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SPECIATION OF LEAD IN ENVIRONMENTAL AND  
BIOLOGICAL SAMPLES

(Technical Report)

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# Speciation of lead in environmental and biological samples (Technical Report)

**Synopsis** - The speciation of lead in environmental and biological samples is reviewed. After a brief outline of the occurrence, chemical transformations and impact of lead species, attention is turned to the various aspects of the analytical chemistry of lead speciation. Sampling, storage and pretreatment methods for water, air, solid and biological samples are discussed before an assessment of currently used analytical techniques and lead speciation schemes is made. Attempts have been directed towards critically choosing the most significant contributions to this subject area, to aid the interested analytical chemist in the selection of the most suitable approaches to lead speciation and to provide a guide to the literature. Further information is readily available in several books [1-5] and review articles [6-17] which provide a much broader and more comprehensive coverage of published work.

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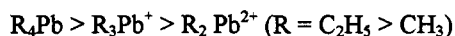
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## 1 INTRODUCTION

Lead has been used at least since Biblical times in a variety of products, but mainly in inorganic form, such that its impact as an environmental pollutant was of little consequence until this century [1-17]. With the development of the internal combustion engine, and the widespread manufacture and distribution of motor vehicles, lead pollution has increased greatly due to its use, in metallic form, in storage batteries and as organic anti-knocking additives (tetraalkyllead) to petrol [18]. Only during the last decade have efforts been made to recover lead from used batteries, and to use alternative petrol additives. Other areas of application for lead compounds include the manufacture of glass, paint, ammunition and insecticides, although legislation in recent years has largely eliminated the last-mentioned use [19,20].

### 1.1 Alkyllead species

Tetraalkyllead (TAL) compounds, which can penetrate the skin and biological membranes and be readily absorbed through the lungs, are highly toxic to the central nervous system and are considered to be some 10 times more harmful than inorganic lead [19-21]. In the environment, TAL pollutants are degraded by sunlight and atmospheric constituents (such as ozone and the hydroxyl radical) into trialkyllead ions [21-23], whereas in biological systems dealkylation occurs through reactions with thiol groups in proteins and enzymes [19,20]. Further conversion finally yields inorganic lead via dialkyllead intermediates [15,21]. The toxicity of alkyllead diminishes in the sequence [24]



but the ionic species are more persistent in the environment [25]. Similarly, methylated lead species are less toxic than the corresponding ethylated compounds, but more stable, volatile and of longer half-lives [15,21,25].

While the major source of alkyllead species in the environment is undoubtedly due to vehicular exhaust fumes, there are also indications that lead can be methylated by biologically mediated mechanisms [26-29], but due to the reportedly low efficiency of such processes, the natural background level of methyllead species is insignificant. Nevertheless, such observations illustrate the bioavailability of lead in the environment, and the need to be able to monitor individual alkyllead species, in order to study the chemical cycling of such compounds and assess exposure risks.

Selected literature data [30-39,164] pertaining to the determination of alkyllead species in a variety of environmental and biological samples are listed in Table 1. Most alkyllead values were obtained using the combination of gas chromatographic separation with atomic absorption spectrometric detection (GC-AAS). This instrumental technique has proven to be the most popular for the identification and quantification of specific alkyllead compounds, and as such is discussed in more detail under section 3.2.2 below.

### 1.2 Inorganic lead

Also included in Table 1 are total or inorganic lead concentrations for these sample types, which demonstrate that, despite the massive consumption of TALs as petrol additives, the major part of the total lead results from inorganic forms. Automobiles emit lead mainly in the form of PbBrCl particles < 0.5  $\mu\text{m}$  in diameter, 90 % of which are respirable [40]. The natural background level of lead is indicated by the concentration found in prehistoric Antarctic ice deposits (0.5 ng/L [39]), which shows that anthropogenic pollution has significantly affected all aquatic eco-systems. The major sources of naturally occurring lead are volcanic activity [41-43], the weathering of lead-containing minerals and silicate dusts [44]. These inputs are, however, negligible compared to annual industrial and domestic inputs [41-44].

Inorganic lead can act as a cumulative poison [45], and may be absorbed through the skin via sweat glands following reaction with lactic or amino acids in sweat [40]. Typical dietary inputs amount to around 10 µg lead per day, absorbed through the gastrointestinal tract with an efficiency of 5-10 % in man [40,46]. The skeleton is the target "organ", containing about 90 % of the total body burden of lead (100-300 mg in industrialised areas) [40,47].

**TABLE 1.** Selected data pertaining to the concentrations of lead species in some environmental and biological samples

Sample description		Concentration				Ref
		Total	Tetra-alkyllead	Ionic alkyllead	ASV-labile fraction <sup>a</sup>	
ng Pb /L						
Ocean water	open	14	-	-	-	[30]
	near shore	250	-	-	62	[30]
Antarctic ice	prehistoric	0.5	-	-	-	[31]
	surface	4	-	-	-	[31]
Potable water (Belgium)		-	-	0.4 - 9.3	-	[32]
Rain water (Antwerp)	suburban	(3-107) · 10 <sup>3</sup>	-	4 - 230	-	[164]
ng Pb /g						
Fish (Ontario lakes)	whole	-	2	4	-	[33]
Chicken	liver	-	-	ca 3.1	-	[34]
	egg	-	-	ca 6.4	-	[34]
Humans (occupationally exposed)	blood urine	130	< 2 - 3	< 3 - 5	-	[35]
		-	-	< 0.43 (Me <sub>3</sub> Pb <sup>+</sup> )	-	[36]
Grass	near petrol station	-	-	10 - 100	-	[37]
	residential	-	-	6 - 11	-	[37]
pg Pb /L						
Air	gaseous	-	0.7 - 11 <sup>b</sup>	-	-	[38]
Urban site	particulate	(45-768) · 10 <sup>3</sup>	0.3 - 36 <sup>b</sup>	-	-	[38]
North Atlantic	particulate	100	-	-	-	[39]
South Atlantic	particulate	1 - 10	-	-	-	[39]
Antarctic	particulate	0.01	-	-	-	[39]

