SCREENING OF ORGANOTIN COMPOUNDS IN THE SWEDISH ENVIRONMENT

SNV Contract: 219 0102

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Abstract

The ecotoxicological effects of organotin compounds (OTC), mainly tributyltin (TBT) and triphenyltin (TPhT) but also their di- and monosubstituted degradation products are well documented. Nowadays, the release of TBT from antifouling paints is recognized worldwide as being one of the main contamination problems for the marine environment, and the use of TBT-based antifouling paints is almost everywhere restricted by law. In order to evaluate the environmental distribution and fate of these compounds, and to control the effectiveness of these legal provisions, many analytical methods have been developed among which gas chromatography coupled to inductively coupled plasma mass spectrometry (GC-ICP-MS) is the most powerful. Previous studies in the Swedish environment have shown the presence of considerable amounts of butyltins in mussel and sediment samples.

In the present study, screening of both butyltins and phenyltins in the Swedish environment is performed using GC-ICP-MS. A method for species specific isotope dilution, (SSID-GC-ICP-MS) was developed using isotopicaly labelled butyl- and phenyltins, synthesized in our laboratory from isotopically enriched metallic ^{116}Sn and ^{124}Sn respectively. Biological samples (clupea harengus, mytilus edulis, salmo salmar, and nassarius reticulatus), sediments, sewage sludge, water samples from purification plants, and harbours were collected from different parts of Sweden and analysed. Generally, the concentrations of OTCs for most samples were found to be lower compared to the amounts reported in 1987. The concentration of TBT, for example, is now 0.1-0.6 ng Sn/L compared to the 260-410 ng Sn/L reported in 1987 for the water samples from Fiskebäckskilsvik. The decrease in OTC concentration was also noticeable for the mussel (mytilus edulis) samples taken from the same area. The concentration of TBT in the mussel samples was found to be between 17-364 ng Sn/g compared to the concentrations 1000-18000 ng Sn/g reported in 1987. Considerable amounts of OTCs were found in the monthly sludge from the water purification plant in Loudden (Stockholm) where monobutyltin (MBT), dibutyltin (DBT), and TBT concentrations were found to be 39, 692, and 32 ng/g respectively. The results from the determination of OTCs in the sediment samples from Gålö, Karlsudd revealed that the sediment layers between 1-3 cm and 39-50 cm had the highest concentrations.

A literature study over relevant topics concerning OTC was performed. More than 140 references organized by the filing programme (Idealist) are presented in a separate file.

Sammanfattning

De ekotoxikologiska effekterna av organotennföreningar (OTC), i huvudsak tributyltenn (TBT) och trifenyltenn (TBT) men också deras mono- och di-substituerade dealkyleringsprodukter är väl dokumenterade. Idag är utsläpp av TBT från skeppsbåttenfärgerna känt för att vara en fara för marina miljöer och användandet har förbjudits i många länder, inklusive Sverige. En rad olika analysmetoder har använts för att utvärdera hur föreningarna är fördelade i miljön, samt för att kontrollera om utsläpp fortfarande förekommer efter införandet av restriktioner. Gaskromatografi kopplad till ”Inductively Coupled Plasma Mass Spectrometry” GC-ICP-MS är en av de kraftfullaste analysteknikerna som finns idag. Tidigare studier om miljöefekter av tennbaserade skeppsbotten-färger har visat att det finns ansenliga mängder av framförallt tributylten, i blåmussla och sediment, i svensk miljö.

I den här studien har vi använt GC-ICP-MS för att undersöka förekomsten av både fenyltenn och butyltenn föreningar. En metod baserad på species-specifik isotoputspäddning (SSID-GC-ICP-MS) har utvecklats genom att använda isotop anrikade organoten föreningar som vi syntetiserade från anrikade ^{116}Sn och ^{124}Sn tenn metaller. Biologiska prover (blåmussla, strömming lax och snäckor), sediment, slam, vatten från reningsverk (både utgående och inkommande vatten) och småbåtshamnar, har tagits från olika delar i landet och analyserats. Koncentration av organotennföreningar i de prover som analyserades var generellt sett lägre än det som rapporterades 1987. Koncentrationen av, till exempel, TBT i vattenproverna från Fiskebäckskilsvik är mycket lägre nu (0.1-0.6 ng Sn/L) jämfört med det som rapporterades 1987 (260-410 ng Sn/L). Haltminskningen av organotennföreningar i mussel proverna, tagna från samma plats, var också avsevärt. TBT koncentrationen ligger mellan 17 och 364 ng Sn/g mot det som raporterades 1987 (1000-18000 ng Sn/g). Högre halter av organotennföreningar har vi funnit i månadsslam från vattenreningsanläggningen i Loudden (Stockholm) där monobutyltenn (MBT), dibutyltenn (DBT) och TBT koncentrationerna bestämdes vara 39, 692, respektive 32 ng Sn/g. Halterna av organotennföreningarna i sediment varierade med sedimentdepositionsdjupet och de högsta halterna av till exempel TBT påträffades mellan sedimentsnittarna 1-3 cm och 39-50 cm.

Literaturstudie över relevanta artiklar har genomförts. Mer än 140 referenser har samlats och förts in i ett referensprogram (Idealist) och de är presenterade i en separat data fil.

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1 Aim of the Project

The Swedish Environmental Protection Agency (Naturvårdsverket) organized a survey for the National Chemicals Inspectorate (Kemikalieinspektionen), during 1987-1988, on the occurrence of organotin compounds in water and biota in Sweden [1]. This survey along with a previously made preliminary investigation performed in 1986-1987 [2] was supposed to be "used as part of the background documentation for decisions on restrictions to use organotin compounds as an active ingredient in antifouling paints" according to Ingvar Björklund [1]. The analysis was that time performed by SIF (Sentrum for Industriforskning) in Oslo.

In 2000 Bo Jansson, from the Institute of Applied Environmental Research (Institutet för tillämpad miljöforskning, ITM) at Stockholm University made a survey on organotin compounds in the Swedish environment and documented that our knowledge on the concentration levels of TBT and TPhT in the Swedish environment is very limited [3]. He proposed that the level of the most common organotin compounds (TBT and TPhT) in the Swedish aquatic environment should be screened and such a screening project should also include method development in order to be able to work with sensitive and reliable analytical methods.

The current study deals with screening of organotin compounds in the Swedish environment and the development of analytical methods for performing the analyses. Emphasis is given to quality assurance and validation of the analytical methods using certified reference materials (CRM), whenever available, when dealing with different sample types (for example mussels, sediments, and water).

2 Introduction

Tin in its inorganic form is generally accepted as being non-toxic, but the toxicological pattern of organotin compounds (OTC) is very complex. The biological effects of the substances depend on both the nature and the number of the organic groups bound to the Sn cation. In general, maximum toxicological activity for aquatic organisms is proved for trisubstituted compounds in any of the R_nSnX_{4-n}–series. The nature of the X-group in R_3SnX derivatives has little or no effect on the biocidic activity, except if X is a toxic component by itself. In this case the biological activity of the OTC may be enhanced [4]. Tributyltin (TBT) and triphenyltin (TPhT) have been extensively used as additives in antifouling paints, wood preservatives, fungicides, biocides, and polymers [5]. TBT-based paints are used on vessels hulls to prevent growth of aquatic organisms that create roughness giving rise to increased drag, resulting in reduced vessel speed per unit energy consumption. The antifouling paint consists of a film-forming material with a biocidal ingredient and a pigment. It works by releasing small amounts of the biocide from the painted hull into the water, forming a thin envelope of highly concentrated TBT around the boat. The toxic concentration repels the settling stages of fouling organisms, like barnacles, seaweeds, or tubeworms on the boat's water-immersed surfaces [4].

However, the constant release of OTC from anti-fouling paints has led to toxic effects for nontarget aquatic species in the aquatic environment, where they cause deleterious effects, such as shell anomalies in oysters and imposex in gastropods, even at concentrations as low as nanograms per liter. In many countries, the use of TBT and TPhT compounds as antifouling paints for small boats is now restricted by law. However organotin compounds (mainly TBT) are still used in paint formulation for large vessels, and about 69 % of all large ships are reported to use them [6]. Once released into the aquatic environment, organotin compounds may undergo a variety of degradation reactions until they are finally adsorbed onto suspended solids and sediments. Sediments are considered to be the ultimate sinks for organotin compounds, thus provide a means to assess the integrated organotin

compound pollution over larger time scale. In addition to being a record of past contamination with organotin compounds of a specific area, sediments also form a potential risk, as remobilization of adsorbed organotin compounds from the sediment may occur [7].

