Organometallic compounds in the environment: What have we learned ?

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The fate of trace metals in the environment and their impact have been a major concern for more than 20 years. Most previous studies have been limited to total metal determination. In general organometallic forms of metals are much more toxic than their inorganic counterparts. Today, the linking of gas or liquid separation techniques with atomic spectrometry detectors will help improve our understanding of the intricate and critical cycles of organometallic forms of metals and metalloids throughout the environment.

There is now a general consensus of concern among the public and the scientific community about the health of the environment. We can be directly aware of atmospheric pollution because we can sometimes smell it. Water contamination by direct input of organic or metallic anthropogenic compounds is somewhat more pernicious since we cannot necessarily see it. Contamination of water by metallic compounds has recently been quoted as one of the major environmental concerns for the near future. The threat arising from the direct release of metals into the environment will in the future be greater than that from organic or even radioactive wastes [1]. Considerable efforts have been made by the international community during the last 20 years to quantify the cycling and impact of trace metals in different parts of the ecosystem. These efforts have mostly used the ins- trumentation generally available, mainly atomic spectrometry, whether by absorption, emission or atomic fluorescence. These techniques determine the total metal content of the sample but provide no information on its chemical identity.

■ The need for speciation techniques

It is interesting to consider recent history and observe how progress of the knowledge of trace metal toxicity and cycling

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is directly dependant on the instrumentation available. Trace metals are generally considered to be toxic when present in excess in the biota. This general scheme can be accepted as a general rule, but now it is of fundamental importance to address some of the real problems associated with the occurrence of specific chemical forms of the metals rather than with the total metal burden present in the sample. This approach is now known as "speciation" which, according to the IUPAC definition, is the process yielding evidence of the atomic or molecular form of an analyte. Let us take for example three of the problems of trace metal contamination most notorious to the public and to the scientific community to outline the need for proper identification of the chemical species of the element in question, respectively mercury, tin and arsenic.

One of the events in the 1950s and 1960s that was most important in triggering international efforts, was the "Minamata" outbreak, in which a Japanese population of fishermen was severely affected by mercury contamination. In this case, the problem was found to be related to the presence of methylmercury $(CH_3)Hg^+$. This organic (organometallic) form of mercury is far more toxic than its inorganic counterpart. It is still generally difficult to determine methylmercury routinely and this is why most research programmes on environmental mercury, even today, employ total mercury determination.

Tin is usually considered to be harmless. Here also, this general statement is valid only for its inorganic forms. However, it became understood that some biocides synthesized based on organometallic tin and directly introduced into the environment were far more toxic than expected. A recent example in the 1980s first noted in the Bay of Arcachon (France) was the effect of TBT (tributyltin, an efficient anti-fouling agent) in seriously harming the development of oysters thereby creating a threat to the important local oyster farming industry. The decision to ban the use of this marine paint on boats < 25 m helped to solve the problem. However, questions are still open relating to the potential toxicity, stability and fate of this chemical accumulated in the sediment.

Finally, although arsenic is to the general public a very toxic element, inorganic forms of arsenic have been found to be far more toxic than organometallic forms. Organisms are able to biosynthesize large organoarsenic compounds such as arsenobetaine and arsenocholine which can be fed at the percent level in animal diets without any toxic effects. Shellfish and seafood such as crab and lobster can present high levels of arsenic but in forms that are chemically totally harmless for human consumption. These compounds, which are particularly resistant to metabolic conversion are rapidity excreted without chemical alteration. The large variety of organoarsenic compounds reported to occur in the environment is tabulated in figure 1.

Knowledge of the biological activity of organometallic compounds is not new. As early as 1310 Peter of Abano stated that "quicksilver made by alchemy is a more deadly poison than natural mercury" [3]. More recently, the pharmacological properties of some organoarsenic derivatives were observed in the early years of the century and were applied as

Table I. Organometallic compounds in industrial processes (4): metals used and their concentration in the compounds as applied

Applications	Level (%)	Metal
Polymer production	(≈ 1 µg/g)	Sn, Zn, Li, W, Mg, Al, Fe, Ru
Coupling agent	0.001 - 0.1	Si
Reaction catalysts	0.01 - 4.0	Sn, Mg, Pb
Cross linking agent	0.01 - 1.0	Si
Stabilizers	1.0 - 10	Sn
Antifouling paints	1.0 - 10	Sn, Cu
Biocides	1.0 - 10	Sn, Ti, Mn, As, Sb, U

Table II. Tin compounds detected to date, or currently suspected to occur in the environment

SnH_4
$Me_xSn^{(4-x)+}$ (x=0-3)
$n\text{-}Bu_xSn^{(4-x)+}$ (x=0-3)
$Ph_xSn^{(4-x)+}$ (x=0-3)

Me_2SnH_2, Me_3SnH ?
$n\text{-}BuSnH_3$?
$n\text{-}Bu_3MeSn$, $n\text{-}Bu_2Me_2Sn$?

a major remedy for syphilis and other infections and contagious diseases.

