

Oceanological and Hydrobiological Studies  
Vol. XXXII, No. 3

Institute of Oceanography

(43-71)  
2003

University of Gdańsk

---

Research Article

**PATHWAYS AND DETERMINATIONS OF ORGANOMETALLIC  
COMPOUNDS IN THE MARINE ENVIRONMENT WITH A  
PARTICULAR EMPHASIS ON ORGANOTIN**

BARBARA RADKE<sup>(1)</sup>, JACEK NAMIEŚNIK<sup>(2)</sup>, JERZY BOLAŁEK<sup>(1)\*</sup>

<sup>1</sup>*University of Gdańsk, Department of Marine Chemistry and Marine  
Environmental Protection, Gdynia  
e-mail: \*jerzyb@sat.ocean.univ.gda.pl*

<sup>2</sup>*Gdańsk University of Technology, Chemistry Department, Analytical Chemistry  
Faculty, Gdańsk*

**Key words:** organometallic compounds, butyl tin derivatives, anti-fouling  
paints, speciation

**Abstract**

Organometallic compounds are widely used in almost all sectors of industry. Some organometallic compounds are very potent toxins. Human activity releases organometallic compounds into the natural environment where they undergo various changes through compound-environment interactions. These compounds have a negative impact on living organisms. The most widespread, man-made organometallic compounds present in the natural environment are those of tin, mercury and lead.

**INTRODUCTION**

Organometallic compounds are described by the following formulas: **MR<sub>n</sub>** or **R<sub>n</sub>MX<sub>m</sub>**, where M is a metal atom, R is alkyl, aryl or a carbonyl group and X is halogen, alcoxyl, hydroxyl or another group. As stated by Pruchnik (1991), **organometallic compounds** are substances which display a metal-carbon bond.

According to this definition, vitamin B<sub>12</sub> (cobalamin), CH<sub>3</sub>Li (methyl lithium), C<sub>6</sub>H<sub>5</sub>Na (phenyl sodium) or (CH<sub>3</sub>)<sub>4</sub>Pb (tetramethyl lead) and similar substances should all be considered organometallic compounds.

The first organometallic compound, **Zeise's salt** ( $KPtCl_3/C_2H_4$ ; Pruchnik, 1991), was produced in 1827. In 1890, Monda, Langer and Quinck synthesized **carbonyl nickel** ( $Ni(CO)_4$ ) - the final product of a chemical reaction between metallic nickel and carbon monoxide. The efforts that followed, including those of Barbier and Grignard in 1900, resulted in the acquisition of more theoretical and practical knowledge regarding the connections between the general theory of chemical compound structure and the chemistry of organometallic substances. The production of organometallic compounds has been slowly increasing since 1920 due to the application of their biochemical features in agriculture (Shim *et al.* 1999). In recent decades, many novel organometallic compounds have been produced and used regularly in industry and agriculture despite the publication of information on their hazardous environmental impact.

The aim of the work presented here is to describe the pathways and analytics of organometallic compounds in the marine environment. The processes and transformations to which these substances are subjected in the marine environment are presented. The influence of environmental conditions on the accumulation of organometallic compounds in living organisms and benthic sediments and their diffusion and re-mobilization from the sediments into the water column are described. Particular emphasis is placed on organotin. Methods for determining organometallic compounds in solid samples are described in brief.

## THE CHARACTERISTIC OF ORGANOMETALLIC COMPOUNDS

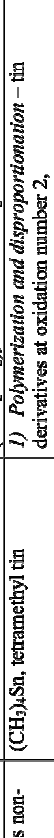
Depending on the type of metal that is present in a molecule, the following groups of compounds can be distinguished: *organolithium* (e.g. methyl lithium, CH<sub>3</sub>Li), *organosodium* (e.g. ethyl sodium, CH<sub>3</sub>CH<sub>2</sub>Na), *organozinc* (e.g. *dimethyl zinc*, (CH<sub>3</sub>)<sub>2</sub>Zn), *organomercury* (e.g. phenyl mercury chloride, C<sub>6</sub>H<sub>5</sub>HgCl).

Organometallic compounds occur in liquid or solid states, and they are soluble in organic solvents *e.g.*, ether, alcohol or toluene. They can be produced during reactions between metals and fluorine derivatives or other organometallic substances (Table 1). Organometallic compounds are synthesized from inorganic substances (*e.g.* methylated mercury compounds, Hg(Me)X) and undergo reduction, substitution, cyclic addition (oxidative coupling) reactions and others. Some show a strong affinity with sulfur which results in the blocking of biologically important thiol groups, therefore many

Table 1

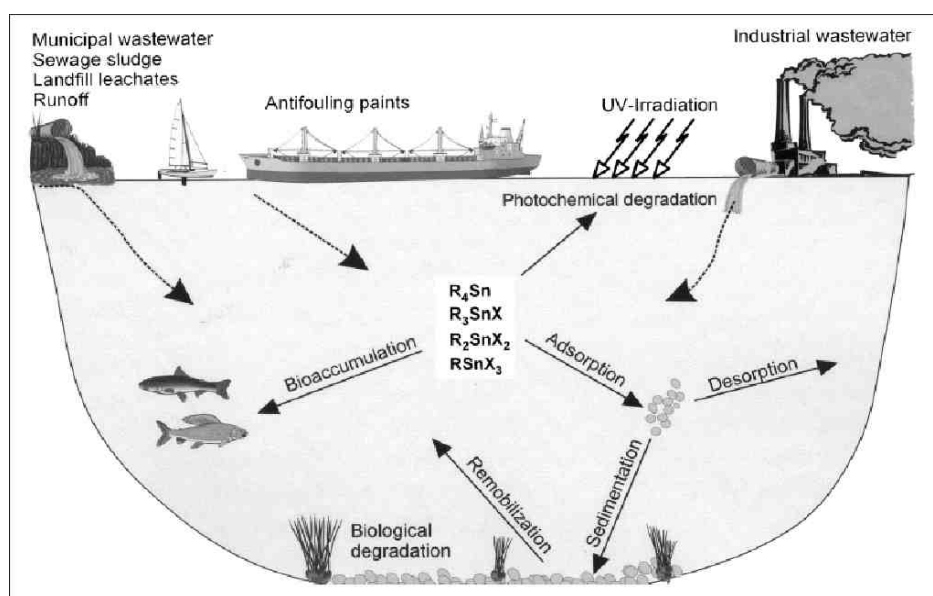
Basic chemical reactions for chosen groups of organometallic compounds.

Organometallic compounds <i>1</i>	Properties <i>2</i>	Example of such compounds <i>3</i>	Main reactions <i>4</i>
*Lithium-containing organic compounds, RLi	Solids (e.g. methyl lithium) or liquids (e.g. butyl lithium)	CH <sub>3</sub> Li, methyl lithium	<p>1) <i>Synthesis</i>: reaction of metallic lithium with halogenated alkanes or halogenated arenes  <math>RX + 2Li \rightarrow RLi + LiX</math>,</p> <p>2) <i>Substitutions</i>: hydrogen substituted with lithium  <math>RH + R_1Li \rightarrow RLi + R_1H</math>,</p> <p>3) <i>Synthesis of hydrocarbons</i>: reactions with oxygen or alcohols (oxidation is a very violent reaction):  <math>RH + H_2O \rightarrow RH + LiOH</math>,  <math>RLi + CH_3OH \rightarrow RH + CH_3OLi</math>.</p>
*Magnesium-containing organic compounds, R <sub>2</sub> Mg or RMgX	Non-volatile, poorly soluble	Grignard reagents CH <sub>3</sub> MgCl (methyl magnesium chloride)	<p>1) <i>Synthesis of Grignard reagents</i>: reaction of metallic magnesium with organic compounds of chlorine, bromine or iodine in anhydrous ether  <math>RX + Mg \rightarrow RMgX</math>,</p> <p>2) <i>Decomposition of Grignard reagents</i>  <math>RMgX + H_2O \rightarrow RH + Mg(OH)X</math>,</p> <p>3) <i>Syntheses</i>: reaction with hydrocarbons of high acidity  <math>R-C \equiv C-H + CH_3MgI \rightarrow R-C \equiv CMgI + CH_4</math>.</p>
*Aluminum-containing organic compounds, RAlX <sub>2</sub> , R <sub>2</sub> AlX or R <sub>3</sub> Al	Occurring as dimers	Trialkyl aluminum compounds (CH <sub>3</sub> ) <sub>3</sub> Al, trimethyl aluminum	<p>1) <i>Synthesis</i>: reaction of aluminum with organomercury compounds  <math>2Al + 3(CH_3)_2Hg \rightarrow (CH_3)_6Al_2 + 3Hg</math>,  or aluminum (aluminum-magnesium alloy) with halogenated alkanes  <math>Al_2Mg_3 + 6C_2H_5Cl \rightarrow (C_2H_5)_6Al_2 + 3MgCl_2</math>,</p> <p>2) <i>Synthesis of triethyl aluminum</i>: reactions of ethylene with hydrogen and metallic aluminum  <math>AlH_3 + C_2H_4 \rightarrow (C_2H_5)_3Al</math>,</p> <p>3) <i>Synthesis of alcohols</i> (oxidation of trialkyl aluminum), <i>synthesis of alkanes</i> (reaction of acids with trialkyl aluminum), <i>synthesis of alkenes</i> (those</p>
<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>

