Speciation of organotins by Gas Chromatography – Inductively Coupled Plasma Mass Spectrometry in marine samples

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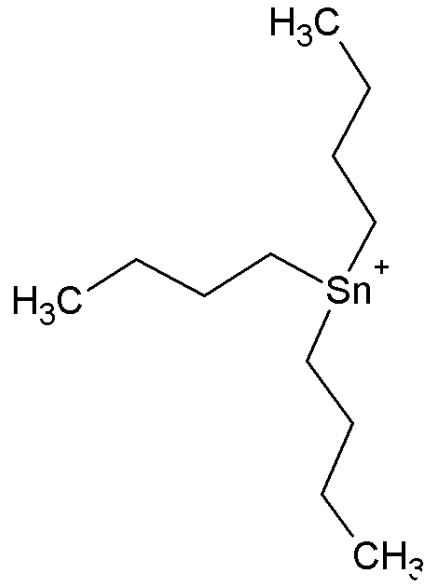
Summary

- Introduction to organotin compounds
- Toxicity of organotins in animals and human
- International and national directives
- Extraction method
- Configuration of GC-ICP/MS
- CRM recovery
- Conclusions

Organotin compounds

- Organotins are chemical compounds, based on tin element bonded to carbon.
- These compounds are classified by the number of organic groups bonded to tin (i.e. mono-, di-, tri-, tetra-organotins).
- The X is an anionic species (i.e. chloride, fluoryde etc.).
- Tri-substituted compounds present the higher toxicity[1].

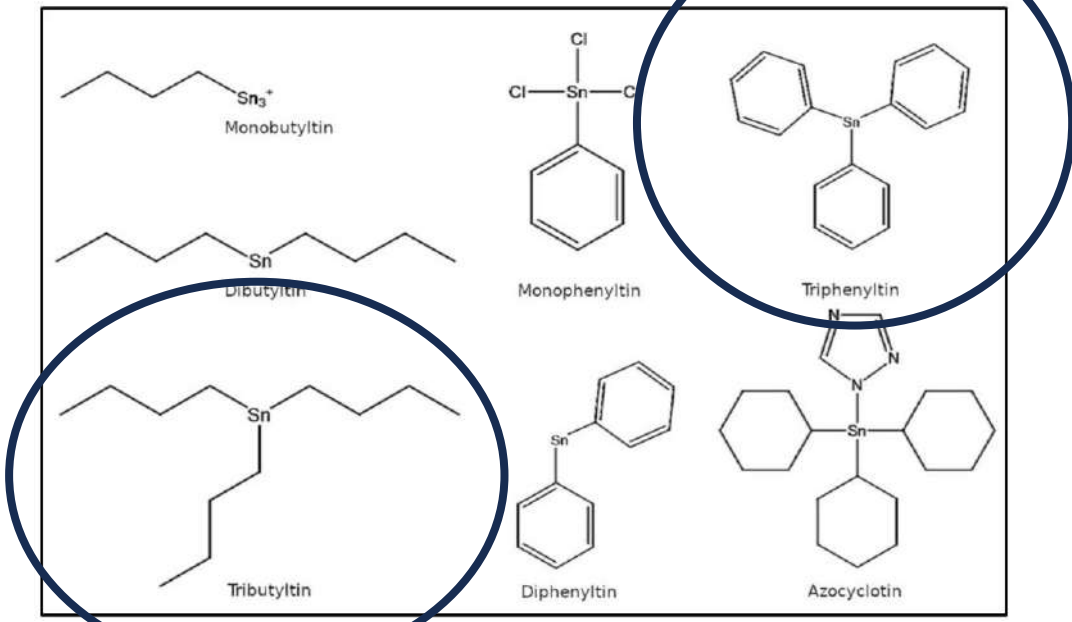


R_4Sn
R_3SnX
R_2SnX_2
$RSnX_3$

[1] Organotin Environmental Fate and Effects, Volume 20, M.A. Champ, P.F. Seligman, Springer, 1996