In water, TBT decomposes into less toxic DBT and MBT species. The problem is that this favourable decomposition takes place far more slowly in sediments, creating an ecotoxicological risk long after its release into a given area, making sediments a source of pollution. TBT and its degradation products DBT and MBT have been detected in different environmental compartments, both marine (waters, sediments, and biota) and terrestrial (waters and soils). The occurrences of the less toxic MBT and DBT compounds in the environment have so far been related to the degradation of TBT caused by microbial activity and/or photochemical reactions, but recently evidence for direct input of MBT and DBT was found by leaching from polyvinyl chlorides (PVC). In fact the major application of organotin compounds (about 70 %) is the use of mono and dialkyltin derivatives as heat and light stabilizer additives in PVC processing [4]. The introduction of relevant environmental legislation in many countries including the European Community has stimulated the development of analytical methods for the determination of the trisubstituted organotin compounds and their degradation products. Figure 1 shows the ionic form of the most extensively studied types of organotin species.

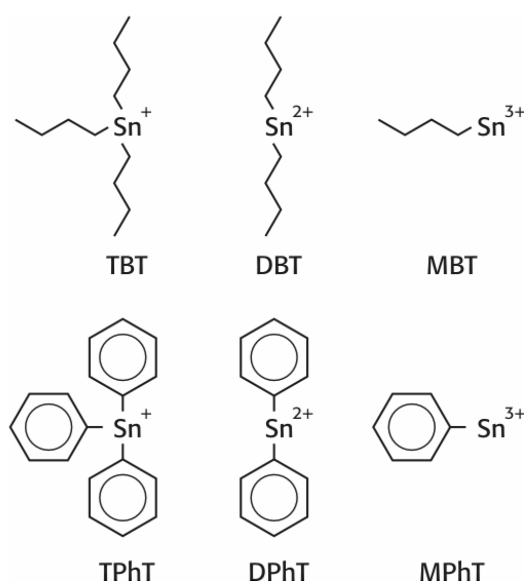

Figure 1. The ionic form of butyltins and phenyltins of varying substitution.

The presently available techniques for the determination of OTCs involve several analytical steps such as extraction, pre-concentration, cleanup, derivatisation (when gas chromatography is used), separation, and finally detection by element- or molecule specific techniques [8-10]. The multitude of analytical steps is causing errors at various levels, making speciation analysis a difficult task.

The extraction step is often accompanied by simultaneous acid leaching (HCl, HBr, acetic acid) and transfer of the released OTC to a solvent of low to medium polarity (e.g., hexane, benzene, toluene), often with the help of a complexing agent such as tropolone or sodium diethyl dithiocarbamate (DDTC). A study on the sorption and desorption characteristics of OTC using pre-deuterated OTC has shown that sorption is a fast and reversible process, involving primarily particulate organic matter (POM) constituents as sorbents [11]. In reality, however, the ease of extracting OTC in general and MBT in particular is dependent on the nature of the sediment and the extraction conditions. Using pre-deuterated internal standards Arnold et al., [12] studied the extractability of OTC in different acetic acid/sodium acetate concentration ratios and found that a combination of 0.25 M sodium acetate and 0.25 M acetic acid enhanced the desorption of mono- and diorganotin compounds. This enhancement is believed to be due to an exchange of sodium ions for MBT and DBT

(which are cationic) on the surface of the sediment, and acetate complexes the leached cationic OTC during extraction with organic solvents. Abalos et al., [13] studied the effect of hydrochloric and acetic acid on the extraction efficiency for MBT, DBT, and TBT. When comparing the two acids they found that acetic acid yielded better recoveries for TBT and DBT, whereas a decrease in their extracted amounts paralleling an important increase in MBT extraction arose from the use of HCl (at 25 % concentration). They then concluded that the increase in MBT was due to degradation of TBT and DBT. In the same study, when evaluating the extraction variables for phenyltins, the authors reported that no degredation takes place in the presence of HCl (25 %) but when pure acetic acid was used an increase in MPhT was observed. This could be due to degradation of DPhT and TPhT, or a better extractability of MPhT in this solvent. The use of solvents with high polarizability was also shown to favour the extraction of the more polar organotins such as MPhT. They also investigated the effect of the extraction time and proposed incorporation of several short extraction period (three times five minutes) with fresh extraction mixtures instead of a single longer extraction, to minimize the possibilities of degradation. Spikes of TPhT could not be recovered when tropolone was used as a complexing agent in a toluene extraction mixture. The authors speculated that the complete loss of TPhT could be associated with a dismutation process, which would lead to the formation of one molecule of DPhT and one of inorganic tin.

In order to enable separation by GC, the ionic organotin compounds need to be extracted from the sample matrix and converted into volatile species such as the hydrides, or their fully alkylated form. For the reduction to hydrides, sodium tetrahydroborate ($NaBH_4$) is commonly used, whereas derivatisation through alkylation can be carried out with Grignard reagents, sodium tetraethylborate ($NaBEt_4$), or sodium tetra(n-propyl)borate ($NaBPr_4$). The use of Grignard reagents has been most common but its sensitivity towards water requires that the organotin species are first extracted into an apolar aprotic solvent by using complexing agents like tropolone or DDTC. *In situ* hydride generation with $NaBH_4$ provides acceptable recovery rates only when applied to methyl- and butyltins, which precludes hydride formation as a means of analyzing organotins of higher boiling point such as the phenyltins [14]. $NaBEt_4$ has become a popular derivatisation reagent during the last years [4]. Derivatisation with $NaBEt_4$ makes the sample preparation faster and easier because it combines an *in situ* derivatisation with extraction of the ethylated organotin compounds into an organic phase.

Several techniques, based on species specific analytical methods, have been developed for the determination of butyltins in environmental matrices. Hyphenated systems, based on on-line coupling of gas chromatography (GC), liquid chromatography (LC), or supercritical fluid chromatography (SFC), to mass spectrometry (MS), inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), and microwave induced plasma atomic emission spectrometry (MIP-AES) are in current use [6]. Among the different techniques, the coupling of GC to ICP-MS appears to be one of the most popular techniques, due to high sensitivity and multi-elemental and multi-isotopic capabilities [10]. Some of the most frequently used sample pretreatment methods for the determination of OTC, in various samples, are summarized in Table 1.

The coupling of chromatography with ICP-MS permits detection of several isotopes. Isotope dilution analysis has been used in organic mass spectrometry for some years, but has only recently been transferred to inorganic mass spectrometry. It is a technique based on isotope ratio measurement whereby the natural isotopic abundance ratio of an analyte is altered by spiking with a standard that has a different isotopic abundance ratio. The prerequisite for the technique is that the analyte of interest should have more than one stable isotope [17]. In the case of tin (10 isotopes) the isotope of highest abundance is ^{120}Sn, usually referred as the reference isotope, and the spike isotope is generally one of the less abundant natural isotopes. For the purposes of speciation analysis, where OTCs are to be determined, there is a requirement for the isotopically enriched element-species to be synthesized. If two interference-free isotopes of a given element can be found, isotope dilution ICP-MS (ID-ICP-MS) can be performed, which generally provides superior accuracy and precision over other calibration strategies, including external calibration and the

method of standard addition, because a ratio rather than an absolute intensity measurement is used in the quantification of the analyte concentration. Once equilibration is achieved between the analyte in the sample and the added spike, ID-ICP-MS is theoretically capable of compensating for any subsequent loss of analyte during sample manipulation, suppression of ion sensitivities by concomitant elements present in the sample matrix, and instrument drift. ID-ICP-MS may therefore be considered as a primary method of analysis and can play a crucial role for quality assurance in trace element chemical speciation of environmental and biological samples. ID-ICP-MS coupled with gas chromatography (GC) ID-GC-ICP-MS [10] and liquid chromatography (LC) ID-LC-ICP-MS [17] have now proven to be the techniques of choice for speciation of organotin compounds. The derivatisation step is not needed in the case of LC (omitting one source of error), but the coupling with GC is superior when it comes to analysing samples of very low concentrations (pg/g) of OTC.