1	2	3	4
<p><i>**Tin-containing organic compounds, e.g. R<sub>4</sub>Sn</i></p>	<p>Sn (IV) is stable; Sn (II) is non-stable</p>	<p>(CH<sub>3</sub>)<sub>4</sub>Sn, tetramethyl tin</p>	<p>with a double bond at the end of the chain) - thermal decomposition of trialkyl aluminium</p> $(RCH_2CH_2)_3Al \xrightarrow{O_2, H^+} 3RCH_2CH_2OH,$ $(RCH_2CH_2)_3Al \xrightarrow{H^+} 3RCH_2CH_3,$ <p>heating</p> $(RCH_2CH_2)_3Al \rightarrow 3RCH=CH_2.$ <p>1) <i>Polymerization and disproportionation</i> - tin derivatives at oxidation number 2,  2) <i>Syntheses of dialkyl cupro-lithium</i> - reaction with alkyl lithium and copper: (I) halides  2RLi + CuI = R<sub>2</sub>CuLi + LiI,  3) <i>Selective and reductive elimination</i> of fluorine-like elements from organic compounds without breaking double bonds or 3-carbon rings (under the condition that organotin contains Sn-H bond).</p> 
<p><i>**Lead-containing organic compounds, R<sub>4</sub>Pb, R<sub>3</sub>PbX or R<sub>2</sub>PbX<sub>2</sub></i></p>	<p>Volatile or liquid toxic substances</p>	<p>(C<sub>2</sub>H<sub>5</sub>)<sub>4</sub>Pb, tetraethyl lead chloride</p>	<p>1) <i>Syntheses of tetraethyl lead</i>  <math>4C_2H_5Cl + Na_2Pb \rightarrow (C_2H_5)_4Pb + 4NaCl</math>  2) <i>** Bio-methylation</i> mediated by microorganisms,  3) <i>**Decomposition of dimethyl lead</i>  <math>2 (CH_3)_2PbX_2 \rightarrow (CH_3)_2PbX + PbX_2 + CH_3X,</math>  where X is acetate, perchlorate, nitrate, chloride, nitrite, bromide, sulphanyl cyanogen or iodide.  4) <i>**Decomposition (CH<sub>3</sub>)<sub>2</sub>PbX</i>:  <math>(CH_3)_2PbX \rightarrow 2 (CH_3)_2Pb + PbX_2 + CH_3X...</math></p>

Source: \* Mastalcz (1996), \*\* Dojlido (1987)

organometallic substances display toxicity (Pruchnik 1993). Organometallic compounds are used in agriculture and forestry as fungicides, insecticides, acaricides and other applications. They are very persistent and characterized by retention times in soil of over 18 months (Korzeniewski 1998). Organometallic compounds are also widely used in dyeing (organotins), wood processing (preservation and conservation), the chemical industry (catalysts in polymerization, oligomerization, reduction of unsaturated organic compounds, and ethylene oxidation, *etc.*), the food industry, medicine and pharmaceutical production (platinum, gold and ruthenium compounds used as medicines) (Wasik and Namieśnik 2001).



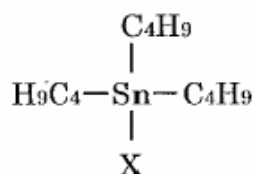
**Fig. 1.** Schematic presentation of the processes influencing pathway dynamics of organotin in the marine environment (Hoch 2001).

Organotins are one of the most common organometallic compounds of anthropogenic origin present in the natural environment. They are transferred into the marine environment by both industry and the communal activities of humankind (Fig. 1). Organotins introduced into the marine environment undergo changes that can possibly result in the production of new chemical compounds that then undergo physico-chemical processes such as adsorption on suspended particular matter, bio-accumulation and sedimentation followed by accumulation in benthic sediments.

However, at present, attention is primarily focused on the **derivatives of butyl tin**. This group of compounds consists of the following components (Hong *et al.* 2002):

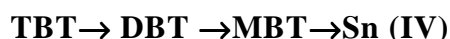
$$\Sigma \text{ BTs} = \text{MBT} + \text{DBT} + \text{TBT}$$

where  $\Sigma$  BTs is total content of butyl tin derivatives; MBT is monobutyl tin; DBT is dibutyl tin; and TBT is tributyl tin (fig. 2.).



**Fig.2.** Chemical formula of tributyl tin (Kawai *et al.* 1998).

The dominant form of butyl tin derivatives present in the marine environment is tributyl tin, TBT. Tributyltins can be described by the following formula:  $\text{Bu}_3\text{SnX}$ , where Bu is butyl; X is OH, Cl or such (Vella *et al.* 1998). Morcillo and Porte (2000) observed that over 90% of butyl tin derivatives accumulated by bivalves of the species *Ruditapes decussata* were present in the form of TBT. A study of the surface layers of benthic sediments in the vicinity of a shipyard in Ulsan, Korea indicated that 69% of the organotin occurring there was comprised of tributyl tin derivatives (TBT), 20% of dibutyl tin (DBT) and 11% of monobutyl tin (MBT) (Shim, 2002). Tributyl tin in the marine environment undergoes degradation in the process of sequential dealkylation which results in dibutyl- and monobutyl tin production and ultimately in the release of non-toxic inorganic tin (Vella *et al.* 1998). Therefore, the schematic presentation of the chemical pathway of TBT can be described as follows:



Tributyl tin degradation in benthic sediments depends on the in situ oxygen conditions. The higher the oxygen content in a given body of water, the faster the degradation processes are.

The influx of tributyl tin into the marine environment stems from industrial activities in ports and particularly in shipyards where ship maintenance and repair activities, such as washing, cleaning and re-painting, are conducted (Kania 1999). The main source of TBT derivatives are the anti-fouling paints used on ship hulls to prevent plant and animal micro- and macroorganisms (*e.g.* barnacles and algae) from growing on them.

Significant financial losses are incurred in the shipping industry due to fouling, which increases water resistance against the hull and higher fuel consumption to compensate for it. Moreover, higher fuel consumption results in increased emissions of NO<sub>x</sub> and SO<sub>x</sub> into the atmosphere.

After tributyl tin derivatives are introduced into the marine environment they dissolve and are either partially absorbed on suspended organic matter or bio-accumulate. This has negative repercussions such as the thickening and delamination of shells (Gomez-Ariza *et al.* 1999), growth and hormonal disorders and changes in the reproductive organs in bivalves (Morcillo and Porte 2000). It has been established that concentrations of these substances in the marine environment decrease as distance from the source of pollution, *i.e.* shipyards and ports, increases (Rajendran *et al.* 2001, Shim *et al.* 1999, Michel *et al.* 2001).

Tributyl tin is also the active ingredient of many bactericides (Diez *et al.* 2002), fungicides, insecticides, acaricides and wood preservatives (Rajendran *et al.* 2001). In 1970 TBT was designated as a marine environment pollutant. Since 1980 many industrialized countries have introduced their own legal regulations regarding the use of TBT in the ship-building industry. These guidelines generally ban the application of TBT-containing anti-fouling paints on hulls shorter than 25 m (Albanis *et al.* 2002, Champ 2000, Alzieu 2000). On January 1, 2003 the International Maritime Organization (IMO) introduced legal sanctions against the use of bactericides based on tributyl tin derivatives for hull protection and also undertook action to fully eliminate such agents from the ship-building industry by January 1, 2008 (Tselentis and Tzannatos 2000). In response to the foregoing, several proposals have been formulated to replace these toxic compounds with alternative substances such as Irgarol 1051 (Biselli *et al.* 2000), dichlofluanid, chlorothalonil, Sea Nine 211 and others (Albanis *et al.* 2002). However, no concrete regulations have been enacted thus far. The replacement of the compounds mentioned above with alternative substances must be thoroughly considered and assessed by scientists. The following questions also arise: Is it possible that in the future the substitute substances introduced into the marine environment will turn out to be even more hazardous to living organisms? What chemical forms will the alternative compounds be

present in? What impact will they have on the marine ecosystem as a whole? Therefore, the idea of alternative compounds is still open for discussion.

