dispersed in acid and organotin extracted by solvent. Specificity for triorganotin compounds is achieved by washing the solvent with sodium hydroxide to partition mono- and diorganotin compounds into the aqueous phase. The solvent extract containing triorganotin compounds is then analysed by EAAS. The limit of detection of the method is approx 0.04 $\mu g\ g^{-1}$ Sn in the original sample.

4.4.2. REAGENTS

Hydrochloric acid, 35.4%, s.g. 1.18 (e.g. AnalaR).

Sodium Hydroxide 99% (e.g. AnalaR).

Hexane (glass distilled grade).

4.4.3. APPARATUS

Top drive all stainless steel homogeniser (e.g. Ultraturrax).

30 ml capacity glass centrifuge tubes with PTFE lined screw-capped lids.

A range of volumetric flasks, graduated measuring cylinders and pipettes.

A wrist action shaker capable of holding the centrifuge tubes described above.

Centrifuge with rotor to accept the centrifuge tubes described above.

An atomic absorption spectrophotometer adapted for electrothermal operation to be operated in accordance with the manufacturers instructions. The use of an autosampler is beneficial.

4.4.4. METHOD

1.5g of wet tissue homogenate is dispersed (by shaking by hand) with 10 ml concentrated HCl in a centrifuge tube. 10 ml of hexane is added and the extract shaken for 30 min, and then centrifuged at 2500 rpm for 15 min. Four ml of the hexane extract is transferred to another centrifuge tube and 8 ml of 3% sodium hydroxide added. The extract is shaken for 10 min, it is then centrifuged at 2500 rpm for 15 min, and an aliquot of the hexane extract analysed using EAAS (Section 3.1.4). Duplicate procedural blanks and a quality control tissue are analysed with each batch of samples. Few certified reference materials are available for TBT analysis (the National Research Council of Canada, Marine Analytical Chemistry Standards Program material PACS1 is certified for TBT, DBT and MBT) alternatively quality control tissues may be prepared in house by collection of a large amount of naturally contaminated shellfish from harbour areas. Tissues spiked with TBTO are used to monitor extraction efficiency.

The sample extracts are reasonably stable, but if not analysed immediately after preparation should be stored in the dark and cool (4°C) to prevent photolytic decay and evaporative loss, and analysed within 24 hours.

4.4.5. SUMMARY OF METHOD

Triorganotins in Shellfish:

1.5 g sample/AQC material/blank/spiked sample

disperse with 10 ml HCl

add 10 ml hexane

shake 30 min.

centrifuge

decant 4ml hexane and add 8 ml sodium hydroxide

shake 10 min.

centrifuge

analyse hexane by EAAS

4.4.6. CALCULATION OF RESULTS

The concentration of organotin (as TBT equivalents) (C) in the sample is given by:

$$C = \frac{T \times v}{W \times Rf} \times 2.5\ \mu g\ g^{-1}\ TBT$$

where:
T = concentration of tin measured in the toluene extract in μgSn ml^{-1}
v = volume of extract in ml
W = weight of sample in g.
Rf = recovery factor for the determination of a known amount of stannous chloride spiked into a tin free sample (see 4.4.7).

4.4.7. PRECISION AND ACCURACY

The recovery of TBTO spiked into shellfish tissue samples was found to be 100% (SD=6 n=8)(Rf=1). In an intercomparison of methods for analysis of mussel tissue, the method gave results at the lower end of the reported range of results, e.g. 0.18 μg g^{-1} SD 0.2. Mean of all samples 0.26 μg g^{-1} (Stephenson et al, 1987).

The above methods for tissue analysis were based on those developed by M & T chemicals Ltd. and are essentially those described by Bryan *et al.* (1986). See also Dooley *et al.* (1986).

5. GAS CHROMATOGRAPHIC METHODS

5.1. SPECIATION OF ORGANOTIN HYDRIDES IN WATER BY GAS CHROMATOGRAPHY

5.1.1. PRINCIPLE

Organotin compounds are reduced to the hydride form (to improve compatibility with capillary GC analysis) and simultaneously extracted into dichloromethane. The extract is reduced in volume and injected into a gas chromatograph fitted with a flame photometric detector (FPD). The limit of detection for TBT compounds is approximately 1 ng l^{-1}. The method was developed by Matthias *et al* (1986). It should be noted that the method is also suitable for the determination of triphenyltin (TPhT) compounds. However the flame photometric detection method is less sensitive for TPhT and detection limits are an order or magnitude higher.

5.1.2. REAGENTS

Sodium borohydride pellets 0.25 g , Atomic absorption spectroscopic grade (e.g. Spectrosol).

Dichloromethane (glass distilled grade).

Authentic standards:
Butyltin trichloride
Dibutyltin dioxide
Bis (tributyltin) oxide
Tripropyltin chloride (Internal standard).
Triphenyltin chloride.

Stock solutions of monobutyltin and dibutyltin compounds are prepared by dissolution in methanol and those for tributyltin, in dichloromethane. Individual stock solutions of standards are prepared by dissolving 60 mg of each compound in 100 ml of solvent to produce a solution containing 0.6 mg ml^{-1} of each compound.

Individual working solutions are prepared by dissolving 0.5 ml of stock solution in 50 ml of dichloromethane to give 6 μg ml^{-1}.

A 20 μl aliquot of internal standard (tripropyltin chloride) is added to each 2 l sample.

A composite working solution may be prepared by mixing mono- di- and triorganotin species. This composite may be deravitized by the extraction method listed below and used to determine the recovery of alkyltin compounds and the relative response factors for each compound detected by the flame photometric detector.

5.1.3. APPARATUS

2.7 l glass "winchester" type plastic coated "safebreak" type sample bottles calibrated at 2l with a PTFE lined screw-capped lid.

A range of volumetric flasks, graduated measuring cylinders and pipettes.

All glass and teflon micropipettes.

30 ml capacity glass centrifuge tubes with PTFE lined screw-capped lids..

5 ml tapered vials with PTFE lined screw-capped lids (e.g."Reactivials").

PTFE tap (see Fig 2).

A wrist action shaker capable of holding the centrifuge tubes described above.

Centrifuge with rotor to accept the centrifuge tubes described above.

Orbital shaker capable of holding the sample bottles.

Gas Chromatograph. Capillary column instrument with temperature programming and flame photometric detection, fitted with a 25 m x 0.32 mm ID fused silica capillary column and coated with a cross-linked 5% phenyl methyl silicone fluid with a film thickness of 0.52 μm. A retention gap consisting of one metre of deactivated, uncoated fused silica (0.53 mm ID) is attached to the injection end of the analytical column in order to allow on column injection and better chromatographic resolution. The use of an autosampler and electronic data capture system is beneficial.

5.1.4. METHOD

A 2 l sample of water is collected in a calibrated 2.7 l bottle. A sodium borohydride pellet (0.25 g) and 40 ml of dichloromethane are added, followed by an appropriate amount of internal standard (20 μl for samples containing 1 to 100 ng l^{-1} TBT). The bottle is capped and shaken by hand for a few seconds. Internal pressure is then released and the bottle is shaken on an orbital shaker for 15 min. ***WARNING* At this stage the vessel is pressurised due to hydrogen evolution and should be handled with care.** The pressure is released and the bottle allowed to stand while the solvent phase separates out. The cap of the bottle is then replaced with the tap shown in Fig.2. The dichloromethane is drawn off into a centrifuge tube, and centrifuged at 2500 rpm for 5 min. Any water in the tube is removed using a Pasteur pipette and the extract blown down to approx 3 ml using a gentle stream of compressed air at ambient temperature (the use of a heating block set at 30°C hastens this step in the procedure). The extract is then transferred to a 5 ml capacity tapered vial and blown down to approximately 200 μl. 1 μl of the extract is then introduced to the GC system.