Sources of ***tri-substituted*** organotins compounds

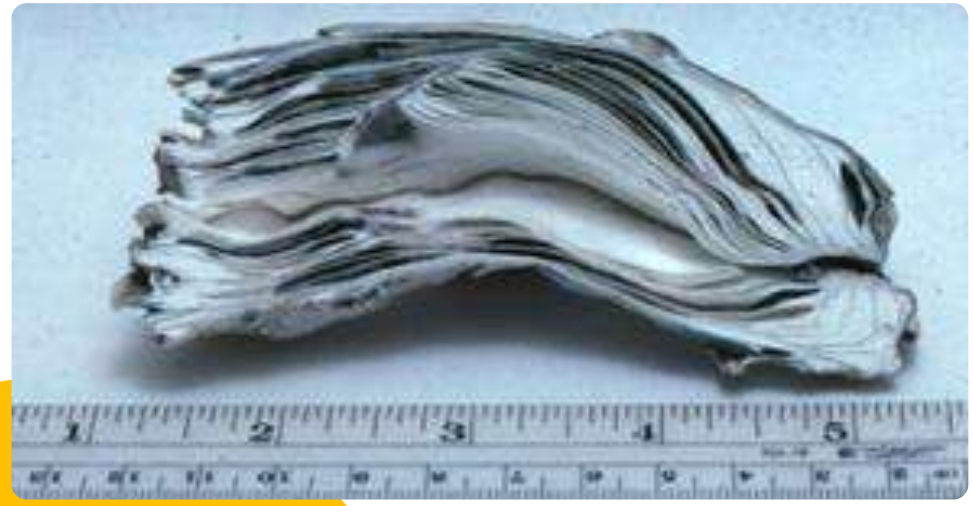
- The first synthesis dates back to the 1850s by Edward Frankland, but only in the early 1940s began a large scale production after a commercial use as a pesticide (**Triphenyltin – TPhT**) and as a biocide in antifouling paint (**Tributyltin – TBT**) [2].



[2] E.D. Burton, I.R. Phillips, D.W. Hawker, In-situ partitioning of butyltin compounds in estuarine sediments, Chemosphere 59 (2005) 585-592.

Toxicity

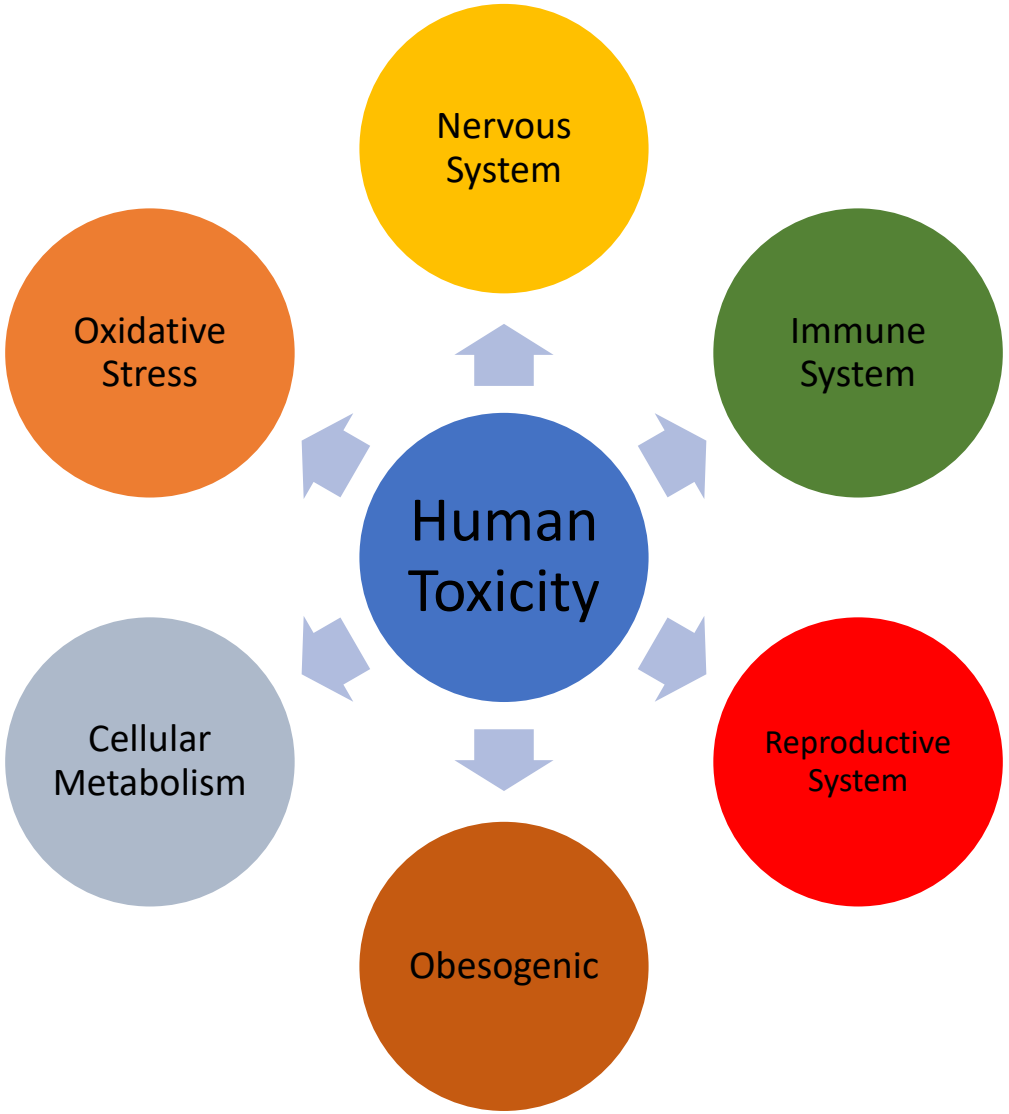
A normal oyster shell



A deformed oyster shell

- Organotin compounds are extremely toxic substances for various organisms, also for human. The first episode of those toxicity effects was dated back in the 70s in a french oyster farm situated on the Atlantic coast [3]. The oyster shell was deformed from birth and it didn't allow to grow properly.