In addition to butyl tin derivatives, other man-made organotin compounds can be found in the marine environment, for example, **methyl tin** (trimethyl tin, TMT; dimethyl tin, DMT; monomethyl tin, MMT), **phenyl tin**, PT (triphenyl tin and the products of its degradation - diphenyl tin, DPT, monophenyl tin, MPT) and others. The derivatives of triphenyl tin, TPT, tributyl tin and TBT, have been widely applied as biocide additives to marine anti-fouling paints (Morcillo and Porte 2000). It should also be noted that the concentrations of TPT derivatives in marine organisms, benthic sediments or the water column are usually lower than TBT concentrations.

Triphenyl tin chloride is used for wood preservation in wooden houses, and it constitutes a potential source of atmospheric organotin pollution (Vella and Vassallo 2001). Methylbutyl tin derivatives are another source of atmospheric pollution (Vella *et al.* 1998).

Organotin compounds are particularly dangerous to living organisms. A frequently cited example are the dramatic events that took place in 1952-1959 in Minamata, Japan, where 3,500 people were poisoned and over 100 died after consuming fish and mollusks containing trace amounts of methyl mercury (Korzeniewski 1998). Symptoms of poisoning included ataxia, muscle spasms, a decreased field of vision, damage to sensory organs, mental disorders and paralysis, while congenital defects were detected in newborns, including serious brain damage.

Inorganic forms of mercury introduced into water bodies undergo a specific cycling and bacteria-mediated transformation into methyl mercury cations ( $\text{CH}_3\text{Hg}^+$ ) and dimethyl mercury ( $(\text{CH}_3)_2\text{Hg}$ ). Those which are most easily absorbed by living organisms are alkylated organomercury compounds such as methyl mercury nitrate, chloride, bromide and phosphate, phenyl mercury chloride and others (Dutkiewicz 1968). Dimethyl mercury ( $(\text{CH}_3)_2\text{Hg}$ ) is transferred to the brain cells very fast, and it blocks the biological functions of thiol, carboxyl and amine groups and amino acids, to which it has affinity (Kabata-Pendias and Pendias 1993). Dimethyl mercury belongs to a group of substances which are classified as highly toxic (*i.e.* poisonous, mutagenic and teratogenic). Because mercury has a high vapor pressure, the risk of mercury poisoning in an industrial environment frequently occurs even at room temperature. Mercury accumulates in the kidneys, liver, brain, spleen, lungs and heart, and death from mercury poisoning occurs from uremia between days six and 10 (Dutkiewicz 1968).

Mercury is used primarily in scientific research and secondly in fungicides and bactericides (phenyl mercury acetate is an active fungicidal ingredient). The

compounds mentioned above are very persistent in the environment with a retention time assessed at over 18 months (Korzeniewski 1998).

**Lead-containing organometallic compounds** are mostly used in the automobile industry for the production of ethyl gasoline and other substances (Kabata-Pendias and Pendias 1993), anti-knock agents for fuels - tetraethyl lead  $(C_2H_5)_4Pb$  and tetramethyl lead  $(CH_3)_4Pb$  (Dojlido, 1987) and  $Pb(CH_3COO)_2$  used in chemical syntheses (Dutkiewicz, 1968) and in dyeing processes. Lead negatively impacts hemoglobin biosynthesis by reacting with proteins in the erythrocyte cell walls which renders them fragile and shortens their life span resulting in hemolytic anemia. Lead tetrachloride, a strong poison, damages the nervous system and causes permanent brain damage in children (Dutkiewicz 1968). Lead toxicity depends on the type and amount of organolead absorbed through the respiratory system.

**Organoaluminum** compounds are mainly used as stereospecific catalysts in the polymerization of olefins. Organic compounds of arsenic and tin are used as fungicides and bactericides. Moreover, arsenic-containing organic compounds are employed in the tanning industry as preservatives. The organoarsenic group includes, among others, diethyl arsine -  $C_2H_5)_2AsH$ , methyl arsine oxide -  $CH_3AsO$  and ethyl arsine oxide -  $(C_2H_5)_2AsO$  (Dutkiewicz 1968). Arsenic and its compounds can cause strong tissue irritation after being absorbed through the respiratory system in the form of dust or gas, through the digestive system and on direct contact with skin. Even small concentrations of arsenic can cause vomiting, while poisoning from exposure to insecticides results in death within hours.

Lithium- and magnesium-containing organic compounds have broad applications in organic synthesis. Particularly important are **organomagnesium** substances, including Grignard reagents that are described by the following formulas:  $RMgX$  or  $ArMgX$ , where R is alkyl, X is Cl, Br or I.

Grignard reactions occur in many inorganic compounds, such as water and carbon dioxide and in the majority of organic substances. These reactions constitute an important laboratory method for obtaining many groups of organic compounds, for example, hydrocarbons (from alcohols or water) and alcohols.

### **Factors influencing the content of organometallic compounds in sediments (TBT as an example)**

Organometallic compounds which are carried into the marine environment undergo sedimentation and accumulation processes in the benthic sediments. At the same time, due to changes in sedimentation conditions (*e.g.* pH, Eh,

increased salinity), these substances may diffuse again from the sediments into the near-bottom water layer.

**Table 2**

Values calculated for  $K_d$  (L/kg), adsorption (%), desorption (%), pH\* and salinity (PSU)\*\* for tributyl tin.

	Montmorillonite			Kaolinite			Illite, kaolinite		
	$K_d$	Adsorption %	Desorption %	$K_d$	Adsorption %	Desorption %	$K_d$	Adsorption %	Desorption %
<b>pH 4</b>	47	47	38	43	45	33	28	32	36
<b>pH 5</b>	72	57	31	41	45	37	47	44	44
<b>pH 6</b>	150	72	24	45	47	26	83	62	ND
<b>pH 7</b>	161	74	22	65	58	23	138	72	27
<b>Salinity 15 PSU</b>	177	76	ND	131	71	ND	138	73	ND
<b>Salinity 0 PSU</b>	529	89	ND	191	78	ND	271	84	ND

ND- undetermined;

$K_d$  – adsorption coefficient; pH was determined for salinity of 32 PSU; salinity (PSU)\*\* was calculated for samples enriched with Montmorillonite, silty minerals and clay at pH 8, and for samples containing kaolinite at pH 7.

Source: (Hoch *et al.* 2002).

The pH value is a significant parameter which controls the adsorption and desorption of organometallic compounds in the aquatic environment. Hoch *et al.* (2002) obtained maximal adsorption values in the material they studied (*i.e.* sample 1 - sediment enriched with montmorillonite; sample 2 - sediment enriched with kaolinite; sample 3 - sediment enriched with illite, kaolinite and silty minerals) within a pH range of 6 to 7 at which the strongest electrostatic interactions between the surface of a given mineral and TBT molecules was observed (Table 2). Desorption processes occurred for each pH value measured and the highest was observed in samples 1 and 2 at pH values of 4, 5 and 8.

Moreover, butyl tin derivatives introduced into the marine environment undergo hydrolysis at a specific pH. At  $\text{pH} < \text{p}K_a$  ( $\text{p}K_a$  is a coefficient calculated for an acid dissociation constant) cations form in seawater. Single-charged cations in an aqueous phase behave like weak acids. The reaction of TBT dissociation can be described as follows (Shoukry 1993):



TBT occurs predominantly as cations at  $\text{pH} < \text{pK}_a = 6.51$ , while it occurs as TBTOH at  $\text{pH} > 6.51$ . Based on the  $\text{pK}_a$  described above, 76% of TBT form cations at pH 6. It should be noted that pH and the dissociation constant depend on **water temperature** and **ionic composition** (Tobias 1978). The high content of  $\text{Cl}^-$  ions in seawater stimulates TBTCI formation. It was also observed that at  $\text{pH} < 7$  the dominant forms of TBT in seawater are  $\text{TBT}^+$  and  $\text{TBT}^+\text{HCO}_3^-$ , while at pH 8 they are TBTCI, TBTOH and  $\text{TBT}^+\text{HCO}_3^-$  (Laughlin *et al.* 1986).

**Salinity** is also a factor which **determines** sorption and desorption. It has been observed that as salinity increases, TBT adsorption in the sediments decreases. The highest adsorption was achieved at a salinity of 0 PSU (Hoch *et al.* 2002).

