Applied Organometallic Chemistry (1989) 3 295–308
© Longman Group UK Ltd 1989
0268–2605/89/03402295/$03.50

The determination of organotins (TBT) in fish and shellfish via gas chromatography–flame photometric detection and direct current plasma emission spectroscopy (GC–FPD/DCP)

Ira S Krull,*† Kenneth W Panaro,‡ John Noonan‡ and Donald Erickson‡
†Department of Chemistry and The Barnett Institute (341MU), Northeastern University, 360 Huntington Avenue, Boston, MA 02115, USA and ‡US Food and Drug Administration (FDA), Winchester Engineering and Analytical Center (WEAC), 109 Holton Street, Winchester, MA 01890, USA

Received 13 December 1988 *Accepted 23 February 1989*

Gas chromatography (GC) has been interfaced very simply and inexpensively with a flame photometric detector (FPD) and a direct current plasma (DCP) atomic emission spectrometer in order to perform highly specific and selective determinations of organotins in fish and shellfish samples. GC–FPD studies employed a fused-silica, megabore column with a thin, immobilized stationary phase of DB-17 (1 μm thickness), with a commercially available GC–FPD instrument. No prior alkylation or hydridization reactions were performed on the organotins; rather they were separated as the original, native species. Separate GC–FPD and GC–DCP injections and quantitative determinations have been performed, though simultaneous FPD/DCP detection on a single injection is suggested. This permitted routine qualitative and quantitative determinations of organotin species in complex food matrices (fish/shellfish) via both element selective detectors. Isothermal GC–FPD/DCP conditions permitted baseline resolution of all four tin species of interest today: monobutyl-, dibutyl-, tributyl- (TBT), and tetrabutyl-tin. Optimization of the GC–DCP interface was accomplished, followed by a determination of detection limits and linearity of the calibration plots, and a comparison of the results with those obtained by the newer GC–FPD approach (which was also developed here). In three sample instances, qualitative and quantitative results for naturally occurring and spiked levels agreed for both the GC–FPD and GC–DCP approaches. Improved sample preparation and extraction procedures have been developed for organotins from fish samples involving extraction with an organic solvent, concentration, saponification, back-extraction, and injection of the eluent onto the GC column. Quantitative levels of organotins (solely TBT) in fish and shellfish are reported for samples from Europe, Korea, Scandinavia, and the USA.

INTRODUCTION

Within the past few decades, the routine use of marine antifouling paints has increased.[1] Their use has spread to major navies around the world, commercial shipping fleets, pleasure craft, power-boat moorings, private and public marinas, ship storage facilities, and even pen nets for aquaculture (raising of fish for commercial profit).[2] There are some very significant advantages in the routine use of these antifouling paints, such as: (1) prevention of the growth of barnacles, shellfish, algae or marine plants on any objects routinely coming in contact with either fresh or salt water; (2) reduced ship or fishpen maintenance and repair; (3) prolonged ship time-at-sea and reduced dockyard/drydock time, out-of-service or inaction; (4) improved fuel efficiency and higher average speed of transit, leading to reduced times between destinations and increased shipping business; and (5) reduced frequency and therefore costs for ship maintenance and repainting. All of these translate into significant economic advantages for the

*Current Science Advisor to the Winchester, Massachusetts, FDA; author to whom correspondence and reprint requests should be addressed.

widespread and continued use of organotin-based marine antifouling paints.

Most commonly used antifouling paints today contain tributyltin (TBT) or a variation thereof, either immobilized on a polymer film or matrix, reversibly bound within a polymer film, or dissolved in an aqueous or oil base. Slow-releasing TBT polymeric materials are also commercially available, though much more expensive than TBT suspended in a paint matrix. As expected, those formulations that release TBT to the environment most rapidly are the least expensive to purchase and use, whilst those which release TBT very slowly into the marine surroundings cost the most to purchase and apply. It seems very clear at this point in time that TBT has become one of the most commonly found pollutants around the word.[3,4]

It is, in a sense, an ultimate pollutant. It is placed onto structures which come in contact with fresh or salt water, and these structures (ships, nets, pilings, etc.) release the TBT into these waters over vast distances over long periods of time. The TBT, once released, is then concentrated in the food chain, showing slow degradation with time, eventually becoming concentrated in man's food supply. Shellfish gradually accumulate TBT in large concentration ratios, by screening the water flowing through and around them. This preconcentration ratio, comparing levels in the water with those in the shellfish, is probably 2–3 orders of magnitude. Deformation of shellfish is a common observation after serious TBT build-up and accumulation. The presence of TBT has now been reported in many coastal enivronments, from France to England, Sweden to Norway. Chesapeake Bay to Seattle (USA), and elsewhere. It is probably safe to assume that TBT has become as prevalent and widespread as any organic or inorganic/organometal pollutant ever previously known to man.[5]

A large number of toxicological studies have been reported that dealt with the various toxic and biological effects that TBT has been shown to have towards marine fauna/flora and mammals.[6] Shellfish deformation is only one more obvious effect, but there are serious mutagenic and teratogenic results for almost all marine life exposed to TBT over time. In our view it is more than likely that similar toxic effects will eventually be demonstrated in higher animals and man. Therefore, the widespread dispersion and distribution of TBT in our food chain is something that deserves continuous monitoring and evaluation. Many reports have shown TBT and other organotins (usually breakdown products arising from TBT in the environment) to be present in environmental samples, including shellfish, fish and flora. However, there are very few studies in which determination of TBT levels in food destined for human consumption has been attempted.

The US Food and Drug Administration (FDA) has a responsibility to monitor continuously foods imported into the USA destined for human consumption. One such effort in this direction was reported by the Seattle Seafood Products Research Center of the US FDA.[7] Unfortunately, there are insufficient data at the moment to conclude at what levels and how often TBT is to be found in our food supply. It was, in part, the purpose of this study to develop improved gas chromatography (GC) element-selective detection (ESD) approaches for organotins in fish and shellfish. It was then intended to utilize such newer, validated approaches for as many real-world marine samples as was possible and practical.

There have been many methods described within recent years for the accurate and precise quantitative determination of TBT and related organotins in foods and water.[5,8–23] Most of these approaches have used gas chromatography, in view of the extreme volatility of derivatives of most butyltin species. However, there have been a number of reports on the possible uses of HPLC, often with element-selective detection for organotins and/or other metal-containing species.[22–29] By and large, most currently employed approaches for the accurate and precise quantitation of TBT and other butyltins rely on GC with some type of element-'selective' or specific detection technique. Most of these use flame photometric detection (FPD) with a tin-specific filter at 600 nm emission. Though somewhat selective for tin-containing species, it is not 100% specific for tin alone.

Recently, we reported an HPLC–direct current plasma (DCP) emission spectroscopic approach for methyltins, using a novel type of paired-ion, reversed-phase HPLC separation.[28] Because direct HPLC–DCP interfacing could not provide suitably low detection limits, we and others have utilized a post-column hydride formation step, after the separation and before introduction into the DCP plume (HPLC–HY–DCP). It is likely that this same approach will prove suitable for butyltins, if an HPLC–HY–DCP confirmatory method were needed or desired. However, for naturally volatile organometals, or for those species that can readily be converted into volatile

derivatives, GC still seemed the most reasonable and practical approach. We have recently described a GC–DCP approach for volatile organomercury species found in fish, especially for methylmercury.[29] Thus, the combination of GC with FPD and DCP appeared to be a very reliable and practical approach to obtain one and/or two 'selective' chromatograms from one or two injections of a fish or shellfish extract.