a ASV = anodic stripping voltammetry

b Total alkyllead

### 1.3 Speciation - definitions and problems

Despite the fact that inorganic lead is present at much higher levels than alkyllead species in all the samples shown in Table 1, attempts to further sub-divide this major fraction have been much less successful. Alkyllead compounds are amenable to extraction procedures, so they can be selectively removed from the sample matrix and preconcentrated [3-5,12,21], and following suitable manipulation (see section 2) the individual species can be quantified by, for example, gas chromatography with element specific detection (section 3.2.2). Such *species specific* determinations of individual inorganic lead compounds are not generally possible, for the following reasons. (i) Inorganic lead compounds cannot be readily extracted from the sample matrix without disturbing its chemical composition and hence the distribution of lead species. (ii) No analytical techniques exist which can directly determine lead forms *in situ* at ultra-trace levels. (iii) Many lead compounds are associated with particulate matter in water samples [1-5] which precludes chromatographic separation at least of the adsorbed fraction. (iv) Although obviously unsuitable for solid phase samples (soils, sediments), liquid chromatography has not yet found wide application in the analysis of liquid samples either, which can be attributed to the previous lack of suitably sensitive and selective dynamic detectors for lead [3] (and most other elements, for that matter). However, the recent introduction and application of inductively-coupled plasma mass spectrometry is beginning to improve this situation. (v) Frequently, the total lead concentration in samples such as natural waters lies at or below the detection limit of most instrumental methods, and so the desire to speciate at such levels is not easy to satisfy.

As a result of the aforementioned, speciation is often defined on a *functional basis* (such as plant available lead species), or *operationally* (by means of a sequence of specific reagents or procedures used to isolate, identify and quantify particular lead phases or forms) [4-9]. To some extent, however, these two definitions may overlap, such as in the use of a single reagent designed to extract the plant available component from soil, where the action of said reagent may depend greatly on other soil characteristics (such as pH, organic matter content, redox potential) and as such be, in effect, operationally defined. It is also unlikely that a single reagent can accurately mimic the complicated processes leading to the biological uptake of soil lead, or any other such micronutrient.

### 1.4 Chemical modelling of aquatic systems

Since inorganic lead speciation is severely constrained by a lack of instruments and techniques which are sufficiently sensitive and selective to unambiguously determine the individual chemical forms at the concentrations normally existing in natural waters, chemical modelling appears to be an attractive, alternative approach. Chemical modelling, based on the concept of thermodynamic equilibrium, has been in use in aquatic chemistry since 1959 [48]. Perhaps the most appealing features of this approach are that (i) only a very restricted number of analytical input data (i.e. the free or total concentration of each reacting component) is needed and (ii) the model has an inherent predictive ability, which means that the consequences of a change in input composition can be foreseen. However, the method has its shortcomings: (i) the thermodynamic data must be accurate and all species present in significant amounts with respect to the equilibrium must be considered, and (ii) the assumption of equilibrium in the calculations may not be reflected in reality, limiting the applicability of the chemical model [49,50].

Recently, efforts have been made to include the effects of organic ligands [51-53], suspended particles [52-54] and kinetics [53,55] in chemical models. Together with ongoing studies to measure and refine the relevant equilibrium constants, and comparisons of calculated and experimental speciation results (insofar as these are feasible), continual improvement is to be expected. Reference should be made to a number of review articles [55,56], books [48-52] and literature cited therein, for further information on this topic.

## 2 SAMPLING, STORAGE AND PRETREATMENT

It is not unknown for elaborate, time-consuming and expensive analytical results to reveal only how much the original sample was contaminated or otherwise adulterated before it even reached the instrument. Many reported results for lead fall into this unfortunate category [6], which in retrospect is not surprising, given the ubiquity of this particular environmental pollutant. In this section, some guidelines will be given to aid in the selection of appropriate sampling, storage and pretreatment protocols for environmental and biological samples. Further details may be found in the cited literature and several books [1,3-5] and review articles [6,12,15,17].

**TABLE 2.** Derivatisation procedures for alkylating ionic organolead species using Grignard reagents

Extract <sup>a</sup>	Procedure <sup>b</sup>	Ref
<i>(i) Butylation</i>		
1. Benzene, n-hexane	Add n-C <sub>4</sub> H <sub>9</sub> MgCl, 1.9-2.3 mol/L in tetrahydrofuran, mix occasionally for 10 min. Wash with tenfold excess 0.5 mol/L H <sub>2</sub> SO <sub>4</sub> to destroy residual Grignard reagent. Separate phases and dry organic layer with anhydrous Na <sub>2</sub> SO <sub>4</sub> .	[58]
2. Pentane	Vacuum evaporate extract to dryness at 20 °C. <sup>c</sup> Add 1 mL n-C <sub>4</sub> H <sub>9</sub> MgCl, 1.9 mol/L in tetrahydrofuran, swirl for 1 min, add 0.25 mL nonane <sup>d</sup> and 10 mL 1 mol/L H <sub>2</sub> SO <sub>4</sub> . Separate organic phase and dry with anhydrous Na <sub>2</sub> SO <sub>4</sub> .	[59]
<i>(ii) Propylation</i>		
3. n-Hexane	Add C <sub>3</sub> H <sub>7</sub> MgCl, 2 mol/L in diethyl ether, shake gently for 8 min. Wash with tenfold excess 0.5 mol/L H <sub>2</sub> SO <sub>4</sub> , separate organic phase and dry with anhydrous Na <sub>2</sub> SO <sub>4</sub> .	[60]