Relations between changes in salinity and pH and TBT adsorption and desorption in coarsely grained material are described in Table 2.

The **organic carbon** content of seawater has a significant influence on the pathway dynamics of organometallic compounds in the aquatic compartment. Due to the selective sorption of pollutants, dissolved organic carbon (DOC) can change non-bioavailable forms of man-made compounds into forms that are assimilated by living organisms.

Humic and fulvic acids also play a significant role in TBT accumulation in the benthic sediments. TBT interacts with humic and fulvic acid, but the mechanisms which cause TBT to bond with humic matter have yet to be elucidated. It has, however, been determined that binding between butyl tin derivatives and humic acids depends on chemical characteristics and increasing hydrophobic properties (Fen and Looser 1995).

In the presence of **aquatic bacteria and microorganisms**, tributyl tin can degrade into DBT, MBT and even inorganic tin (Kawai *et al.* 1998). The half-life of TBT ranges from a few days to two weeks depending on the conditions the water samples are subjected to (*i.e.* temperature, incubation, initial TBT concentration in the water and other factors). For example, Harino *et al.* (1997) estimated that the half-times for TBT, DBT and TPT were 15, 10 and 60 days, respectively, at an initial TBT concentration of  $9.3 \mu\text{g}/\text{dm}^{-3}$  and at initial DBT and TPT concentrations of  $8.0 \mu\text{g}/\text{dm}^{-3}$ . Steward and Mora (1990) reported that the half-life of TBT undergoing biodegradation in coastal waters ranges from three to 15 days depending on temperature and other natural factors.

TBT can be also degraded in the benthic sediments. However, its degradation rate in the sediments is slower than in the water column and depends on in situ oxygen conditions. It has been estimated that the TBT

half-life in sediments ranges from four to eight years (Steward and Mora 1990).

Geological factors are also an important aspect which influences the content of organometallic compounds in benthic sediments. It was established that, in general, TBT concentrations in sediments are higher than in the water column. Under simulated estuarine conditions, it was determined that from 57 to 95% of the TBT present in the water column is absorbed onto the sediment surface (Randall and Weber 1986). A significant factor in this process may be the grain-size of the sediments. It was determined that sediment fractions of smaller grain-size have high sorptive properties. The explanation of this phenomenon is simple; as grain-size decreases the surface proper of the sediment layer, defined as the ratio of the total surface area of particles to their mass, increases (Pempkowiak 1997). Therefore, it can be expected that higher organometallic molecule accumulation will occur in the sediments in those water bodies where mud, silt and silty minerals (*i.e.* illite, kaolinite and montmorillonite) are present.

Increased concentrations of organometallic compounds can be caused by the transport and sedimentation of terrestrial material. Both factors depend on the bottom type and the dynamic conditions in a given body of water (Håkanson 1988).

On the other hand, feeding phytoplankton organisms may eliminate organometallic compounds naturally by incorporating them into the food chain, therefore decreasing their concentrations in the marine environment. A similar role is played by the benthos and organisms which feed on mud. The accumulation level of organometallic compounds in living organisms depends on metabolism, age and the particular characteristics of a given species (*e.g.* breathing or feeding mode).

The bioaccumulation level can vary among different species of zooplankton. For example, the TBT content in zooplankton (*Daphnia*), measured for the same pH and similar TBT concentrations, can reach values ten-fold lower in comparison with those in juvenile fish (Fent and Looser 1995). On the other hand, the accumulation level of butyl tin derivatives in bivalves can depend on the growth rate and developmental stage of a given individual. It has been determined that higher concentrations of these compounds were present in individuals of average and low growth rates, while lower concentrations were found in larger bivalves. Smaller individuals are capable of assimilating larger amounts of pollutants thanks to higher filtering rates (Hong *et al.* 2002).

Another factor which controls bioaccumulation in the marine compartment is pH. The accumulation rate increased significantly at pH 8, and this, in turn, caused an increase in the mortality rate of *Daphnia*. This phenomenon was not detected at pH 6. Higher pH values stimulate bioaccumulation in fishes (Tsuda *et al.* 1990), because at different pH ranges various butyl tin compounds occur in different chemical forms which, depending on pH, can be more or less bioavailable. For example, hydrophobic TBTOH penetrates the plasmalemma more easily than the hydrophilic TBT<sup>+</sup> cation (TBT<sup>+</sup> dominates in seawater at pH 6, while TBTOH does so at pH 8 (Fent and Looser 1995).

Another issue also worth mentioning is that DBT and MBT accumulate in bivalves (*Ruditapes decussata*) in smaller amounts than TBT (Morcillo and Porte, 2000) and that both compounds are less toxic to microorganisms in comparison with tributyl tin derivatives (Gadd 2000, Tsang *et al.* 1999).

The bioaccumulation coefficient for butyl tin derivatives is defined as follows (Fent and Looser 1995, Gomez - Ariza *et al.* 1999, Hong *et al.* 2002):

$$\text{BCF} = \frac{\text{TBT concentration in live tissues}}{\text{TBT concentration in sea water}}$$

The bioavailability of TBT reflects the length of time in which fluctuations in concentrations of the compounds mentioned above occurred as well as the constant rate of absorption and excretion by a given organism and the organism-specific TBT half-life.

### **Speciation analysis of organometallic compounds**

Biochemical and toxicological research have shown that the toxicity of elements depends not only on their content but also on the number of physico-chemical forms in which they were introduced into the natural environment and in which they presently occur. Determining the total amounts of a given element in investigated samples seems insufficient to assess the hazard it poses to living organisms. In order to obtain additional information on the toxicity of an element, it is necessary to identify and evaluate the share of particular chemical and physical forms of a given element in the environment. According to IUPC guidelines, the term "speciation" can be defined as the identification and determination of different chemical and physical forms of a given element in an

Table 3

## The application of chosen coupled techniques used in speciation analysis

Technique	Comments on the coupling device	Possible problems	Examples of application
GC – MS	Usually heating of analyte transfer line is required.	Analytes should be volatile derivatisation before final determination.	Determination of lead organic compounds in benzene, metalloporphyrins in petroleum, organotin compounds in environmental matrices.
LC – MS	Considerable numbers of interfaces are built up; the most common are particle beam, thermospray, atmospheric pressure chemical ionization, electrospray.	Restriction concerning flow rates and the composition (e.g., additives – buffers) of the mobile phase.	Speciation of organotin compounds.
GC – AAS (quartz and graphite furnace)	GC – FAAS: usually narrow – bore tube between the two instruments, tube should be as short as possible to avoid peak broadening, transfer tube has to be heated to the highest temperature of the oven temperature program.	Due to insufficient sensitivity for environmental analysis, a silica tube furnace electrically heated to the atomizing temperature is needed – a feature that commercial furnaces do not have.	Suitable for organometals (those of Pb, Sn, As, Se). Determination of tributyltin in sea water. Determination of trimethyllead.
HPLC - FAAS	Simple coupling. The end of capillary is connected with a nebulizer. In the case of ICP, it is necessary to use device for the separation of analytes and mobile phase solvent.	The outlet of the column can contain molecules which block the nebulizer. Organic compounds in the outlet can undergo incomplete combustion in the burner and then block it. Solvents from the mobile phase can cause plasma extinguishing in ICP.	Determination of organotin compounds in water, sediments and shellfish tissue. Speciation analysis of As, Sb, Se, Hg, Pb, Cr.
HPLC - EFAAS	The HPLC effluent is volatilized to an aerosol in a heated silica capillary and enters the furnace through a vitreous graphite tube.	Limitation in the use of solvents and buffer solution in the eluent (buffer are highly volatile). Only well – separation can be distinguished.	Determination of tin species. Analysis of organolead compounds.
FIA - FAAS	Very simple coupling.	The outlet of the column can contain molecules which block the nebulizer. Organic compounds in the outlet can undergo incomplete combustion in the burner and the block. Solvents from the mobile phase can cause plasma extinguishing in ICP.	Determination of inorganic Se and Cr compounds in water.
Capillary electrophoresis - FAAS	Technically, the coupling is quite difficult. The flow rate of the mobile phase in CZE is usually not compatible with the flow rate of the sample in AAS spectrometry.		Only several applications of these techniques are reported in the literature due to the connection problems.