Note: Sample extracts stored in dichloromethane revert to alkyl tin chlorides within hours of

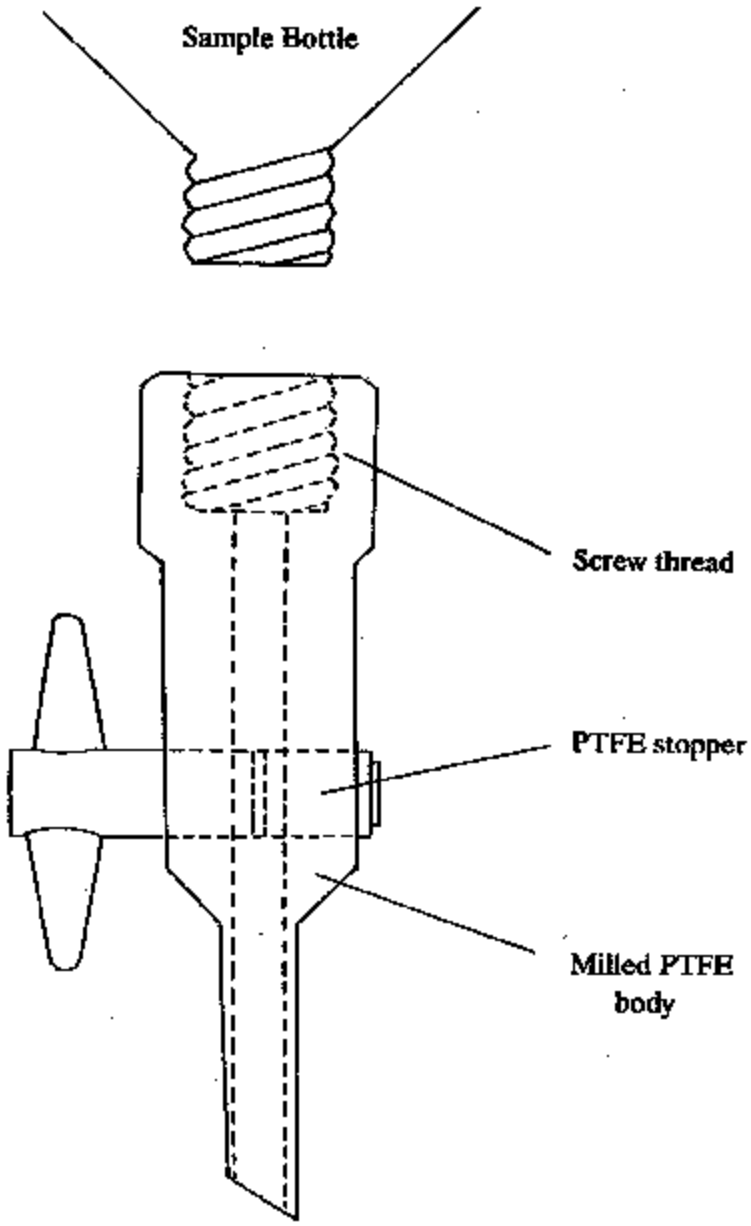

Fig.2. PTFE tap for water sample bottles.

preparation. Extracts may be stored at -20°C for several weeks. The hydrides may be reformed by shaking extracts with 300 µl of 5% aqueous sodium borohydride. An example of a GC separation of standards is shown in Fig. 3. and that of a sample in Fig.4.

Conditions for GC:

It is difficult to describe optimal operating conditions for GC. Different manufacturers instruments require different gas flows for fine tuning of both detector and carrier gas conditions. The sulphur filter in the detector should be removed and replaced by a narrow band filter of 610 ± 10 nm. Pure oxygen is not required and the flame is hydrogen rich. As a general rule, flows of around 160 ml min^{-1} hydrogen, and 90 ml min^{-1} air are satisfactory . Nitrogen is used as a make-up gas at 20 ml min^{-1}. Both direct on-column and splitless injections have been used successfully. Helium or hydrogen at approx 2 ml min^{-1} is used as carrier gas. The recommended oven programme is 40 to 200°C at 15°C min^{-1}, with the detector set at 225°C Examples of traces for standards are shown in Fig.3 .***WARNING*** **If hydrogen is used as the carrier gas the GC should be fitted with a leak detector to reduce the risk of explosion.**

5.1.5. SUMMARY OF METHOD

Organotins in water by GC:

2 l water sample/blank/spiked sample

add sodium borohydride

add 40 ml dichloromethane

add internal standard

cap and shake and release pressure

shake for 15 min

release pressure

stand

decant dichloromethane

centrifuge

remove water

blow down to 200 µl

analyse by GC/FPD

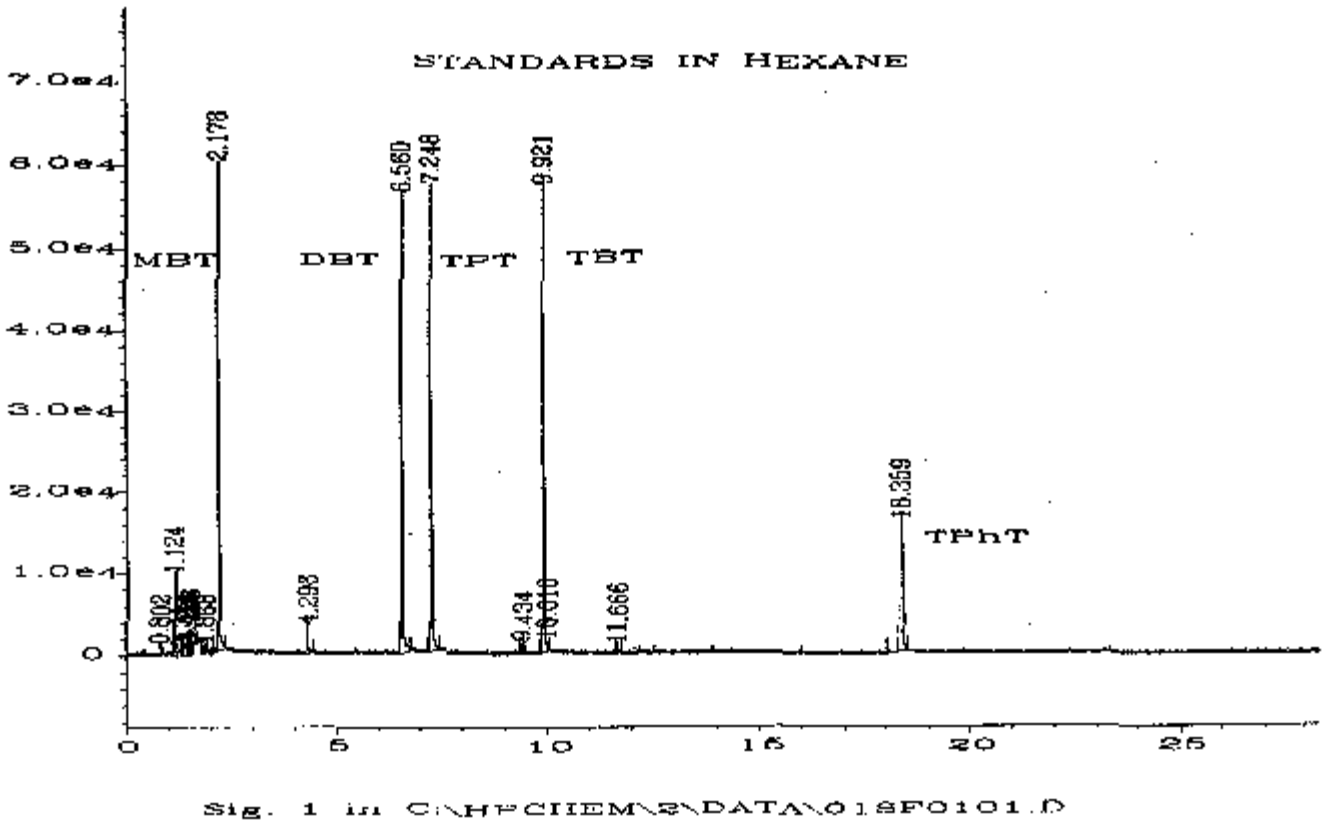

Fig.3. GC/FPD trace of organotin hydrides MBT, 0.27 ng on column, DBT, 0.24 ng on-column, TPT; 0.26 ng on column , TBT, 0.26 ng on column and TPhT, 0.55 ng on column.