a Organic extract containing TALs, and complexed ionic alkyllead and Pb<sup>2+</sup>

b Exact volumes of reagents tend to vary slightly depending on sample type

c Permits preconcentration of ionic alkyllead species, but TALs present in pentane phase are lost during solvent evaporation

d Heptane was found to be superior in Ref. [35]

The various sample types generally have differing requirements regarding sampling, storage and pretreatment procedures, and hence will be treated separately in appropriate sections below. However, as far as the speciation of ionic alkyllead compounds goes, there is one common feature involved in their determination by gas chromatography with lead-specific detection, namely the need to produce non-ionic alkyl derivatives (see also section 3.2.2). This is most frequently performed using a Grignard reagent as originally proposed for ionic alkyllead derivatisation by Estes et al. [57]. With further modification and improvements, this is now the method of choice, the most popular schemes being shown in Table 2 [58-60]. Some controversy exists regarding the efficiency of butylating dialkyllead species [25,60]. For this reason it may be preferable to propylate, although the efficiency of the procedure chosen should be experimentally determined in each case. Another area of concern arose from the work of Blais and Marshall [61], who reported that high levels of inorganic lead present in the sample extracts were conducive to transmetallation and reequilibration reactions during derivatisation. Thus alkyllead species, in

particular the highly unstable monoalkyllead compounds, could be formed as artifacts of the analytical method. For this reason it would appear that masking of inorganic lead or selective extraction of the alkyllead species are beneficial to the procedure as a whole (see also Tables 4 and 5).

It is also possible to use phenylmagnesium chloride [61,62] or sodium tetraethylborate [63] for alkylation. However, the former results in less volatile derivatives which lengthen and degrade the chromatographic separation, and the latter does not allow unambiguous interpretation of the analytical data, due to the occurrence of ethyl and mixed ethyl/methyllead species arising from pollution sources [63]. Consequently, the procedures given in Table 2 are most widely employed. It is interesting to note that, following an appropriate extraction procedure (depending on the sample type), alkylated derivatives (and possibly TALs as well) are amenable to speciation using the same instrumental system (independent of sample type), thus the methodology is quite universal in its applicability to environmental and biological samples. However, on a cautionary note, it should be realised that with the advent of instrumentation providing much improved detection limits (see section 3.2.2), the risks for obtaining biased results at low analyte concentrations has increased. Such bias may result from impurities in the Grignard reagent used causing artifact formation, as well as from contamination of the reagents employed in the sample preparation steps [176].

## 2.1 Water samples

For the determination of total lead concentrations it is normal to acidify the sample in order to prevent losses by adsorption on the walls of the sample vessel [64,65]. Acidification, however, changes the physicochemical distribution of lead species, and therefore must not be used prior to speciation. Ideally, the sample should be analysed immediately, otherwise it should be stored in Teflon or polyethylene containers, which are generally considered most suitable [64-68]. Initial cleaning of the containers is very important to avoid sample contamination, and several suitable procedures are outlined in Table 3 [66-68].

**TABLE 3.** Recommended cleaning procedures for polyethylene and Teflon containers for water sampling

Bottle	Cleaning procedure	Purpose	Ref
1. Polyethylene	Immerse in 10 % HCl, 70 °C for 4 days, repeat 3 times, second time with 2 % high-purity acid, and finally with 1 % HCl. Store filled with 0.1 % HCl until use.	Initial cleaning	[66]
2. Polyethylene	48 hour soak with 10% HNO <sub>3</sub> , followed by deionised, distilled H <sub>2</sub> O rinse and drain drying.	Initial and routine cleaning	[67]
3. Polyethylene and Teflon	Fill with 4 mol/L HNO <sub>3</sub> , place in sealed polyethylene bag, treat for 7 hours (or overnight) with ultrasound. Repeat with 1 mol/L and then 0.05 mol/L HNO <sub>3</sub> . Rinse between treatments with pure H <sub>2</sub> O and store containing 0.01 mol/L HNO <sub>3</sub> .	Initial cleaning	[68]
	Ultrasonically clean for 2 hours in 0.01 mol/L HNO <sub>3</sub> and store containing same acid after rinsing	Routine cleaning	
4. Teflon	Preliminary treatment with 50 % analytical reagent grade HNO <sub>3</sub> at 80-90 °C for 1 week. Then proceed as in 1.	Routine cleaning	[66]

An important point to consider when choosing a cleaning procedure is the expected lead concentration in the water samples of interest. For example, deep ocean seawater will have extremely low lead levels, and

therefore a rigorous cleaning of the sample vessels will be necessary (procedures 1 and 4 in Table 3 [66]). It should also be noted that acid leaching can activate surface sites on container walls and lead to losses of analyte through adsorption [6]. To avoid this potential source of error, containers are often well rinsed with sample, or left to equilibrate with natural levels of heavy metals prior to sampling [64]. Alternatively, conditioning salt solutions containing calcium and magnesium sulfates for inland water sampling, and a mixture of sodium chloride, calcium sulfate, and magnesium sulfate for seawater, may be used to deactivate acid-leached vessels [69]. Since lead may be present at ng/L levels in many types of water (see Table 1), care must be taken to ensure that the conditioning salt solutions do not contaminate the sample vessels.