1	2	3	4
Hydride generation - AAS	Coupling relatively simple.	This technique does not identify species that do not give rise to volatile derivatives during reduction.	Highly sensitive determination of As, Se, Sn, Bi, Te, Sb, Pb and Hg species.
GC - AES (with plasmas ICP or MIP excitation source)	The effluent from GC can be introduced directly into the flame without extinguishing it.		Speciation analysis of organolead in rainwater, snow, harbour, river, lake and trap water.
GC - ICP - MS	As for MIP, GC effluent is usually transported to the plasma via a heated line which is either glass-lined or has an analytical column which passes through the center.		Speciation of Hg, Se, Sn. Speciation of Sn, Pb, Hg. Determination of Sb, Se, Sn, Te, Hg, Pb, Bi in natural waters, landfill and fermentation gas after purge and trap pre-concentration.
GC - ICP - AES	Large plasma gas flow rate causes dilution of the analytes.	Low detection limits because the sensitivity of an atomic emission spectrometer does not overcome dilution with plasma gas; large dead volume; poor excitation/ionization of non-metals.	Speciation of Hg.
LC - ICP - MS	The necessity of using a nebulizer spray chamber (the plasma technique is sensitive to organic solvents present in LC effluents). Different sample introduction techniques are investigated (glass frit nebulizers, thermospray vaporizers, ultrasonic nebulizer).	Low efficiency of sample nebulization, which has a major impact on sensitivity. Instability of the plasma to organic vapors. Deposition of the carbon on the sampling cone and torch.	Speciation of organoarsenic and organochromium. Determination of tributyltin in sea water. Determination of organolead.
LC - ICP - AES	As above.	As above.	Speciation of organoarsenic compounds. Determination of Cr(III) and Cr(VI). Determination of metalloprotein. Analysis of organotin compounds in marine mussel samples.

where: AAS – atomic absorption spectrometry, EFAAS – electrothermal atomic absorption spectrometry (graphite furnace), FAAS – flame atomic absorption spectrometry, MIP- microwave – induced plasma, ICP – AAS – inductively coupled plasma atomic emission spectrometry, ICP – MS – inductively coupled plasma mass spectrometry.

Source: (Kot and Namiestnik, 2000)

investigated sample, while the term “speciation analytics” refers to the source of information regarding the form which occurs. A few types of speciation analysis can be found in the literature, *e.g. physical speciation* deals with determining different forms of the same element in the same sample (*i.e.* dissolved, suspended, adsorbed, complex and others), while *chemical speciation* addresses the division of the total element content into various chemical forms (Namieśnik 2001). Chemical speciation can be further divided into *screening speciation* (the identification and determination of one specific analyte), *group speciation* (determining the concentration level of a given group of compounds or of an investigated element at a specific oxidation state), *individual speciation* (identifying and determining all the individual molecules of a given element by fractionation and isolation; this is the most difficult technique), *distribution speciation* (determining the same chemical components within various ranges of the investigated subject) and *chiral speciation* (determining the enantiomers of a given chemical compound).

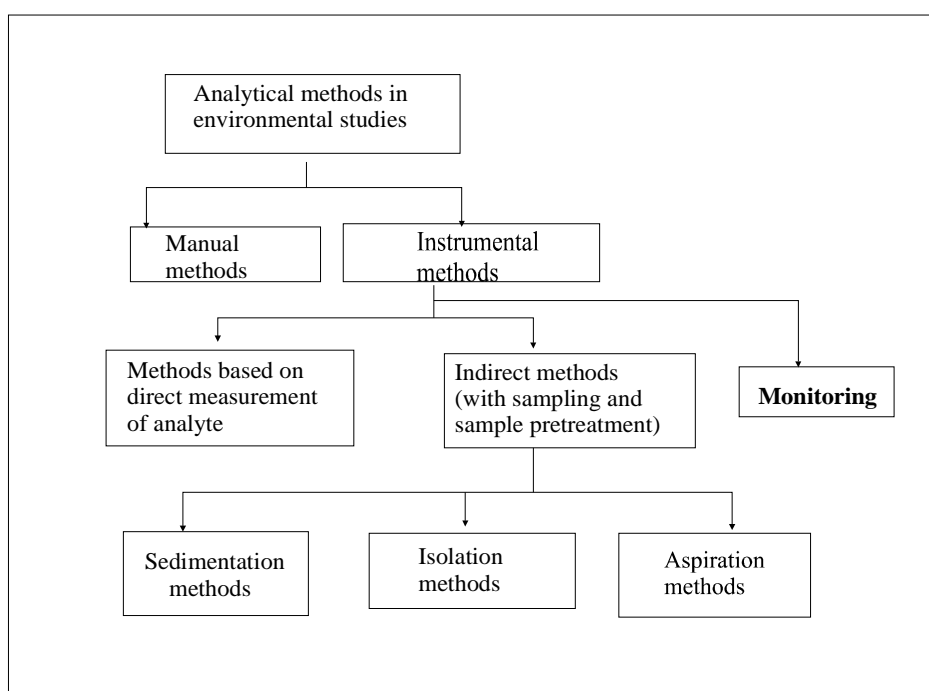
In consideration of the biochemical aspects, speciation analysis is a very significant stage of research. Knowledge regarding specific physico-chemical forms of elements allows researchers to assess the risk of poisoning with a given element (*e.g.* with the example of tin, mono-tin and dialkyl tin are less hazardous than trialkyl tin). The importance of the role of speciation analysis in research is supported by the following examples (Kot and Namieśnik 2000):

- studies on the biogeochemical cycles of particular elements;
- assessment of toxicity and ecotoxicity of particular elements;
- quality control of food products;
- control of pharmacological products, including medicines;
- control of technological processes;
- environmental impact assessment of technological installations;
- research on occupational exposure;
- clinical analysis.

Speciation methods are significantly different from the methods applied to analyze total metal content. Various separation techniques are employed in speciation analytics, including gas and liquid chromatography, electrophoresis, extraction, flotation and supercritical fluid chromatography. Gas chromatography is usually used to determine the organometallic compounds Sn, As, Pb and Hg (Wasik and Namieśnik 2000). Coupled techniques are also employed to analyze environmental samples; these include **GF – AAS** for the speciation analysis of Cr in seawater (Obiols *et al.* 1987), **HPLC-ICP-MS** in organolead speciation (Pereiro and Diaz 2002), **GC – ETAAS** and **GC - QFAAS** in the

speciation analysis of trace metals (Smedes *et al.* 2000) and **HPLC-HG-AAS** for determining Sb (III) and Sb(V) (Krachler and Emons 2000). Organotin is determined with the use of, among other methods, **GC – AAS** (Ritsema *et al.* 1998, Forsyth *et al.* 1992), **GC-FPD** (Lespes *et al.* 1996; Harino *et al.* 1992, 1997), and **HG-GC-QFAAS** (Pannier *et al.* 1996). The application of chosen coupled techniques in speciation analysis is presented in Table 3.

The first step in speciation analysis is particularly important. Sample collection and storage must ensure the integrity of the chemical composition of the investigated material. A change in the ratio of the physico-chemical forms of the elements present in a sample can produce results which paint an inaccurate picture of environmental conditions. Therefore, modern instrumental methods have an ever increasing importance in the development of analytical chemistry. The development of the branch of science dedicated to designing and implementing measuring instruments is linked to the increasing use of artificial intelligence and neural networks.



**Fig.3.** Classification scheme of the basic analytical methods used in environmental research (Namieśnik *et al.* 2000).

At the same time, the development of automation in methodology and research procedures, including laboratory automation through the installation of modern technical equipment, allows analytical measurements to be made with a high degree of precision and accuracy. An additional advantage of implementing technical advances in chemical analysis is that the number of analytical steps is reduced, which, in turn, shortens the total time of analysis and lends a certain measure of protection from analyte loss and sample contamination.

Analytical methods can be divided generally into manual and automated procedures; the classification scheme of analytical methods used in environmental studies is presented in Figure 3.

The term “manual analytical methods” encompasses procedures in which most of the operations are performed either directly by a technician or with the use of a simple instrument handled by the technician.

The results of manual analyses are usually obtained with a significant time lag. The automation of analytical instruments allows data to be obtained immediately following environmental sample collection since the analytical procedure, from sample collection to the identification and determination of sample components, is fully automated.

Comparative studies of the methods noted above indicate that these methods are of equal value and allow data to be obtained with a similar level of accuracy and precision (Namieśnik and Górecki 2001).

The analytical methods discussed above can also be divided into direct (analyte determination directly in the sample) and indirect methods (analyte determination in a secondary matrix prepared from the original sample used when *e.g.* analytes are present in trace amounts).

Preparing environmental samples for analysis is a complex process, and the operations performed at this stage may significantly influence the final results. The diversity of collected environmental samples results from their chemical and physical properties and depends on the physical state of the sample, its chemical composition and the concentration of the collected analyte. The number of operations performed during *in situ* sample collection and sub-sample preparation in the laboratory following transport also have a significant influence on the amount and quality of the material collected for analysis.