Arsenic being an exception, the toxicity of organometallic compounds is in general much higher than that of their inorganic counterparts and is due to their ability to penetrate cell membranes and modify intracellular chemistry. Further, their high liposolubility can lead to very marked accumulation in biological tissues. For example, most of the mercury present in fish tissues is methylated.

Organometallic compounds in the environment

Organometallic compounds occur in the environment either because they are naturally formed, or because of direct anthropogenic inputs. Some organometallic species of mercury, arsenic and now tin have been observed to have potent biocidal properties and this has been applied to the synthesis of a large variety of pesticides which has led to their direct introduction into the environment. Organometallic compounds are also used in many industrial processes (table I) sometimes at the per cent level. Yet, there is still little known about their later stability and fate when dispersed in the environment, though these concerns are not completely novel.

Natural methylation in the environment of a metal by a methylating agent is still quite a controversial process today. These processes can affect metals differently. Mercury was one of the first elements to be thoroughly studied for its methylation potential and the toxicity associated with the final product. Natural alkylation of arsenic and tin are now well established facts. Analytical or chemical evidence of environment methyl metal formation also exists for selenium, tellurium and germanium. The occurrence of antimony methylation is now questioned due to possible analytical errors. Lead methylation is still controversial, one of the main reasons being the widespread use of methyllead as an antiknock additive to gasoline and the resulting dispersion of this compound worldwide; indeed lead species (alkylleads) can be traced even in the snows of Greenland [5].

A wide diversity of methylating routes has been suggested. Microbiological methylation has often been mentioned and direct chemical methylation is also possible. It is difficult to distinguish between biological or chemical reaction pathways. However, in the final reactive stage, two reagents are always present and the methyl transfer takes place between a methyl-donor (carbanion or carbonium ion) and the available metal if present in the proper oxidation state. This underlines the importance of redox reactions in natural methylation processes. One of the first and best known reaction pathways attempting to explain the biomethylation of mercury was proposed by Woods *et al* [6], who reported that methyl cobalamin (a natural methylating agent and derivative of vitamin B_{12}) could rapidly transfer its methyl group to the mercuric ion Hg^{2+} yielding methylmercury. Other pathways have been described [7] but it is difficult to assess their real environmental impact.

It is beyond the scope of this paper to review the possible pathways but we must mention that it is very likely that many other routes are also possible. Me-
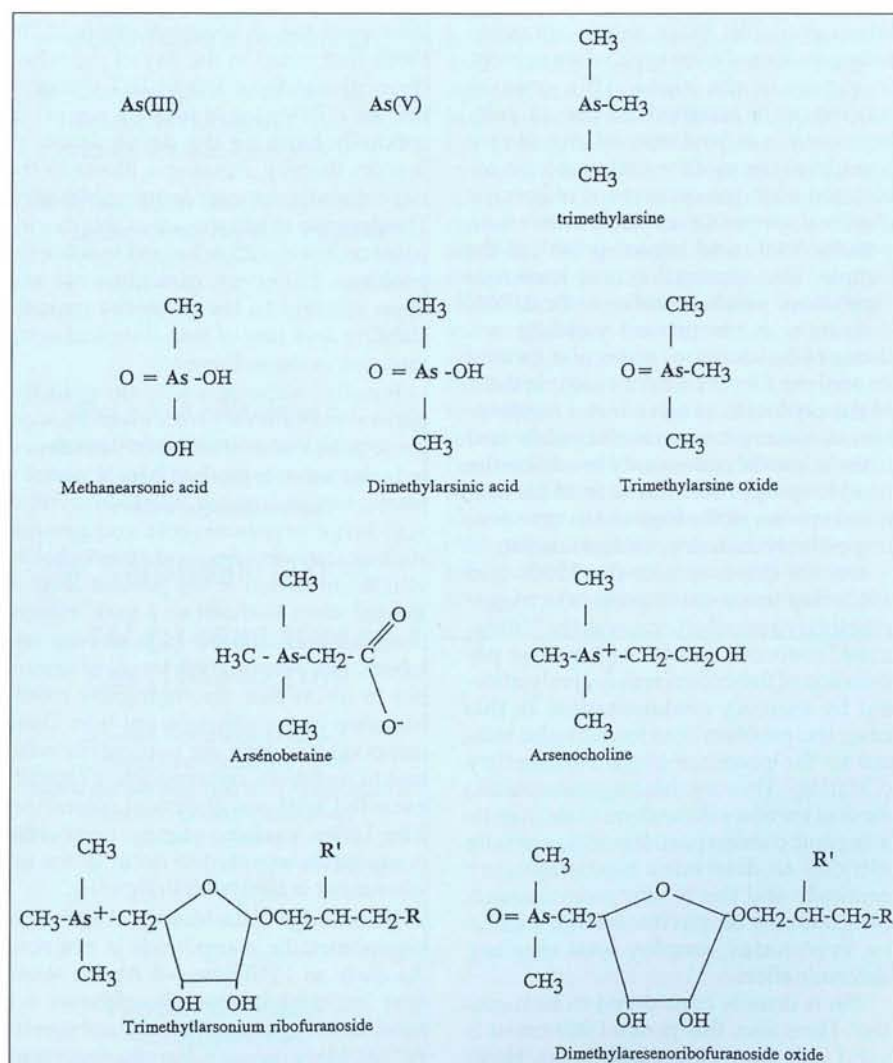