It has been pointed out [17] that in the presence of organometallic compounds such as tetraalkyllead, preconditioning with sample solution has to be avoided, to prevent the adsorption and accumulation of these compounds on the container surface. Instead the container is treated with an organic solvent to release adsorbed organolead species after emptying out the sample. This organic phase may then be analysed for its organic lead content by gas chromatography with element specific detection (see section 3.2.2).

Using properly cleaned containers, unacidified natural water samples may be stored at 4 °C, in the dark, for up to 3 months without any measurable changes in the distribution of lead species [64,67,68,70,71].

**2.1.1 Filtration.** The question of whether or not to filter water samples remains largely unresolved. On the one hand, if the sample is not filtered, changes in the distribution of lead may occur with time due to adsorption and desorption processes at particle surfaces. In addition, the risk of sampling errors is increased, due to inhomogeneity in the distribution of particles in the water column.

On the other hand, filtration may introduce errors since its efficiency changes with the filter load [72]. Variable concentration values might be obtained if (i) some lead is initially associated with colloids in the sample (colloids pass through the filter with a small but not a large load) and (ii) lead species in solution can be retained by colloids and particles trapped on the filter. It has been suggested that consecutive fractions of filtered solutions should be collected and analysed individually to ensure a constant lead concentration or at least to identify the maximum tolerable sample load before a change in composition is observed [17]. To avoid rapid sedimentation and changes in filtration efficiency, Mart [66] has suggested bubbling nitrogen through the sample solution in the filter holder unit. This swirls up suspended matter and reduces the sedimentation rate, allowing larger samples to be filtered before the problems noted above become evident.

To avoid rupturing of phytoplankton, which could lead to elevated trace metal concentrations, the pressure difference across the filter should not exceed 26 kPa [66]. A slight nitrogen overpressure is advantageous for filtering, both with respect to minimising overloading as discussed above [17,66] and maintaining the redox conditions in the samples.

It goes without saying that the filtration equipment must be scrupulously cleaned before use [66] by first soaking in 50 % HCl for two two-day periods, rinsing with ultrapure water several times, and soaking again in 10 % sub-boiling distilled HCl for 1 week. After thorough rinsing, and storage in 1 % HCl, the equipment should be rinsed again and allowed to equilibrate with a seawater sample (having a very low lead concentration) for a few days before being used. If proper precautions are taken, even deep ocean seawater samples may be filtered without introducing significant levels of contamination [66]. In general, however, sea water samples need not be filtered as the levels of particulate and suspended matter are negligible [65]. Mart [66] considers that filtration is necessary for turbid waters as found in rivers, estuaries, shallow coastal areas and eutrophic lakes.

**2.1.2 Lead complexes with humic substances and colloids.** Humic substances, including humic and fulvic acids, represent the predominant organic constituents found in river water. They are derived from soils and sediments, where they form through the chemical and biologically mediated breakdown of biological tissue [73]. Various heavy metals, including lead, interact and form humic complexes which may either exist



alone or be associated with colloidal or suspended particles. The presence of humic complexes is believed to reduce metal uptake by organisms [74], and is thus an important factor in speciation work.

Hiraide [75] has recently summarised efforts made to separate lead and other metals [76,77] present as humic colloids or associated with other negatively charged species (inorganic colloids such as hydrated iron(III) oxide-clay and silica aggregates). River water samples are first filtered, then separate portions are passed through columns packed with either indium-treated XAD-2 resin or DEAE-Sephadex A-25 anion exchanger (16 mm i.d. x 5 mm). The former column material collects only humic complexes, the latter all negatively-charged species [75]. Lead is then desorbed batchwise with nitric acid, and determined by a suitable technique, such as electrothermal atomic absorption spectrometry (ETAAS) [76] or differential pulse anodic stripping voltammetry (DPASV) [77].

Following filtration to remove suspended particulate matter, Benoit et al. [177] employed ultrafiltration through a cross-flow, hollow fibre cartridge (nominal molecular weight cut-off 10 kDa) to separate colloids from 100-200 L water samples collected from Galveston Bay, Texas. The colloids were de-salted via diafiltration, frozen, freeze-dried and weighed. Colloid samples (30-40 mg) prepared in this fashion were then subjected to microwave digestion in 6 g ultrapure concentrated HNO<sub>3</sub> using closed Teflon vessels. The digestion vessel contents were then evaporated to dryness and the residues dissolved in 15 g of 5 % HNO<sub>3</sub> prior to lead determination by ETAAS. Data obtained indicated that most of the lead passing through 0.4 µm polycarbonate membrane filters was associated with colloids [177].

These procedures are attractive since the separation of lead complexes can be performed at the sample collection site, and the columns or filtrates transferred to the laboratory for subsequent analysis. This avoids potential contamination problems and changes in speciation which could occur if samples had to be stored prior to separation. However, it should be noted that recent work by Cescon et al. [178] has indicated that freezing and storage at -20 °C will preserve the speciation of lead (as well as cadmium and copper) as determined by the operationally-defined, DPASV-based techniques discussed in section 3.1. It is hoped that future studies will compare results obtained for lead speciation following the various schemes outlined here and in section 3.1.

