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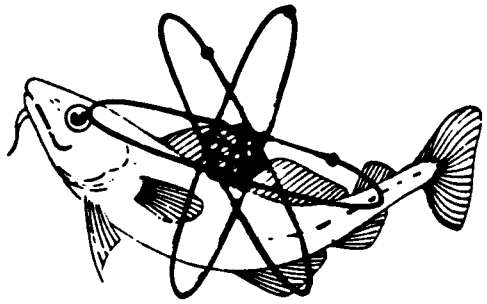
MINISTRY OF AGRICULTURE FISHERIES AND FOOD

DIRECTORATE OF FISHERIES RESEARCH

AQUATIC ENVIRONMENT PROTECTION: ANALYTICAL METHODS



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The determination of total tin and organotin compounds in environmental samples

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LOWESTOFT, 1989

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Copies of this report are available from the Fisheries Laboratory, Remembrance Avenue, Burnham-on-Crouch, Essex, CM0 8HA.

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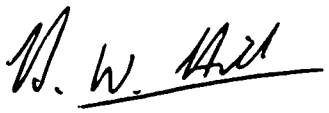
CONTENTS

Foreword

As part of its responsibilities under various Acts of Parliament, MAFF Directorate of Fisheries Research has a duty to carry out a substantial programme of monitoring, surveillance and research in relation to the quality of the aquatic environment in and around the United Kingdom. In the course of that programme, a wide variety of methods of analysis are used for a wide variety of contaminants, both inorganic and organic, stable and radioactive. This series of publications describes the main methods used in the course of this work and parallels the existing Aquatic Environment Monitoring Report series, in which much of the resulting data is published. Regardless of whether the analytical procedure relates to a radionuclide or a non-radioactive contaminant, each report contains a step-by-step guide to analytical procedures and an explanation of the calculation of results.



H. W. Hill

Director of Fisheries Research
Ministry of Agriculture, Fisheries and Food

Background

The Burnham-on-Crouch Laboratory of the Directorate of Fisheries Research (DFR) of the Ministry of Agriculture, Fisheries and Food has well-equipped analytical chemistry facilities and is called upon to provide a comprehensive range of analytical services. Although some of the requirements arise from customers elsewhere in the DFR, these are usually single or small-volume requests. The vast majority of the analyses undertaken stem from programmes generated in-house by DFR's Aquatic Environment Protection Division 2, which is responsible for assessing the distribution and the impact, or potential impact, of many types of discharge other than radioactive discharges, on both marine and freshwater fisheries. Some of this work arises from the Division's special responsibility for the licensing, enforcing and monitoring of the impact of wastes dumped at sea. In addition, there is a major programme of general environmental quality monitoring which is used to back-up pollution prevention and environmental protection activities.

Over the years, analyses have been conducted on a wide variety of sample types, both freshwater and marine, environmental and non-environmental (effluents, mine tailings, drill-muds, dredgings) for a large range of contaminants. Many of the analyses are of a routine or semi-routine nature and set procedures have been developed for their conduct. These procedures have been thoroughly tested and proven by the DFR and in most cases have been subjected to intercomparison tests with other laboratories and to quality assurance schemes involving the use of standards and reference materials.

It is believed that other laboratories may find details of the methods used at the Burnham-on-Crouch Laboratory of interest. Consequently, a suite of publications has been prepared. The present publication relates to methods for analysis of organotin compounds. Others in the series give details of the methods used for the determination of hydrocarbons, metals and pesticides. Each publication gives details of the procedures followed for the analyses of a wide variety of sample types. Although fairly complete details are provided, the intention of the publication is to inform other analysts of the general procedures, apparatus and analytical instrumentation required. If difficulties in applying the methods are encountered, contact should be made with the leading author from whom further details can be obtained.

Generally, details are provided of any special sampling or sample preservation procedures. However, where the necessary steps have been laid down by international agreement and apply in common to all of the determinands, details are not provided in the individual method section. This applies particularly to the analyses of fish and shellfish for either temporal or spatial trend purposes or for human health risk assessment purposes. For these objectives, the International Council for the Exploration of the Sea (ICES) has laid down guidelines and these are reproduced in Appendix 1.

1. Introduction

Anthropogenic inputs of alkyltin compounds to the aquatic environment arise from the use of trialkyltins as biocides and dialkyltins as catalysts and stabilisers in the plastics industry. Environmental damage has been demonstrated to occur when tributyltin compounds are used in antifouling formulations on large numbers of boats in enclosed waters, on antifouled nets used to contain salmon, and at sites of spills of timber treatment biocides. Since alkyltins 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.

Methods fall into two categories: (a) those using atomic absorption spectrophotometry (AAS); and (b) those using gas chromatography (GC). Whilst the former allows more rapid processing of samples, the latter is recommended for sensitivity and detailed speciation.

2. General considerations

2.1 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.

2.2 Preparation of glassware

Before use, all glassware is soaked in 10% Pyroneg for 8 h, then rinsed with tap water and soaked for a further 8 h in concentrated hydrochloric acid (Analar). Finally, it is rinsed in double distilled water and dried in a glass oven at 50°C.

2.3 Sample collection and storage

Water samples are collected in 2.7l glass bottles with PTFE-lined, screw-capped lids. Before use, they are rinsed with concentrated hydrochloric acid, 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 Figure 1). Water samples are stored in a cool room and extracted within 48 h of collection. Extracts have been held at -20°C for several months with no measureable breakdown of tributyltin (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 be stored by freezing in cleaned glass jars. No measurable breakdown of TBT in stored samples has been recorded after holding at -20°C for six months.