Fig 1. Summary of the diversity of inorganic and organometallic forms of arsenic in the environment (adapted from (2)).

thylation reactions can also possibly proceed "by accident", as first suggested by Lars Ladner in 1971 [3], who proposed that complexes of mercury and homocysteine can intercept CH_3^+ groups in a "mistaken" synthesis of methionine. Methylation reactions are also very common in the chemistry of life. This hypothesis can be further supported since several metals or metalloids that can be methylated may replace essential elements in the process of life, such as arsenic for phosphorus, germanium for silicon, selenium for sulphur and most recently, tin for carbon [8].

Solving the reaction pathway of the methylation of a metal is important but the results must always be considered with caution. The chemistry of life uses so many chemicals that a descriptive route using only one ligand, is an excessively reductionist view. Finally, we must add to the list of potential routes, trans-alkylation reactions between inorganic forms of metal and anthropogenic compounds present in the environment, such as organosilanes.

Analytical Chemistry; state of the art, limitations and perspectives

Except when under the influence of direct anthropogenic inputs, organometallic compounds occur in the environment as ultra-low traces. The yield of natural methylation mechanisms is always small (very often far less than a few percent). As a result, methylated species only comprise a few percent of the total metal concentration. One of the first goals of environmental organometallic research is to be able to detect and quantify species, *ie* the fraction of the total metal concentration in each species. Two major analytical challenges are put forward. We have to determine ultra-traces of some metals ranging from nanograms to picograms in the sample (parts per trillion) without alteration of the chemical form in which the metal is present. Both requirements, that is species identification and sensitivity, are far away from most contemporary commercial instrumentation. This problem has been solved most generally by using a combination of different analytical processes and of analytical techniques [9], which has provided the necessary techniques but has also multiplied the analytical steps. It therefore requires a strict control for the proper determination of the species present. Part of the success of speciation techniques depends on the design quality and efficiency of the interfaces between the different stages of the analytical procedure.

All techniques use a primary preconcentration step. This procedure can be performed "off line" from the instrumentation, or "on line" as a part of the instrumentation set-up. The first range of techniques using "off line" preconcen-

tration most often use standard chromatography procedures (gas or liquid). Modern separation techniques such as high resolution capillary gas chromatography (HRCGC) or high performance liquid chromatography (HPLC) have enabled a very good control of the separation and thus of the species identification stage. The transformation of the analytes into volatile forms is by derivatization with Grignard reagents.

For "off line" gas separation techniques, the analytical protocol requires tedious preanalytical steps where the preconcentration stage can be included. Some of the major drawbacks of this approach are related to the large number of steps involved in the derivatization/preconcentration stage. Interfacing is in general straightforward and the gaseous nature of the effluent can be easily adapted to various atomic spectrometry techniques such as atomic absorption, atomic emission, atomic fluorescence or even mass spectrometry. Liquid chromatography separation techniques are very powerful for they generally allow one to bypass the derivatization stage and provide a large panel of possible chromatography procedures enabling a wider range of organometallic compounds. However, the small amount of eluent delivered leads to an overall low sensitivity. Interfacing is more complex in this case since the liquid eluent must be transformed into the gaseous state prior to detection. This stage is technically difficult and usually leads to an overall loss in sensitivity. This can now be overcame by the simple and direct interfacing of liquid chromatography techniques to the high sensitivity multielemental detector

of inductively coupled plasma/mass spectrometry (ICP/MS).