*2.1.3 Alkyllead species in water.* Blaszkewicz et al. [78] determined trialkyllead species in a variety of natural water samples using high performance liquid chromatography (HPLC) with a chemical reaction detector (see section 3.2.1). To minimise wall adsorption and potential analyte deterioration, 1 mL concentrated HCl was added per litre of water sample directly in the amber glass collection vessel. Prior to analysis, the samples were stored at 4 °C in the dark to avoid photochemical degradation of the trialkyllead species. Due to the very low concentrations present in precipitation and river, creek, harbour and drinking water samples, extensive off-line preconcentration was required (see Table 4). Using an initial sample volume of 500 mL, detection limits for trimethyl- (TML) and triethyllead (TEL) of 15 and 20 ng/L, respectively, were reported.

Blais and Marshall [79] also used HPLC to separate ionic alkyllead species in water samples, and compared a variety of extraction procedures. Table 4 includes the procedure yielding the highest recovery of ionic alkyllead added to water samples. Analytical conditions are discussed later in section 3.2.1. It would appear that both the sample preparation and analytical procedures described by Blais and Marshall [79] are preferable to those of Blaszkewicz et al. [78] considering that the former enable the dialkyllead species to be determined simultaneously. Furthermore, Blais and Marshall [79] employed a lead-specific detector based on atomic absorption spectrometry (AAS) with inherently better sensitivity than the spectrophotometric, chemical reaction detector used by Blaszkewicz et al. [78]. However, it should be pointed out that no analytical results have so far been presented, although the feasibility of determining sub-µg/L concentrations of alkyllead species in 10 mL water samples was claimed [79].

Tetraalkyllead (TAL) species may be easily extracted from water samples using organic solvents [80]. Recoveries are quantitative provided the water does not contain suspended particles [25]. Otherwise, recoveries as low as about 60 % may result for unfiltered samples [25] due to adsorption losses on

TABLE 4. Collection, storage and pretreatment methods for water samples facilitating the determination of lead species by chromatography (section 3.2)

Sample type(s)	Compounds studied <sup>a</sup> (extraction efficiency, %)	Sample collection <sup>b</sup>	Storage	Sample pretreatment <sup>c</sup>	Separation technique <sup>d</sup>	Ref.
Rain, drinking and harbour waters, snow	$\text{Me}_3\text{Pb}^+$ , $\text{Et}_3\text{Pb}^+$	Narrow-necked, 2 L, amber glass bottles. Add 1 mL conc. HCl per litre sample	In dark at 4 °C	Complex metal ions in 500 mL sample with $10^{-3}$ moles EDTA, adjust pH to 10 with NaOH and pump (10 mL/min) through extraction column (8 mm i.d.) packed with 500 mg silica gel (40-63 $\mu\text{m}$ ). Elute adsorbed lead species with 2 mL acetate buffer (pH 3.7) containing 10 % methanol. Add 4 mL borate buffer (pH 10) to dilute and adjust pH to 8.	HPLC (Table 9, entry 1)	[78]
Water (unspecified origin)	$\text{Me}_3\text{Pb}^+$ (98); $\text{Et}_3\text{Pb}^+$ (95); $\text{Me}_2\text{Pb}^{2+}$ (92); $\text{Et}_2\text{Pb}^{2+}$ (94); $\text{Pb}^{2+}$ (only inefficiently extracted)	—	—	Add 10 mL 0.2 mol/L phosphate to 10 mL sample and extract three times with 5 mL hexane/benzene (1+1 v/v) containing 1 % m/v dithionite. Back extract with three 5 mL 0.15 mol/L $\text{HNO}_3$ washes, basify to pH 9, re-extract three times with 5 mL hexane + 1 mL 5 mmol/L $\text{NH}_4\text{TMDTC}$ in $\text{H}_2\text{O}$ . Combine hexane extracts, dry over anhydrous $\text{Na}_2\text{SO}_4$ , and evaporate to 0.2 mL under a gentle stream of $\text{N}_2$ .	HPLC (Table 9, entry 3)	[79]
Lake water	$\text{R}_4\text{Pb}$ (89)	Note: Do not suction filter! Volatile $\text{R}_4\text{Pb}$ species will be lost	Add 5 mL hexane per 200 mL sample and shake for 5 min immediately after collection. At 4 °C, samples are then stable for 1 week	Place sample (200 mL water + 5 mL hexane) in 250 mL separating funnel. Shake rigorously for 30 min in a reciprocating shaker, wait 20 min for phase separation. Drain of 195 mL of water and transfer remaining mixture into a capped tube. Analyse hexane phase immediately.	GC	[80]
Rain water	$\text{Me}_3\text{Pb}^+$ ; $\text{Et}_3\text{Pb}^+$ ; $\text{Me}_2\text{Pb}^{2+}$ ; $\text{Et}_2\text{Pb}^{2+}$	Polyethylene containers, samples protected from daylight	In dark at 20 °C	Filter 500 mL sample on type RA Millipore filter (1.2 $\mu\text{m}$ ) and add 4 g citric acid + 1 g EDTA + $\text{NH}_4\text{OH}$ to give pH 9. Transfer to 1 L separating funnel, add 2 mL 0.25 mol/L NaDDTC + 10 mL pentane and shake for 2 min. Collect organic layer in 50 mL flask and combine with additional 10 mL pentane extract of sample. Proceed according to Table 2, entry 2	GC <sup>e</sup>	[59]
Rain and lake surface water, snow	(according to Ref. [60], extraction efficiencies are > 95%)					[84]

TABLE 4. (continued)