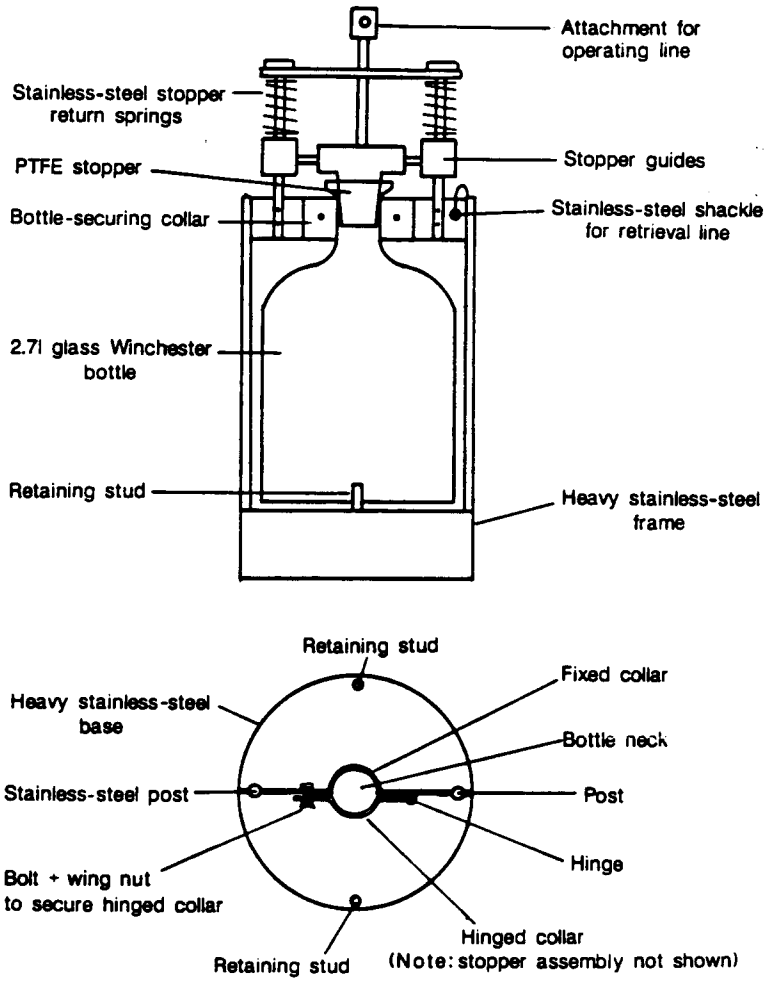


Figure 1 The stainless-steel and PTFE water sampler.

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.

3. Determination of total tin concentrations in water by atomic absorption spectrophotometry (AAS)

3.1 Basis of the method

Water samples are acidified, and compounds containing tin are bound to a complexing agent (tropolone) and extracted into toluene. The extract is analysed by electrothermal atomic absorption spectrophotometry (EAAS). The detection limit is approximately 100 ng l^{-1} Sn in the original sample.

3.2 Reagents and equipment

Hydrobromic acid (Aristar)
Toluene/tropolone reagent, 1.25 g tropolone dissolved in 2.5l toluene (Analar).

Bis (tributyltin) oxide [Hexabutyldistannoxane] (TBTO).
One-litre glass sampling bottles with PTFE-lined, screw-capped lids.
'Eppendorf' centrifuge tubes.

3.3 Procedure

Water (750 ml) is collected in a calibrated 1 l glass sampling bottle, 20 ml of hydrobromic acid is added, mixed and allowed to stand for 15 min. Toluene/tropolone reagent (25 ml) 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 an 'Eppendorf' 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 EAAS.

Calibration standards in the range 0.005 $\mu g\ ml^{-1}$ to 0.2 $\mu g\ ml^{-1}$ Sn in toluene are prepared from a stock solution of 1 $\mu g\ ml^{-1}$ Sn as TBTO in glacial acetic acid. Duplicate reagent blanks are prepared with each batch of samples, and spiked samples are prepared to monitor extraction efficiency. Mean recovery of stannous chloride was found to be 81% (s.d. = 7.1, n = 8).

3.4 AAS operating conditions

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

Wavelength 286.3 nm
Deuterium background correction

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

Uncoated graphite tubes.

4. Determination of organotin concentrations in water by atomic absorption spectrophotometry (AAS)

4.1 Basis of the method

By omission of the tropolone complexing agent in the previous method, organotin compounds are extracted but inorganic tin compounds are not extracted. In this procedure, glacial acetic acid has been demonstrated to give better extraction efficiency for organotins than hydrobromic acid. The detection limit of the method is approximately 40 ng^{-1} Sn in the original sample.

4.2 Reagents and equipment

Toluene (Analar).
Glacial acetic acid (Analar).
TBTO.
Glass sampling bottles (2.7 1) with PTFE-lined, screw-capped lids.
'Eppendorf' centrifuge tubes.

4.3 Procedure

The procedure is essentially the same as that described for total tin. Water (2 l) is collected in a calibrated 2.7 l bottle. Glacial acetic acid (50 ml) is added, and 25 ml of toluene is substituted for the toluene/tropolone reagent used in Section 3.