Table 1. Some of the most commonly used methods for the determination of OTCs in various samples along with the analysis results obtained.

Sample type	Sample pretreatment	Compound studied (amount found / ng^{-l})	Separation and detection technique	Ref
Marine sediment from contaminated harbour area in Mar piccolo (Italy)	Add 3 mL HCl and 6 mL methanol to 1 g of sample. Shake and sonicate for 15 min in ultrasonic bath. Add 3 mL of acetate buffer (pH 5.3) and centrifuge the leached sediment at 4000 rpm for 15 min. Pipette 1 mL supernatant into a 15 mL glass vial and add 6 mL of HOAc/NaOAc buffer (pH 5.3). Close the vial with a septum and add 1 mL of $NaBEt_4$ solution with a syringe. Sonicate the reaction mixture and pierce the SPME needle into the septum and expose the fiber into the headspace.	MBT = 8 DBT = 10 TBT = 1	Headspace microextraction GC/MS	8
Sediment samples Samples 1 and 2 from the harbour of Ostend, sample 3 from a dry dock in the harbour of Antwerp, and sample 4 from a leisure craft maintenance place located in the province of Limburg, all in Belgium	Add 2 ml of HCl (32 %) and 8 ml H_2O to 1 g sample in a centrifugation vessel. Add 25 ml of hexane-ethyl acetate mixture (1:1) containning 0.05 % tropolone. Sonicate the mixture for 1 h, followed by centrifugation at 3000 rpm for 5 min. Transfer the organic phase into an extraction vessel and evaporate to dryness using rotary evapo-ration. Add 0.5 zml of hexane containing Pe_3SnEt as an internal standard and derivatize by adding 1 ml of $NaBEt_4$ solution together with 50 ml of acetate buffer solution. Shake the mixture manually for 5 min and seperate the hexane phase. Introduce the extract into a clean-up column (a pasteur pipette filled with alumina to form a plug of 5 cm). Add an additional volume of 1 ml diethyl ether and evaporate the added diethyl ether using a gentle stream of nitrogen.	**Sample 1** MBT= 0.14 $\pm$ 0.02 DBT= 0.44 $\pm$ 0.03 TBT= 0.14 $\pm$ 0.02 **Sample 2** MBT= 0.36 $\pm$ 0.02 DBT= 1.11 $\pm$ 0.12 TBT= 2.33 $\pm$ 0.14 **Sample 3** MBT= 8.13 $\pm$ 0.33 DBT= 10.0 $\pm$ 0.6 TBT= 26.4 $\pm$ 1.8 **Sample 4** MBT= 1.55 $\pm$ 0.09 DBT= 1.67 $\pm$ 0.20 TBT= 6.60 $\pm$ 0.18	GC interfaced with AAS and AES	12
Sediment samples Two samples (1&2) from the harbour of Ostend (Belgium).	Add 4 ml H_2O, 1 ml acetic acid (96 %), 1 ml DDTC in pentane, and 25 ml hexane into a 100 ml Erlenmeyer flask containing 1 g sample. Sonicate the mixture for 30 min and decant the organic phase into a 100 ml beaker. Repeat the extraction with 25 ml of hexane and stir magnetically for 30 min. Centrifuge the mixture for 5 min at 3000 rpm. Dry over Na_2SO_4 and evaporate to dryness on a rotary evaporator. Add 250 µl of n-octane, containing Pr_3SnPe and pentylate with 1 ml of 1 M n-PeMgBr. Destroy excess Grignard reagent by adding 10 ml of 0.5 M H_2SO_4. Introduce the octane layer into a clean-up column (a pasteur pipette filled with alumina to form a plug of 5 cm). Add an additional volume of 1 ml diethyl ether and evaporate the added diethyl ether using a gentle stream of nitrogen.	**Sample 1** DBT= 0.43 $\pm$ 0.02 TBT= 0.31 $\pm$ 0.03 **Sample 2** DBT= 1.39 $\pm$ 0.06 TBT= 2.67 $\pm$ 0.08	GC interfaced with AAS and AES	12

Sample type	Sample pretreatment	Compound studied (amount found / ng^{-l})	Separation and detection technique	Ref
Water 1. Sea water from Sahrm el Sheikh harbour in South Sinai (Egypt) 2. Harbour water from Wädenswil, Lake Zurich (Switzerland)	Add 0.5 mL of acetic acid/acetate buffer solution (pH 5) and 1.45 g of sodium chloride to 50 mL water sample. After shaking spike with 100 µl of deuterated standard solution mixture (12.5 ng/L of each species in MeOH). Shake and add 150 µl of 1.5 % (w/v) $NaBEt_4$ aqueous solution. Add 1 ml of hexane and shake in the dark for 12 h at 25 ºC. Transfer 180 µl of the hexane extract to a 1 mL autosampler vial and spike with 10 µl of 0.2 ng/µl TeBT in hexane. Inject 50 µl in to the GC.	**1. Sahrm el Sheikh** MBT = 3.4 ± 7.6 DBT = 2.1 ± 24 TBT = 2.6 ± 17 MPhT= 1.5 ± 6.7 DPhT = 0.5 ± 76 TPhT = 4.8 ± 2.1 **2. Wädenswil** MBT = nd DBT = 3 ± 17 TBT = nd MPhT = 37 ± 3 DPhT = 23 ± 6 TPhT = 353 ± 3	LLE, Large volume injection GC/MS	15
Sediment Certified reference material PACS-2 (0.98 ± 0.13 TBT ng/L)	Put 0.5 g of PACS-2 in a Prolabo microwave digester and spike with 0.04 mL of ^{117}Sn-enriched TBT solution. Add 5 mL of acetic acid and heat at 60 % power for 3 min. Centrifuge at 2000 rpm for 5 min. Transfer 1 mL volume of the supernatant to a reaction vial and add 1 mL of deionized water. Adjust to pH 5-6 with 1.2 mL ammonium hydroxide. Buffer the content with 0.8 mL of ammonium citrate (2 mol L^{-1}) and dilute to 10 mL with methanol.	TBT = 1.018 ± 0.0315	Microwave extraction, ID-HPLC-ICP-MS	16
Sediment/Sludge 1. Sediment pore water from (1-20 cm) from Stansstaad harbour, Lake Lucerne (Switzerland) 2. Sewage sludge from four wastewater treatment plants in the Zurich canton (Switzerland)	Weigh 2.5 g freeze dried sediment or sludge in a beaker. Spike homogeneously with 500 µl of deuterated standard solution mixture (12.5 ng/L of each species in MeOH). Mix with 9 g of quartz sand and transfer the mixture to 11 mL extraction cells. After two hours fill the extraction cells with a mixture of 1 M sodium acetate and 1 M acetic acid in MeOH, using ASE. Extract with three to five static cycles of 5 min. Renew 4 mL of solvent between each static extraction. Rinse the cells with 4 mL of solvent and purge with nitrogen. Transfer the combined extracts to 250 mL volumetric flasks containing 7.3 g of NaCl. Add water and adjust the pH to 5 with 1 M NaOH. Add 1 mL of aqueous solution of 5 % (w/v) $NaBEt_4$ and fill the bottles to 250 mL with water. Add 2 mL of hexane and shake for 12 h. Transfer 500 µl of the hexane extract to 2 mL GC vials and spike with 10 µl of TeBT (5 ng/µl). For sewage sludge transfer the hexane extract to 10 mL centrifuge tubes containing 0.9 g of deactivated silica gel and 2 mL water. Shake vigorously and centrifuge.	1) Sediment pore water MBT = 11.0± 1.9 DBT = 4.5 ± 4.7 TBT = 9.6 ± 4.4 MPhT = 12.3 ± 4 DPhT = 2.5 ± 14 TPhT = 4.1± 3 2) Sewage sludge MBT = 300 ± 4 DBT = 253 ± 5 TBT = 45 ± 5 MPhT = 7 ± 21 DPhT = nd TPhT = 7 ± 38	ASE, Large volume injection GC/MS	15

Sandrine Aguerre et al. evaluated the performance of four specific detectors, hyphenated with gas chromatographic separation, in terms of sensitivity, selectivity, linearity and operation costs [18]. The relative performance of flame photometric detection (FPD), microwave-induced plasma atomic emission spectrometry (MIP-AES), pulsed flame photometric detector (PFPD), and ICP-MS are presented in Table 2.