[3] M. Hoch, Organotin compounds in the environment - an overview, Appl. Geochem. 16 (2001) 719-743.


Nervous System
Immune System
Reproductive System
Obesogenic
Cellular Metabolism
Oxidative Stress
Human Toxicity

European regulation

- The first European Directive was **89/677/CEE** which introduce organotin compounds in the hazardous substances under restriction list.
- In 2001 **IMO (Internation Maritime Organization)** banned TBT antifouling paints from 2003 and its presence on the ships from 2008.
- **European Commission Parliament** included the recommendations of IMO and adopted the **Regulation 782/2003** on the prohibition of organic compounds on ship.
- **Directive 2013/39/UE (Water Framework Directive)** of the European Parliament and of the Council of 12 August 2013 (amending **Directives 2000/60/EC** and **2008/105/EC**) establish a framework for Community action in the field of water policy.

Environmental quality standards (EQS) for *priority substances* and *certain other pollutants* are provided by **Directive 2013/39/EU.**

Italian regulation of organotins

- Decreto legislativo **13 ottobre 2015, n. 172**

  Attuazione della direttiva 2013/39/UE, che modifica le direttive 2000/60/CE per quanto riguarda le sostanze prioritarie nel settore della politica delle acque.

- Decreto legislativo **3 aprile 2006, n. 152**

  Norme in materia ambientale

Tabella 1/A Standard di qualità ambientale nella colonna d'acqua e nel biota per le sostanze nell'elenco di priorità.

N.	Denominazione della sostanza	Numero CAS[1]	SQA-MA[2] Acque superficiali interne[3]	SQA-MA[2] Altre acque di superficie	SQA-CMA[4] Acque superficiali interne[3]	SQA-CMA[4] Altre acque di superficie	SQA Biota[12]	Identificazione sostanza[15]
(30)	Tributilstagno (composti) (tributilstagno-catione)	36643-28-4	0,0002	0,0002	0,0015	0,0015		PP

Tabella 1, Allegato 5, Parte IV, D.Lgs 152/06.

		A SITI AD USO VERDE PUBBLICO, PRIVATO E RESIDENZIALE (MG KG-1 COME SS)	B SITI AD USO COMMERCIALE E INDUSTRIALE (MG KG-1 COME SS)
	COMPOSTI INORGANICI		
13	Stagno [*]	1	350

[*] Con la Legge n. 116 del 11.08.2014, si stabilisce che "3-bis. Alla tabella 1 dell'allegato 5 al titolo V della parte quarta del decreto legislativo 3 aprile 2006, n. 152, al punto 13, la parola: **"Stagno"** è sostituita dalle seguenti: **"Composti organo-stannici"**.

Organotin compounds in sediments

NUMERO CAS	PARAMETRI	SQA-MA[1] [2]
	Metalli	**mg/kg s.s**
7440-43-9	Cadmio	0,3
7439-97-6	Mercurio	0,3
7439-92-1	Piombo	30
	Organo metalli	**µg/kg**
	Tributilstagno	5
	Policiclici Aromatici	**µg/kg**
120-12-7	Antracene	24
91-20-3	Naftalene	35
	Pesticidi	
309-00-2	Aldrin	0,2
319-84-6	Alfa esaclorocicloesano	0,2
319-85-7	Beta esaclorocicloesano	0,2
58-89-9	Gamma esaclorocicloesano lindano	0,2
	DDT[3]	1
	DDD[3]	0,8
	DDE[3]	1,8
60-57-1	Dieldrin	0,2

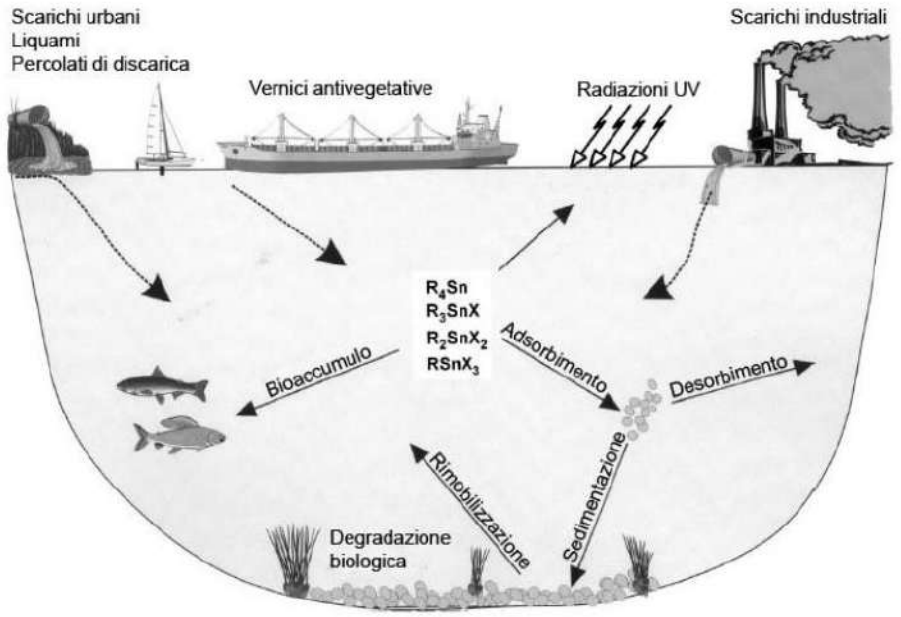
Tabella 2/A Standard di qualità ambientale nei sedimenti nei corpi idrici marino-costieri e di transizione.
D.Lgs 13 ottobre 2015, n. 172