In a recent intercalibration exercise, the US National Bureau of Standards, Gaithersburg, supplied a reference water containing an unknown concentration of TBT. After 1000-fold dilution, the above method produced a result of 1.06 $\mu g\ l^{-1}$ of TBT (s.d. = 0.05, n = 5). The standard was later certified to contain 1.06 ± 0.05 $\mu g\ ml^{-1}$ of TBT, (Blair *et al.*, 1986).

Recovery of dibutyltin by this method is approximately 16%.

The AAS methods described in Sections 3 and 4 above were developed by M&T Chemicals Inc. (see Trachman, Tyberg and Branigan, 1977 for background), and use of the organotin method has been described recently 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 (J. J. Cleary personal communication) (see also Parks, Blair and Brinkman, (1985) for descriptions of possible matrix interferences). The Department of the Environment (DOE) Standing Committee of Analysts has also recommended lanthanum-coated furnace tubes to increase sensitivity (DOE, in preparation).

5. Speciation of alkyltin hydrides in water by gas chromatography (GC)

5.1 Basis of the method

Alkyltin compounds are reduced to the hydride form and simultaneously extracted into dichloromethane. The extract is reduced in volume and injected into a gas chromatograph fitted with a flame photometric detector. The limit of detection for TBT compounds is approximately 1 ng l^{-1}. The method was developed by Matthias *et al.*, (1986).

5.2 Reagents and equipment

Sodium borohydride pellets (Analar).
Dichloromethane (glass distilled grade).
Tripropyltin chloride (TPTCl).
Alkyltin standards.
All-glass and PTFE micropipette.
'Soveril' capped centrifuge tubes.
'Reactivials'.
Glass and PTFE tap (see Figure 2).

5.3 Procedure

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 TPTCl internal standard. The bottle is capped and shaken on an orbital shaker for 15 min. 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 Figure 2. The dichloromethane is drawn off into a 'Soveril' 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 approximately 3 ml using a gentle stream of compressed air at ambient temperature. The extract is then transferred to a 'Reactivial' and blown down to 100-200 µl. One microlitre of the extract is then introduced to the GC system.

GC analysis is carried out using a 25 m x 0.32 mm ID fused silica capillary column, coated with a cross-linked 5% phenyl methyl silicone fluid, with a film thickness of 0.52 µm. Hewlett Packard 5880A, 5890 or Carlo Erba 4160 GC's equipped with flame photometric detectors have been used successfully for analysis of alkyltin compounds at the Burnham-on-Crouch Laboratory. All instruments are linked to a 3357 laboratory automation system based on a Hewlett Packard 1000 minicomputer.

Sample extracts stored in dichloromethane revert to alkyltin chlorides. Hydrides may be reformed by shaking extracts with aqueous sodium borohydride.

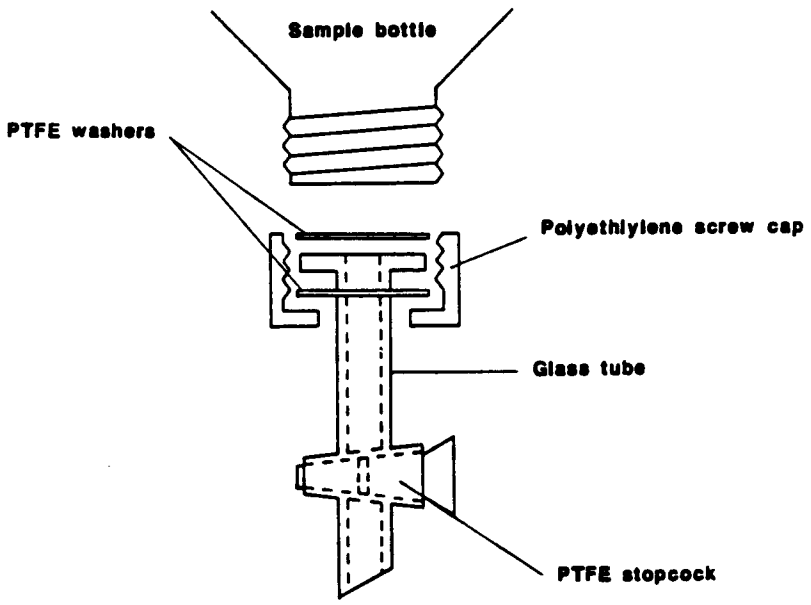


Figure 2 The glass and PTFE tap for water sample bottles.

5.4 Conditions for GC

It is difficult to describe optimal operating conditions for GC. The Carlo Erba and HP machines require different gas flows for fine tuning of both detector and carrier gas conditions. For initial studies, the sulphur filter in the detector was removed to give a non-specific detector. The present procedure employs a narrow-band filter of 610 nm (Ealing Electro-optics). No additional oyxgen is required and the flame is hydrogen rich. As a general rule, flows of around 50 ml min^{-1} of hydrogen, 100 ml min^{-1} of air and 15 ml min^{-1} of make-up gas are satisfactory for the HP 5890. The 4160 requires 100 ml min^{-1} of hydrogen and

70 ml min^{-1} of air. Both direct on-column and splitless injections have been used successfully. Hydrogen at approximately 2 ml min^{-1} is used as a carrier gas. The oven programme is 40 to 200°C at 15°C min^{-1}. An example of a chromatogram is shown in Figure 3. At the 50 ng l^{-1} level, the mean recovery for TBTO is 88% (s.d. = 15, n = 8); $DBTCl_2$ is 77.9% (s.d. = 5.8, n = 8); $MBTCl_3$ is 83.6% (s.d. = 5.3, n = 8).