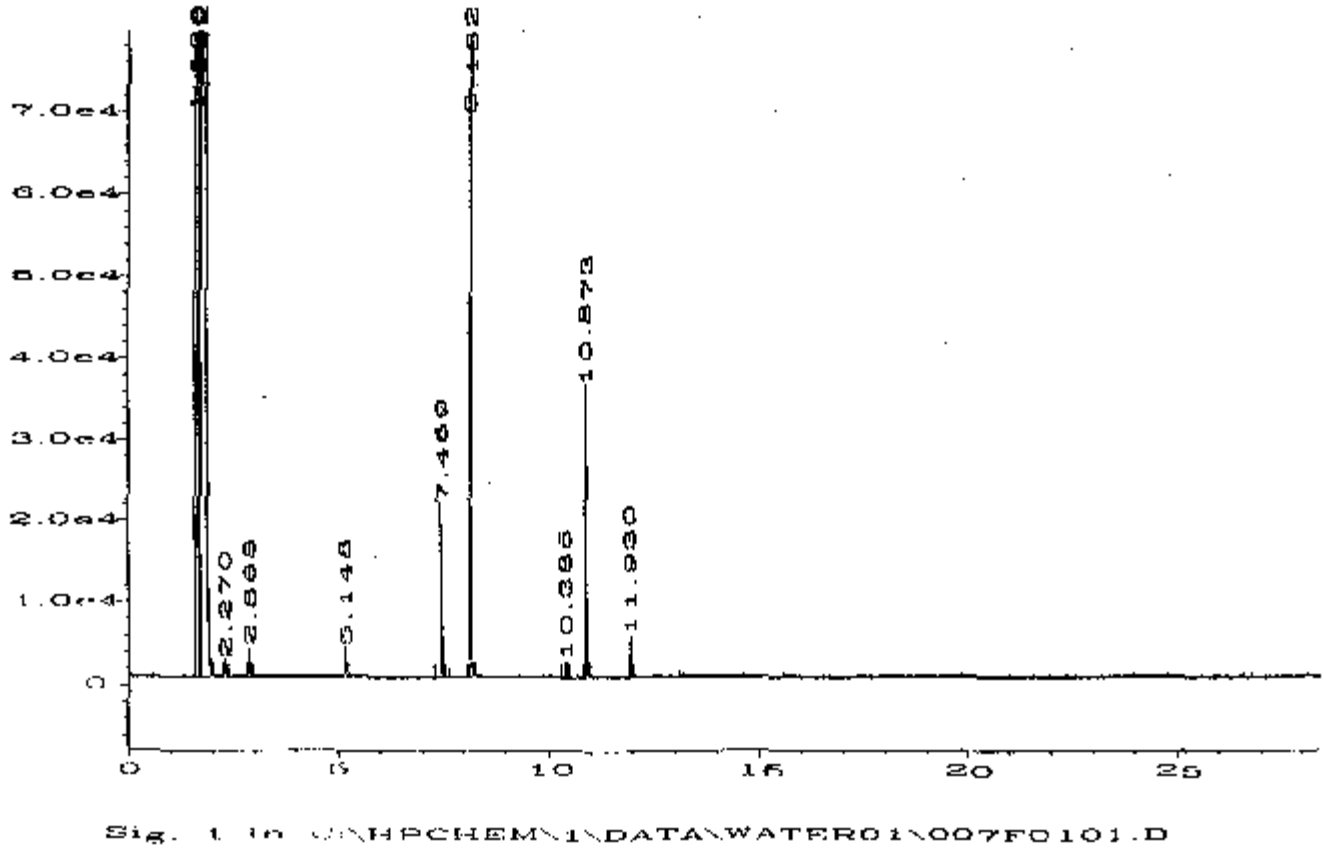

Fig.4. GC/FPD trace of dockyard water sample containing alkyltin compounds. MBT (retention time2.27 min.) 7 ng l^{-1}, DBT (rt 7.46 min.) 18 ng l^{-1}, TBT (rt 10.87 min.) 36 ng l^{-1}. TPhT (r.t. 20.66 min.) was not detected.

5.1.6. CALCULATION OF RESULTS

The retention times of standards are used for identification purposes. The peak heights or areas of known quantities of standards are then used to calculate a response factor for each compound to the tripropyltin internal standard. Where a variety of forms of the compounds are compared (e.g. oxide, chloride), it is advisable to express the results in terms of the cation only. The amounts of each standard used are expressed as the cation by multiplying the concentration used by a correction factor. i.e. for tripropyltin chloride Mol. wt.=283.2. (tripropyltin cation Mol. wt.=247.8), the factor =0.875 (247.8 ÷ 283.2). Hence the concentration of TPT cation present in the sample is: 20 μl of a 0.6 ng ml^{-1} solution i.e. 120ng x 0.875 = 105 ng in the 2 l sample, or 52.5 ng l^{-1}.

The concentration of each alkyltin cation in the sample (Cx) is then given by:

$$Cx = \frac{Hx \times Ci \times R}{Hi} \quad ng\ l^{-1}$$

where:
Hx = the peak height or area given by the alkyltin cation
Ci = the concentration of the internal standard cation in each litre of sample.
R = the relative response of the internal standard cation (RI) to that of the alkyltin cation (RX)

$$R = \frac{Ri}{Rx}$$

Hi = peak height or area given by the internal standard (cation)

The linearity of the detector response should be assessed for each GC. Typically the Hewlett Packard 5890 FPD shows a linear response for concentrations of 50 pg to 900 pg alkyltin compounds on-column.

5.1.7. PRECISION AND ACCURACY

The tripropyltin internal standard behaves very much like TBT occurring in the sample, and therefore loss of some of the sample on extraction and derivatization may be tolerated without compromising the precision and accuracy of the final determination. Hence only a one step dichloromethane extraction is employed, rather than sequential extraction steps. At the 50 ng l^{-1} level the mean recovery for TBTO relative to TPT has been found to be 88% (SD= 15; n=12), $DBTCl_2$ 88% (SD=5.8; n=8), $MBTCl_3$ 84% (SD=5.3; n=8). The method has been evaluated in a number of laboratory intercalibrations. A low level intercalibration was organised recently by the National Institute of Science and Technology in the USA. The concentration of the sample provided was undisclosed but later the mean was given as 6.2 ng l^{-1} (SD 0.05), the above method gave a value of 6.6 (SD= 0.05), markedly better in this case than the general performance values quoted above (Blair *et al,* 1992). In an intercalibration organised by the Community Bureau of Reference within the EEC, at a higher concentration, the method provided a result of 2.64 μg l^{-1} (SD= 0.15). The mean of all values submitted was 2.84 μg l^{-1} (SD= 1.62).