Sediment accumulation of organotins

Sediments are known to act as "reservoirs" for organotin compounds in ecosystems water, favoring both the persistence of these pollutants over time and their continuity release in the water column following the action of natural agents such as currents, tides, or anthropic, such as dredging, fishing gear, etc.

Organotin compounds tend to accumulate in the sediment due to the high affinity for the organic and inorganic fraction.

The evaluation of the accumulation of organotin compounds in the sedimentary compartment and the control of their dispersal in aquatic ecosystems is an issue highly relevant to the environment[4].



[4] Weidenhaupt et al., 1997; Hoch, 2001

EPA Method 8323/2003 - Extraction

METHOD 8323

DETERMINATION OF ORGANOTINS BY MICRO-LIQUID CHROMATOGRAPHY- ELECTROSPRAY ION TRAP MASS SPECTROMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the use of solid-phase extraction (SPE) discs, solvent extractions (for biological tissues), and micro-liquid chromatography (μLC) coupled with electrospray ion trap mass spectrometry (ES-ITMS) [this technique would also be applicable to ES-quadrupole mass spectrometry (ES-MS)] for the determination of organotins (as the cation) in waters and biological tissues. The following compounds can be determined by this method:

Compound Name	CAS No. [a]
Tributyltin chloride[b]	1461-22-9
Dibutyltin dichloride	683-18-1
Monobutyltin trichloride	1118-46-3
Triphenyltin chloride	668-34-8
Diphenyltin dichloride	1135-99-5
Monophenyltin trichloride	1124-19-2

[a] Chemical Abstract Service Registry Number.
[b] The organotins are listed as the chloride salt, however this method is designed to detect the free cation, whether from the chloride salt, oxide, etc., of the organotin.

7.0 REAGENTS AND STANDARDS

7.1 Reagent grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 Organic-free reagent water. All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

7.3 Acetic acid, CH_3CO_2H

7.4 Hydrochloric acid (12N), HCl

7.5 Solvents

The choice of solvent will depend on the analytes of interest and no single solvent is universally applicable to all analyte groups. Whatever solvent system is employed *including* those specifically listed in this method, the analyst *must* demonstrate adequate performance for the analytes of interest, at the levels of interest. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in Method 3500, using a clean reference matrix. Method 8000 describes procedures that may be used to develop performance criteria for such demonstrations as well as for matrix spike and laboratory control sample results.

All solvents should be pesticide quality or equivalent. Solvents may be degassed prior to use.

7.5.1 Methanol, CH_3OH - HPLC quality or equivalent.

7.5.2 Solution for standards and water extraction: Methanol/1% acetic acid, v/v

7.5.2.1 Solution for tissue extractions: Hexane (99%)/Acetic acid (1%)/tropolone (0.1%). Example: For a 250mL extraction solution you would have 247.5 mLs of hexane, 2.5 mLs of acetic acid and 250 mg of tropolone.

7.5.3 Mobile phase solution: 80% methanol/14% water/6% acetic acid/0.1% tropolone, v/v/v/w.

7.5.4 50:50 methanol:water, solution for mass calibration tuning standard.

7.6 Standard materials - pure standard materials or certified solutions of each analyte targeted for analysis.

ARPAT current extraction method for total organotins in sediments and biota

Extraction	**Biota:** 1 g sample + 10 ml hexane/tropolone solution [hexane (99%)/acetic acid (1%)/tropolone (0,1%)] **Sediments:** 1 g sample + 20 ml hexane/tropolone solution [hexane (99%)/acetic acid (1%)/tropolone (0,1%)]
Sonication	Water bath sonicator 45 minutes
Acidification	Adjust pH to approx 2.0 with HCl conc.
Centrifugation	Centrifuge at 4000 rpm for approx 30 minutes
Evaporation	Evaporate the solvent volume to dryness using a gentle stream of clean, dry Nitrogen
Acid mineralization	1 ml Hcl conc. + 1 ml HNO3 conc. + 8 ml Milli Q water in oven at 105°C for 1 hour
Revelation	ICP-MS determination