Most literature reports have utilized some type of pre-injection derivatization for TBT and the other butyltin species. Such approaches have generally used hydridization or alkylation, in order to provide improved GC performance characteristics of the original species. Improvements in the off-column derivatization methods generally reported have quite recently used reaction GC to form the hydrides of TBT and its analogs prior to FPD detection.[7,9]

There have been very few reports on the direct GC determination of butyltins without some type of pre-column derivatization.[30,31] In general, those reports that have used direct injection have shown broadened peak shapes, serious tailing of peaks, poor column efficiencies and less-than-ideal peak resolution or capacity. Clearly, an ideal GC–element selective detection (ESD) method might involve the following steps: (1) simple sample work-up and extraction from fish/shellfish with high recovery efficiencies; (2) no artetact formation of the butyltin species of interest during sample work-up or GC–ESD; (3) no prior derivatization off-line or on-line before GC injection; (4) good chromatographic performance properties with modern fused-silica capillary or megabore columns; (5) high analyte selectivity and identification via dual element selective detectors (FPD/DCP); (6) element ratios obtainable via multiwavelength (element) identification; (7) good accuracy, precision, and reproducibility of quantitations; and (8) high sample throughput and fast turn-around times.

We report here an analytical method for volatile organotins that utilizes direct sample extract injection onto a fused-silica, megabore GC column containing a thin film (1 μm thickness) of the immobilized stationary phase on the column walls. Separation was followed by either FPD or DCP detection, in order to provide two chromatograms whose peak heights and/or areas could be used for subtraction or ratioing to improve analyte identification further. It would also be feasible to make a single injection and obtain simultaneously both FPD/DCP chromatograms. The overall method, GC–FPD/DCP, has been optimized with regard to the standard analytical figures of merit. Validation has been derived from single blind spiked fish samples, as well as recovery studies on several of the real-world samples studied. The FPD and DCP results have, in general, been in excellent agreement. Finally, the overall methodology was applied to as many fish and shellfish samples as were obtainable, food destined for the marketplace and eventual human consumption.

EXPERIMENTAL

Chemicals, reagents, and solvents

Standards of n-butyltin trichloride, di-n-butyltin dibromide, tri-n-butyltin chloride, and tetra-n-butyltin, all of the highest purity available, were obtained from Morton Thiokol Corp., Alfa Division (Danvers, MA, USA). These were used without further purification. Purity was checked in-house via different GC–FPD/DCP conditions and total tin content via AA. Solvents, analytical grade, including acetone, methylene chloride, chloroform, hexane, and others, were all obtained as the Burdick and Jackson brand, distilled-in-glass (Doe & Ingalls, Medford, MA, USA). Amercian Chemical Society (ACS) certified grade concentrated hydrochloric acid was purchased from Fisher Scientific (Boston, MA, USA). The NBS samples of mussel and oyster composites were obtained from the National Bureau of Standards (US Department of Commerce, Gaithersburg, MD, USA).

Apparatus, instrumentation and operating conditions

A Tracor (Tracor Instruments Corp., Austin, TX, USA) model 560 gas chromatograph was used for most of the GC–FPD studies, containing a Melpar FPD unit with two tin (600 and 365 nm) filters. A separate Tracor model 560 gas chromatograph was used for the GC–DCP studies, wherein the column effluent was now directed to only the DCP detector. This unit was mounted directly beneath the Spectrametrics (Applied Research Laboratories, Dearborn, MI, USA) model Spectraspan IIIb DCP system, so that the GC column effluent was sent via a heated quartz tube into the normal viewing zone of the DCP. This was operated in the active diagnostic mode (repeat dial = 0). Other operating conditions included a sleeve pressure of

50 psi (345 kPa); zero nebulizer pressure (nebulizer, spray chamber, sample tube and peristaltic pump removed); a gain of 30; PMT voltage setting of 8 or 9 (900 or 950 V); input slit settings (vertical × horizontal) or 200 × 200 or 300 × 200; and an emission line of 303.4 nm.

The megabore GC column was a DB-17, 30 m × 0.53 mm × 1.0 μm coating from J&W Scientific Inc. (Folsom, CA, USA). A direct injection kit was used to fit the megabore column to the particular GC instrument used, and this was obtained from Supelco Inc. (Supelco Park, Bellefonte, PA, USA). A small plug of glass wool was placed in the injection liner, to protect the megabore column from contamination due to unwanted, extraneous sample components.

Standard operating conditions for the GC included a column temperature which was isocratic, or 160°C; an FPD temperature of 250°C; an injection port temperature of 200°C; a helium flow rate of 5 cm^3 min^{-1}; make-up cell gas of 25 cm^3 min^{-1} helium; a chart speed of 0.5 in min^{-1} (1.3 cm min^{-1}); and FPD gas flows of 200 cm^3 min^{-1} hydrogen, 120 cm^3 min^{-1} air, and 20 cm^3 min^{-1} oxygen.

Methods and procedures

Extractions of organotins from fish and shellfish involved an initial extraction, back-extraction, saponification, another extraction, and direct injection onto a megabore GC column. In general, this provided final fish samples that were compatible with repeated, routine, megabore GC requirements, without overloading or contaminating the head of the column after many repeat injections. Certain fish samples, those containing more fat, required more extensive sample work-up and preparation than others.

The organotin standards were prepared by using stock solutions made up by first dissolving the appropriate metal standard in acetone. The actual TBT external standard used for injection into GC was diluted to an appropriate volume with 0.2 mol dm^{-3} hydrochloric acid (HCl) in acetone, in order to improve GC peak shape and reproducibility. Mixed external standards containing DBT were made up in 2.0 mol dm^{-3} hydrochloric acid in acetone, again to improve peak shape of this organotin species.

The general procedure started with 50 g of fish/shellfish in a centrifuge bottle. To this was added about 150 cm^3 2% hydrochloric acid in acetone, and the mixture was homogenized with a Polytron homogenizer for about 1 min. This was followed by centrifugation at 1500 rpm for 5 min, decantation of the liquid portion into a 500 cm^3 round-bottom flask, and one additional extraction of the fish sample with another 150 cm^3 portion of 2% hydrochloric acid in acetone. All acetone extracts were combined. The acetone was removed on a rotary evaporator using a 45°C water bath. The remaining aqueous solution was then extracted with 150 cm^3 hexane. The hexane solution was dried through sodium sulfate (anhydrous) and collected in a 300 cm^3 round-bottom flask. The aqueous layer was re-extracted with 50 cm^3 hexane, and this was combined with the first hexane extract, after drying through the same sodium sulfate. The total hexane extracts were rotary-evaporated to less than 5 cm^3. The resulting residue was then quantitatively transferred to a 5 cm^3 volumetric flask and brought to exactly 5 cm^3. For high fat-containing fish (e.g. striped bass, salmon), 2 cm^3 from the 5 cm^3 sample was taken for saponification. If the sample was not high in fat content, the total 5 cm^3 sample was taken directly for saponification.

Saponification was crucial in order to prolong the lifetime of the GC column, and to provide good reproducibility of TBT peak shape, peak height, and peak area. The sample was transferred to a 125 cm^3 flat-bottom flask and treated with 50 cm^3 of 2.0 mol dm^{-3} potassium hydroxide in 60% methanol. The solution was heated for 1 h using the standard procedure for saponification. The sample was quantitatively transferred to a 1000 cm^3 separatory funnel containing 750 cm^3 of distilled/de-ionized water. To this funnel was added 100 cm^3 of a 1:1 mixture of chloroform/methylene chloride. The total solution was shaken for 1 min. The layers separated, and the lower, milky-white layer was transferred to a second 1000 cm^3 separatory funnel containing 750 cm^3 of de-ionized/distilled water and 60 cm^3 of saturated sodium chloride (NaCl) solution. No shaking of this second separatory funnel was necessary, for the milky-white layer when added to it immediately separated into a clear organic layer (bottom layer). If this did not happen immediately, an additional 10 cm^3 of saturated sodium chloride solution was added and the funnel was just swirled. The clear organic layer was dried through sodium sulfate and collected in a 500 cm^3 round-bottom flask. This extraction procedure was performed three more times, each time

using the 100 cm^3 of the 1:1 mixture. The organic layers were combined and evaporated just to dryness. The resultant residue was quantitatively transferred to a volumetric flask and brought to exactly the same volume as that originally taken for the saponification step (2 or 5 cm^3) with 0.2 mol dm^{-3} hydrochloric acid in acetone. If the saponification step was not included, some of the fats would pass through the entire procedure along with the organotins and end up being injected into the megabore column. This seriously degraded column performance and decreased overall lifetime and reproducibility.