5.2. SPECIATION OF ORGANOTIN HYDRIDES BY GC/MS

GC/MS forms another suitable detection system for alkyltin hydrides extracted by the method described above. The flame photometric detector is replaced with a mass spectrometer set up in the electron impact mode with unit resolution.

Fig.5 (a-e) show a GC/MS separation and the mass spectra of di and triorganotin compounds. Characteristic tin isotopic clusters give readily identifiable fragmentation patterns. The prominent peaks around masses 119 and 177 provide a useful confirmatory technique in identification of the presence of tributyltin at single nanograms per litre in water samples.

5.3. SPECIATION OF ALKYL TIN HYDRIDES IN SEDIMENTS AND ANIMAL TISSUES BY GAS CHROMATOGRAPHY

5.3.1. PRINCIPLE

Alkyltin compounds are extracted from animal tissues or sediments by sodium hydroxide and methanol, converted to hydrides and partitioned into hexane. The derivatives are then analysed by gas chromatography with flame photometric detection. The method was developed by Waldock *et al.* (1989).

5.3.2. REAGENTS

Sodium borohydride powder. Purity 96% or better

Hexane (glass distilled grade).

Double distilled water.

Sodium hydroxide and methanol reagent. Prepared by dissolving 1 g sodium hydroxide (98% pure or better e.g. AnalaR) in 1 l of methanol (99.8% pure or better e.g. AnalaR) to give a 1% solution.

Authentic Standards:
Butyltin trichloride.
Dibutyltin dioxide.
Bis (tributyltin) oxide.
Tripropyltin chloride (Internal standard).
Individual stock solutions of standards are prepared by dissolving 0.1 g of each compound in 100 ml of methanol to produce a solution containing 1 mg ml^{-1} of each compound.
Individual working solutions are then prepared by dissolving 0.5ml of stock solution in 50 ml of methanol to provide a 10 μg ml^{-1} solution.

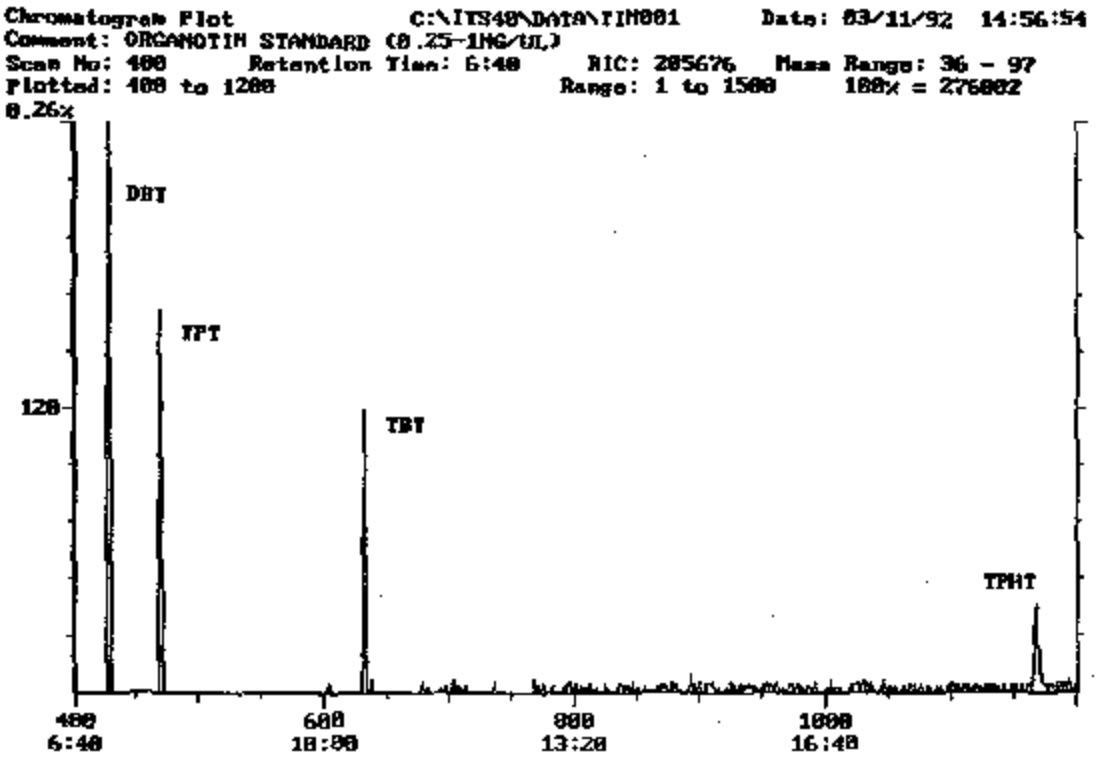

Fig 5a. Scanning GC/MS of di and triorganotin hydrides.
(a) Total ion current (concentrations as Fig.4).