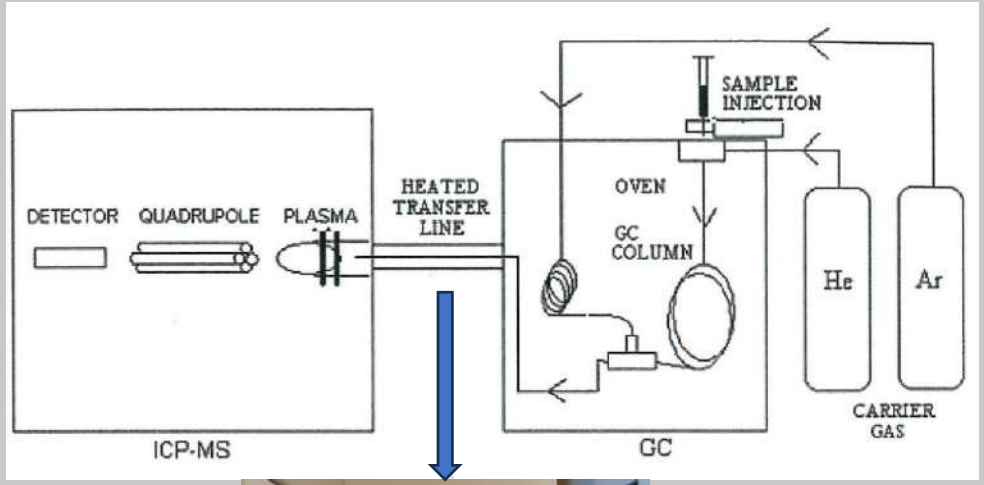
Speciation of organotins… why?

- The aim of this study is to develop an analytical method suitable for the determination and speciation of organotin compounds in marine sample, in particular in sediments.

- NO DERIVATIZATION.

Configuration of GC-ICP/MS system

*



(Illustration from Thermo Fisher Scientific Operating Manual)

Settings of GC and ICP/MS

For the instrumental analysis, first of all, it's necessary to tune the GC-ICP/MS system.

The steps are:

- Ignite the plasma and wait approximately 10 minutes;
- Select the 129, 132 and 143 masses which are the major isotopes of Xenon dissolved in the Argon gas.

ICP conditions:

- Cool Gas: 14,0 L/min
- Plasma Power: 1550 W
- Auxiliary Flow: 0,8L/min
- Nebulizer Flow: 1,0L/min

MS conditions:

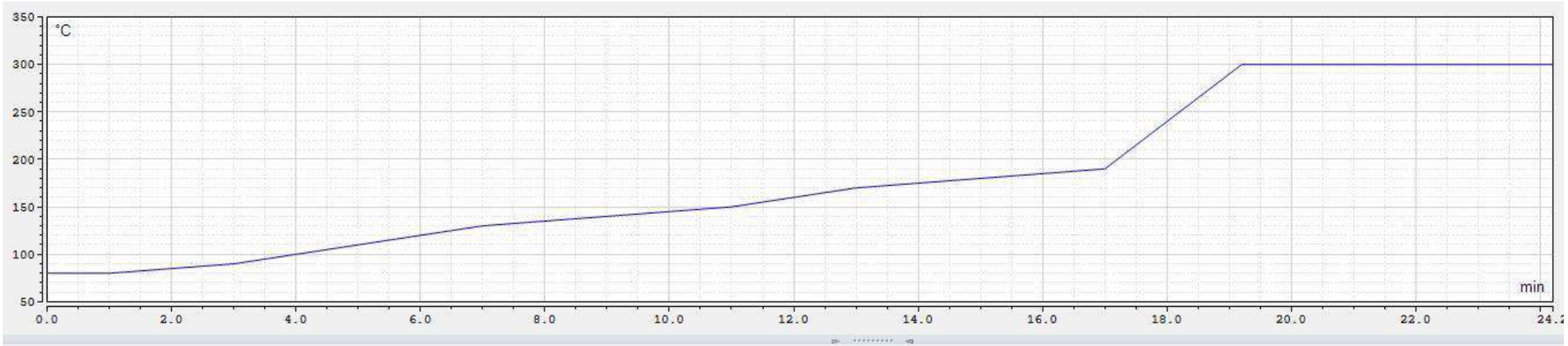
Selected masses: 118 & 120

Boiling Temperature of organotin compounds

Tabella 1. Proprietà chimico-fisiche di alcuni composti organostannici (Gmelin, 1978; Bluden e Chapman, 1986). Abbreviazioni: nessuna informazione (ni), dati non disponibili (nd). (a): solubilità in acqua di mare; (b): solubilità in acqua distillata.