Sample type(s)	Compounds studied <sup>a</sup> (extraction efficiency, %)	Sample collection <sup>b</sup>	Storage	Sample pretreatment <sup>a,c</sup>	Separation technique <sup>d</sup>	Ref.
Rainwater run-off	R <sub>4</sub> Pb (no recovery data reported); Me <sub>3</sub> Pb <sup>+</sup> (102); Et <sub>3</sub> Pb <sup>+</sup> (96); Me <sub>2</sub> Pb <sup>2+</sup> (44); Et <sub>2</sub> Pb <sup>2+</sup> (73); Pb <sup>2+</sup> (no recovery data reported but very low)	—	—	Add 5 mL 0.2 mol/L phosphate buffer (pH 9.0) to 30 mL <sup>l</sup> water sample. Extract R <sub>4</sub> Pb with 3 mL hexane, wash twice with 2 mL H <sub>2</sub> O and dry over anhydrous Na <sub>2</sub> SO <sub>4</sub> . Extract ionic alkylleads three times with 5 mL hexane containing 10 % 0.15 mol/L dithizone in THF. Reduce volume under a gentle flow of N <sub>2</sub> . Extract Pb <sup>2+</sup> three times using 5 mL IBMK, back-extract combined organic phase with HNO <sub>3</sub> . <sup>e</sup>	GCC <sup>e</sup>	[61]
River, estuarine, lake, sea, potable, road surface and run-off waters	R <sub>4</sub> Pb (60); Me <sub>3</sub> Pb <sup>+</sup> ; Et <sub>3</sub> Pb <sup>+</sup> ; Me <sub>2</sub> Pb <sup>2+</sup> ; Et <sub>2</sub> Pb <sup>2+</sup> (all ionic alkyllead species recovered to > 95 %)	2 L amber glass bottles, filled manually or left in the dark in the field for rain water sample	Extracted on day of collection	Add 50 g NaCl per litre sample + 5 mL 0.5 mol/L NaDDTC + 5-20 mL hexane to unfiltered sample (0.5-2 L) in original collection vessel, and place on mechanical shaker for 30 min. Separate organic phase and proceed according to Table 2, entry 1 or 3 if ionic alkyllead species are to be determined	GCC <sup>e</sup>	[85]
Rain water						[38]

<sup>a</sup> R = CH<sub>3</sub> (Me) or C<sub>2</sub>H<sub>5</sub> (Et)

<sup>b</sup> It is to be recommended that the organic solvent is added directly following sample collection as R<sub>4</sub>Pb compounds rapidly decompose in water [83]

<sup>c</sup> EDTA = ethylenediaminetetraacetic acid, NH<sub>4</sub>TMDTC = ammonium tetramethylene-dithiocarbamate, NaDDTC = sodium diethyldithiocarbamate, THF = tetrahydrofuran, IBMK = isobutyl methyl ketone

<sup>d</sup> HPLC = high performance liquid chromatography (see section 3.2.1), GC = gas chromatography (section 3.2.2) with lead-specific detection (Table 10)

<sup>e</sup> Derivatisation of ionic alkyllead species prior to determination (see Table 2)

<sup>f</sup> Larger sample volumes will be required for typical analytical work at trace levels

<sup>g</sup> Hexane-dithizone extract is then derivatised (Table 2, entry 1 or 3). Inorganic lead in HNO<sub>3</sub> back-extract is determined by electrothermal atomic absorption spectrometry

particulate matter [81], a problem which appears to be accentuated if aqueous samples are filtered. It is also recommended to perform the extraction in the sample collection bottle, since TALs are readily adsorbed on vessel walls [17,82] and to add organic solvent as soon as possible after collection, as TALs decompose rapidly in aqueous solutions [83]. Similar complexometric extraction procedures for ionic alkyllead species in water, ice and snow samples have been described by a variety of workers [38,59,61,84,85,164-167] (Table 4) using gas chromatography for separation and a lead-specific detector for quantification, see section 3.2.2.

Recently, van Cleuvenbergen et al. [168] reported that the degradation of trialkyllead species in deionised water yielded inorganic lead directly. Similarly, TALs decomposed to inorganic lead via trialkyllead, with only minor formation of dialkyllead species (< 2 %). It was noted that dialkyllead species are too stable to be involved as short-lived intermediates, and thus their presence in many environmental water samples might be attributed to their direct deposition from the atmosphere [168]. However, it should be noted that in natural waters, other environmental factors could lead to the formation of dialkyllead.

Solid-phase extraction (SPE) techniques have been applied to the sampling of ionic alkyllead species from water samples [179,180]. Hewitt et al. [179] used 10 mL bed volumes of Amberlite IR-120 resin in the Na<sup>+</sup> form and clinoptilolite (a silica-rich zeolite) ion exchangers to quantitatively extract trimethyl-, triethyl- and diethyllead from solutions pumped at 2 mL/min. Elution was achieved using 150 mL 2.7 mol/L NaCl solution at a flow rate of 1-1.5 mL/min. The eluates were further manipulated as described in the final entry in Table 4, the ionic alkyllead species then being derivatised and determined by coupled gas chromatography - atomic absorption spectrometry (GC-AAS) as discussed in section 3.2.2. Trimethyllead was completely adsorbed from solutions containing less than 10 mmol/L NaCl, but the efficiency dropped to 50 % at 1 mol/L NaCl. Thus, such ion exchange columns are less than ideal for seawater and other saline samples [179].