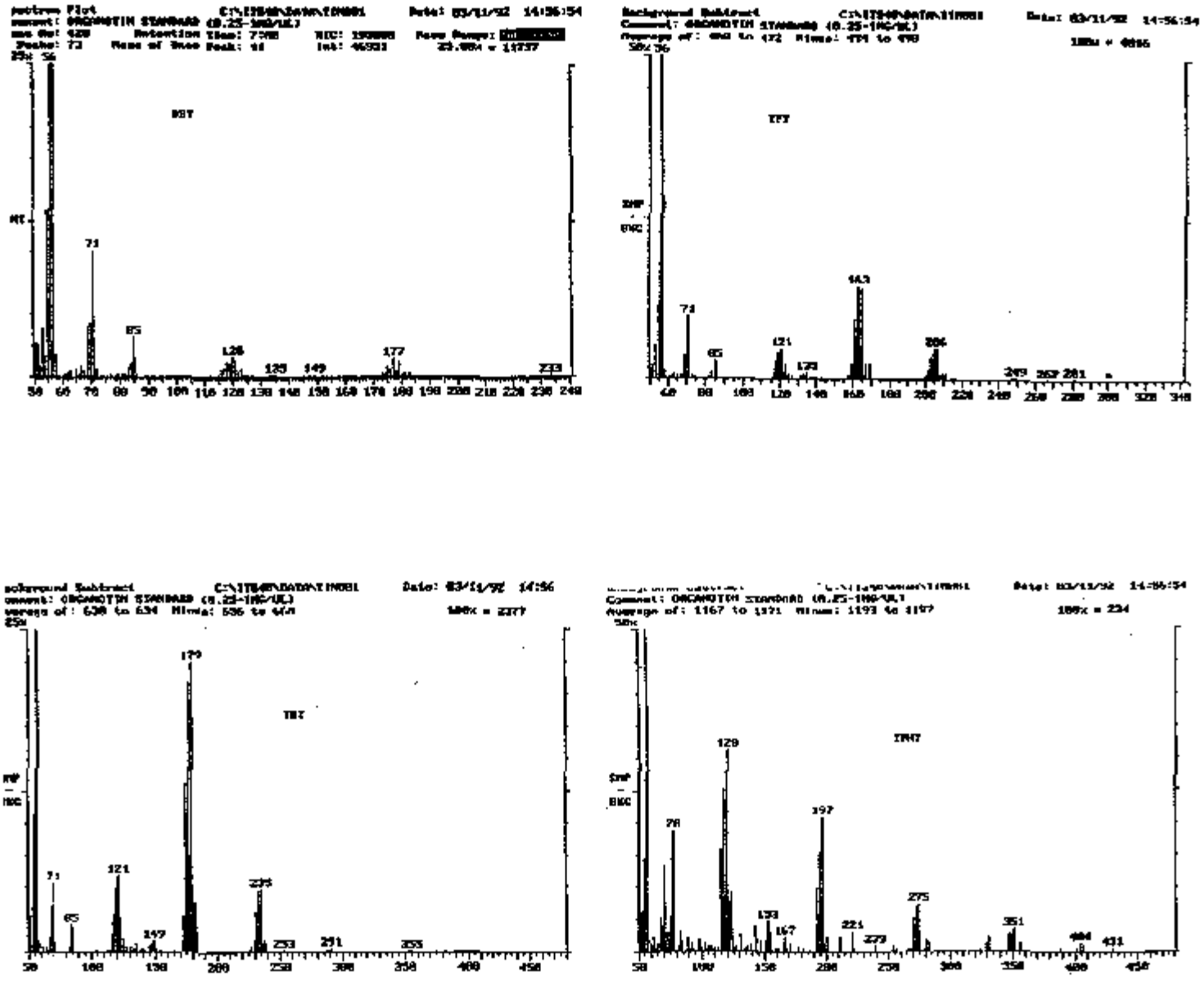

Fig 5b. Scanning GC/MS of di and triorganotin hydrides.
(b) Mass spectrum for DBT, (c) Mass spectrum for TPT,
(d) Mass spectrum for TBT, (e) Mass spectrum for TPhT.

A composite working standard may be prepared by adding 50 μl of each working standard to a centrifuge tube containing 2 ml of distilled water, 8 ml of sodium hydroxide/methanol reagent, 100 mg of sodium borohydride and 2 ml hexane. The tube is then shaken for 15 min, and centrifuged at 2500 rpm. This composite may be used to determine relative response factors for each compound detected by the flame photometric detector.

5.3.3. APPARATUS

Top drive all stainless steel homogeniser (e.g. Ultraturrax)

A range of volumetric flasks, graduated measuring cylinders and pipettes.

All glass and teflon micropipettes.

30 ml capacity glass centrifuge tubes with PTFE lined screw-capped lids..

5 ml capacity tapered vials with PTFE lined screw-capped lids (e.g."Reactivials").

A wrist action shaker capable of holding the centrifuge tubes described above.

Centrifuge with rotor to accept the centrifuge tubes described above.

Gas Chromatograph. Capillary column instrument with temperature programming and flame photometric detection, fitted with a 25 m x 0.32 mm ID fused silica capillary column and coated with a cross-linked 5% phenyl methyl silicone fluid with a film thickness of 0.52 μm. A retention gap consisting of one metre of deactivated, uncoated fused silica (0.53 mm ID) is attached to the injection end of the analytical column in order to allow on column injection and better chromatographic resolution. The use of an autosampler and electronic data capture system is beneficial.

5.3.4. METHOD

Sediment preparation: Sub samples of 4 g of sediment are oven dried to constant weight at 105°C to determine moisture content. Extraction of alkyltin is then carried out on up to 4g of wet sediment sample, ensuring that there is at least 2 ml of water (either natural or added) in the sample.

Animal tissues (shellfish and fish) are homogenised and 4 g of the homogenate is used for each analysis.

An appropriate amount of internal standard (tripropyltin chloride) is added to the sediment or tissue in a centrifuge tube; e.g. 50 μl for samples containing 0.01 to 1 $\mu g\ g^{-1}$ TBT. Double distilled water is added to the sample if necessary (see above). Sufficient 0.1% sodium hydroxide in methanol is then added to the sample to obtain a 4:1 MeOH:water (v/v) ratio. The minimum volume of methanol reagent is therefore 8 ml and the maximum, below 16 ml for sediment samples. For shellfish, 10 ml of sodium hydroxide/methanol reagent is added for wet samples. The tube is capped and shaken

for 45 min, then 2 ml of hexane and approximately 100 mg of sodium borohydride are added. The sample is then shaken for a further 15 min. After centrifugation (4000 rpm for 20 min.), the hexane phase is analysed by GC/FPD (see Section 5.1.4). Examples of chromatograms are shown in Figs 6 and 7.

The method does not employ a "clean up" procedure, and is therefore rapid, extremely robust and suitable for a high throughput of monitoring samples. Residues of extracted organic material do however tend to accumulate at the injection end of the analytical column/retention gap and degrade the performance of even the highest quality capillary columns. The retention gap should be frequently changed and 15 cm of fused silica periodically removed from the injection end of the analytical column.

Stored extracts will revert to alkyl tin chlorides within days of preparation. Extracts may be stored at -20°C for several weeks. The hydrides may be reformed by shaking extracts with 300 µl of 5% aqueous sodium borohydride.

5.3.5. SUMMARY OF METHOD

Alkyl tin hydrides in tissues and sediments:

4g tissue/sediment/AQC material/blank/spiked sample

add internal standard

adjust water content if necessary

add 4 volumes of sodium hydroxide/methanol reagent

shake 45 min.

add 2 ml hexane and 100 mg sodium borohydride

shake 15 min.

centrifuge

analyse hexane by GC/FPD.