RESULTS AND DISCUSSION

GC–FPD/DCP instrumentation arrangements

Although DCP has been interfaced with GC in several instances, especially to perform trace organometal speciation, most of this work has involved a heated transfer line.[32,33] This would be used to transfer the GC effluent into the DCP plume, but it provided additional, unwanted extra-column effects, especially band broadening and potential loss of sample (< 100% recovery of injected sample). We have not found any literature reports that have placed the DCP plume directly above the GC column exit, other than our own.[29] In our earlier study, emphasizing methylmercury, we had constructed a low-cost, independent, isothermal GC that slipped directly under the DCP. In the current studies, we actually lifted the DCP over the GC column exit (Fig. 1a), so that the effluent directly entered the DCP plume region. This final instrumental arrangement would allow us to split the GC effluent, and obtain both FPD and DCP chromatograms simultaneously from a single injection. This could in principle provide additional analyte confirmation using different detector peak height/area ratioing on a single injection. However, such split effluent results are not yet available.

Figure 1b, with the DCP top removed, illustrates the arrangement of the column exit, DCP plume region, and location of two other detectors available with this GC, and ECD and an FPD. However, for the present studies, we have used only a single detector per injection, and thus all of the chromatograms reported

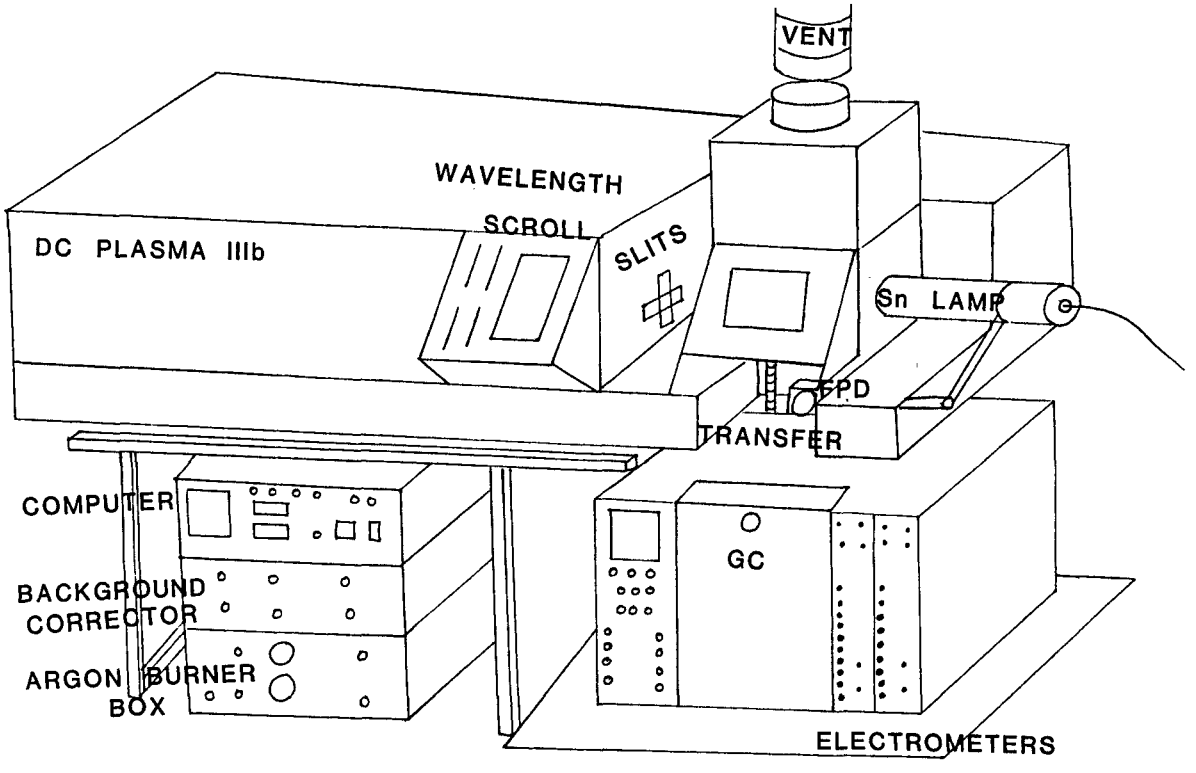

Figure 1a Schematic diagram of instrumentation used for GC–FPD/DCP determinations of organotins in fish/shellfish.

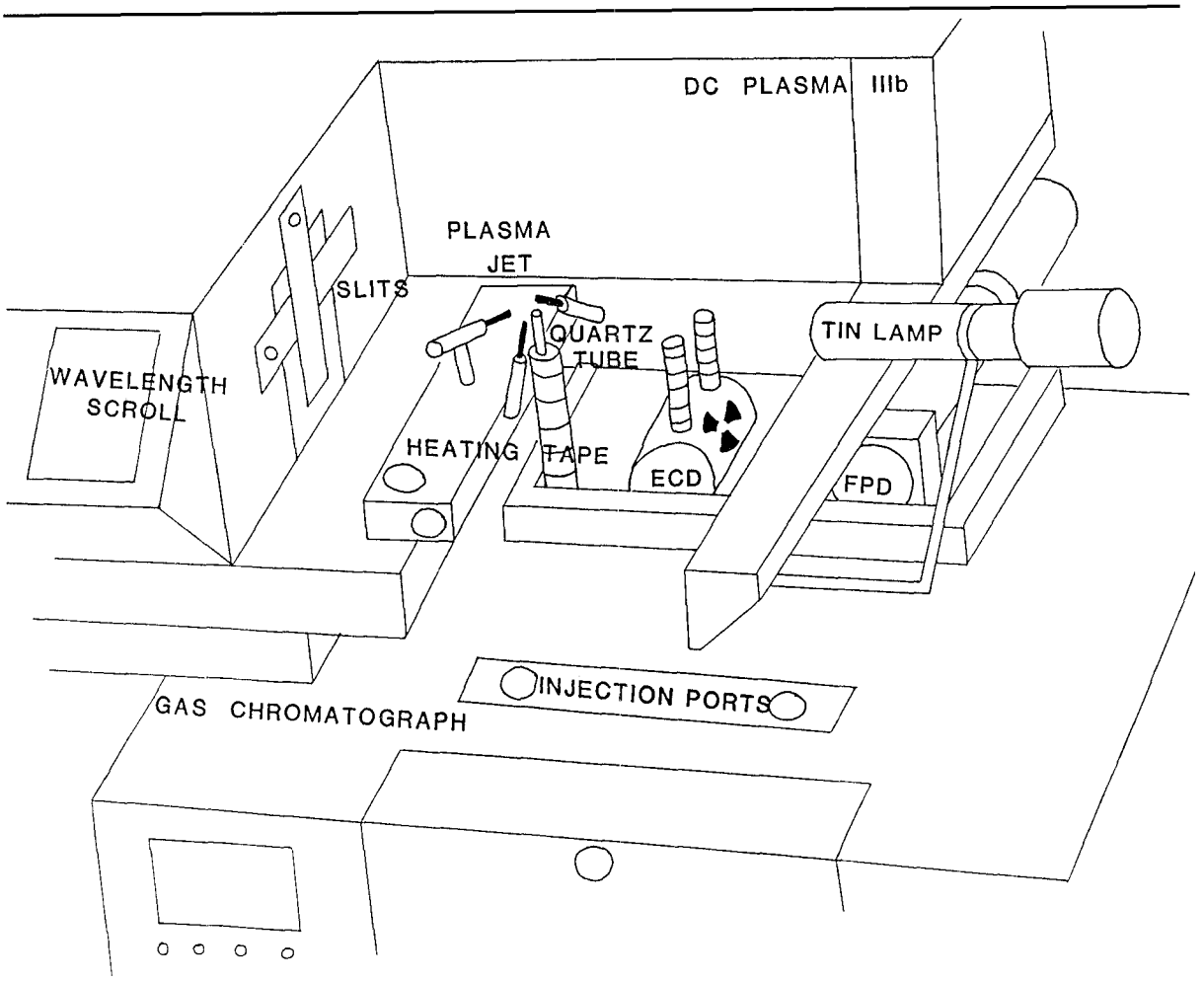

Figure 1b Arrangement of DCP plume, ECD and FPD detectors on top of the GC; DCP top housing removed.