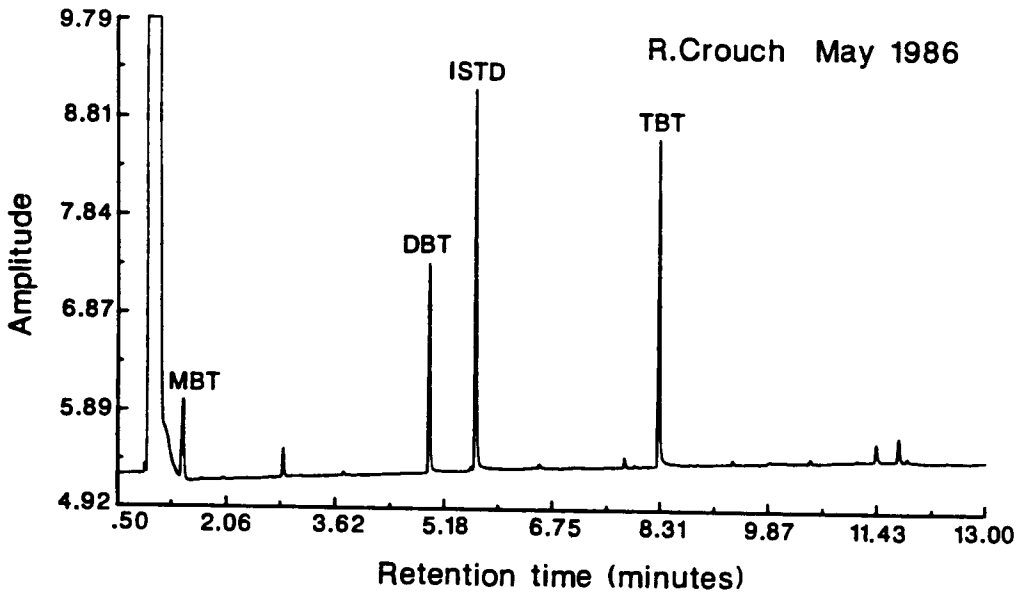


Figure 3 A capillary gas chromatogram of alkyltin hydrides from a sample of estuarine water.

6. Speciation of alkyltin hydrides by gas chromatography/mass spectrometry (GC/MS)

Coupled gas chromatography mass/spectrometry (GC/MS) forms another suitable detection system for alkyltin hydrides extracted by the method described above. At the Burnham-on-Crouch Laboratory, a Carlo Erba 4160 is directly coupled to the ion source of a Finnigan 3200 quadrupole mass spectrometer interfaced to, and controlled by, an Incos 2300 data system. The mass spectrometer is operated in the electron impact mode at 50eV.

6.1 Scanning MS (broad scan 40-450 amu)

Figure 4 shows the mass spectrum of tributyltin hydrides. Characteristic tin isotopic clusters give readily identifiable fragmentation patterns. However, only water samples taken at the sites of spills of organotins, or in marinas containing TBT-antifouled yachts, yield sufficient signal for scanning runs (µg l^{-1} concentrations).

6.2 Multiple ion detection

The prominent peaks around masses 119 and 177 provide a useful confirmatory technique in identification of the presence of alkyltins at single nanograms per litre concentrations in water samples. Figure 5 shows comparative traces for dibutyltin and tributyltin, measured using only masses 119 and 177 against authentic standards (the slight offset is

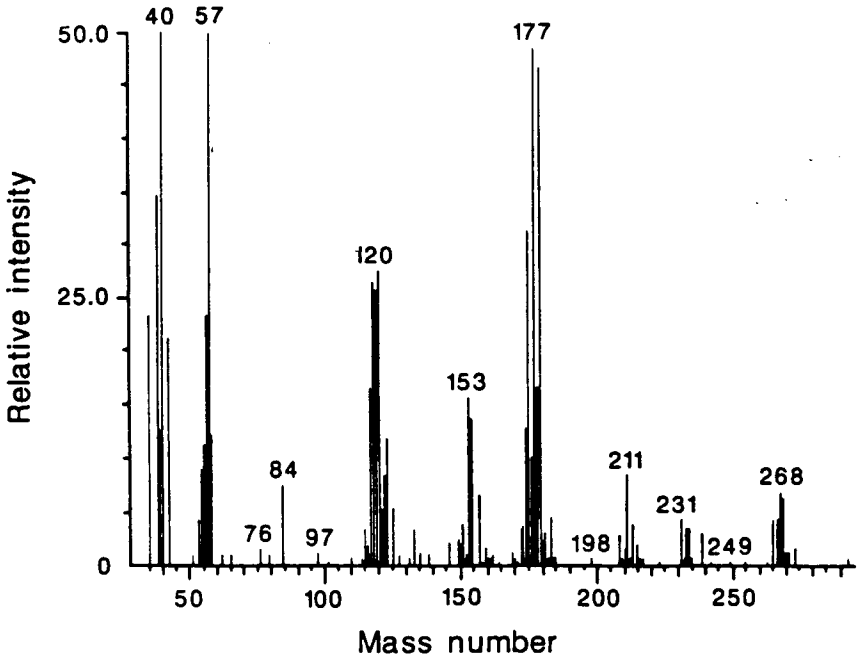


Figure 4 Mass spectrum of tributyltin hydride.