Table 2. Comparison of the analytical performances of four different detection principles coupled with GC in the determination of organotin compounds.

Analytical Parameter		FPD	MIP-AES	PFPD	ICP-MS
Limit of Detection (LOD) (pg Sn l^{-1})	MBT	31	42	4	2
	DBT	7	11	1	0.7
	TBT	6	9	1	0.6
	MPhT	114	53	8	4
	DPhT	167	58	13	6
	TPhT	583	415	200	20
Repeatability (%)	MBT	3	5	4	8
	DBT	3	4	5	9
	TBT	5	6	7	16
	MPhT	9	5	5	14
	DPhT	8	8	9	8
	TPhT	16	18	18	25

The limit of detection (LOD) is expressed as three times the standard deviation of the noise. Repeatability is expressed as relative standard deviation (RSD) for the whole procedure and calculated from six replicates.

3 Experimental

3.1 Sampling

3.1.1 Site selection and sample preparation

The choice of the sampling locations was made in collaboration with Britta Hedlund (SNV) and other experts who were involved in previous studies [1]. The sampling sites for the different samples are shown in Figure 2.

3.1.1.1 Water samples

Pre-cleaned brown glass bottles (amber DURAN) equipped with polypropene screw closures, suprapure HCl (in house distilled), pre-cleaned Pasteur pipettes, and an instruction leaflet for sampling (see appendix 1) was sent to the personnel at the different sampling locations for collecting the water samples.

The samples from the yacht harbour in Hinsholmskil (Gothenburg) were collected by submersing the glass bottles at a depth of 0.5 m, approximately 500 m away from deck M, S, and Y (see figure 2b). The samples were then acidified on-site to pH 2 using suprapure HCl, to increase the solubility of the organotins and thereby avoid adsorption to the glass walls, and stored in a refrigerator (4 °C).

Inlet and outlet water samples from water purification plants were obtained from Gryaab (Gothenburg) and Loudden (Stockholm Water AB).

3.1.1.2 Sediment samples

The sediment samples, sliced at 1 cm intervals, were taken from one site (Fig 2a), using a sampling probe at position N590665 E181968 (33 m depth), Swedish position system RT90, in cooperation with Professor Per Jonsson from ITM. The samples where then stored at -18 °C and freeze-dried.

3.1.1.3 Mussel (mytilus edulis) samples

The mussel samples were collected from different sites in the bay of Fiskebäckskil (the inner part, the middle, and the mouth of the bay) and were collected in collaboration with Professor Åke Granmo, Kristineberg Marine Research Station. The mussel samples were shipped from the

sampling area in styrofoam boxes, filled with dry ice cubes, and stored in the refrigerator upon arrival to our laboratory.

3.1.1.4 Herring (clupea harengus) and Salmon (salmo salar) samples

Three kilograms of herring was purchased from a local fisherman at "Obbola By" (2001-06-11) and kept on ice. The herring was caught the previous day at N 63˚ 37.5', O 20˚ 15.91' and kept on ice. Bones and head were removed and 30 % of the fillets (with skin) were collected from all fishes and homogenized and frozen (-22 °C) the same day and later freeze-dried without thawing. Part of the sample was kept unfrozen for the determination of the water content.

Twenty-five pieces of filet (3 kg) from 9 different salmons from the same batch were collected at a fish shop (Domus) in Umeå (2002-06-12). The salmons had a weight of 5-6 kg each and had been farmed in Norway by Nergard AS, farmer: TSK-04. The sample taken was part of order 1953/21, N.136. Sub-samples were taken from each piece (total of about 1 kg) and homogenized and frozen (-22 °C) for freeze drying. Part of the sample was kept unfrozen to determine the water content.

3.1.1.5 Mollusc (nassarius reticulatus) samples

A total of 21 mollusc samples, collected from 6 different sampling sites [19], were received from Kristineberg Marine Research Station. The samples were stored in the refrigerator upon arrival to our laboratory.

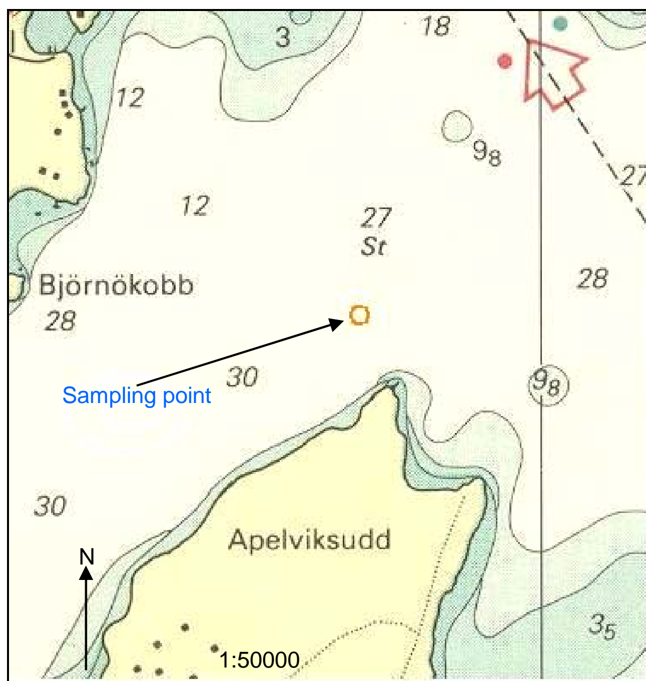

Figure 2a: The samplig site at Sandemarsfjärden, Karlsudd Gålö, in Stockholms skärgård

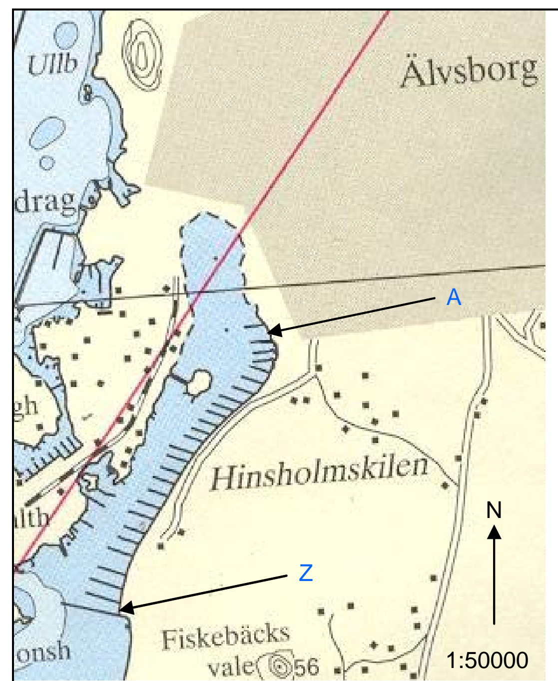

Figure 2b: The samplig site at Hinsholmskilen, Gothenburg

Figure 2c: The samplig site at Norrbyn, Västerbotten

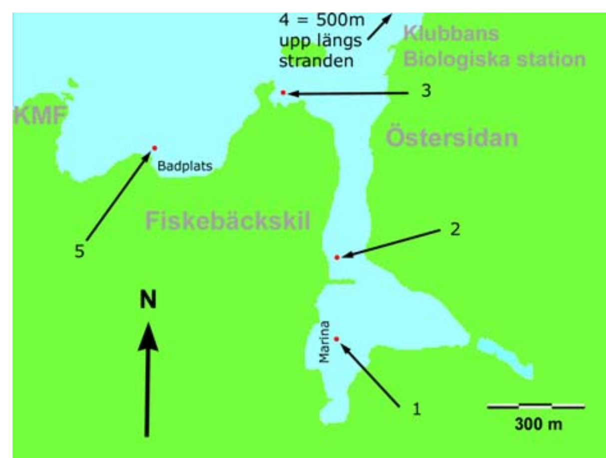

Figure 2d: The samplig sites in the Bay of Fiskebäckskil, Västkusten

NOTE: Maps are not to scale!