	Temperatura di fusione (°C)	Temperatura di ebollizione (°C)	Densità (g/cm^3)	Solubilità (mg Sn/L)
Tetrabutilstagno	-97	145	1.06	ni
Tributilstagno clouro	-16	172	1.21	30-70 [a] 5-17 [b]
Dibutilstagno cloruro	39-41	135	nd	4-50 [a]
Monobutilstagno cloruro	ni	93	1.69	ni
Trimetilstagno cloruro	37-39	154	nd	
Dimetilstagno cloruro	106-108	188-190	nd	
Monometilstagno cloruro	48-51	171	nd	2000 [a]

Settings of GC and ICP/MS



Mode: Ramped temperature

No	Retention time [min]	Rate [°C/min]	Target value [°C]	Hold time [min]
1	0.000	Run		
2	1.000	0.00	80.0	1.00
3	3.000	5.00	90.0	0.00
4	7.000	10.00	130.0	0.00
5	11.000	5.00	150.0	0.00
6	13.000	10.00	170.0	0.00
7	17.000	5.00	190.0	0.00
8	24.200	50.00	300.0	5.00
9		New Row		
10	24.200	StopRun		

Sistema Nazionale per la Protezione dell'Ambiente

[redacted]

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Date Received: _____

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Catalog No.:	Lot No.:	Storage:	Solvent:	Exp. Date:	Description:
010933-03	499179	≤ -10 °C	Methylene Chloride	18-Mar-2028	Di-n-butyltin Dichloride, 1000 mg/L, 1 ml

Compound	CAS No.	Purity (%)	Neat Material Lot No.	Concentration
Di-n-butyltin dichloride	683-18-1	99.3	933.7.1P	995 ± 7.36 mg/L

Certified By: Briana Smith

Briana Smith
Manufacture Date 20-Mar-2023

Follow all storage requirements, keep tightly closed when not in use, and use good laboratory practices when handling.
This Reference Material was manufactured, produced, and/or certified under a quality management system that is accredited to ISO 9001:2015.

All weights are traceable through N. I. S. T. Test No. 822/264157-00.
Concentration (correct for purity) and uncertainty (95% confidence) values listed are determined gravimetrically.
The stated uncertainty is the expanded uncertainty with a coverage factor of two to give a 95% confidence level.

ARPAT
Agenzia regionale per la protezione ambientale della Toscana

[redacted]

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Catalog No.:	Lot No.:	Storage:	Solvent:	Exp. Date:	Description:
010935-02	499178	≤ -10 °C	Methylene Chloride	18-Mar-2028	N-butyltin Trichloride Solution, 1000 mg/L, 1 ml

Compound	CAS No.	Purity (%)	Neat Material Lot No.	Concentration
n-butyltin trichloride	1118-46-3	100	935.7.1P	1008 ± 24.78 mg/L

[redacted]

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Catalog No.:	Lot No.:	Storage:	Solvent:	Exp. Date:	Description:
012523-01	482523	≤ -10 °C	Methylene Chloride	4-Jun-2025	Tri-n-propyltin Chloride Solution, 1000 mg/L, 1 ml

Compound	CAS No.	Purity (%)	Neat Material Lot No.	Concentration
tri-n-propyltin chloride	2279-76-7	99.1	2523.421.2.2P	1001 ± 22.98 mg/L

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[redacted]

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Rev 0

Certificate of Analysis Page 1 of 3

Catalog No.	Lot No.	Storage	Solvent	Date Received	Exp. Date
G34-012289-01 -5PAK	500126	≤ -10 °C	Methanol	_____	11-Apr-2025

Description: Diphenyltin Dichloride Solution, 1000 mg/L, 5 x 1 ml

Container: 1 ml Ampule, Amber Glass

[redacted]

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Date Received: _____

Certificate of Analysis Rev 0 Page 1 of 1

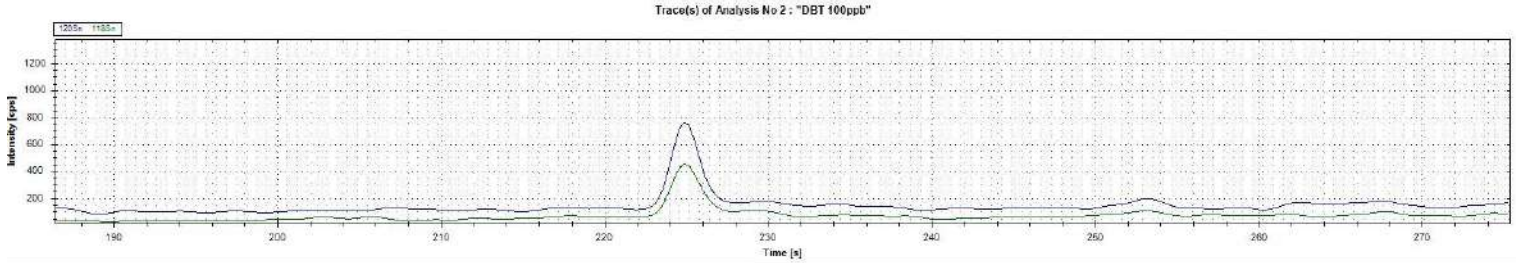
Catalog No.:	Lot No.:	Storage:	Solvent:	Exp. Date:	Description:
010934-15	499175	<4 Degrees C	Methanol	20-Sep-2024	Tri-n-butyltin Chloride Solution, 1,000 mg/L, 1 ml