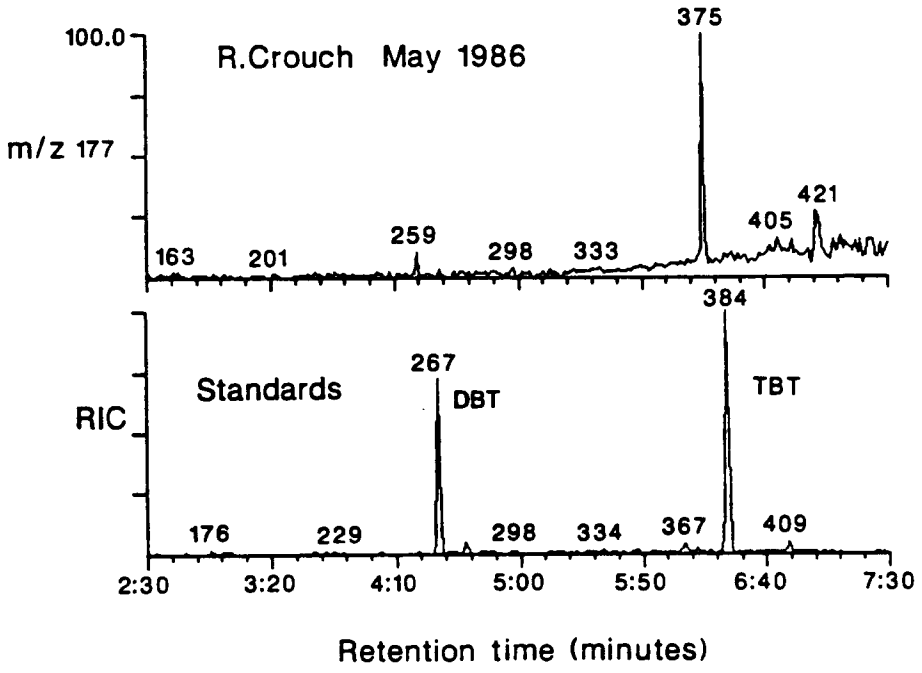


Figure 5 MID mass spectrometry of tributyltin hydride comparing m/z 177 of an environmental sample with authentic standards: RIC = reconstructed ion chromatogram.

due to manual 'start time' error). The estuarine water contained approximately 20 ng l^{-1} of TBT.

Grignard reagents have also been used to prepare organotin derivatives for GC/MS in this laboratory (see Waldock and Miller, 1983), and a variety of other techniques have been used in a recent laboratory intercalibration exercise (Blair et al., 1986).

7. Determination of total tin concentrations in shellfish tissue

7.1 Basis of the method

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

7.2 Reagents and equipment

Nitric acid (Analar).
Sulphuric acid (Analar).
Perchloric acid (Analar).
Hydrobromic acid (Analar).
Toluene/tropolone reagent (1.25 g tropolone in 2.5 l toluene).
"Soveril" centrifuge tubes.

7.3 Procedure

Wet tissue homogenate (1 g) is digested with 5 ml 1:1 concentration of nitric/sulphuric acid in a heated 'Soveril' tube (100°C for 4 h). Perchloric acid (0.5 ml) 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 with 5 ml of toluene/tropolone reagent by shaking for 20 min. The toluene extract is then analysed by EAAS (see Section 3). Duplicate procedural blanks and quality-control tissues are analysed with each batch of samples. Tissues spiked with TBTO are used to monitor extraction efficiency. Mean recover of TBTO is 95% (s.d. = 16, n = 8).

8. Determination of tributyltin in shellfish tissue

8.1 Basis of the method

As in the method above, the tissue is dispersed in acid and organotin is extracted by solvent. Some specificity is achieved by washing the solvent with sodium hydroxide to partition mono-organotin and diorganotin compounds into the aqueous phase. The solvent extract is then analysed by EAAS. The limit of detection of the method is approximately 0.4 $\mu g\ g^{-1}$ Sn in the original sample.

8.2 Reagents and equipment

Hydrochloric acid (Analar).
Sodium hydroxide (Analar).
Hexane (glass distilled grade).
'Soveril' centrifuge tubes.

8.3 Procedure

Wet tissue homogenate (1.5 g) is dispersed with concentrated hydrochloric acid in a 'Soveril' centrifuge tube. Hexane (10 ml) is added and the extract shaken for 30 min, and centrifuged at 2500 rpm for 15 min. Four millilitres of the hexane extract is transferred to another

centrifuge tube and 8 ml of 3% sodium hydroxide is added. The extract is shaken for 10 min, centrifuged, and an aliquot of the hexane extract is analysed using EAAS as above. Duplicate procedural blanks and a quality-control tissue are analysed with each batch of samples. Tissues spiked with TBTO are used to monitor extraction efficiency. Recovery of TBTO is 100% (s.d. = 6, n = 8).

The above methods for tissue analysis were based on those developed by M & T Chemicals Inc. (1982) and are essentially those described by Bryan et al. (1986), (see also Dooley and Vafa (1986)).

9. Speciation of alkyltin hydrides in tissues by gas chromatography (GC)

9.1 Basis of the method

Alkyltin compounds are extracted from animal tissues by dichloromethane and methanol, converted to hydrides by sodium borohydride and analysed by GC.

9.2 Reagents and equipment

Sodium borohydride powder (Analar).
Dichloromethane (glass distilled grade).
Methanol (glass distilled grade).
Tripropyltin chloride (TPTCl).
Alkyltin standards.
All-glass and PTFE micropipette.
FEP centrifuge tubes.