Johansson et al. [180] constructed a sampling system for the preconcentration of lead (and mercury) species from seawater. The samples were first pumped through a tubular functional membrane [181] at a flow rate of 2 mL/min to adjust the pH to the optimal value of 7 for subsequent, on-line enrichment by SPE in a microcolumn (60 µL) containing diethyldithiocarbamate (DTC) groups immobilised on a resin. The sorbed lead species were eluted using 1 mL 66 mmol/L HCl at a flow rate of 3.3 mL/min. Further sample work-up involved adjustment of the eluate pH to 9, complexometric extraction of the lead compounds into hexane, derivatisation and analysis by GC-AAS. Trimethyl-, triethyl-, diethyl- and inorganic lead were adsorbed on the DTC resin with efficiencies of 75-95 % [180].

The latter SPE approach [180] appears to be more attractive for seawater samples, and also permits the lead species to be preconcentrated to a greater degree (elution volume 1 mL compared with 150 mL in the work of Hewitt et al. [179]), simplifying the following liquid-liquid extraction stage. One drawback, not considered by Hewitt et al. [179], which limits the general applicability of SPE in a flowing stream is that the presence of humic substances severely reduces the enrichment efficiency. This results from the high stability of complexes formed between lead species and humic substances in natural waters, and the short time available for transfer of the former to the resin in a flow system [180].

## 2.2 Sediment, soil and particulate matter

Solid materials are characterised by their heterogeneity and complexity of interactions with their surroundings (water and air), and thus care is required to minimise alterations in lead speciation resulting from changes in the environmental conditions of the system on sampling [7,86]. Sub-surface soils and sediments are naturally isolated from the oxidising influence of air and water, and such anoxic material must be protected from the atmosphere at all times following sampling. This will obviously complicate the subsequent separation and determination of lead in the various solid fractions, as exposure to air leads to a rapid redistribution of lead species [87,88]. Relatively little work has been devoted to studying the peculiarities of anoxic soil and sediment sampling, storage and speciation, so no specific approaches can be recommended. However, freezing of anoxic sediments has been shown to cause very little change in the fractionation pattern [87] and may be a useful storage procedure.

It is more typical for the speciation of lead in surface soils (to a depth of 5 cm [89]) and sediments to be studied, since the uppermost layer provides most relevant information regarding atmospheric pollution, bioavailability and exchange of lead with overlying water [7,86,90]. Immediate analysis is to be recommended, as storage may significantly change the distribution of lead species. Generally speaking, however, some form of pretreatment and storage is involved (wet storage at room temperature, moist frozen, air dried or oven dried [91]) which may affect the results of subsequent speciation. Thus the experimenter must consider the validity of the results and conclusions derived from them. Details of the exact sampling and storage procedures must be recorded as meticulously as for the fractionation and analytical stages.

Filtration is normally applied to effect the removal of particulate matter from natural water or air samples. This approach is somewhat limited in utility for solid speciation studies due to problems in quantitatively removing accumulated particles from the filter substrate [86], although techniques such as ultrasonic scrubbing [92] and filter dissolution in a solvent extraction procedure [93] appear promising. In air sampling (see below), filters are used to separate particulate matter from gaseous alkyllead species, and usually the total lead concentration in the collected aerosol fraction is measured. Hiraide [75] described the collection of suspended particles from 250 mL river water samples by centrifugation at 5000 rpm for 5 minutes. The collected particles were then transferred to a small Teflon vessel, 0.7 mL of 0.1 mol/L NaOH was added, and the mixture was left to stand for one hour with occasional shaking. After centrifugation, the supernatant was collected and combined with a further 0.7 mL of water used to rinse the particles. Lead present in the combined phase was determined by electrothermal atomic absorption spectrometry (ETAAS), the result giving the concentration of humic complexed lead associated with suspended particles. Total lead was determined in a separately collected sample of suspended particles using ETAAS following decomposition in a mixture of perchloric, nitric and hydrofluoric acids.

Suspended sediments in natural waters can also be recovered by continuous-flow centrifugation [94] and sediment traps [95]. The aforementioned sampling techniques are not, however, equivalent, particularly with respect to small, low-density organic particle fractions collected [96].

As the lead concentrations in the solid materials under consideration are generally much higher than those found in natural waters (see Table 1), the requirements on sampling and handling procedures are less rigorous from the contamination point of view. Nevertheless, reasonable caution should always be exercised, to avoid the sample coming into contact with, for example, items coloured by lead-containing pigments. In general, Teflon or polyethylene-coated sampling devices are to be preferred, and can be cleaned according to the procedures given in Table 3, where appropriate, or rinsed with ethylenediaminetetraacetic (EDTA) acid and copious amounts of distilled deionised water. Sediment corers of poly(vinyl chloride) are useful for small scale sampling, and can be easily cut into sections (under an inert atmosphere) for lead speciation depth profile studies [64].

Soil and sediment samples contain substantial quantities of interstitial waters which can be removed by centrifugation. This procedure is not, however, to be recommended when it is the speciation in the solid material that is of interest. Interstitial waters contain the most mobile lead phase in equilibrium with the solid. During centrifugation, active sites freed on the surface may selectively re-adsorb some of the water-soluble metals, resulting in a change in the speciation [64].

Particulate matter suspended in natural waters or in atmospheric aerosols can be collected by filtration (0.45  $\mu\text{m}$ ). Filters should be cleaned by soaking in acid, preferably with sonication, and careful rinsing. For representative samples, very large volumes should be filtered, which, while feasible for particles in air (1000 L/min using Hi-Vol apparatus), is impractical in aquatic milieu due to the problems with filter clogging encountered in handling the large volumes of about 100 L required [17,72].