3.1.1.6 Samples from sewage purification stations

The sludge sample (collected over a month) was taken from a sewage treatment station in Loudden, in collaboration with Cajsa Wahlberg, at the water purification station in Stockholm (Stockholm Vatten).

4 Development of the Analytical Methodology

Much has happened on speciation of organotins, since we started to work on this project. The absence of suitable certified reference materials (CRMs) has until recently been one of the stumbling blocks in the validation of analytical methods for the speciation of organotin compounds. The commercially available CRMs, BCR646 and PACS-2 have been used throughout this work. BCR646 is a freshwater sediment and is certified with respect to MBT, DBT, TBT, MPhT, DPhT, and TPhT while only butyltin values are available for PACS-2 (marine sediment). There is still much to be done to understand the inter-conversion processes (alkylation and dialkylation) that takes place during sample work up. Synthesis of organotin species from isotopically enriched tin has currently improved the reliability for species-specific determination of the butyltins using MS-detection [16]. Methods based on isotope dilution have generally proven to be very promising in the determination of OTCs and the manufacturing of more reference materials is also underway. Our laboratory is participating in the production of candidate CRMs as well as in intercomparison studies including OTCs.

As part of our work here, we have synthesized isotopically enriched organotin species for the development of more reliable extraction and derivatisation procedures for the determination of OTCs in sediments, using ID-GC-ICP-MS. Two manuscripts are now published in the Journal of Analytical Atomic Spectrometry (JAAS) [20, 21] and are attached to this report as appendix 2 and 3.

4.1 Instrumentation

A Varian 3300 gas chromatograph (Palo Alto, CA, USA) fitted with an on-column injector liner and a methyl silicone capillary column (30 m by 0.53 mm i.d., 1.5 μ film thickness; SPB-1, Supelco, Bellafonte, PA) was used for separation of Sn species and an Agilent 7500 ICP-MS (Foster City, CA) was used for detection. The GC was coupled to the ICP-MS *via* a custom made interface. The operating parameters of the ICP-MS were selected by optimizing the sensitivity for ^{129}Xe, by adding Xe gas at 0.5 ml/min to the Ar carrier gas flow. Oxygen was added to the plasma to prevent carbon deposits on the sampler/skimmer Pt cones. The operating parameters of the GC and ICP-MS are given in Table 3.

Table 3. Operating conditions for the GC and ICP-MS.

GC parameters	
Injection volume	1 µL
Carrier helium gas flow	22 mL min^{-1}
Injector temperature	180 °C
Oven temperature	130 °C → 40 °C min^{-1} → 210 °C, hold 0.5 minutes
	210 °C → 7 °C min^{-1} → 225 °C, hold 0.5 minutes
	225 °C → 40 °C min^{-1} → 280 °C, hold 2.0 minutes
Transfer line temperature	200 °C
ICP-MS parameters	
ICP RF power	1200 W
Plasma argon gas flow	15 L min^{-1}
Nebulizer argon gas flow	1.0 L min^{-1}
Auxiliary argon gas flow	0.9 L min^{-1}
Auxiliary oxygen gas flow	3 mL min^{-1}
Sampler/Skimmer cones	Platinum
Dwell time	100 ms for $^{116}Sn^+$, $^{117}Sn^+$, $^{118}Sn^+$, $^{119}Sn^+$, $^{120}Sn^+$ and $^{124}Sn^+$

4.2 Reagents

Dichloromethane, diethyl ether, toluene, and acetic acid (guaranteed reagent grades) were obtained from Merck, Germany. Methanol (HPLC gradient grade) and HBr (pro analysis grade) were from J.T. Baker, whereas the HCl was in-house distilled. Sodium tetraethyl borate (98 %) was obtained in sealed septum vials from Galab, Geesthacht, Germany. The solution used for derivatisation (25 % w/v sodium tetraethyl borate) was prepared by injecting an appropriate volume of tetrahydrofuran (Merck, p.a.), which was kept dry by keeping it in contact with 3 Å molecular sieve granules, through the septum. In this way the reagent could be used for at least 4 months without excessive degradation through exposure to ambient air or water vapour. Deionised water was prepared by an Ultra-Q water purification system, Millipore, Bedford, MD, USA. Tropolone (98 %) was obtained from Aldrich (Steinheim, Germany).

Butyl tin stock-standards (1000 µg/g as Sn) in methanol with natural isotope distribution were prepared from monobutyl- (95 %), dibutyl- (96 %) and tributyl (96 %) tin chloride (Aldrich). All organotin stock and diluted solutions were kept in 20 mL glass vials provided with silicon rubber septum caps lined with poly(tetrafluoroethene) (PTFE; Coricon, Knivsta, Sweden). Solutions recovered after extraction and derivatisation were stored in 1.5 mL vials with PTFE-lined silicon rubber septum caps. Solutions were withdrawn using dedicated glass syringes (Hamilton, Reno, NE) for each isotope. Tin contents in standards with natural isotope distribution are based on gravimetric measurements, corrected for reagent purity. Isotopically enriched butyl- and phenyltins, synthesised in our laboratory, were used for the determination of OTCs using isotope dilution. The concentrations of butyl- and phenyltins in the isotopically enriched standards were determined by

reversed isotope dilution, using the equation proposed by Fassett et al. [22]. All gases used (O_2, He, Ar, N_2) had at least 99.995 % purity.

4.2.1 Preparation of spikes for the determination of OTCs using isotope dilution

Two separate mixtures, one for the butyltins and another one for the phenyltins were prepared from the synthesized isotopically enriched butyl- and phenyltins [20, 21]. The concentration of the butyltins in the first spike solution (referred as ID-spike 1) was 0.5, 9.8, and 50 µg/g with respect to MBT, DBT, and TBT. In the second spike solution (referred as ID-spike 2) the concentration of MPhT, DPhT, and TPhT was 10, 23, and 15 µg/g, respectively.

4.2.2 Investigation of the stability of phenyltins during sample preparation

A mixture of standard solutions containing inorganic tin, MPhT, DPhT, and TPhT was used as spike to study the extraction efficiency and occurrence of interconversion when different extraction reagents are used for water and sediment samples. Detailed information on the comparison of 5 different extraction procedures is given in appendix 3.

4.3 Extraction and derivatisation

A modified method proposed by J.W. Wegener [23] was used for the extraction and derivatization of OTCs in the water and biological samples.

4.3.1 Water samples

Add 5.0 g sodium sulfate to 500 mL sample and adjust the pH to 5 with 25 % aqueous ammonia and concentrated acetic acid as required. Transfer the mixture to a separatory funnel (rinsed with acetone and n-hexane before use) and add 0.1 mL of a 0.3 % (w/v) aqueous solution of sodium tetraethylborate. Add 10 ml of n-hexane and 10-50 µl of 1ppm TeBT (internal standard). Add 10-50 µl of the standard solution containing 1 ppm of each tin species.

Shake during 5 minutes. Collect the top layer in a centrifuge tube (rinsed with acetone and n-hexane before use) and repeat the extraction with 10 mL of n-hexane.

Centrifuge the collected n-hexane layers at 3000 rpm during 4 min. Transfer the top layer to a test tube and concentrate to a volume of 0.3-1 mL using a gentle nitrogen stream. Transfer the final solution to 1.5 mL glass tubes, provided with septa, and inject 1 µL of the sample into the GC.