9.3 Procedure

An appropriate amount of internal standard (TPTCl) in methanol is added to 1 to 2 g of homogenised tissue in an FEP centrifuge tube and agitated on a mechanical shaker for 60 min. Ten millilitres of dichloromethane/methanol 1:1 v/v is added and the tube shaken for a further 30 min, centrifuged at 2000 rpm for 5 min and the supernatant liquid is decanted to another FEP centrifuge tube. The extract is blown down to 5 ml using a gentle stream of compressed air. Approximately 0.1 g of sodium borohydride and 3 ml of hexane are then added and the extract is shaken for 15 min. An aliquot of the hexane layer is removed, dried over anhydrous sodium sulphate and analysed by GC/FPD (conditions as for Section 5 but with the upper temperature for GC oven increased to 300°C). Results show reasonable agreement with EAAS methods (see Table 1). The limit of detection for TBT in oyster tissue is 0.01 $\mu g\ g^{-1}$. Recovery for TBTO relative to the internal standard is 99.6% (s.d. = 5.6, n = 13).

10. Speciation of alkyltin hydrides in animal tissues and sediments by gas chromatography (GC)

10.1 Basis of the method

Alkyltin compounds are extracted from animal tissues or sediments by sodium hydroxide and methanol, converted to hydrides and partitioned into hexane. The derivatives are analysed by gas chromatography.

10.2 Reagents and equipment

Sodium borohydride.
Sodium hydroxide (Analar).
Methanol (glass distilled grade).
Tripropyltin chloride.
Alkyltin standards.
All-glass and PTFE micropipette.
FEP centrifuge tubes.

10.3 Procedure

Samples of sediment are sieved through a 710 μm sieve to remove coarse material. Sub-samples of 4 g of sediment are then oven dried to constant weight at 105°C to determine moisture content. Extraction of alkyltin is then carried out on 2 g of wet sample, ensuring that there is at least 1 ml of water (either natural or added) in the sample.

An appropriate amount of internal standard (tripropyltin chloride) is added to the sample in an FEP centrifuge tube. 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 tube is capped and shaken for 45 min, then 3 ml of hexane and approximately 100 mg of sodium borohydride are added, and the sample shaken for a further 10 min. After centrifugation (2000 rpm for 5 min), the hexane phase is analysed by GC/FPD (see Section 5). The limit of detection for TBT in estuarine muds is 0.01 $\mu g\ g^{-1}$.

Tissues are prepared by homogenisation as in the previous method (Section 9). The extraction procedure is similar to that for sediments, but 10 ml of 0.1% sodium hydroxide in methanol is used to extract each 2 g sample, regardless of the water content of the tissue.

11. Quality assurance

11.1 Water

The use of a relatively large volume of water (750-2000 ml) prohibits the storage of a quality-control sample. Analytical quality control is monitored by the addition of a standard (50 ng l^{-1} TBTO) to either a 0.43 μm filtered sample of water from the laboratory seawater supply, or to tap water, for saltwater and freshwater samples respectively. A single 'spiked' sample is analysed with each batch (usually 10) of environmental samples. A single blank sample of water from the same source is analysed with each batch of environmental samples.

11.2 Tissues and sediments

Adult oysters held at sites known to be contaminated with TBT provide analytical quality-control tissue. Several hundred grams of oyster flesh are homogenised and 1.5 g aliquots in FEP centrifuge tubes are stored by freezing at -20°C. A single sample of quality-control tissue is analysed with each batch (usually 12) of environmental samples. Typical quality-control performance figures for the method described in Section 8 are: mean = 1.22 $\mu g\ g^{-1}$, s.d. = 0.21, n = 20. For the method given in Section 9, mean = 1.68 $\mu g\ g^{-1}$, s.d. = 0.09, n = 12. A less-contaminated analytical quality-control sample, used for the method outlined in Section 10, gave a

mean of 0.43 μg g^{-1}, s.d. = 0.04, n = 14. Using the same method, the sediment analytical quality control gave values of 9.08 μg g^{-1}, s.d. = 0.37, n = 14.

The performance of methods given in Sections 8, 9 and 10 is compared in Table 1. Oyster (Crassostrea gigas) tissue taken each month in 1986 provided a range of tissue concentrations of TBT.

Table 1 Butyltin content (μg g^{-1} wet weight) of Pacific oysters from Creeksea, River Crouch, using three different analytical methods

Collection date (1986)	Method-Section 8	Method-Section 9			Method-Section 10		
	TBT	MBT	DBT	TBT	MBT	DBT	TBT
26 Mar	0.13	<0.01	0.02	0.07	<0.01	0.02	0.08
28 April	0.30	0.01	0.04	0.34	<0.01	0.04	0.38
27 May	0.77	0.01	0.06	0.94	<0.01	0.07	1.25
25 June	0.93	0.01	0.07	1.16	0.01	0.08	1.55
23 July	0.82	0.01	0.08	1.11	0.01	0.08	1.29
22 Aug	1.12	0.01	0.08	1.45	0.01	0.06	1.72
19 Sept	0.83	0.01	0.10	1.04	0.01	0.05	1.24
6 Nov	0.72	0.01	0.10	0.84	0.01	0.04	1.13
3 Dec	0.58	<0.01	0.09	0.79	0.01	0.03	0.91

11.3 Intercalibrations

In addition to internal analytical quality-control procedures, wherever possible samples from other laboratories have been analysed in intercalibration exercises. Since most methods of analyses for organotins in environmental samples have been developed recently, such intercalibrations have been relatively simple (i.e. analysis of standards at higher than environmental concentrations). Nevertheless, in all such intercalibrations the above methods have given results close to the mean of results submitted by all participants.