depict a single detector's response, either FPD or DCP. Future studies will emphasize a variable-ratio, post-column effluent splitter, providing simultaneous dual chromatograms, FPD/DCP (organotins) or ECD/DCP (organomercury determinations). Placing the DCP above the GC column exit has provided for virtually zero extra-column variance; there is but a short segment of narrow internal diameter (i.d.), heated quartz tube to transfer the column effluent directly into the DCP plume.

Optimization of GC–FPD conditions for trace detection of organotins

All four organotins have been baseline-resolved, without prior derivatization, using the GC-FPD conditions indicated in Fig. 2 (see Experimental section). Similar chromatograms, not depicted here, have also been obtained via GC–DCP. A 1–μm film thickness of DB-17 in a megabore column of 0.53 mm i.d., 30 m long, was able to produce the separation, and peak shape, indicated. We suspect that this has been possible because of the thin film thickness now available with immobilized, wall-coated open tubular capillary (megabore) type columns. Though some peak asymmetry is evident, perhaps due to the FPD itself (compare Figs 2 and 6–8), all four peaks are baseline-resolved within 5 min. It has proved crucial that the standard organotins and sample extracts were made up in 0.2 mol dm^{-3} hydrochloric acid in acetone, in order to improve peak shape and reproducibility of injections. In the absence of the added hydrochloric acid, both peak shape and resolution suffered.

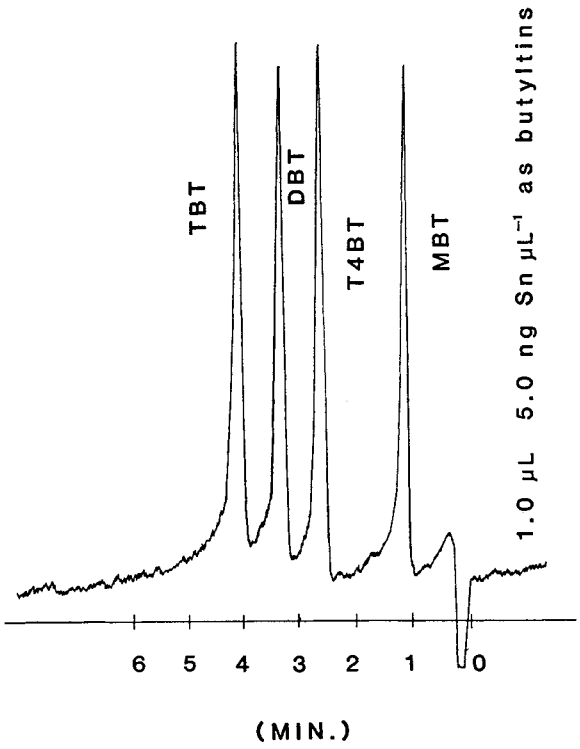

Figure 2 GC–FPD chromatogram for a mixture of four standard organotins under optimized GC conditions: megabore 1 μm DB-17 column, 30 m × 0.53 mm, operated at 160°C, FPD at 220°C, injection port at 200°C, flow rate 5 cm^3 min^{-1} helium. Melpar FPD unit; tin filter at 600 nm. FPD gas flows; hydrogen 200 cm^3 min^{-1}; air 120 cm^3 min^{-1}; oxygen 20 cm^3 min^{-1}. 1.0 μL 0.1 mol m^{-3} HCl in acetone followed injection, in same syringe. TBT, tributyltin; DBT, dibutyltin; T4BT, tetrabutyltin; MBT, monobutyltin.

Detection limits, calibration plots and linearity of calibration plots for DBT and TBT by FPD and DCP

Because tributyltin (TBT) and dibutyltin (DBT) are the two most commonly found organotins in the environment, we have emphasized their analytical figures of merit. Figure 3 illustrates a typical GC–FPD chromatogram for single injections of 0.05 ng tin as DBT and 0.05 ng tin as TBT, with 1μL injections of each on-column. Though baseline noise is considerable, each peak is obvious and peak shape is adequate for quantitation at these levels. Tables 1 and 2 summarize the determination of the percentage RSD (relative standard deviation) at the 0.05 ng level of tin as DBT or TBT. These levels represent a concentration injected of 50 ppb (parts per billion). Table 3

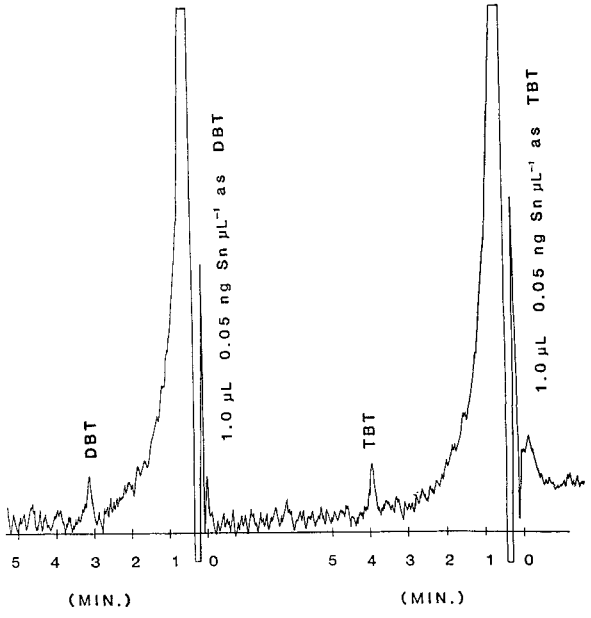

Figure 3 GC–FPD chromatogram of tributyltin and dibutyltin at the limits of detection; conditions as in Fig. 2.

Table 1 Detection limits of dibutyltin at the 0.05 ng/injection level by GC–FPD[a]

Injection no.	Analyte peak ht (mm)	Background peak ht (mm)
1	20.0	5.0
2	21.5	5.5
3	21.5	5.0
4	18.0	4.0
5	20.0	5.0
6	16.0	4.5
7	17.0	4.0
8	19.0	4.5
9	20.0	6.0
10	17.0	5.0
Mean	19.0	4.9
Standard deviation	±1.9	±0.65
RSD (%)	10.0	13.2

[a] Signal/noise ratio = 3.9:1.

summarizes the determination of percentage RSD at the 0.25 ng level of tin as TBT using GC–DCP, with a signal/noise ratio of 3:1. This level again corresponds to a concentration of 0.05 ng μL^{-1} of tin as TBT, or 50 ppb.

Calibation plots were obtained for both DBT and TBT via GC–FPD and TBT via GC–DCP, ranging from the detection limits, shown above, up to and

Table 2 Detection limits of tributyltin at the 0.05 ng/injection level by GC–FPD[a]

Injection no.	Analyte peak ht (mm)	Background peak ht (mm)
1	20.0	5.0
2	20.5	4.5
3	18.5	5.0
4	16.0	6.0
5	19.5	5.0
6	16.0	6.0
7	21.5	6.0
8	18.0	5.0
9	22.0	6.0
10	18.0	5.5
Mean	18.9	5.4
Standard deviation	±2.1	±0.56
RSD (%)	11.1	10.4

[a] Signal/noise ratio = 3.5:1.