4.3.2 Extraction of biological samples

Place 5 g of homogenized sample in a 30 mL centrifuge tube, pre-rinsed with diethyl ether and with a PTFE-lined screw cap. Add 5 mL of water (extracted with half of its volume of n-pentane to which 0.02 % (w/v) tropolon has been added) to the centrifuge tube. Homogenize during 15 seconds. Add carefully and stepwise 37 % HCl (extracted with equal volume of n-pentane) avoiding overfoaming until the pH reaches a level between 1.5 and 2.2. Ultrasonicate for 5 minutes to assist the foam breaking. Add 0.5 g of sodium chloride (treated by heating at 250 °C during 8 hours). Add 12 mL of a freshly (daily) prepared 0.02 % (w/v) solution of tropolon in diethyl ether. Mix for 30 s, ultrasonicate for 5 minutes, and finally agitate during 5 minutes on an orbital shaker at 250 rpm. Separate the layers by centrifuging at 3000 rpm during 4 minutes. Add some sodium sulfate (treated by heating at 250 °C for 8 hours) in a new 30 mL centrifuge tube. Transfer the organic layer from the first tube to this second tube. Evaporate the diethyl ether layer to dryness by a gentle flow of N_2(g). The procedure described above was used for the mussel, herring, salmon, and mollusc samples. The herring, salmon, and mollusc samples were spiked with 50 mg of ID-spike 1 and 40 mg of ID-spike 2, and allowed to equilibrate for one hour prior to extraction.

4.3.3 Derivatisation of biological samples

After drying, the residue was redissolved in 1 mL toluene. There upon 40 µL of 25 % (m/m) sodium tetraethyl borate in THF was added for derivatisation. The derivatisation was assisted by placing the glass vials in a water bath (90 °C). The excess sodium tetraethyl borate was then destroyed by adding 1 mL HCl. The glass vials were cooled with ice while adding the HCl in order to avoid vaporization. The vials were then centrifuged at 3000 rpm for 3 minutes before transferring the organic phase to 1.5 mL GC vials equipped with septa. Inject 1 µL of the sample in the GC.

4.3.4 Extraction of sediment samples

To 0.5-0.7 g aliquots of freeze dried samples were added 50 mg of ID-spike 1 and 40 mg of ID-spike 2. After equilibration for 1 h, 10 ml of 4.5 M HBr was added and the suspensions were then sonicated for 15 minutes. The samples were thereafter extracted with 20 ml of dichloromethane containing 0.04 % (w/v) Tropolone for 1 hour. The mixture was then centrifuged for 5 minutes at 3000 rpm to separate the organic layer, which was transferred to a new vessel containing 3.5 g sodium sulfate. The dichloromethane was then evaporated to about 10 mL by a gentle stream of $N_2(g)$. The liquid was then transferred to a new glass vial and evaporated to dryness by further purging with $N_2(g)$.

4.3.5 Derivatisation of Sediment samples

After drying, the residue was dissolved in 1 mL toluene. There upon 40 µL of 25 % (m/m) sodium tetraethyl borate in THF was added for derivatisation. The mixture was left at room temperature for 2 h for completion of the reaction and centrifuged at 5000 rpm for 2 min to settle the decomposition products. Part of the organic layer was then transferred to 1.5 mL GC vials and closed with septa.

5 Results and Discussion

The water, mussel, and sludge samples were analyzed before our recent method development, a calibration system based on species specific isotope dilution (SSID-ICP-MS) for the determination of OTCs. These samples were therefore analysed using the GC-ICP-MS standard addition method and employing tetrabutyltin (TeBT) as internal standard. The determinations of OTCs in sediment, and the remaining biological samples were performed using the SSID-GC-ICP-MS methods.

5.1 Study on the stability of phenyltins during sample preparation

In order to get accurate results for OTCs interconversion of species during sampling, sample storage and analysis has to be avoided. If this is not possible interconversions can be accounted for by spiking samples with isotopically enriched species of interest. For the determination of butyltin species interconversions do not constitute big problems during sample workup [20]. However the binding strength of the phenyl group/groups to tin is relatively week and phenyltin species can be easily decomposed during sample workup. This constitutes a big problem for specieation analysis. In order to get efficient extraction, relatively strong reagents are needed (leading to decomposition) but if weeker reagents are used the extraction will be incomplete. This dilemma makes determination of phenylt tin species unreliable. The issue is discussed in appendix 3.

5.2 Method validation

Recovery test for the butyltins was made using certified reference materials and samples of interest. Two CRMs, PACS-2 and BCR646 (sediment) and one candidate CRM (oyster tissue) were used for this study. Different extraction and derivatisation methods were compared, of which the extraction and derivatisation procedures described in sections 4.3.4 and 4.3.5 offered the best results, as shown

in Table 4. For additional details on the comparison of procedures for the determination of butyltins and phenyltins in sediments see Appendix 2-3.

Table 4. Determined and certified values using the analytical methods used for the determination of organotins in different CRMs.

Certified Reference Material	MBT	DBT	TBT	MPhT	DPhT	TPhT
PACS-2	496 ± 36	1038 ± 26	951 ± 61	NA	NA	NA
Certified value	450	1050	980	NA	NA	NA
BCR-646	399 ± 10	422 ± 4	195 ± 6	39 ± 7	19 ± 4	11± 2
Certified value	410 ± 74	395 ± 41	195 ± 20	42 ± 10	16 ± 3	9.8 ± 3.1
BCR-710	25 ± 3	39 ± 5	46 ± 9	NA	NA	NA
Certified value	34 ± 9	42 ± 8	54 ± 8	NA	NA	NA

NA = not available.

5.3 Water samples

5.3.1 Fiskebäckskil bay

Compared to the measurements made during the years 1987 [1-2], the concentration of OTC found in the present study are substantially lower (Table 5).

Table 5. Comparison of butyltin concentrations found in water samples from Fiskebäckskil by the current study, with measurements made in 1987.

Sampling site	Concentration (ng Sn/L)	
	Sampled 1987-05-18	Sampled 2001-06-01
Station 1		
MBT	< 50	4.31
DBT	97	0.77
TBT	260	0.3
Station 2		
MBT	< 50	5.9
DBT	81	0.6
TBT	410	0.6
Station 3		
MBT	< 50	3.8
DBT	< 12	0.4
TBT	< 12	0.1

For the measurements performed during 1987 a GC coupled to a mass selective detector (SIF, Oslo) was used. The detection limits obtained with this system were 50, 12, 12 ng Sn/L for MBT, DBT, and TBT, respectively, in a 500 mL water sample. The detection limits (interpreted as three times the standard deviations of the blank) obtained with the GC-ICP-MS used in the present work were about three order of magnitude lower; 20, 5, 16, 40, 27, and 78 pg/L for MBT, DBT, TBT, MPhT, DPhT, and TPhT, respectively, using the same sample volume. It is not easy to make comparison on the analytical performance of the two different techniques used since detailed information on the detection system is not available for the measurements done in 1987 [2]. The sensitivity of GC-ICP-MS for the determination of OTCs is however known to be superior to other analytical techniques, as was discussed in the introduction section. In the case of MBT a direct comparison cannot be made since the detection limit for the compound was high in the previous study. The levels found in the present study were all about ten times below the limit of quantification reported in 1987. The

DBT levels at station 1 and 2 were both about 100 times lower than in 1987, whereas the TBT concentrations appear to have decrease by a factor of nearly 1000.

5.3.2 Harbour water from Fiskebäck (Gothenburg) and Norrby (outside Umeå)

In the literature it is documented that the leach rate of TBT from surfaces treated with modern TBT-based copolymer paints are designed to reach a constant TBT leach rate of 1.6 μ g cm^{-2} per day reported as elemental Sn [4]. For, example, during a 3-day harbour stay, a commercial ship leaching TBT at the constant leach rate can release more that 200 g TBT into water. If freshly painted, the amount can reach 600 g. This can result in a dissolved TBT concentration in the water adjacent to the ship ranging between 100-200 ng (Sn) l^{-1}. One can thus expect to find major sites of TBT pollution in harbours used by large commercial vessels, and near docks or other facilities where shipbuilding, repair, and repainting occurs. In the yacht harbour in Gothenburg, the concentration of OTC was not high. Relatively higher concentrations of OTC were found in Norrby, outside Umeå (Table 6). This can be associated with the number of small boats (1000–2000) moored down by the jetties in connection to the maritime recreation areas along the coast.

Table 6. Concentrations of OTC found in water, mussel, fish, and sludge samples. The OTCs in the biological samples are given as dry weight (dw)concentrations.