12. Reagent and equipment suppliers

Hydrobromic acid. Glacial acetic acid. Nitric acid. Suplphuric acid. Perchloric acid. Hydrochloric acid. Sodium hydroxide. Toluene. Stannous chloride. Sodium borohydride pellets: All BDH Ltd., Poole, Dorset.

Dichloromethane. Hexane: Rathburn Chemicals Ltd., Walkerburn, Scotland.

Tropolone. Bis (tributyltin) oxide. Dibutyltin dichloride. Monobutyltin trichloride. Tripropyltin chloride: Merck, c/o BDH Ltd., Poole, Dorset; Aldrich Ltd. Gillingham, Dorset; and Sigma Ltd., Poole, Dorset.

'Eppendorf' polypropylene centrifuge tubes: Scientific Supplies Ltd., Vine Hill, London.

'Soveril' centrifuge tubes: V A Howe Ltd., Wandsworth, London.

FEP centrifuge tubes: BDH Ltd., Poole, Dorset.

GC columns: Hewlett Packard, Winnersh Triangle, Wokingham.

'Reactivials': Aldrich Ltd., Gillingham, Dorset.

GC/FPD filters: Ealing Electro-optics, Inc., Natick, M.A., USA.

Pyroneg: Diversy Ltd., Northampton.

Disclaimer: The reference to proprietary products in this report should not be construed as an official endorsement of those products, nor is any criticism implied of similar products which have not been mentioned.

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Appendix 1 - Details to be followed for sample collection, preparation and analysis in the conduct of cooperative monitoring

Monitoring, using fish or shellfish as indicator species, may be conducted for one of the following three purposes:

(1) The provision of a continuing assurance of the quality of marine foodstuffs with respect to human health.

(2) The provision over a wide geographical area of an indication of the health of the marine environment in the entire ICES North Atlantic area.

(3) The provision of an analysis of trends over time in pollutant concentrations in selected areas, especially in relation to the assessment of the efficacy of control measures.

Sampling

Samples to meet Objective 1 (to be collected every second year; started in 1982)

(a) A sample should consist of 25 fish or large crustaceans such as crabs or lobsters, 50 mussels or other molluscs, and 100 small crustaceans such as shrimps.

(b) The sample should be selected in a way that reflects the size distribution of the commercially exploitable portion of the catch of that species within that particular geographical area. This distribution may be determined from previous data or on board the sampling vessel but, having been established, should only be amended if a significant change in the distribution can be demonstrated.

(c) Sampling should be conducted prior to spawning of the species concerned.

(d) Samples should be collected from at least the following areas: the estuaries of the Forth, Thames, Rhine, Scheldt and Clyde, the Skagerrak, Kattegat and Oslo Fjord, the Irish Sea, German Bight and Southern Bight of the North Sea, certain parts of the Gulf of St Lawrence and the US middle Atlantic Bight, and the area off Portugal.

Samples to meet Objective 2 (to be collected every 5 years; started in 1985)

(a) A sample should consist of 25 fish or 50 mussels.

(b) Selected fish samples should be representative of the area in question, i.e. they should not be very recent immigrants to the area or on passage through it. Each sample should consist of the same or similar sized fish.

(c) Mussels should be between 20 and 50 mm in size and preferably as close as possible to the lower end of this range.

(d) Sampling should take place prior to spawning of the species concerned.

(e) Samples should be collected from as many locations as is practicable throughout the ICES area.

Samples to meet Objective 3 (to be collected every year; started in 1982)

(a) A sample of fish should consist of at least 25 individuals, and preferably more than 25 individuals. The sample should be collected in a length-stratified manner, i.e. the sizes of the fish should span a length range as wide as possible and there should be an equal number of individuals in each length grouping.

The stratification should be based upon an equidistant logged length interval, i.e. the log (upper bound) minus log (lower bound) should be equal for each length interval. The length range of the entire sample should be selected so that the individuals in the lower bound yield sufficient tissue for the chemical analyses, while the upper bound should be selected so that at least 5 fish can readily be found in the sampled catch. The length range should be divided into 5 (or more) length intervals of equal size (after log transformation). (See notes on length stratification on pages 24 and 25 for an example.) Once the length stratification for a particular species and area has been agreed, this stratification should be strictly adhered to for a number of years. No length interval should be less than 2-3 cm. If the length range is smaller than 2-3 cm, the species is not ideally suited for the proposed analysis.

(b) A sample of mussels should span a size range as wide as possible and should consist of sufficient individuals to provide material for analysis in groups of the different sizes. The number of individuals in each length range should be recorded for each site and, thereafter, this distribution should be used for that site each year.

(c) Sampling should be conducted annually from the same areas, from the same stock and at the same time each year; mussel samples should be collected at the same position in relation to tidal height each year.

(d) Samples should be collected in such a way that at least the following areas are adequately covered: the estuaries of the Forth, Thames, Rhine, Scheldt and Clyde, the Skagerrak, Kattegat and Oslo Fjord, the Irish Sea, German Bight and Southern Bight of the North Sea, certain parts of the Gulf of St Lawrence and the US middle Atlantic Bight and the area off Portugal.