Before scientific investigations begin, it is necessary to design a detailed plan in order to avoid making errors during the various analytical steps. The plan should include the choice of an appropriate, representative site for environmental sample collection, a description of the geological, geographical and other characteristics of the site (*e.g.* longitude and latitude, depth, type of

benthic sediment, presence of organic matter in the water column, etc.) and the identification of chemical pollution sources in the environment.

There are several operational modes with regard to the locations of measuring equipment and the sampling site, as follows: *on-line* - a measuring device, attached to the shunt of technological line or equipment, is used to distribute the analyzed medium, the sample inside the analyzer can be brought back to the transfer line or treated as waste; *in-line* - a measuring instrument is attached directly to the transfer line or technological equipment; *off-line* - the collected sample is transported to the laboratory where it undergoes analysis (Namieśnik *et al.* 2000).

Knowledge of the physico-chemical properties of a given material, the choice of a proper sampling technique and appropriate sample storage should ensure that the natural composition of the sample remains intact until analysis begins. With some products, it is required that the sample preservation procedure safeguard against dust, smoke, air, humidity, light or temperature changes.

In some cases, it is necessary to freeze samples prior to analysis (*e.g.* solid state samples). This is done to prevent changes in the chemical and biological properties of the investigated material caused by environmental factors or contamination. It should be remembered that each analytical step in sample preparation carries with it the risk of error resulting from analyte contamination or its partial loss. Therefore, precautionary measures should be taken to avoid such events. Firstly, emphasis should be placed on the cleanliness and deactivation of surfaces of vessels and equipment that come into contact with samples since these factors are the most frequent sources of error in chemical analysis. Other sources of bias stem from differences in the volatility of particular sample components, the adsorption and desorption of sample components on the walls of containers and measuring instruments, sample contamination from contact with the environment, change in the chemical composition of samples caused by reagents and other factors (Namieśnik and Górecki 2001).

In order to avoid other errors, attention should also be paid to the condition of the measuring instruments (*e.g.* automatic zeroing and calibration, protection against sudden damage, emergency power supply, calibration of modules, protection against flame extinguishing, *etc.*). The instruments should have the required characteristics, *i.e.* high resolution and sensitivity, long autonomic cycles and, if needed, the capability of delivering sample data in real time (Namieśnik 2001).

In general, the following analytical steps are necessary in the preparation of solid samples of marine origin: preservation; transport to the laboratory; proper storage in the laboratory; physical handling

(drying, fragmentation and sieving); chemical treatment (freeing of analytes, derivatisation and purification); separation, detection (derivatisation and calibration) and identification of the elements and their specific forms, *i.e.* speciation analysis (Namieśnik *et al.* 2000). The basic operations performed during the pre-treatment of environmental samples are presented in Table 4.

**Table 4**

Basic operations and processes in sample preparation prior to analysis proper.

Operations	Type of sample		
	Gaseous	Liquid	Solid
<b>1. Performed <i>in situ</i></b>			
Removal of dust	+		
Drying	+		
Removal of interfering substances	+		
Removal of suspended matter		+	
Chemical preservation		+	
Derivation		+	
Isolation and/or enrichment	+	+	+
Transport	+	+	+
<b>2. Performed in laboratory</b>			
Drying		+	
Fragmentation			
Homogenization	+	+	+
Chemical preservation		+	+
Sieving			+
Mineralization		+	+
Isolation and/or enrichment	+	+	+
Derivatisation	+	+	+
Purification and removal of interfering substances	+	+	+
Fractionation and sample splitting	+	+	+
Calibration and verification of instruments	+	+	+
Detection	+	+	+

Source: (Namieśnik and Górecki 2001)

Another important aspect of chemical analysis is the quality control and quality assurance (QC/QA) of the data obtained based on Good Laboratory Practice (GLP). The following goals are incorporated into the *Quality Assurance/Quality Control* (QA/QC) system: monitoring and assessment of the precision of data obtained through the periodic analysis of control samples; accuracy assessment based on certified reference materials; comparison of data obtained with results from a reference

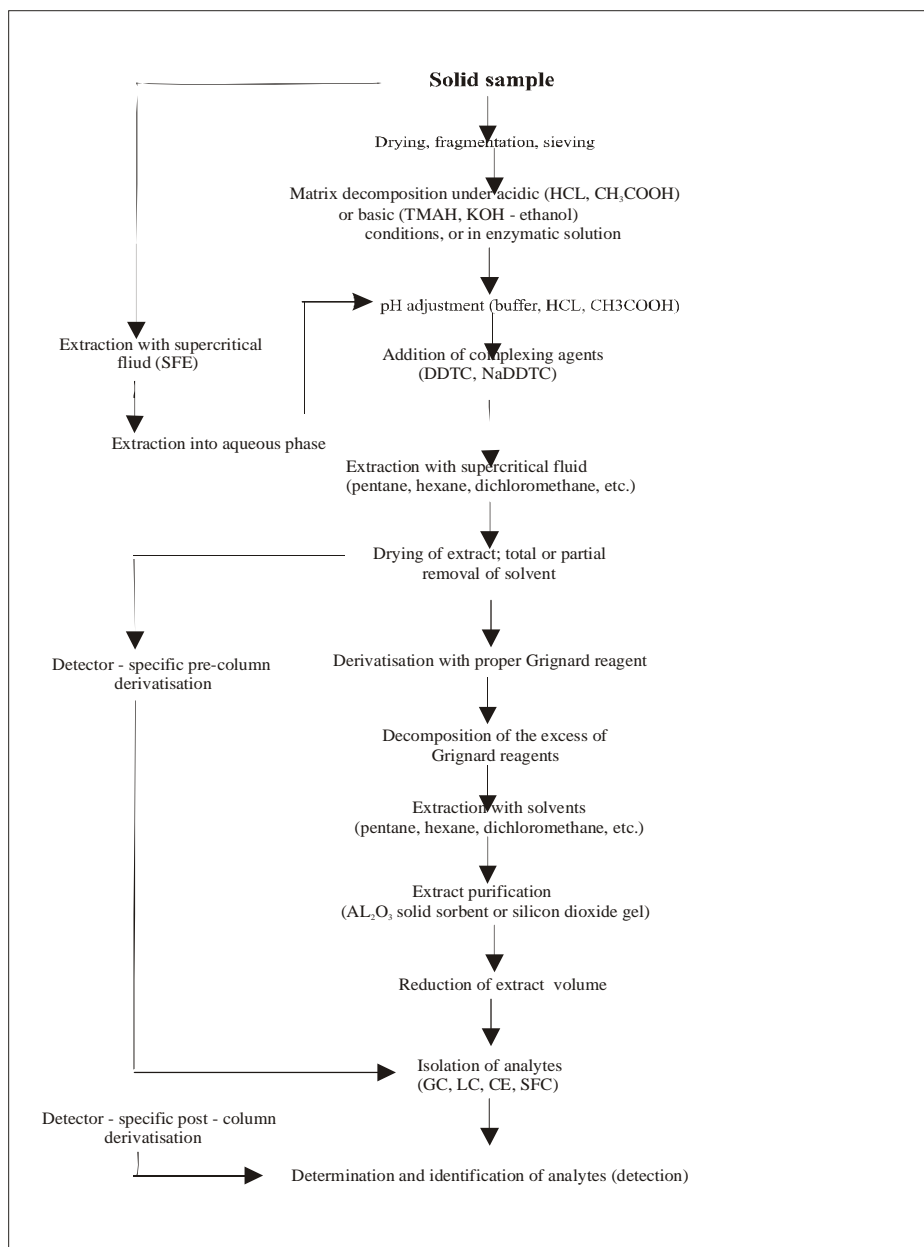
method for the same sample; analysis of samples spiked with a standard; the use of control charts; auditing (Namieśnik, Górecki 2001a).

At present, the quality of laboratory procedures is safeguarded by Good Laboratory Practice (GLP), laboratory accreditation according to EN 45001 or ISO Guide 25 and ISO 9000 certification. Analytical procedures contain the following calibration-related operations: periodic checks of the reliability of analytical equipment using standard mixes (*e.g.* a mix used for zeroing instruments and dilution series of standard mixes containing specified concentrations of analytes); validity check of the entire analytical procedure by spiking the analyzed samples with a standard; using reference materials (Namieśnik, Górecki 2001). In both cases the analytical procedure must be exactly the same as that used for the real samples. There are three types of standards – chemical and physical standards and those used for special purposes (Namieśnik and Górecki 2001a). Moreover, standard materials used in analyses should fulfill the following criteria: homogeneity; the long-term stability of chemical compounds; certified content of the highest possible number of analytes.