Table 3 Detection limits of tributyltin at the 0.25 ng/inection level by GC–DCP[a]

Injection no.	Analyte peak ht (mm)	Background peak ht (mm)
1	36.0	10.0
2	30.0	8.5
3	29.0	10.0
4	31.5	12.0
5	39.0	12.5
6	34.0	11.0
Mean	33.3	10.7
Standard deviation	±3.81	±1.47
RSD (%)	11.4	13.7

[a] Signal/noise ratio = 3:1. Detection limit determined for 0.25 ng tin on column as tributyltin, 0.05 ng μL^{-1} tin as tributyltin injected, 5 μL injection.

including 5 or 300 ppm concentrations, (Table 4). The data points fit the equation for a straight line in each case, with correlation coefficients and coefficients of determination indicated. Though not demanding a straight line for such plots, the data are entirely consistent with linearity, suggesting that good quantitations are possible over the concentration ranges studied.

Comparison of FPD detection at 600 nm versus 365 nm for TBT

All of the data reported here and elsewhere have generally used FPD with a 600 nm emission filter. Though others have suggested that this is a more sensitive flame emission line for tin compounds, our own results suggest otherwise. Thus, Fig. 4 makes a direct comparison, on a single injection, of both wavelengths of DBT and TBT at the levels and GC conditions indicated. It is clear that improved sensitivity and detection limits could result by the future use of 365 nm as the preferred emission wavelength. Sensitivity is approximately double at the lower wavelength.

Comparison of GC–FPD and GC–DCP chromatograms for organotins

Figure 5 illustrates a set of three chromatograms via GC–FPD, with conditions indicated. The right-hand chromatogram is that of an NBS mussel composite sample, known to contain only TBT at low levels (ppb). The second (middle) chromatogram is that for a standard of TBT at the concentration indicated (100 ppb). The left-hand chromatogram is that for a typical sample blank, using the same extraction procedure that was applied to the original mussel sample (experimental). Peak shape and reproducibility,

Table 4 Summary of line equations of calibration plots for dibutyltin and tributyltin by GC–FPD/DCP

Organotin	Concentration range (ppm)	Correlation coefficient[a]	Coefficient of determination[a]
Dibutyltin (FPD)	0.05–100	0.9998	0.9997
Tributyltin (FPD)	0.05–100	0.9999	0.9999
Tributyltin (DCP)	0.005–5.0	0.9987	0.9974

[a] These represent the coefficients of a least-squares fit to a linear equation.

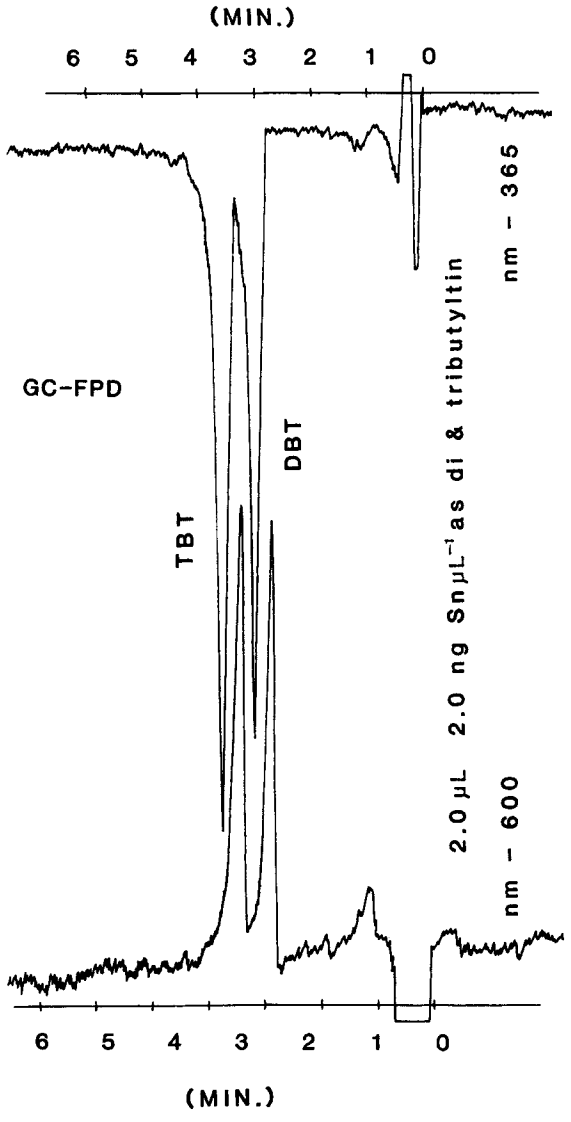

Figure 4 GC–FPD dual chromatograms for dibutyltin and tributyltin at the levels indicated, using two simultaneous detectors set at 600 nm and 365 nm for improved tin speciation. GC conditions: column of DB-17, 30 m × 0.53 mm, 1.0 μm film, column temperature 160°C, FPC cell temperature 220°C, injection port 200°C, column flow rate 5 cm^3 min^{-1} helium, Melpar FPD unit at 600 nm and 365 nm, hydrogen 200 cm^3 min^{-1}, air 120 cm^3 min^{-1}, oxygen 20 cm^3 min^{-1}; other conditions as indicated in Fig. 2. Note improved signal/noise response for the 365 nm chromatogram.

day-to-day or within-day, have all been excellent, using the now-optimized sample extraction procedure developed here for these GC–FPD/DCP studies.

Figure 6 illustrates a typical GC–DCP chromatogram for TBT, with conditions indicated. Except for a different carrier gas and increased flow rate, the GC conditions were identical to those above for GC–FPD. The retention time for TBT is about half that in the GC–FPD chromatograms, mainly because of the higher carrier gas flow rate. Under these particular GC conditions, TBT is not adequately resolved from other possible tin-containing species. Since we first proved that only TBT was present via the GC–FPD results, there was no need to use GC–DCP conditions that would also separate TBT from tin species not present in any of these particular samples. However, if needed, we have shown that the other three normally occurring organotins (DBT, MBT, Bu_4Sn) all separate from TBT under GC–DCP conditions approaching those used in GC–FPD. The final peak shape and asymmetry factor for TBT via GC–DCP were somewhat improved over those found by GC–FPD analysis (Fig. 5). This may have been due to the differences in detector response times, dead volumes, or contact time for FPD versus DCP. It may also have to do with the faster flow rate and shorter retention time in the GC–DCP mode.

Single-blind spiked results for TBT in fish via GC–FPD

In order to validate, in part, this newer approach, both sample work-up and GC–FPD detection, we have performed three separate, single-blind, spiking studies with different levels of TBT. Table 5 summarizes all of the data, wherein two flounder and one whiting sample were separately spiked by one analyst at the levels indicated, and these were then analyzed by a second analyst, levels unknown. Percentage recoveries represent the agreement between the spiked and found levels, which ranged from 92.6 to 98.6%. Reproducibility of these determinations was quite good, with standard deviations of ±1.08–3.60 for levels spiked below 200 ppb in TBT. These particular samples of fish contained no incurred (natural) levels of TBT (Table 6).