WATER		MBT	DBT	TBT	MPhT	DPhT	TPhT
		ng Sn/L	*ng Sn/L*	*ng Sn/L*	*ng Sn/L*	*ng Sn/L*	*ng Sn/L*
Bromma water purification plant (outlet)		2.5 ± 1.9	0.06 ± 0.11	ND	1.2 ± 0.95	1.14 ± 0.72	ND
Gryaab water purification plant (inlet)		17 ± 8.9	5.1 ± 3.5	0.8 ± 0.4	ND	ND	ND
Karlsudd (Gålö)		3.9 ± 2.9	1.7 ± 1.0	0.9 ± 0.5	ND	ND	ND
Norrby, outside Umeå in Västerbotten		6.0 ± 3.2	4.4 ± 2.4	4.7 ± 2.1	4.0 ± 2.3	4.70 ± 2.20	4.16 ± 2.90
Fiskebäckskilsvik,	Sampling site 1 C	4.3 ± 3.4	0.77 ± 0.42	0.3 ± 0.4	0.7 ± 0.6	0.56 ± 0.28	ND
	Sampling site 2 C	5.9 ± 3.3	0.60 ± 0.38	0.6 ± 0.4	ND	ND	0.23 ± 0.20
	Sampling site 3 C	3.8 ± 3.2	0.40 ± 0.30	0.1 ± 0.2	ND	ND	ND
Hinsholmskil,	Deck Y	0.05 ± 0.09	0.01 ± 0.04	0.1 ± 0.2	0.01 ± 0.08	0.02 ± 0.03	0.01 ± 0.09
	Deck M	0.24 ± 0.28	0.15 ± 0.19	0.2 ± 0.3	0.03 ± 0.07	0.19 ± 0.09	0.17 ± 0.14
	Deck S	0.98 ± 0.45	0.77 ± 0.38	0.9 ± 0.5	0.13 ± 0.29	0.94 ± 0.48	0.82 ± 0.57
MUSSEL		**MBT**	**DBT**	**TBT**	**MPhT**	**DPhT**	**TPhT**
		ng Sn /g (dw)	*ng Sn /g (dw)*	*ng Sn /g (dw)*	*ng Sn /g (dw)*	*ng Sn /g (dw)*	*ng Sn /g (dw)*
Fiskebäckskil,	Sampling site 1B	410 ± 162	190 ± 65	250 ± 71	ND	ND	ND
	Sampling site 1C	44 ± 28	98 ± 32	360 ± 48	ND	ND	ND
	Sampling site 2C	18 ± 10	33 ± 11	170 ± 29	ND	ND	ND
	Sampling site 3A	48 ± 16	13 ± 7	37 ± 12	ND	ND	ND
FISH		**MBT**	**DBT**	**TBT**	**MPhT**	**DPhT**	**TPhT**
		ng Sn /g (dw)	*ng Sn /g (dw)*	*ng Sn /g (dw)*	*ng Sn /g (dw)*	*ng Sn /g (dw)*	*ng Sn /g (dw)*
Salmon	Sample 1	0.27	1.12	10.95	15.50	1.15	10.45
	Sample 2	0.42	1.83	9.72	ND	0.22	8.51
Herring	Sample 1	0.27	0.70	6.26	ND	0.31	ND
	Sample 2	0.18	0.53	4.29	0.86	0.10	6.30
SLUDGE		**MBT**	**DBT**	**TBT**	**MPhT**	**DPhT**	**TPhT**
		ng Sn /g	*ng Sn /g*	*ng Sn /g*	*ng Sn/g*	*ng Sn/g*	*ng Sn/g*
Loudden monthly sludge		39 ± 14	692 ± 180	32 ± 18	ND	ND	ND

The ± values represent uncertainties based on the standard addition method (n = 3). The fish sample concentrations have been determined using SSID-GC-ICP-MS with only two replicates, where both measurements are listed in the table above. The general measure of uncertainty obtained with this technique at the concentration levels reported here is 10-25 % residual standard deviation (% RSD). ND, not detected.

5.3.3 Inlet and outlet water samples from two purification plants

Higher levels of MBT (7 times) and DBT (85 times) were found in the inlet water of the purification plant in Gryaab compared to the outlet water from Bromma water purification plant. While no TBT could be detected in the Bromma outlet water an amount of 0.8 ng/L was found in the inlet water of the Gryaab plant.

5.4 Biological samples

5.4.1 Mussel (mytilus edulis), Herring (clupea harengus) and Salmon (salmo salar)

The concentrations of OTCs found in the mussel, herring, and salmon samples are generally lower compared to the amounts reported earlier. TBT concentrations in the mussel samples (dry weight) from Fiskebäckskilsvik were found to be in the range from 17 to 364 ng Sn/g, compared to the higher concentrations (1-18 µg Sn/g) reported in 1987. In the 1987 Björklund report [2] the TBT concentrations in Baltic herring from Trälhavet at Vaxholm and Norra Farleden at Växlet were found to be 0.25 µg/g and 0.59 µg/g respectively. In 2002, when estimating the OTC exposure levels in fishes from Holland, Frank Willemsen [24] reported an average concentration of 20 ng/g TBT (based on dry weight) with a range that spans between 2 to 56 ng/g TBT. The concentrations of TBT found in the present investigation are much lower compared to the amounts reported by Björklund and are within the concentration range found in 2002 [24]. It can be concluded that the fishes analysed this time do not seem to have been exposed to the same level as those reported by Björklund, due to the effect of restriction for using TBT as antifouling paint.

5.4.2 Mollusc (nassarius reticulatus)

The concentrations of OTCs found in the mollusc samples are shown in table 7. Detailed discussion on these results is given in [19].

Table 7. Results from the determination of OTC in Mollusc (nassarius reticulates). The values given represent concentrations in dry weight (dw).

Location (station)	MBT	DBT	TBT	MPhT	DPhT
	ng Sn /g (dw)	*ng Sn /g (dw)*	*ng Sn /g (dw)*	*ng Sn /g (dw)*	*ng Sn /g (dw)*
Burholmarna (1)	3.0	5.3	14.6	ND	3.5
(2)	3.0	7.3	18.4	ND	ND
(3)	3.1	6.7	4.8	ND	1.8
Kalvhagerfjorden (1)	2.3	3.3	5.9	ND	ND
(2)	1.5	1.7	ND	ND	ND
(3)	3.4	4.4	ND	ND	6.6
Malmö hamn (1)	13.6	15.9	9.8	ND	3.0
(2)	24.3	35.1	39.3	ND	ND
(3)	32.2	52.2	45.9	ND	68.5
Brofjorden South (1)	5.3	9.5	5.7	ND	1.1
(2)	14.6	10.2	48.4	0.6	ND
(3)	4.8	9.3	9.7	ND	ND
Brofjorden North (1)	15.0	16.3	30.5	ND	6.4
(2)	5.4	6.2	0.9	ND	7.1
(3)	6.9	10.7	9.6	ND	4.7
Göteborg South (1)	2.8	5.4	7.9	ND	ND
(2)	5.8	10.3	7.5	ND	1.4
(3)	5.4	12.6	9.7	0.2	3.5
Göteborg South (1)	6.4	6.4	23.4	0.3	1.8
(2)	5.5	9.7	3.1	0.4	2.5
(3)	4.7	9.7	9.3	ND	1.7

5.5 Sediments

The concentrations of OTCs found in the different sediment sections (the same core) from Gålö, Karlsudd (Table 7) indicated an accumulation in the uppermost layers (1-3 cm) and between 39-50 cm.

Table 8. Results from the determination of OTC in the sediment samples from Gålö, Karlsudd.

Sediment depth (cm)	MBT	DBT	TBT	MPhT	DPhT
	ng Sn /g	*Ng Sn /g*	*ng Sn /g*	*Ng Sn /g*	*ng Sn /g*
1-2	13.7	43.7	22.1	14.9	2.6
2-3	19.0	45.0	28.9	1.4	11.0
5-6	3.0	7.1	5.7	0.7	1.9
6-7	2.7	3.6	1.4	1.3	6.0
9-10	4.3	5.2	16.9	2.5	0.5
15-16	2.0	8.4	3.6	4.5	ND
24-25	2.9	1.1	ND	ND	ND
39-40	7.7	12.7	24.4	1.4	ND
44-45	1.88	3.4	ND	0.032	3.1
49-50	4.0	6.9	21.7	0.4	ND
54-55	2.2	1.55	ND	ND	ND
59-60	0.8	ND	ND	0.77	1.8

The samples have been analysed using SSID-GC-ICP-MS with single determination at each depth level. The general measure of uncertainty obtained with this technique at the concentration levels reported here is 5-15 % RSD. ND, not detected.