It is recommended that solid samples collected for the determination of organic derivative content be stored in Teflon or polyethylene containers at lowered temperatures and away from light. Humid samples can be air-dried, sublimated, dried in ovens at 50<sup>0</sup> C or stored frozen at -20<sup>0</sup> C without previous water removal (Wasik and Namieśnik 2001). In addition, samples containing volatile components should be extracted with an appropriate solvent immediately after collection.

Following drying, the next step in solid sample preparation is homogenization; grinders or mortars are employed to obtain fully homogenous material. Some procedures require the dissolution of the sample matrix; this is done to free analytes that are bonded to proteins (biological samples), humic substances (soil samples enriched with humic acids) or adsorbed, complexed or otherwise bound by other sample components. Acids such as HCl and CH<sub>3</sub>COOH, or mixes of acids with alcohols, solutions of NaOH, KOH, TMAH (tetramethyl ammonium hydroxide) in water or alcohol or enzymatic solutions are used for this purpose. The resulting pH in the treated sample is adjusted to the desired level with acid or buffer. The analytical procedures proposed for organic compounds of lead, tin and mercury are presented as a flow chart in Fig. 4.

In the professional literature particular attention is paid to describing various isolation and sample enrichment techniques. This interest is due,



**Fig. 4.** Simplified flow chart with preparation procedures that are applied to solid samples containing organometallic compounds of Pb, Sn and Hg before speciation analysis (Wasik and Namieśnik 2001).

in part, to the low concentrations of analytes in the samples, the complexity of the sample matrix and the incompatibility of the physical state of sample matrix with regard to chosen analytical techniques.

According to the IUPAC definition, enrichment is the term applied to each operation or process that results in an increase in the ratio of the concentration or analyte content to the concentrations of other matrix components in the analyzed sample (Namieśnik *et al.* 2000).

Analyte enrichment is included in all the analytical procedures as it is significant in detecting organometallic compounds which are present in very low concentrations. Enrichment is performed by employing extraction with liquid solvents and gas (stripping) and solid phase or supercritical fluid extraction (SFE). Applying extraction in analytical procedures later allows analyte levels to be determined with chromatographic techniques.

The application of extraction in analysis is advantageous because of the following reasons (Namieśnik *et al.* 2000):

- a. enrichment of the analyzed components due to the increase of analyte in the secondary matrix to a level higher than the detection limit; used for final determinations;
- b. transfer of analytes onto a matrix that has a much simpler composition than the original sample matrix; this simplifies final determinations (in chromatographic techniques, organic solvents or the appropriate gas are “friendly” to columns and detectors),
- c. removal of interfering substances which leads to a decrease of interference at further analytical steps thanks to the selective transfer of some sample analytes onto the secondary matrix.

The term “**derivatisation**” describes chemical operations through which analytes with particular properties that are suitable for a given analytical method are obtained (Namieśnik *et al.* 2000). Derivatisation is recommended in most of the procedures for organometallic compound determinations (*e.g.* Hg, As, Sn, Sb, Pb, Ge, Se and Cd). Its purpose is to change the chemical form of the analyte into one that enables and simplifies further analysis. In addition, derivatisation simplifies the extraction of analytes through the use of stripping, extraction with solvents, solid phase extraction or stationary phase microextraction (Wasik and Namieśnik 2001). Derivatisation can be performed before, during or after extraction with solvents.

The last analytical step is the **identification** of the final analytes. There are several methods of metal identification, and the choice of the proper technique depends on separating the analytes. Electron Capture Detectors (ECD) and Flame-Photometric Detectors (FPD) are most widely applied in metal analysis. Spectroscopic methods such as mass spectroscopy, ICP-MS and ICP-AES are the most universal, sensitive and selective detection methods used in speciation analytics as well as in total content determinations of metals in organometallic compounds (Wasik and Namieśnik 2001).

## CONCLUSIONS

Organometallic compounds are widely used in almost all branches of industry, and some are very potent toxins. Investigations of the toxicity of organometallic compounds indicate that tributyl tin derivatives and their metabolites are highly toxic to living organisms. Moreover, tributyl tin (TBT) is the dominant form among the butyl tin derivatives in the marine environment. TBT derivatives are the active ingredients of many bactericides, fungicides, insecticides, acaricides and wood preservatives. However, the main source of TBT derivatives in the marine compartment are anti-fouling paints used to protect ship hulls.

Great financial losses are incurred in the shipping industry due to fouling. From an environmental point of view, the increased fuel usage necessary to counteract the additional resistance of the fouled hull produces higher pollution emissions into the atmosphere. The application of anti-fouling covers seems to be the best solution; however, paints containing organotin that have been used to date cause increased pollution in the marine environment. Since these paints are highly toxic, regulations have been introduced which limit the use of tributyl tin derivatives. Nevertheless, in Korea, Vietnam, India and China scientific studies have confirmed that the concentrations of TBT derivatives in the marine compartment are still very high due to the strong development of the shipyard industry. There is an urgent need to substitute toxic organotin compounds with alternative substances as the demand for anti-fouling agents, particularly in the shipyard industry, is on the rise. The present study on the toxicity of elements indicates that the determination of the total content of a given element in the collected material is insufficient in assessing the risk to living organisms.

For this purpose, speciation methods are employed in combination with different separation techniques (*e.g.* gas chromatography, supercritical

fluid chromatography, liquid chromatography, electrophoresis, extraction and flotation). Gas chromatography is used in determinations of organometallic compounds of Sn, As, Pb and Hg. Spectroscopic methods of detection such as mass spectroscopy, ICP-MS and ICP-AES are the most versatile, sensitive and selective ones; they are used in speciation analyses of organometallic compounds as well as for total metal content determinations. The preparation of environmental samples for analysis is a complex process, and the operations performed in this analytical step may significantly influence the final results. A set of regulations describing the steps in sample preparation is available.

## REFERENCES

- Albanis T.A., Lambropoulou D.A., Sakkas V.A., Konstantinou I.K., 2002. *Antifouling paint booster biocide contamination in Greek marine sediments*. Chemosphere, **48**, 475-485.
- Alzieu C., 2000. *Environmental impact of TBT: the French experience*. Sci. Total. Environ., **258**, 99-102.
- Biselli S., Bester K., Hühnerfuss H., Fent K., 2000. *Concentrations of the antifouling compound Irgarol 1051 and of organotins in water and sediments of German North and Baltic Sea marinas*. Mar. Pollut. Bull., **3**, 233-243.
- Champ M., A., 2000. *A review of organotin regulatory strategies pending actions, related costs and benefits*. Sci. Total. Environ., **258**, 21-71.
- Diez S., Abalos M., Bayona J.M., 2002. *Organotin contamination in sediments from the Western Mediterranean enclosures following 10 years of TBT regulation*. Water Res., **36**, 905-918.
- Dojlido J., 1987. *Chemia wody*. Arkady, Warszawa, 107-112, 119-124.
- Dutkiewicz T., 1968. *Chemia toksykologiczna*. Zakład Wydawnictw Lekarskich, Warszawa, 282-292.
- Fent K., Looser P.W. 1995. *Bioaccumulation and bioavailability of tributyltin chloride: influence of pH and humic acids*. Environ. Sci. Technol., **29**, 1631-1637.
- Forsyth D.S., Weber D., Barlow L., 1999. *The determination of organotin compounds in fruit juices using gas chromatography – atomic absorption spectrometry*. Appl. Organometal. Chem., **6**, 579-585.
- Gomez-Ariza J.L., E. Morale E., Giraldez L., 1999. *Uptake and elimination of tributyltin in clams, Venerupis decussata*. Mar. Environ. Res., **47**, 399-413.