Compound	CAS No.	Purity (%)	Neat Material Lot No.	Concentration
tri-n-butyltin chloride	1461-22-9	96.6	934.421.2P	1005 ± 21.36 mg/L

Standard solutions

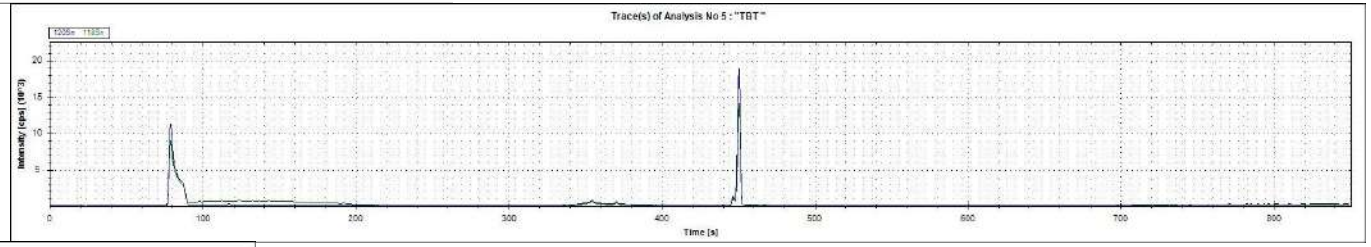
Every single standard solutions are with a purity higer than 96% and a concentration of 1000 mg/L, all from chloride salts.

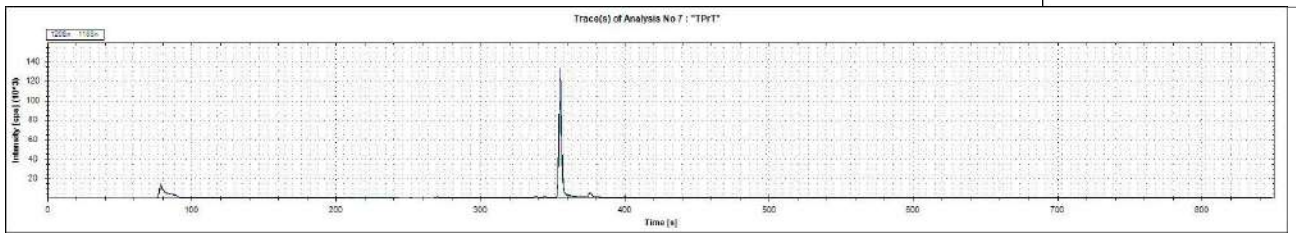
DBT, TBT, TPrT and TPhT speciation



DBT (RT=225s)

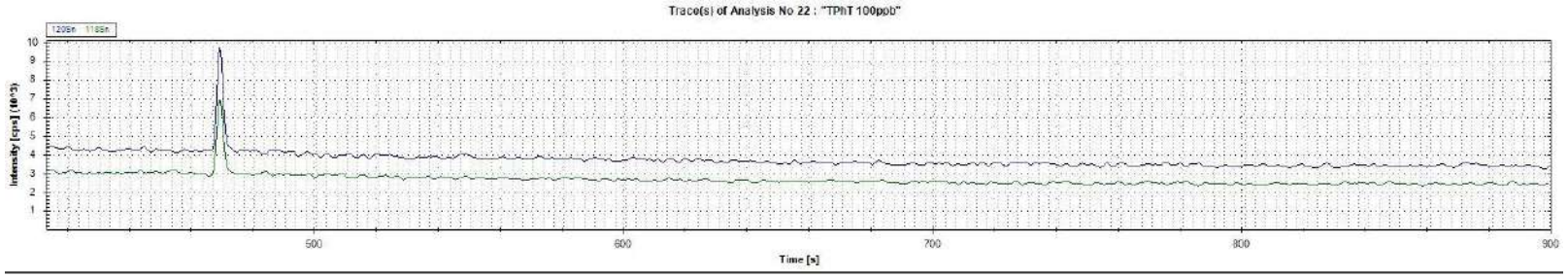
TBT (RT=453s)





TPrT (RT=358s)

TPhT (RT=470s)



Certified Reference Material – Fresh Water Sediment

EUROPEAN COMMISSION
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CERTIFIED REFERENCE MATERIAL
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CERTIFICATE OF ANALYSIS