An "on line" technique using direct interfacing between hydride generation methods, and combining simultaneously the preconcentration step by cryofocussing and later chromatography by gentle warming of the trap can very easily be interfaced with atomic absorption spectrometry or atomic fluorescence. Techniques derived from these procedures achieve the highest sensitivity and ease of operation in comparison to other methods using "off line" preconcentration methods with considerably fewer analytical steps. However, they are limited to low boiling point organometallic species (methyl, ethyl, butyl-) and have a low resolution capacity (fig 3). Nonetheless, the high sensitivity of these techniques has suggested the occurrence of unexpected species in the environment. These species include the natural occurrence of stannane SnH_4, hydrides of organotins, $(CH_3)_xSnH_{4-x}$, n-$(C_4H_9)_xSnH_{4-x}$, ($x = 1 - 3$). Some reports have mentioned the possible presence of mixed methyl-butyltin compounds. Species of organotins detected or suspected to occur in the environment are listed in table II. This question is still controversial and needs further investigation. Finally, dimethylmercury, $(CH_3)_2Hg$ has recently been found in oceanic [10] and in methanogenic mangrove environments [11].

It is important to state that there are no preferred techniques overriding all others. At this level, the finding of a new specie should be confirmed by an independent method, as the occurrence of new molecules may suggest unexpected biochemical pathways. Natural methylation reactions may lead, after full alky-
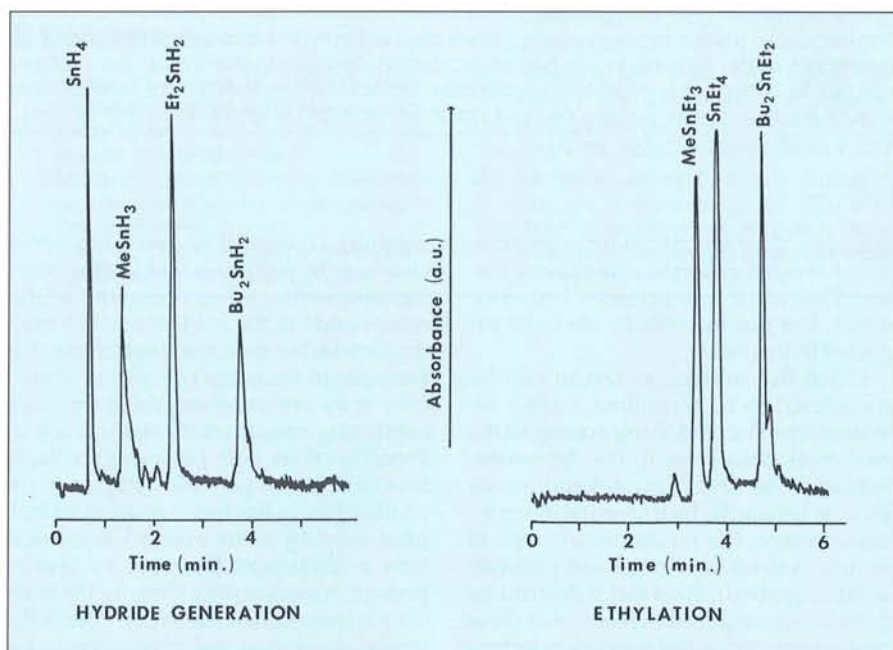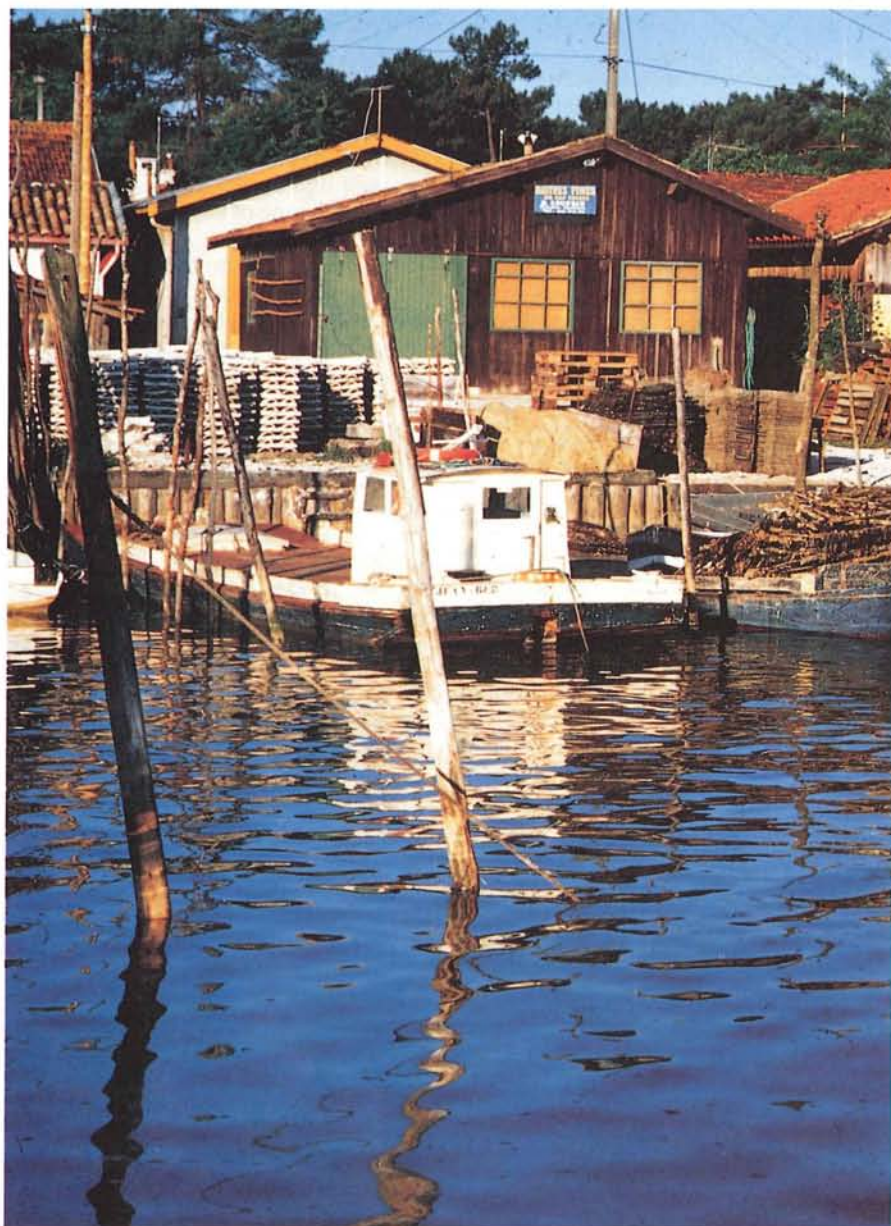
Fig 2. Chromatograms of low boiling point organotin compounds determined by aqueous derivatization, cryogenic trapping, chromatography and atomic absorption detection. Derivatization was by hydride generation using ($NaBH_4$) or aqueous ethylation ($NaBEt_4$) (10 ng as Sn, Donard & Martin, unpublished results).

Fig 3. Chromatogram of high boiling point organotin compounds by capillary gas chromatography and flame photometric detection using a simultaneous derivatization/extraction technique by NaBEt₄ in water (500 pg as Sn; Donard, Lalere & Garrigues, unpublished results, adapted from (16)).

The dispersion of toxic tributyltin compounds originating in anti-fouling paints threatened traditional oyster farming in the Bay of Arcachon (France) in the 1980s. This problem helped to arouse the international concern about the environmental fate of firstly organotin and organometallic compounds in general (photograph from G D'Auzac).

lation of the element under consideration, to volatilization to atmospheric water. This route has yet seen little evidence, but positive results are to be expected in this field.