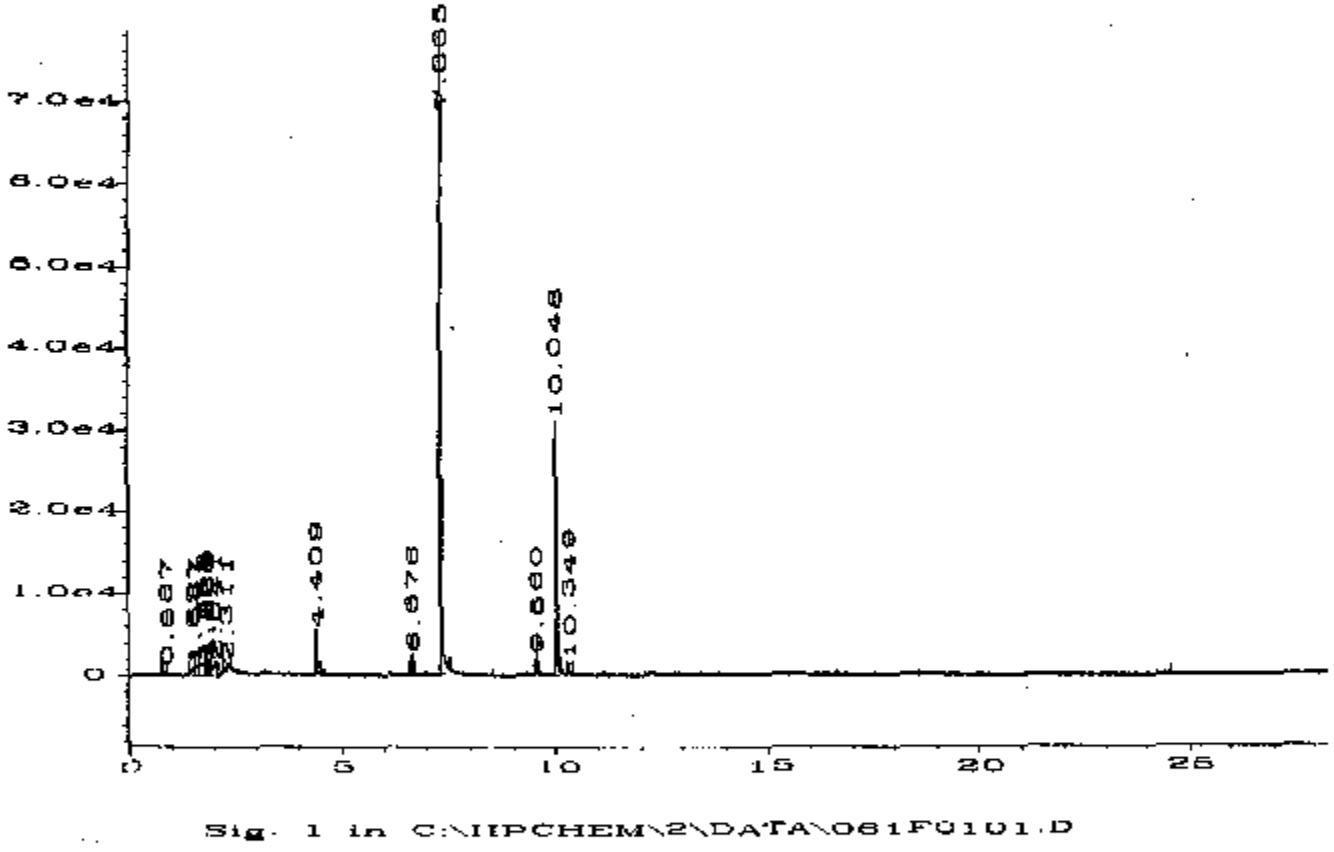

Fig 6. GC/FPD trace of an oyster extract. MBT (rt 2.31 min.) not detected, DBT (rt 6.68 min.) 0.009 μg g^{-1} wet wt., TBT (rt 10.05 min.) 0.13 μg g^{-1} wet wt.

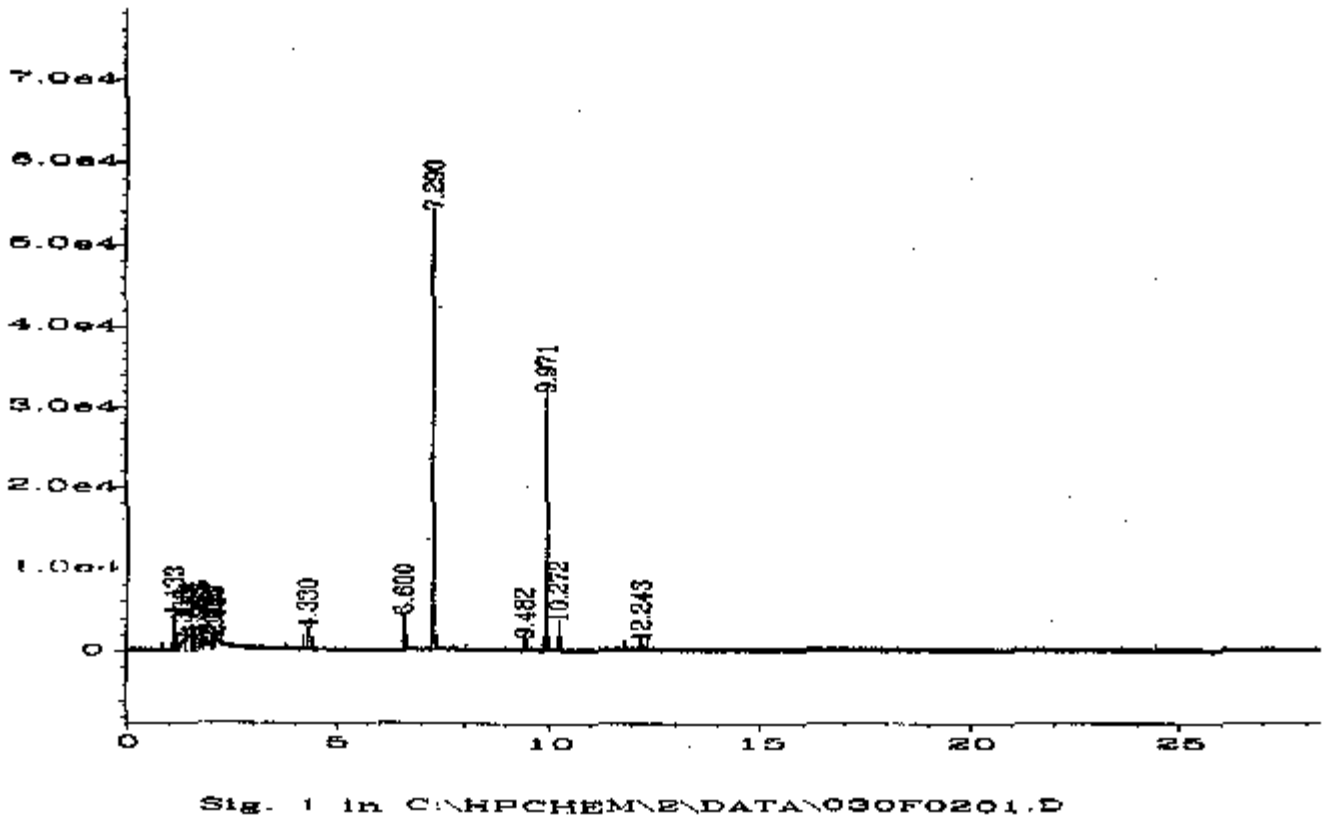

Fig.7. GC/FPD trace of a marina sediment extract. MBT (rt 2.20 min.) not detected, DBT (rt 6.60 min.) 0.02 µg g^{-1} dry wt., TBT (rt 9.97 min.) 0.24 µg g^{-1} dry wt.

5.3.6. CALCULATION OF RESULTS

The retention times of standards are used for identification purposes. The peak heights or areas of known quantities of standards are then used to calculate a response factor for each compound to the tripropyltin internal standard. The concentration of each alkyltin compound in the sample (Cy) is then given by:

$$Cy = \frac{Hy \times Ci \times R}{Hi \times W} \quad \mu g\ g^{-1} \text{ (dry wt. sediment, wet wt. tissue)}$$

where:
Hy = the peak height or area given by the alkyltin compound
Ci = concentration of the internal standard (as the cation) in the tissue or sediment sample in $\mu g\ g^{-1}$
R = the relative response of the internal standard (Ri) to that of the alkyltin compound Rx

$$R = \frac{Ri}{Rx}$$

Hi = peak height or area given by the internal standard
W = weight of sample in grams (dry weight for sediments, wet weight for tissues).

5.3.7. PRECISION AND ACCURACY

The recovery of tributyltin was determined by standard additions of tributyltin hydride to samples previously spiked with TBTO, and comparing the resultant GC peak areas over a range of spiked concentrations. The calculated recovery ranged from 90-97% (mean 93%) for sediment and 95-104% (mean 98%) for shellfish tissue. However the use of an internal standard (tripropyltin chloride) which behaves in a similar way to TBT allows for loss of sample without compromising the precision and accuracy of the determination. The relative recovery for TBT, DBT and MBT to the internal standard is close to 100%. The detection limit for the method is 0.005 $\mu g\ g^{-1}$ for TBT, 0.010 $\mu g\ g^{-1}$ for DBT and 0.02 $\mu g\ g^{-1}$ for MBT. These limits may be reduced by concentration of the hexane extracts before GC analysis, however since a "clean up" procedure is not employed, concentrated samples more rapidly degrade the performance of the capillary column.

5.4. SPECIATION OF ORGANOTIN COMPOUNDS IN DIFFICULT MATRICES

5.4.1. PRINCIPLE

In the case of heavily contaminated or fatty samples such as sewage sludges it may be necessary to apply a clean up procedure before gas chromatographic separation of organotin compounds. Hydrocarbons for example are known to quench the FPD response.