Figure 7 (conditions indicated) illustrates a GC–DCP chromatogram for a typical fish (smoked salmon from Denmark) extract containing high levels of TBT (Table 7). Figure 8 (conditions indicated) illustrates another typical GC–DCP chromatogram for a mussel (Maryland, USA) extract, at very low levels (Table 7). Thus, using the sample work-up procedure developed here specifically for megabore GC columns,

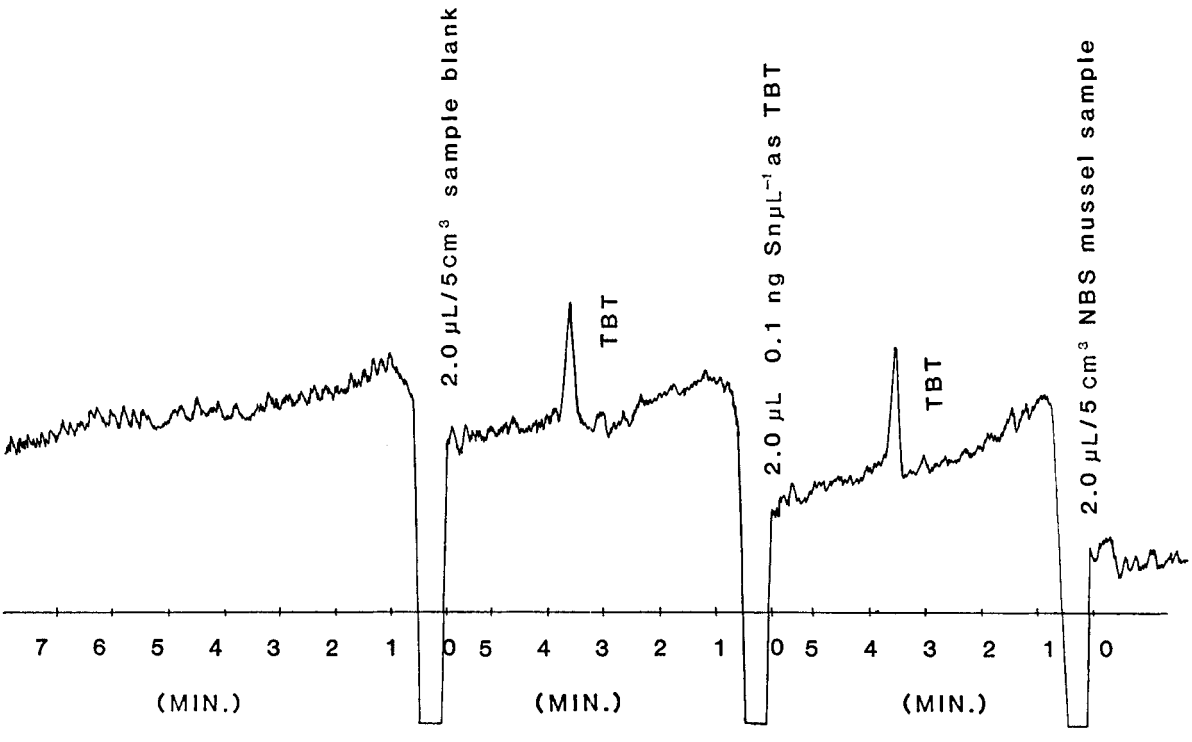

Figure 5 GC-FPD chromatograms of an NBS mussel sample extract containing tributyltin standard at the level indicated, and sample extract blank. GC-FPD conditions: DB-17, 30 m × 0.53 mm × 1 µm film, column temperature 160°C, FPD temperature 220°C, injection temperature 200°C, chart speed 0.5 in min^{-1} (1.3 cm min^{-1}), column flow rate 5 cm^3 min^{-1} helium, Melpar FPD unit used tin filter 600 nm, flow rate of hydrogen 200 cm^3 min^{-1}, air 120 cm^3 min^{-1}, oxygen 20 cm^3 min^{-1}.

without prior analyte derivatization, but with direct injection of cleaned-up extracts, peak shapes and chromatographic performance factors by either FPD or DCP detection have proved extremely useful and practical for real-world samples. This has been one of the few times that both standards and real-world samples have been analyzed by capillary (megabore) GC without prior analyte (tributyltin) hydridization or alkylation.

It should be clear that the direct injection of an appropriately worked-up sample extract is indeed very feasible and practical, providing (as below) highly accurate and precise quantitation at all incurred levels down to about 10 ppb. Though our detection limits by both FPD and DCP have been indicated as 50 ppb for a standard solution (Tables 1–3), in actual fish samples detection limits were below about 5 ppb. This was because of an effective, overall preconcentration of the TBT as a result of extraction. We have been able to detect tributyltin accurately and precisely at levels in fish or shellfish as low as 13 ppb (Table 6).

Determination of TBT in fish and shellfish

One of the major goals of this program was to determine levels, if any, of tributyltin and other organotins, if present, in various fish and shellfish samples from all parts of the world. Thus, any samples that came into the FDA laboratories in Boston/Winchester were worked-up and analyzed by GC–FDP and/or GC–DCP for levels of tributyltin. Table 6 summarizes all of the data available, for fish such as salmon, striped bass, flounder, and whiting, as well as shellfish, such as clams, quohogs, and oysters. In addition, NBS composites of oyster and mussel were analyzed for tributyltin. In six of the samples reported, we have performed recovery studies, and these ranged from 91.6 to 109.9%, within an acceptable range of 100 ± 10%. Most shellfish samples contained tributyltin at levels below 50 ppb. The flounder (USA) and whiting (Uruguay) contained no detectable tributyltin at our current limits of detection in the fish (*ca* 5 ppb).

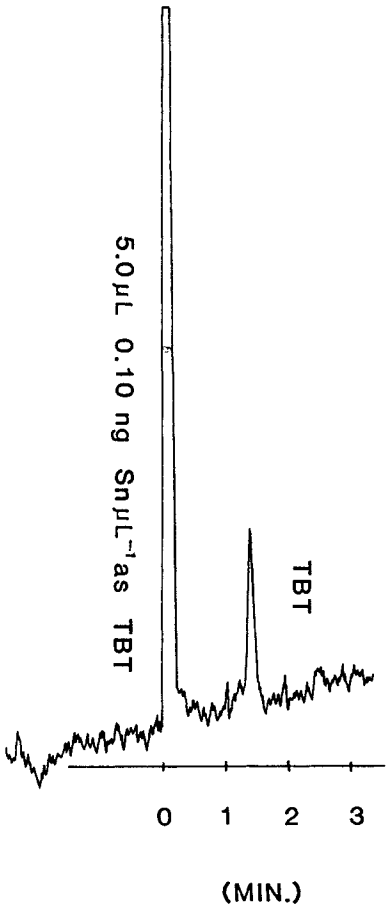

Figure 6 GC–DCP chromatogram for a single injection of standard tributyltin, at the level indicated, under optimized GC–DCP conditions, megabore 1 μm DB-17 column, 30 m × 0.53 mm, operated at 150°C, injection port 180°C, column flow rate 35 cm^3 min^{-1} argon, DCP at 303.41 nm, gain setting 40, PMT 9, sleeve pressure 50 psi (345 kla) active diagnostic mode, recorder chart speed 0.5 in min^{-1} (1.3 cm min^{-1}, 10 mV FSD).

However, one salmon sample from Denmark contained considerable levels of tributyltin, as high as 825 ppb. Other samples of salmon from Scotland, depending on the region of origin, contained levels of nil to less than 100 ppb. More than 13 samples were studied here, from all parts of the world; nine of these contained measurable levels of tributyltin. These results can be compared with those recently reported by another FDA laboratory in Seattle, WA, USA, which studied salmon from the northwest region of the USA.[7] In general, those fish contained tributyltin at levels below 100 ppb, but for a single specimen.

It is impossible to come to any worldwide conclusions based on the data presented here. It is of interest, however, that two of the salmon samples studied have significant levels of tributyltin, higher than those reported previously.[7] Much additional work is needed in order to estimate the level of man's tributyltin exposure via marketplace fish and shellfish, which are the major expected sources of TBT intake.