Once the instrumentation can be considered to be controlled, further attention must be paid to improving all the analytical steps prior to the determination step. All analytical determinations include before the instrumental determination stage, the fundamental steps of sample collection, storage and pretreatment (digestion). Total metal determination already required the control of these preliminary steps, but speciation techniques, owing to the low abundance and high instability of organometallic compounds will require extra control of them. The whole procedure must be very carefully controlled to avoid important errors in the pathways and in the understanding of the cycling of organometallic compounds in the environment. Analytical artefacts can lead to such errors. For example, in the subject of cycling of mercury in the environment, there are many conflicting results on its methylation or demethylation. Both processes are likely to occur but it is possible that part of the conflict lies in the fact that most techniques used by some authors determine only a fraction of the mercury species present in the sample. Often, methylmercury is determined indirectly after differential separation and preconcentration using for example the resin technique and the results are quoted as "organic mercury". Significant progress in this difficult field will be made when the de-termination of all forms of mercury can be performed simultaneously.
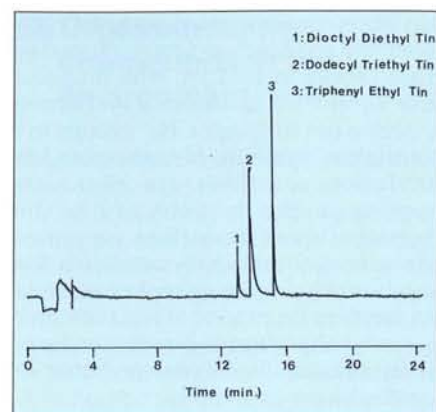
The recent introduction of aqueous ethylation of lead and mercury compounds [12] to substitute for the standard and unsatisfactory hydride generation step is a good illustration of the type of progress to be expected. When this new derivatization route is completely mastered, we may expect significant improvement in our understanding of mercury cycling in the environment.

Another example is the persistence of toxic organotin compounds, such as butylated tins. Tributyltin was at first reported to be degraded rapidly in the environment. The published range of half-lives, from a few days to several months, suggests that the analytical stage may also contribute to the conflicting results. Making progress in this field will be of considerable future importance to obtain a correct estimate of the fate of anthropogenic organometallic species. Finally, it is also important that we obtain a good agreement between speciation methods for organometallic species and total metal determination in order to have an accurate estimation of the fraction of metal in alkylated forms. One of the best illustrations of this important analytical need is the rapid development of environmental quality level defined for the toxic tributyltin species (table III). At first, estimations based on observation and toxicity tests recommended tributyltin (TBT) levels of 69 ng Sn/l salt water. Less than two years later, after the discovery of the imposex phenomenon leading to the decline of a population of the marine snail *Nucella lapillus*, most national authorities recommended the acceptable level of TBT in water as below 2 ng Sn/l. This is close to the detection limit of most analytical set-ups and illustrates the drastic need for ultra-trace organometallic detection.

Table III. Evolution of the water quality criteria for tributyltin concentration in environmental waters (adapted from (13–15)). Proposed or adopted upper limits for tributyltin (expressed as g Sn/l water) in various water samples

Concentration	Sample	References
0.069	Saltwater	13
0.303	Freshwater	13
0.0016	Saltwater	14
0.026	Freshwater	14
0.020 → 0.002	Estuarine	15

■ Perspectives

In 1981, "Biomethylation: pollution amplified" was the title of an article summarizing the issues addressed by natural transformation of metals [3]. "Nature may have ways of turning relatively harmless inorganic chemicals into dangerous organic compounds. If so, we may have to revise our attitude to pollution control" [3]. This statement is still valid, and the attitude towards organometallic dispersion in the environment is still quite fuzzy. Some of the important questions raised 20 years ago are not satisfactorily answered and will still need major progress to achieve a realistic view of the problem. One simple example lies in the fact that biogeochemical properties (*eg* adsorption, bioaccumulation) of organometallic compounds differ considerably from their inorganic counterparts thus modifying their persistence and pathways in the environment.

It is obvious the years to come will see a trend in environmental trace metal research away from determination of the total metal to that of the metal species. This trend is reflected in the adoption of the term "speciation" from which is inferred the efforts of the scientific community in addressing the specificity of the action of some metallic species. If we have not yet necessarily made marked progress on fundamental issues such as what are the main biogeochemical pathways leading to the formation of organometallic compounds in the environment, their stability and persistance, we certainly have made significant steps in the development of the analytical technology required to determine ultra- trace concentrations of organometallic compounds in environmental matrices. This has allowed a better estimate of the potential problems and their definition associated with the widespread occurrence of these elements. But still, much work is required since, in comparison to total metal determination, the technology and the protocols required to determine organometallic compounds are in general only available to research institutions able to develop their own instrumentation.

Progress in analytical development will most probably come from the improvement of preanalytical schemes. Preconcentration and new digestion procedures, along with new derivatization chemistry, will certainly contribute to significant improvement here. The necessity of reference standard materials is of prime importance since it will allow interlaboratory comparison. It is very likely that some future environmental regulations will be based on species determination rather than on the total metal content of the sample. ■

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