The organotin hydrides formed using methods 5.1 and 5.3 are not stable, and it is difficult to achieve reproducible recoveries from clean up columns. The alternative approach of forming an alkyl derivative has the advantage of being more amenable to column chromatographic clean up, but the disadvantage of being a more labour intensive technique. The following method was developed by Fent and Muller (1991) and describes the use of an ethyl Grignard reagent to ethylate alkyl and aryl tin compounds (however methyl, propyl or hexylmagnesium bromide may be substituted in the same method). The procedure has been developed for sewage sludge, but may be used for animal tissues and sediments.

5.4.2. REAGENTS

Methanol 99.8% pure or better e.g.AnalaR.

Diethyl ether 99.5% pure or better e.g. AnalaR.

Grignard reagent ethylmagnisium bromide 2.0 M solution in tetrahydrofuran.

Diethyl ether/tropolone reagent. 0.25% tropolone (pure m.p. 49-51°C) in diethyl ether.

Hydrochloric acid, 35.4%, s.g. 1.18 (e.g. AnalaR).

Hexane (glass distilled grade).

Calcium chloride 99% (e.g. AnalaR)

Sodium sulphate 99.5% (e.g. AnalaR)

Silica gel 60 pesticide residue grade

Authentic standards:

Butyltin trichloride
Dibutyltin dioxide
Bis (tributyltin) oxide
Tripropyltin chloride (Internal standard).
Triphenyltin chloride.

Individual stock solutions of standards are prepared by dissolving 0.1 g of each compound in 100 ml of methanol to produce a solution containing 1 mg ml^{-1} of each compound.
Individual working solutions are prepared by dissolving 0.5ml of stock solution in 50 ml of methanol to provide a 10 μg ml^{-1} solution.

A composite working standard may be prepared by adding 50 μl of each working standard to 2 ml of diethyl ether and forming the ethyl derivative as outlined in Section 5.4.4.

5.4.3. APPARATUS

A range of volumetric flasks, graduated measuring cylinders and pipettes.

All glass and teflon micropipettes.

Stainless steel top drive homogeniser e.g Ultraturrax.

30 ml capacity glass centrifuge tubes with PTFE lined screw-capped lids..

5 ml tapered vials with PTFE lined screw-capped lids (e.g."Reactivials").

A wrist action shaker capable of holding the centrifuge tubes described above.

Centrifuge with rotor to accept the centrifuge tubes described above.

Gas Chromatograph. Capillary column instrument with temperature programming and flame photometric detection, fitted with a 25 m x 0.32 mm ID fused silica capillary column and coated with a cross-linked 5% phenyl methyl silicone fluid with a film thickness of 0.52 μm. A retention gap consisting of one metre of deactivated, uncoated fused silica (0.53 mm ID) is attached to the injection end of the analytical column in order to allow on column injection and better chromatographic resolution. The use of an autosampler and electronic data capture system is beneficial.

5.4.4. METHOD

Sludge preparation: Sub samples of 4 g of sewage sludge are oven dried to constant weight at 105°C to determine moisture content. Extraction of organotin compounds is then carried out on 10g of sludge which is placed in a centrifuge tube. An appropriate volume of internal standard is added (50 μl for samples containing 0.01 to 1 μg g^{-1} organotins) and the pH of the sample adjusted to 2 by addition of HCl. The sample is then homogenised for 3 min. 10 ml of diethyl ether/tropolone reagent is added and the tube capped and shaken for 10 minutes. The sample is then centrifuged at 2500 rpm for 10 min and the ether phase decanted and placed in a 50 ml capacity flat bottomed flask contaning 5 g calcium chloride. The ether extraction step is repeated two more times and the extracts bulked. After swirling the flask to dry the ether phase, the solvent is decanted to a clean centrifuge tube and blown down to approximately 2ml. The extract is then combined with 0.5 ml of ethylmagnesium bromide reagent in a 5 ml capacity tapered vial, capped and left for 10 min. The

Grignard reaction is then stopped by careful addition of 2 ml 2M HCl. The aqueous phase is removed, discarded and the extract dried by addition of a small amount (approximately 100 mg) of sodium sulphate. The volume of the sample is then reduced to approximately 0.5 ml by blowing down with a gentle stream of nitrogen.

A small chromatographic column is prepared using a Pasteur pipette containing 0.5 g of silica gel. The sample is then carefully added to the top of the column and organotins eluted using 10 ml of 10% ether in hexane. The eluatent is reduced in volume to approximately 200 μl and 1 μl introduced to the GC/FPD. For GC conditions see Section 5.1.4.

5.4.5. SUMMARY OF METHOD

Organotins in sewage sludge:

10g sewage/AQC material/blank/spiked sample

add internal standard

adjust pH

homogenise

extract with ether/tropolone three times

dry extract with calcium chloride

blow down to 2 ml

add 0.5 ml Grignard and leave for 10 min

stop reaction with 2 ml 2M HCl

dry ether phase with sodium sulphate

reduce volume to 0.5 ml

clean up on silica gel column, eluting with 10 ml ether/hexane

reduce volume

analyse by GC/FPD

5.4.6. CALCULATION OF RESULTS

The retention times of standards are used for identification purposes. The peak heights or areas of known quantities of standards are then used to calculate a response factor for each compound to the tripropyltin internal standard. The concentration of each alkyltin compound in the sample (Cy) is then given by:

$$Cy = \frac{Hy \times Ci \times R}{Hi \times W} \quad \mu g\ g^{-1} \text{ (dry wt. sludge)}$$

where:
Hy = the peak height or area given by the organotin compound
Ci = the concentration of the internal standard (as the cation) in the sewage sludge sample in $\mu g\ g^{-1}$
R = the relative response of the internal standard (Ri) to that of the alkyltin compound (Rx)

$$R = \frac{Ri}{Rx}$$

Hi = peak height or area given by the internal standard
W = weight of sample in grams dry weight .

5.4.7. PRECISION AND ACCURACY

Recoveries for monoorganotin compounds by this method were found to be 50 -60 %, 60 - 80% for diorganotin compounds and 50-60% for triorganotin compounds. The detection limits for butyl and phenyltins in sewage sludge were 0.01 to 0.1 μg cation g^{-1} dry weight.

6. QUALITY ASSURANCE

Water:

The use of a relatively large volume of water (750 to 2000 ml) prohibits the storage of a quality control sample. Analytical quality control is monitored by the addition of a standard (200 ng l^{-1} TBTO for Method 4.1 or 50 ng l^{-1} TBTO for Method 5.1) to either a 0.43 μm filtered sample of seawater, or to tap water, for salt and freshwater samples respectively. Three "spiked" samples are analysed with each batch (usually ten) of environmental samples. A single blank sample of water from the same source is analysed with each batch of environmental samples.

Tissues and sediments:

There are few sources of reference materials of certified organotin content. The National Research Council in Canada have produced a sediment with a certified TBT content (see Section 4.4.4.) and the Community Bureau of Reference are in the process of producing reference materials. "In house" reference materials can be prepared and calibrated against certified materials. If necessary bivalves may be artificially contaminated with TBT by dosing holding tanks with TBT or those taken from sites known to be contaminated with TBT provide analytical quality control tissue. Similarly sediments may be either artificially contaminated or taken from naturally contaminated sites. Several hundred grams of tissue are homogenised and stored by freezing at -20°C. A single sample of quality control tissue is analysed with each batch, usually 12, of environmental samples. Analysts should refer to Reference Methods for Marine Pollution Studies Number 57 in this series to instigate a quality assurance programme. Figures 8 and 9 show typical quality control diagrams for the methods described in Section 5.3.4.