Comparison of GC–FPD and GC–DCP results for tributyltin in fish and shellfish

Though FPD is somewhat selective for tin-containing compounds, which is why it has always been the preferred, general detection method in GC, it is possible that DCP can be more selective, perhaps specific. The FPD operates on the basis of a filter or filters, meant to select only a narrow range of wavelengths, as emitted by tin-containing compounds having burned in the flame. The DCP, on the other hand, is somewhat more specific for tin, in that it allows a much narrower range of wavelengths to be detected by its photomultiplier tube, because of the improved monochromator and optics inherent in the DCP over the FPD. Basically, the FPD has no monochromator, only a set of one or more filters. Depending on the nature of the sample, extraction efficiency for organotins alone, and other factors, in principle the DCP can indeed be more specific for tin compounds. Our own improved sample work-up procedures, developed here, have allowed us to use either FPD or DCP detection and to see only a single peak in every sample for tributyltin, whether present or incurred. When sample preparation is less rigorous, or FPD-responding interferents (non-tin-containing) remain in the sample extract, we would expect the DCP chromatograms to be simpler, cleaner, and less ambiguous in both qualitative and quantitative terms. The spectral bandwidth in the FPD 600 nm filter was ±5 nm (595–605 nm), whilst the effective bandwidth of the DCP at 303.41 nm was ±0.05 nm.

In order to compare directly the advantages possible, as well as accuracy, precision, and reproducibility, we have studied three samples of salmon, mussel, and whiting for tributyltin by both GC–FPD and GC–DCP. Table 7 summarizes the levels of tributyltin found by both GC–FPD and GC–DCP. We have never found any evidence for other organotins present in any of the samples studied by either FPD or DCP. In general, the GC–DCP numbers are in good to excellent agreement with those for the GC–FPD approach. Percentage recoveries were reported above

Table 5 Single-blind,[a] spiked results for tributyltin in fish by GC–FPD

Fish species	TBT added (ppb)	TBT recovered ± SD (ppb)	Recovery (%)
Flounder	46.84	45.62 ± 1.08 (n=3)	97.4
Flounder	93.68	86.79 ± 3.42 (n=3)	92.6
Whiting	187.36	184.8 ± 3.68 (n=3)	98.6

[a] Single-blind protocol was followed; levels spiked were unknown to the analyst doing the TBT determinations. Values were compared through a third party.

Table 6 Summary of tributyltin present in fish and shellfish by GC–FPD

Fish species	Origin	Found ± SD[a] (ppb)	Spiked ppb	Recovery (± SD (ppb)	Recovery (%)
Smoked salmon	Scotland	85.22 ± 6.25 (n=9)	–	–	–
Smoked salmon	Scotland	Nil (n=3)	93.68	95.16 ± 2.46 (n=3)	101.6
Smoked salmon	Ireland	Nil (n=3)	–	–	–
Smoked salmon	Denmark	824.5 ± 111 (n=9)	–	–	–
Smoked salmon	Norway	71.34 ± 5.06 (n=9)	93.68	97.05 ± 3.0 (n=3)	103.6
Smoked salmon	Norway	213.7 ± 13.6 (n=9)	–	–	–
Striped bass	Rhode Island, USA	84.08 ± 3.56 (n=9)	140.52	128.72 ± 7.96 (n=3)	91.6
Flounder	Rhode Island, USA	Nil (n=3)	–	–	–
Whiting[b]	Uruguay, S. America	Nil (n=3)	93.68	92.81 ± 0.80 (n=3)	99.1
Oysters	Korea	49.91 ± 1.08 (n=9)	46.84	51.50 ± 1.64 (n=3)	109.9
Quohogs	Rhode Island, USA	13.07 ± 1.31 (n=9)	140.52	127.12 ± 2.34 (n=3)	90.5
Clams	Maryland, USA	15.90 ± 1.90 (n=9)	–	–	–
Clams	Maryland, USA	19.30 ± 1.90 (n=9)	–	–	–
NBS oyster	Composite (USA)	17.07 ± 0.37 (n=3)	–	–	–
NBS mussel	composite (USA)	34.03 ± 0.56 (n=3)	–	–	–

–, Not determined.

[a] Determined in terms of tributyltin present, rather than tin.

[b] Single blind, spiked sample; no tributyltin incurred. GC–FPD recovery=98.6%.

Table 7 Summary of tributyltin present in fish and shellfish using GC–FPD and GC–DCP detection, separately on the same sample

Fish species	Origin	GC–DCP found ± SD[a] (ppb)	GC–FPD found ± SD[a] (ppb)
Smoked salmon	Denmark	801.9 ± 58.1 (n=9)	824.5 ± 111.9 (n=9)
Mussel	Maryland, USA	32.88 ± 2.3 (n=6)	34.03 ± 0.56 (n=3)
Whiting[b]	Uruguay	185.3 ± 4.7 (n=3)	184.8 ± 3.6 (n=3)

[a] Determined in terms of tributyltin present, rather than tin.

[b] Single-blind, spiked sample, no tributyltin incurred. GC–FPD recovery = 98.6%, GC–DCP recovery = 98.9%.

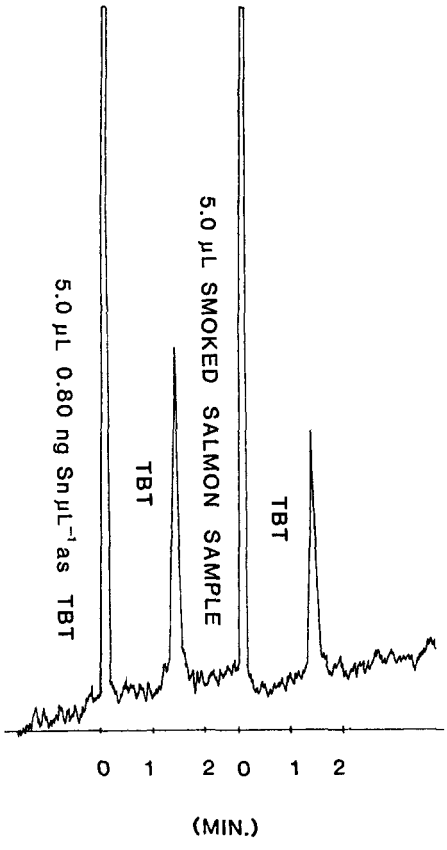

Figure 7 GC-DCP chromatogram for an extract of smoked salmon from Denmark and standard injection of tributyltin, with tributyltin peak as indicated. Specific conditions: megabore 1 μm DB-17 column, 30 m × 0.53 mm, operated at 150°C, injection port 180°C, column flow rate 35 cm^3 min^{-1} argon, DCP at 303.41 nm, gain setting 30, PMT 8 sleeve pressure 50 psi (345 kla), active diagnostic mode, recorder chart speed 0.5 in min^{-1} (1.3 cm min^{-1}, 10 mV FSD).

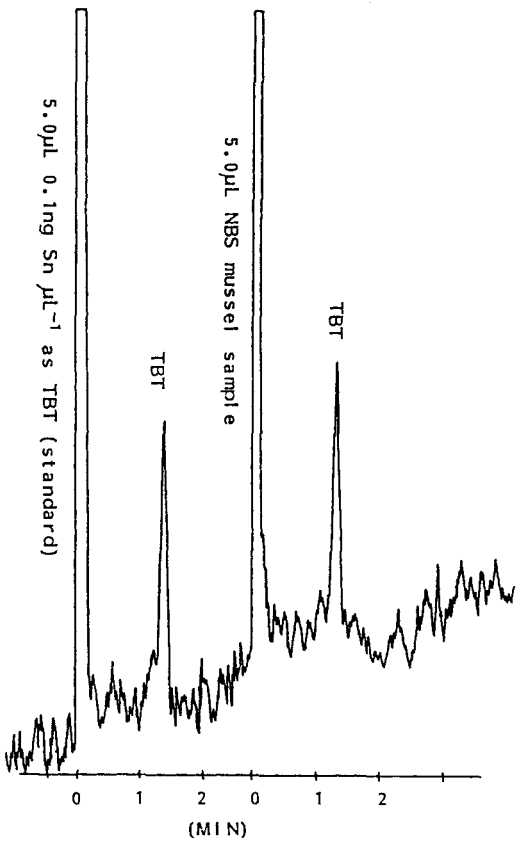

Figure 8 GC-DCP chromatograms for an extract of shellfish (mussel) from Maryland, USA, with tributyltin peak as indicated. Specific conditions megabore 1 μm DB-17 column, 30 m × 0.53 mm, operated at 150°C, injection port 180°C, column flow rate 35 cm^3 min^{-1} argon, DCP at 303.41 nm, gain setting 40, PMT 9, sleeve pressure 50psi (345 kla), active diagnostic mode, recorder chart speed 0.5 in min^{-1} (1.3 cm min^{-1}, 10 mV FSD).