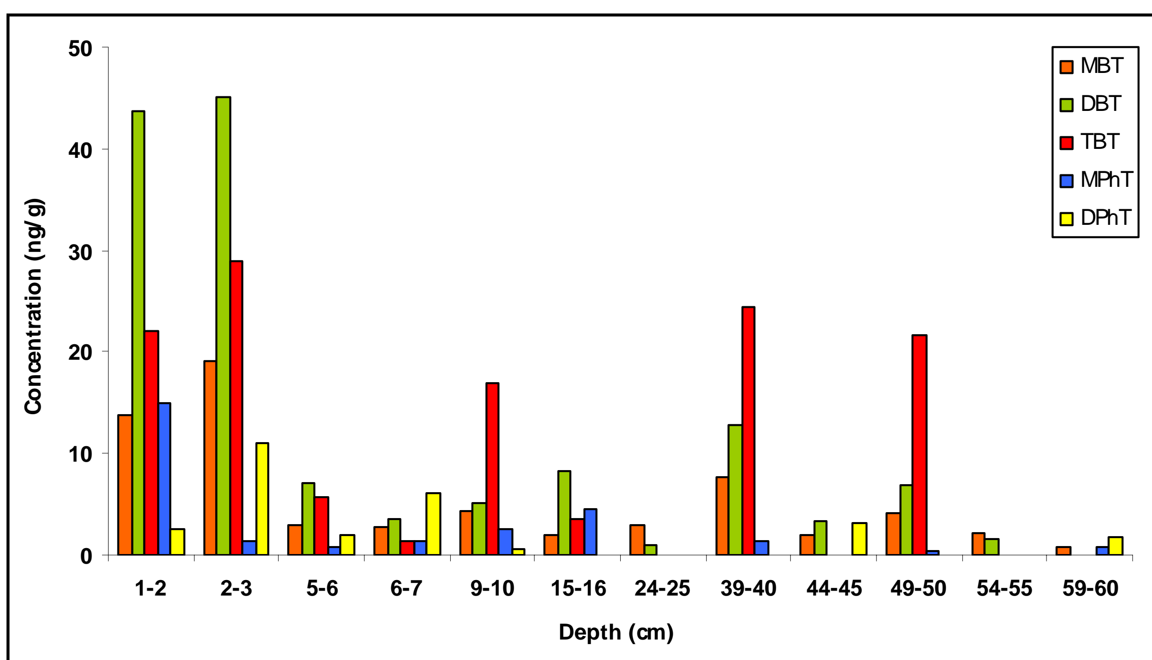

Figure 3. Concentration profile of OTC in a sediment core from Gålö, Karlsudd

Compared to a recent report on concentration profiles of TBT in sediment cores from the harbour of Wädenswil in Switzerland [12] the variation of the OTC concentrations with depth does not reflect the same dependency. In the depletion profile for the harbour sediment from Wädenswil, the highest concentrations were found in the sediment layers between 14-20 cm (corresponding to values measured in 1986 [12]). Thereafter the concentration declined towards the outermost and the deeper cores, resulting in a more or less Gaussian shape. It is puzzling that the concentration of OTCs is higher in the uppermost layers and the depletion profile shown in Figure 3 does not reflect the situation in Wädenswil. One plausible explanation is that we have contamination on the surface

or/and degradation of, for example TBT, might have taken place in the deeper sediment layers. A possible explanation to the higher concentration in the layers between 39-50 cm (corresponding to the period from 1970 to 1980, if 1.5 cm sediment depletion is assumed to be equal to 1 year) is that more TBT was released during those times when it was still used as antifouling paint. One should also keep in mind that the Wadenswil samples reflect freshwater sediments, whereas the Galö sample is a seawater sediment.

6 Conclusions

The use of isotopically enriched organotins for species specific determination of OTC in the various samples makes the analysis more precise and accurate. The application of isotope dilution ICP-MS to species-specific determination is however still limited by the lack of a commercially available enriched standards, which we now have managed to synthesize. This of course is partly initiated by the current screening study.

7 Future Aspects

In the field of speciation analysis there is still a large uncertainty of reported results, making it difficult to compare data obtained by different groups and in different regions. An important task is therefore to develop analytical methods with traceability to SI units, with known total measurement uncertainty. Within this project the synthesis of isotopically enriched OTCs is a step in this direction. In addition our laboratory is at present involved in an international co-operation project to determine total measurement uncertainties for the determination of TBT in sediment samples. These efforts should lead to a better transparency of methodology and will make it easier to arrive at reliable conclusions when comparing data from different sources.

8 References

1 Björklund I., Rapport från Kemikalieinspektionen, **1988**, 6/88.

2 Björklund I., Rapport från Kemikalieinspektionen. **1987**, 7/87.

3 Jansson B., Institutet för tillämpad miljöforskning (ITM), *"Tennorganiska föreningar i svensk miljö – behöver vi ytterligare kunskaper?"*, Final draft, **2000**.

4 Hoch M., Applied Geochemistry, **2001**, *16*, 719.

5 Encinar J. R., Villar M. I. M., Santamaria V. G., Alonso J. I. G., Sanz-Medel A., *Anal. Chem*. **2001**, *73*, 3174

6 Tao H., Rajendran R. B., Quetel C. R., Nakazato T., Tominaga M., Miyazaki A., *Anal.Chem*., **1999**, *71*, 4208

7 Schubert P., Rosenberg E., Grasserbauer M., *Intern. J. Environ. Anal. Chem*., **2000**, *78*, 185.

8 Cardellicchio N., Giandomenico S., Decataldo A., Di Leo A., *Fresenius J. Anal. Chem*. **2001**, *369*, 510.

9 Ndungu K., Mathiasson L., *Anal. Chim. Acta* **2000**, *404*, 319.

10 Encinar J. R., Alonso J. I. G., Sanz-Medel A., *J. Anal. Atom. Spectrom*. **2000**, *15*, 1233.

11 Berg M., Arnold C. G., Muller S. R., Muhlemann J., Schwarzenbach R. P., *Environ. Sci. Technol*. **2001**, *35*, 3151.

12 Arnold C. G., Berg M., Muller S. R., Dommann U., Schwarzenbach R. P., *Anal. Chem*. **1998**, *70*, 3094.

13 Abalos M., Bayona J. M., Quevauviller P., *Applied Organomet. Chem*. **1998**, *12*, 541.

14 Kuballa J., Jantzen E., Wilken R. D., *"Organotin compounds in sediments of the rivers Elbe and Mulde, Sediments and toxic substances"*, Springer: Berlin, **1996**.

15 Ceulemans M., Adams F. C., *Anal. Chim. Acta* **1995**, *317*, 161.
16 Yang L., Mester Z., Sturgeon R. E., *Anal. Chem*. **2002**, *74*, 2968.
17 Hill S. J., Pitts L. J., Fisher A. S., *Trends Anal. Chem.* **2000**, *19*, 120.
18 Aguerre S., Lespes G., Desauziers V., Potin Gautier M., *J. Anal. At. Spectrom*, 2001, **16**, 263.
19 Magnusson M., Granmo Å., Investigation of imposex on the Swedish west coast using Nassarius reticulatus as bioindicator species, Report Swedish Natural Protection Agency **2004**.
20 Kumar S. J., Tesfalidet S., Snell J., Frech W., *J. Anal. Atom. Spectrom.* **2003**, *18*, 714. **Appendix 2**.
21 Kumar S. J., Tesfalidet S., Snell J., Nguyen Van D., Frech W., *J. Anal. Atom. Spectrom.* **2004**, *19*, Advanced Article. **Appendix 3**.
22 Fasset J. D., Paul J., *Anal. Chem.* **1989**, *61*, 641A.
23 Wegener J. W., Institute for Environmental Studies (IVM), Vrije Universiteit, Amsterdam, private communication.
24 Willemsen F. Risk analysis in OT-SAFE, OT-SAFE meeting, Pau, France, January 2002.