(e) The species of interest can only be selected in the light of information on fish stock composition and history and the known or perceived problems which define national priorities. It is preferable to use a fish species which continues to grow throughout its life. Species which are of particular interest in an ICES context are: cod or hake; plaice; flounder; mackerel (Scomber scombrus); mussels; shrimps; but data relating to other species are also required.

Storage and pretreatment of samples prior to analyses

To meet all three objectives:

(a) Fish samples should be collected ungutted and preserved (deep frozen) as soon as practicable after collection; length and weight should be determined before freezing.

(b) Mussels should be held live in clean (settled) sea water from the area of collection for 12-14 h to allow discharge of pseudo-faeces. The length of each individual, even if used as part of a composite, should be measured as a maximum value regardless of direction or orientation.

(c) After cleaning and measuring the mussels, the individual animals should be carefully freed from their shells by cutting the adductor muscle. The shell cavity liquor can then be drained and discarded by placing the opened shells vertically in a filter funnel for 5 min. The remaining shell contents may then be preserved either individually or as pooled samples.

(d) Since a wide variety of factors can affect the total body burden of a pollutant in shrimps, the only useful objective in analysing shrimps is Objective 1. For this purpose, the shrimps should be boiled whole in sea water from the area of collection for 10 min. The tails should then be removed, peeled and thoroughly homogenised in preparation for storage or analysis.

To meet Objectives 1 or 2

(a) In order to reduce the number of analyses which have to be performed, pooled samples may be used. These should be prepared as described below and analysed in duplicate.

(b) An equivalent quantity of muscle tissue must be taken from each fish, e.g. a whole fillet of every fish. If the total quantity of yielded tissue is too large to be handled conveniently, the tissue may be sub-sampled, but a fixed proportion of each tissue must then be taken, e.g. 10% of each whole fillet or 10% of each whole liver, the sub-sample being taken after homogenisation of the whole fillet/liver or in the form of complete longitudinal sections.

To meet Objective 3

(a) Each fish should be analysed individually and the following biological variables should always be recorded when sampling for time trend analysis purposes: age; total weight; total length; liver weight when contaminants in liver are determined (if another fatty organ is used, the weight should be recorded); sex; and degree of sexual maturation, where applicable.

(b) Mussels may be pooled in small groups to provide enough tissue for analysis but different sized groups should be analysed separately.

Reporting of results

For Objective 1

Results should be reported on a wet weight basis along with details of the size range of the sample and details of site, date and method of collection, preservation details (if appropriate) and brief details of the methods of analysis used; if PCBs were analysed for, these details should include the formulation or isomer(s) and the method of quantification used.

For Objective 2

(a) Results should be reported as for Objective 1. In addition, results of analyses of mussels for metals should also be reported on a dry weight basis. All results of analyses for organochlorine compounds must be reported also on an extracted fat weight basis or as a minimum be accompanied by a fat weight determination result.

(b) Dry weight determinations should be carried out in duplicate by air-drying to constant weight at 105°C of sub-samples of the material analysed for the contaminants.

(c) Fat weight should be determined on a sub-sample of the extract used for the organochlorine compound analyses. The results should be accompanied by a brief description of the method used for extraction.

For Objective 3

Results should be reported as for Objective 2, but the individual analysis figures should be given together with full details of the size, age, weight, sex, etc., of the individual fish analysed.

In reporting these data to ICES, the ICES Interim Reporting Format for Contaminants in Fish and Shellfish must be used for such data so as to allow machine handling and statistical analysis of the data.

General details

Results should be submitted to the ICES Environment Officer not later than 30 June of the calendar year following collection of the sample. These results should be accompanied by the name of the contributing laboratories and the name of an individual contact in the event of queries. The contributors should specify the most recent ICES intercalibration exercise in which they took part. A brief commentary on the data is also required, at least in relation to that supplied for the purposes of Objectives 1 and 3.

Notes on length stratification

The main finding from the statistical analyses of data on contaminants in fish tissue is the gain in precision which can be obtained from stratification using biological variables. Although several biological parameters

have been shown to be significant as stratification variables in different materials, length appears to be the only parameter which is simple to apply at sea and which shows up as being significant in most analyses.

Much discussion has been devoted to whether simple linear or log-linear (multiplicative) models give the better fit. General experience with other fish and other types of data indicate preference for the log-normal model at least for the present. As the length dependence of the contaminant level is not well understood, sampling should keep the length-contaminant relationship under constant surveillance, i.e. the entire length range should be covered evenly. The length range should be defined from practical considerations, the lower bound ensuring that enough tissue is available for chemical analysis and the upper bound such that at least 5 fish in the largest length interval can readily be found. The length stratification should be determined in such a way that it can be maintained over many years. The length interval should be at least 2-3 cm in size.

It is suggested that the length range be split into 5 length intervals which are of equal size after log transformation. For example, if the length range is 20-70 cm, then the interval boundaries could be (rounded to 0.5 cm) as follows:

cm	No. of fish	Log upper - log lower
20.0-25.5	5	0.243
25.5-33.0	5	0.258
33.0-42.5	5	0.253
42.5-54.5	5	0.249
54.5-70.0	5	0.250
Total	25	

Care should be taken to ensure that samples are not unduly clustered within each stratum (length interval). More length intervals could be used and the test of the hypothesised contaminant-length relationship becomes stronger if the lengths are evenly distributed. But the item of major importance is to keep the length stratification identical from one year to the next.