(Table 6) for GC–FPD, and only one of these has been repeated by GC–DCP. For the single-blind, spiked sample of whiting (nil incurred tributyltin, percentage recovery by GC–FPD was 98.6% and by GC–DCP 98.9%, again in excellent agreement. The actual level spiked was indicated in Table 5 (187.36 ppb). There does not appear to be an artefact present by either detection approach.[29]

In the future, dual FPD/DCP chromatograms will be generated for a single sample injection, using a fixed-ratio effluent splitter with the GC oven. This will then permit additional analyte identification via dual detector responses, using a single retention time, as opposed to two different retention times used here via two separate injections. The dual chromatograms thus generated will directly provide peak height/area ratioing and comparison with the same data for standards injected under identical GC conditions.[34] The same approach could be used here with two different injections under slightly different GC conditions, and comparisons made between standards and suspected TBT peaks in samples has been based on two different retention times, peak shapes, and tin-specific/selective responses in FPD/DCP. Additional confirmation has been possible via quantitation in recovery and single-blind, spiked studies.

Acknowledgements This work was performed at the Boston, MA, and Winchester, MA, District Offices of the US Food and Drug Administration (FDA). We are grateful to the FDA for the opportunity to perform this work and to report the results. Acknowledgement is made to colleagues within the FDA who prepared blind, spiked fish samples for method validation purposes, including M Lookabaugh and A Gelasco. W S Adams, L Gershman, and M Finkelson provided encouragement, time, and guidance during these studies. T Gilbert at Northeastern University made valuable comments on reading earlier drafts of the manuscript. I S Krull is a Science Advisor to the Winchester Engineering and Analytical Center District Office of the US FDA. Ken Panaro was the Trace Metals Specialist at the WEAC District Office of the US FDA. This is contribution number 383 from The Barnett Institute at Northeastern University.

REFERENCES

1. Champ, M A and Pugh, W L in *Oceans '87 Proceedings, The Ocean – An International Workplace*, vol 4, International Organotin Symposium, Halifax, Nova Scotia, Canada, September 28–October 1, 1987, Wm MacNab & Son, Halifax, Canada, 1987, p 1296 (copies available from: The IEEE Service Center, 445 Hoes Lane, Piscataway, NJ 08854, USA)
2. Schatzberg, P In Ref., 1, p 1324
3. Ref. 1, pp 1352–1398
4. Clark, E A, Sterritt, R M and Lester, J N *Environ. Sci. Technol.*, 1988, 22(6): 600
5. Ref. 1, pp 1334–1352
6. Ref. 1, pp 1421–1524
7. Sullivan, J J, Torkelson, J D, Wekell, M W, Hollingsworth, A, Saxton, W L, Miller, G A, Panaro, K W and Uhler, A D *Anal. Chem.*, 1988, 60: 626
8. Blair, W W, Olson, G J, Brinckman, F E, Paule, R C and Becker, D A *An International Butyltin Measurement Methods Intercomparison: Sample Preparation and Results of Analyses*, NBSIR 86-3321, US Department of Commerce, National Bureau of Standards, Gaithersburg, MD, 1986
9. (a) Clark, S, Ashby, J and Craig, P J *Analyst (London)*, 1987, 112: 1781; (b) Clark, S and Craig, P J *Organomet. Chem.*, 1988, 2: 33
10. Sasaki, K, Ishizaka, T, Suzuki, T and Saito, Y *J. Assoc. Off. Anal. Chem.*, 1988, 71(2): 360
11. Muller, M D *Anal. Chem.*, 1987, 59: 617
12. Nakahara, T *Appl. Spectrosc.*, 1983, 37(6): 539
13. Chau, Y K, Wong, P T S and Bengert, G A *Anal. Chem.*, 1982, 54: 246
14. Ebdon, L, Hill, S J and Jones, P *Analyst (London)*, 1985, 110: 515
15. Tsuda, T, Hakanishi, H, Morita, T and Takebayashi, J *J. Assoc. Off. Anal. Chem.*, 1986, 69(6): 981
16. Unger, M A, MacIntyre, W G, Greaves, J and Huggett, R J *Chemosphere*, 1986, 15(4): 461
17. Blair, W R, Parks, E J, Olson, G J, Brinckman, F E, Valeiras-Price, M C and Bellama, J M *J. Chromatogr.*, 1987, 410: 383
18. Matthias, C L, Bellama, J M, Olson, G J and Brinckman, F E *Environ. Sci. Technol.*, 1986, 20: 609
19. Donard, O F X, Rapsomanikis, S and Weber, J H *Anal. Chem.*, 1986, 58: 772
20. Jewett, K L and Brinckman, F E *J. Chromatogr. Sci.*, 1981, 19: 583
21. Jackson, J A, Blair, W R, Brinckman, F E and Iverson, W P *Environ. Sci. Technol.*, 1982, 16: 110
22. Uden, P C *Chromatogr. Forum*, November–December, 1986, **17**
23. Uden, P C Specific element detection in chromatography by plasma emission spectroscopy. In *Environmental Speciation and Monitoring Needs for Trace Metal-Containing Substances from Energy-Related Processes*, Brinckman, F E and Fish, R H (eds), National Bureau of Standards, Washington, DC, 1981, p 184
24. Krull, I S Trace metal analysis by high performance liquid chromatography. In *Environmental Analysis by Liquid Chromatography*, Lawrence, J F (ed), The Humana Press, Clifton, NJ, 1984, Chapter 5
25. Krull, I S *Trends Anal. Chem. (TrAC)*, 1984, 3(3): 76
26. Bushee, D S, Krull, I S, Demko, P R and Smith, S B, Jr *J. Liq. Chromatogr.*, 1984, 7(5): 8671
27. Krull, I S and Panaro, K W *Appl. Spectrosc.*, 1985, 39(6): 960
28. Krull, I S Ion chromatography and liquid chromatography in inorganic speciation studies. In *The Dahlem Conference on The Importance of Chemical Speciation in Environmental Processes*, Bernhard, M, Brinckman, F E, and Sadlar, P J (eds) West Berlin, FRG, September 1984, Springer-Verlag, Berlin and Heidelberg, 1986, p 579
29. Panaro, K W, Erickson, D and Krull, I S *Analyst (London)*, 1987, 112: 1097
30. Junk, G A and Richard, J J Tributyltin chloride in water: solid phase extraction capillary GC and separation and EC detection. In *Oceans '86 Proceedings*, vol. 4, Organotin Symposium, September 1986, Washington, DC, p 1160 (Copies available from: The IEEE Service Center, 445 Hoes Lane, Piscataway, NJ 08854, USA)
31. Panaro, K and Krull, I S *Anal. Lett.*, 1984, 17(A2): 157
32. Cappon, C J *Liquid chromatogr./Gas chromatogr.*, 1987, 5(5): 400
33. Ebdon, L, Hill, S and Ward, R W *Analyst (London)*, 1986, 111: 1113
34. Krull, I S, Swartz, M E and Driscoll, J N Multiple detection in gas chromatography. In *Advances in Chromatography*, vol. 24, Giddings, J C, Grushka, E, Cazes, J and Brown, P R (eds), Marcel Dekker, New York, 1984, Chapter 8