- Gadd G.M., 2000. *Microbial interactions with tributyltin compounds: detoxification, accumulation, and environmental fate*. Sci. Total. Environ., **258**, 119-127.
- Håkanson L., 1988. *Metal monitoring in coastal environments* [in:] Seelinger U., de Lacerda L.D., Patchineelam S.R., 1988. *Metals in Coastal Environments of Latin America*, Springer - Verlag, Berlin 297, 239-137.
- Harino H., Fukushima M., Kurokawa Y., Kawai S., 1997. *Susceptibility of bacterial populations to organotin compounds and microbial degradation of organotin compounds in environmental water*. Environ. Pollut., **98**, 157-162.
- Harino H., Fukushima M., Tanaka M., 1992. *Simultaneous determination of butyltin and phenyltin compounds in the aquatic environment by gas chromatography*. Anal. Chim. Acta., **264**, 91-96.
- Hoch M., Alonso – Zacarate J., Lischick M., 2002. *Adsorption behavior of toxic tributyltin to clay rich - sediments under various environmental conditions*. Environ. Toxicol. Chem., **21**, 1390-1397.
- Hoch M., 2001. *Organotin compounds in the environment – an overview*. Appl. Geochem. **16**, 719-743.
- Hong H.K, Takahashia S., Min B.Y., Tanabe S., 2002. *Butyltin residues in blue mussels (Mytilus edulis) and arkshells (Scapharca broughtonii) collected from Korean coastal waters*. Environ. Pollut., **117**, 475-486.
- Kabata-Pendias A., Pendias H., 1993. *Biochemia pierwiastków śladowych*. Wyd. PWN, 159.
- Kania C., 1999. *Szkodliwe skutki stosowania farb przeciwporostowych w budownictwie okrętowym*. Ochrona przed korozją, **42**, 588-592.
- Kawai S., Kurokawa Y., Harino H., Fukushima M., 1998. *Degradation of tributyltin by a bacterial strain isolated from polluted river water*. Environ. Pollut. **102**, 259-263.
- Korzeniewski K., 1998. *Ochrona środowiska morskiego*. Wyd. UG, 78-85.
- Kot A., Namieśnik J, 2000. *The role of speciation in analytical chemistry*. Trends Anal. Chem. **19**, 69-79.
- Krachler M., Emons H., 2000. *Potential of high performance liquid chromatography coupled to flow injection hydride generation atomic absorption spectrometry for the speciation of inorganic or organic antimony compounds*. J. Anal. At. Spectrom., **15**, 281-285.
- Laughlin Jr. R.B., Guard H.E., Coleman III W.M, 1986. *Tributyltin in seawater: speciation and octanol-water partition coefficient*. Environ. Sci. Technol., **20**, 201-204 [in:] Fent K., Looser P.W.1995. *Bioaccumulation and bioavailability of tributyltin chloride: influence of pH and humic acids*. Environ. Sci. Technol., **29**, 1631-1637.

- Lespes G., Carlier – Pinasseau C., Potin – Gautier M., Astruc M., 1996. *Direct determination of butyl – and phenyltin compounds as chlorides using gas Chromatography and flame photometric detection*. *Analyst.*, **121**, 1969-1973.
- Mastalerz P., 1996. *Podręcznik chemii organicznej*. Wyd. Chemiczne, Wrocław, 209-216.
- Michel P., Averty B., Andral B., Chiffolleau J., Galgani F., 2001. *Tributyltin along the Coasts of Corsica (Western Mediterranean): A persistent problem*. *Mar. Pollut. Bull.*, **42**, 1128-1132.
- Morcillo Y., Porte C., 2000. *Evidence of endocrine disruption in calms – Ruditapes decussata- transplanted to a tributyltin – polluted environmental*. *Environ. Pollut.* **107**, 47-52.
- Namieśnik J., 2001. *Modern trends in monitoring and analysis of environmental pollutants*. *Pol. J. Environ. Stud.*, **10**, 6405-413.
- Namieśnik J., Górecki T., 2001. *Preparation of environmental samples for the determination of trace constituents*. *Pol. J. Environ. Stud.*, **10**, 77-84.
- Namieśnik J., Górecki T., 2001a. *Quality of analytical results*. *Rev. Roum. Chem.* **46**, 953-962.
- Namieśnik J., Jamrógiewicz Z., Pilarczyk M., Torres L., 2000. *Przygotowanie próbek środowiskowych do analizy*. WNT, Warszawa, 15 -91.
- Obiols J., Devesa R., Garcia – Berro J., Serra J., 1987. *Specjacja of chromium in waters by coprecipitation – AAS*. *Inter. J. Environ. Anal. Chem.*, **30**, 197–207.
- Pannier F., Astruc A., Astruc M., 1996. *Determination of butyltin compounds in marine biological samples by enzymatic hydrolysis and HG-GC-QFAAS detection.*, *Anal. Chim. Acta.*, **327**, 287-293.
- Pempkowiak J., 1997. *Zarys geochemii morskiej*. Wyd. UG, 155-158.
- Pereiro I.R., Diaz A. C., 2002. *Speciation of mercury, tin, and lead compounds by gas chromatography with microwave – induced plasma and atomic – emission detection (GC-MIP-AED)*. *Anal. Bioanal. Chem.*, **372**, 74 - 90.
- Pruchnik F., 1993. *Preparatyka związków metaloorganicznych: metale przejściowe*. PWN, Warszawa, 15-47.
- Pruchnik F., 1991. *Chemia metaloorganiczna*. PWN, Warszawa, 17 – 39.
- Rajendran R.B, Tao H., Miyazaki A., Ramesh R., Ramachandran S., 2001. *Determination of butyl-, phenyl-, octyl- and tributylmonomethyltin compounds in marine environment (Bay of Bengal, India) using gas chromatography – inductively coupled plasma mass spectrometry*. *J. Environ. Monit.*, **3**, 627-634.
- Randall L., Weber J.H, 1986. *Adsorptive behavior of butyltin compounds under simulated estuarine conditions*. *Sci. Total. Environ.*, **57**, 191-203.

- Ritsema R., de Smaele T., Moens L., de Jong A.S., Donard O.F.X., 1998. *Determination of butyltins in harbour sediment and water by aqueous phase ethylation GC-ICP-MS and hydride generation GC-AAS.* Environ. Pollut., **99**, 271-277.
- Smedes F., de Jong A. S., Davies I. M., 2000. *Determination of (mono-, di- and) tributyltin in sediments. Analytical methods.* J. Environ. Monit., **2**, 541-549.
- Shim W.J, Hong S.H, Yim U.H., Yim N.S., Kim N.S., Oh J.R., 2002. *Horizontal and vertical distribution of butyltin compounds in sediments from Shipyards in Korea.* Environ. Contam. Toxicol., **43**, 277-283.
- Shim W., Oh J.R., Kahng S.H., Shim J.H, Lee S.H, 1999. *Horizontal distribution of butyltins in surface from an enclosed bay system, Korea.* Environ. Pollut., **106**, 351-357.
- Shoukry M.M, 1993. *Equilibrium study of tributyltin (IV) complexes with amino acids and related compounds.* Bull. Soc. Chim. Fr. 1230, 117-120 [in:] Fent K., Looser P.W.1995. *Bioaccumulation and bioavailability of tributyltin chloride: influence of pH and humic acids.* Environ. Sci. Technol., **29**, 1631-1637.
- Steward C., De Mora S.J., 1990. *A review of the degradation of tri(n-butyl)tin in marine environmental.* Environ. Technol., **11**, 565-570.
- Tobias R.S., 1978. *The chemistry of organometallic cations in aqueous media.* [in:] *Organometals and organometalloids. Occurrence and fate in the environment.* (Edited by Brinckman P.E and Bellama J.M)., ACS Symposium Series, pp. 130-148 [in:] Fent K., Looser P.W.1995. *Bioaccumulation and bioavailability of tributyltin chloride: influence of pH and humic acids.* Environ. Sci. Technol., **29**, 1631-1637.
- Tsang C.k., Lau P.S., Tam N.F.Y., Wong Y.S., 1999. *Biodegradation capacity of tributyltin by two Chlorella species.* Environ. Pollut., **105**, 289-297.
- Tselentis B.S., Tzannatos E.S., 2000. *Butyltin compounds in coastal sediments of areas associated with maritime facilities in Greece.* Fresenius Envir. Bull., **9**, 499-507.
- Tsuda T., Aoki S., Kojima M., Harada H., 1990. *The influence of pH on the accumulation of tri-n-butyltin chloride and triphenyltin chloride in carp.* Comp. Biochem. Physiol. **95**, 151-153 [in:] Fent K., Looser P.W.1995. *Bioaccumulation and bioavailability of tributyltin chloride: influence of pH and humic acids.* Environ. Sci. Technol., **29**, 1631-1637.
- Wasik A., Namieśnik J., 2001. *Speciation of organometallic compounds of tin, lead and mercury.* Pol. J. Environ. Stud., **10**, 405-413.

- Vella A.J, Vassallo R., 2001. *Emission to air of volatile organotins from tributyltin contaminated harbour sediments*. Environ. Biol. Toxicol., **16**, 239-244.
- Vella A. J., Mintoff B., Axiak V., Agius D., Cassone R., 1998. *Organotin pollution in Malta Coastal zone*. Toxicol. Environ. Chem., **67**, 491-510.