FRESH WATER SEDIMENT		
	Mass fraction based on dry mass	
	Certified value [1] [µg/kg]	Uncertainty [2] [µg/kg]
TBT: $Sn(C_4H_9)_3^+$	480	80
DBT: $Sn(C_4H_9)_2^{2+}$	770	90
MBT: $Sn(C_4H_9)^{3+}$	610	120
TPhT: $Sn(C_6H_5)_3^+$	29	11
DPhT: $Sn(C_6H_5)_2^{2+}$	36	8
MPhT: $Sn(C_6H_5)^{3+}$	69	18

1) Unweighted mean value of the means of 6-14 accepted sets of data. The certified value is valid for the cation indicated. Unweighted mean of accepted mean values, independently obtained by 6 - 14 laboratories. The value is traceable to the International System of Units (SI).
2) The certified uncertainty is the expanded uncertainty with a coverage factor k = 2, corresponding to a level of confidence of about 95 %, comprising uncertainties from the characterisation and inhomogeneity studies.

This certificate is valid for one year after purchase.
Sales date: 14 NOV 2022
The minimum amount of sample to be used is 600 mg.

NOTE
This material has been certified by BCR (Community Bureau of Reference, the former reference materials programme of the European Commission). The certificate has been revised under the responsibility of IRMM.

Brussels, December 2000
Latest revision: April 2015

Signed:

Prof. Dr. Hendrik Emons
European Commission
Joint Research Centre
Institute for Reference Materials and Measurements
Retieseweg 111
B-2440 Geel, Belgium

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TBT CRM recovery and calibration curve

Concentration in ppb	Intensities (cps)
1	425
10	4978
100	71674

$Y=7,28*10^2X -1,26*10^3$

$R^2=0,9997$

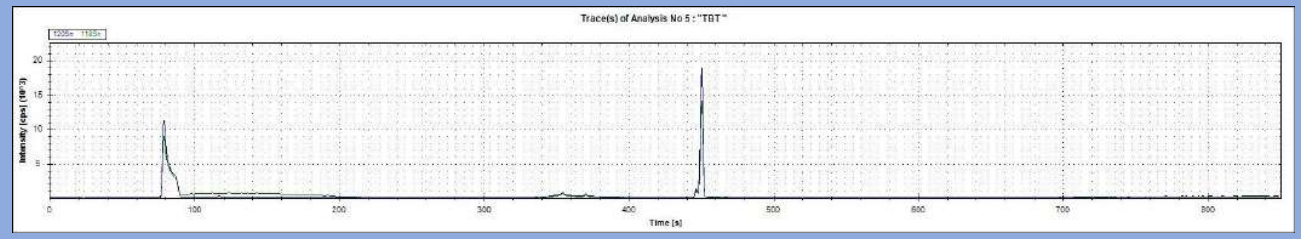
Sample	Certified Value (ppb)	Value obtained (ppb)	Recovery in %
BCR-646	480 ± 80	267 ± 27	55,6

Conclusions

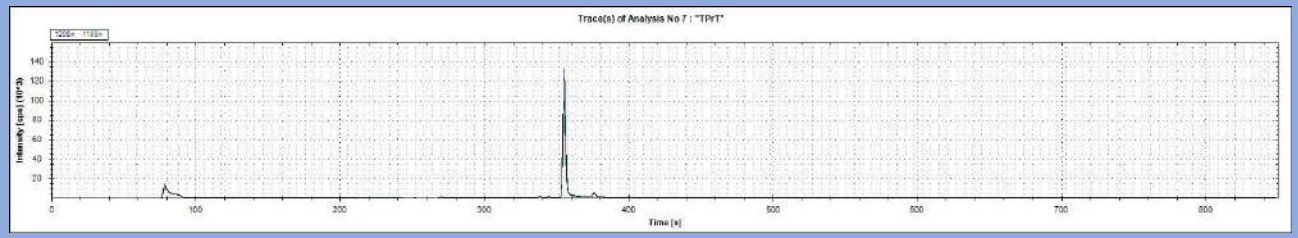
In this study we have developed a new method for the separation of organotins **without the derivatization** after extraction. The conditions of the gas chromatography and ICP/MS analysis enabled us to **achieve a high sensitivity** for tin detection.

The quantification limit is **1ug/Kg of TBT** (as TBT chloride).

To ensure accurate quantification, we employed Tripropyltin (TPrT) as an **internal standard**.



TBT (RT=453s)



TPrT (RT=358s)

Next Steps…for a better analysis

- **Optimize the extraction method** following the ISPRA guide lines («I composti organostannici in ambiente marino e lagunare» Quaderni – Ricerca Marina 8/2016);
- **Reduce** the GC **run time**;
- Introduce TPrT as an **Internal Standard** and evaluate its recovery at different concentration to ensure an accurate quantification;
- Evaluate the **recoveries** and possible **interferences;**
- Using a PTV injector to get a higher intensity of the signals.

Thanks to all the staff of ARPAT AVL Laboratory of Livorno. A special thanks to Dr. Franco Castellani Tarabini and a wish to celebrate his new retired life, and to Dr. Carlo Cini, former chief of AVL Chimica I, for his continuously support.