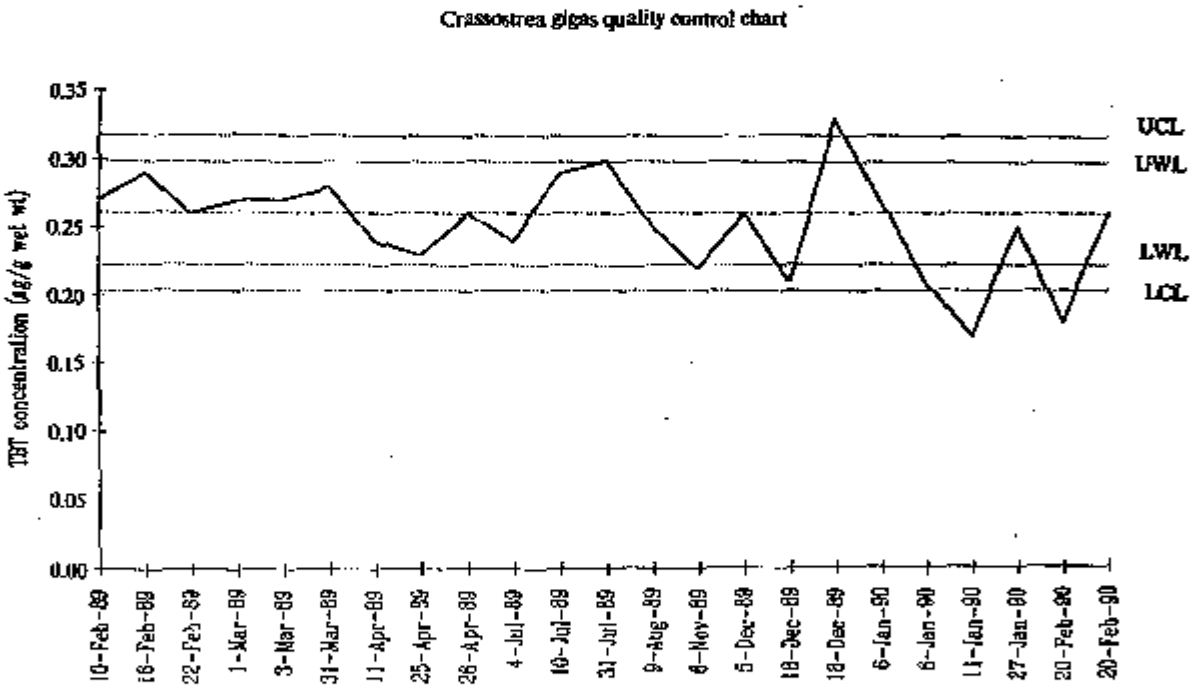

Fig.8. A quality control chart for TBT determinations in oyster tissue.

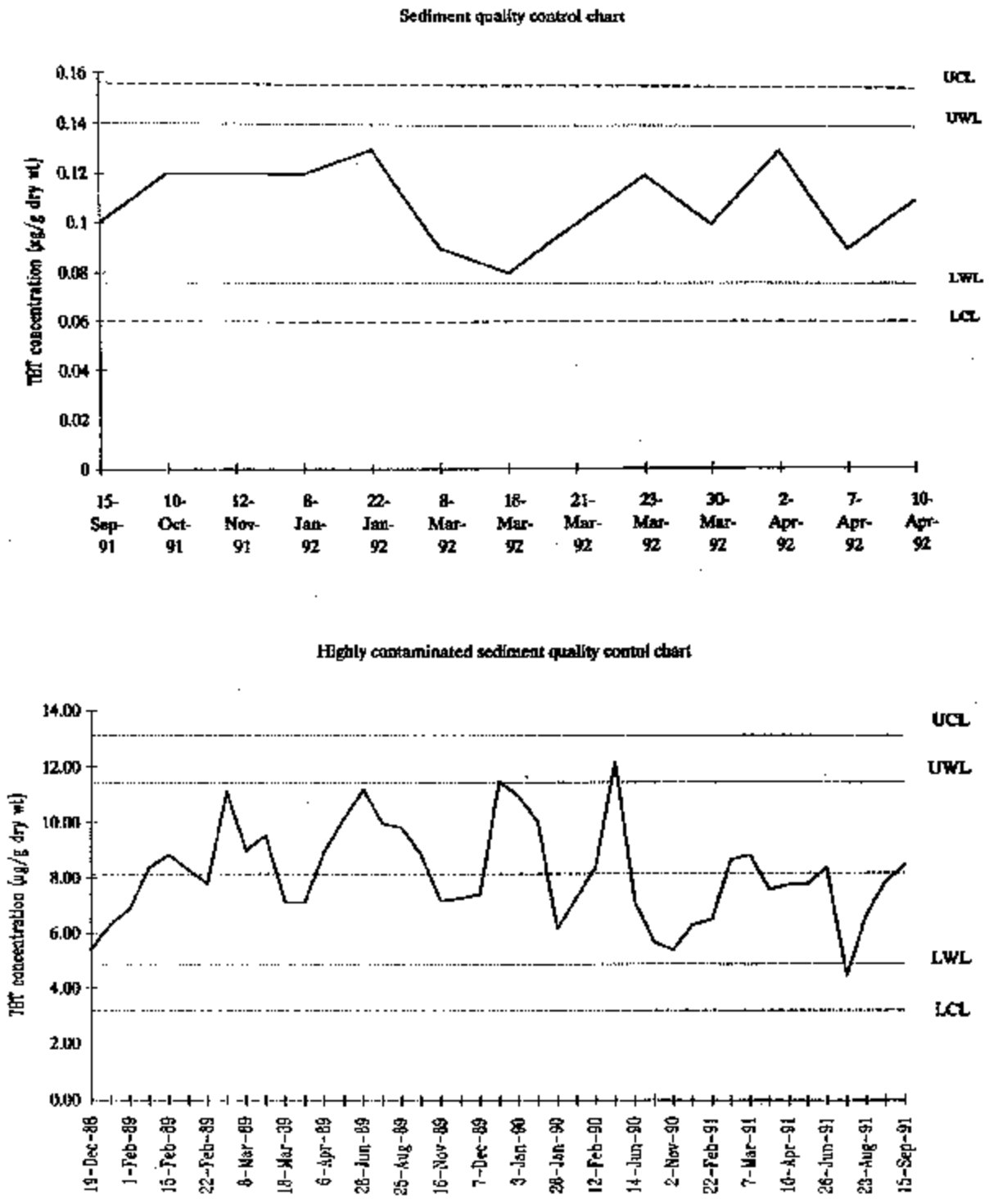

Fig 9. Quality control charts for TBT determinations in sediments. (a) outer marina sediment, (b) spiked sediment.

8. SOURCES OF REAGENTS AND APPARATUS.

Some of the reagents and apparatus listed in the above methods are difficult to obtain. The following list includes known suppliers in the United Kingdom but is not exhaustive. Listing of a product does not suggest an endorsement of quality.

Alkyl and aryl tin standards:
Fluka Chemical Company, Peakdale Road Glossop Derbyshire
Aldrich Chemical Company, The old Brickyard, New Rd, Gillingham, Dorset.

Tropolone and AnalaR grade reagents:
Merck Ltd., Merck House, Poole, Dorset.

Silica gel 60: Merck (as above).

Grignard reagents:
Aldrich (as above).

Teflon Winchester tap: Cowey Scientific, Southerby Rd., Middlesbrough.

Narrow Band 610 nm filter for FPD:
Ealing Electro Optics, Greycaine Rd., Watford.

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