An interesting development for the study of trace elements in specific, sub-micrometre size-fraction particulates is the use of sedimentation field-flow fractionation (SdFFF) for separation and inductively coupled plasma - mass spectrometry (ICP-MS) for detection [158]. Although still in its infancy, the ability to separate size-fractions by SdFFF coupled with the high multi-elemental specificity and detection capability of ICP-MS provides an attractive approach to investigate the chemistry of clays and other

**TABLE 5.** Procedures for the extraction of alkyllead species from solid phase samples, suitable for subsequent chromatographic separation and lead specific detection of the individual compounds (see section 3.2. below)

Sample	Compounds extracted <sup>a</sup> (extraction efficiency, %)	Extraction procedure <sup>c</sup>	Separation <sup>f</sup> technique	Ref.
Sediment	$\text{Me}_3\text{Pb}^+$ (90); $\text{Et}_3\text{Pb}^+$ (86); $\text{Me}_2\text{Pb}^{2+}$ (72); $\text{Et}_2\text{Pb}^{2+}$ (75)	Slurry 5 g sediment with 5 mL $\text{H}_2\text{O}$ + 10 mL 0.2 mol/L phosphate buffer (pH 9.0) and extract three times with 5 mL hexane/benzene (1 + 1 v/v) containing 1 % m/V dithizone. Back extract with three 5 mL 0.15 mol/L $\text{HNO}_3$ washes, basify to pH 9.0, re-extract three times with 5 mL hexane + 1 mL 5 mmol/L $\text{NH}_4\text{TMDTC}$ in $\text{H}_2\text{O}$ . Combine hexane extracts, dry over anhydrous $\text{Na}_2\text{SO}_4$ and evaporate to 0.2 mL under a gentle stream of $\text{N}_2$ .	HPLC (Table 9, entry 3)	[79]
Soil	$\text{Me}_3\text{Pb}^+$ (83); $\text{Et}_3\text{Pb}^+$ (83); $\text{Me}_2\text{Pb}^{2+}$ (73); $\text{Et}_2\text{Pb}^{2+}$ (74)	Slurry 5 g soil with 5 mL $\text{H}_2\text{O}$ + 10 mL 0.2 mol/L phosphate buffer (pH 9.0) and extract three times with 5 mL hexane + 1 mL 5 mmol/L $\text{NH}_4\text{TMDTC}$ in $\text{H}_2\text{O}$ . Combine hexane extracts, dry over anhydrous $\text{Na}_2\text{SO}_4$ and evaporate to 0.2 mL under a gentle stream of $\text{N}_2$ .	HPLC (Table 9, entry 3)	[79]
Aerosol particles	$\text{R}_4\text{Pb}$ (93); $\text{Me}_3\text{Pb}^+$ (77 [82], 89 [25]); <sup>b</sup> $\text{Et}_3\text{Pb}^+$ (100); $\text{Me}_2\text{Pb}^{2+}$ (30 [82]; 101 [25]); $\text{Et}_2\text{Pb}^{2+}$ (15 [82]; 104 [25])	Place filter <sup>d</sup> in glass-stoppered tube, add 30 mL $\text{H}_2\text{O}$ + 5 mL n-hexane + 1.5 g $\text{NaCl}$ + 1.5 mL 0.5 mol/L $\text{NaDDTC}$ , shake for 30 min and separate organic phase.	GC <sup>e</sup>	[82]
Aerosol particles	$\text{R}_4\text{Pb}$ removed in first hexane extraction (> 93); $\text{Me}_3\text{Pb}^+$ (98); $\text{Et}_3\text{Pb}^+$ (91); $\text{Me}_2\text{Pb}^{2+}$ (90); $\text{Et}_2\text{Pb}^{2+}$ (95)	Cut up filter <sup>d</sup> and place in 1 L beaker, add 30 mL hexane and stir for 10 min to extract TALs, add 300 mL $\text{H}_2\text{O}$ and stir before filtering, wash filter with 100 mL $\text{H}_2\text{O}$ , and combine liquid in 1 L separating funnel. Transfer aqueous phase to 1 L beaker, add 4 g citric acid + 1 g EDTA, stir for 90 min and filter. Add 10 g EDTA, adjust pH to 9.0 with ammonia, add 5 mL 0.25 mol/L $\text{NaDDTC}$ and extract twice with 30 mL pentane and rinse funnel with 10 mL pentane. Dry combined pentane layers with anhydrous $\text{Na}_2\text{SO}_4$ . Rotovaporate to dryness in Erlenmeyer flask at 20 °C and dissolve dry residue in 0.25 mL nonane.	GC <sup>e</sup>	[97]
Sediment	$\text{R}_4\text{Pb}$ (no recovery data reported); $\text{Me}_3\text{Pb}^+$ (111); $\text{Et}_3\text{Pb}^+$ (94); $\text{Me}_2\text{Pb}^{2+}$ (113); $\text{Et}_2\text{Pb}^{2+}$ (93)	Dried (1-2 g) or wet (5 g) sediment placed in capped glass vial, add 10 mL $\text{H}_2\text{O}$ + 6 g $\text{NaCl}$ + 1 g $\text{KI}$ + 2 g $\text{Na benzoate}$ + 3 mL 0.5 mol/L $\text{NaDDTC}$ + 2 g coarse glass beads + 3 mL benzene. Shake for 2 h and separate organic phase.	GC <sup>e</sup>	[98]

TABLE 5. (continued)

Sample	Compounds extracted <sup>a</sup> (extraction efficiency, %)	Extraction procedure <sup>c</sup>	Separation <sup>f</sup> technique	Ref.
Sediment, dust, soil	R <sub>4</sub> Pb (55) Me <sub>3</sub> Pb <sup>+</sup> (71-112); Et <sub>3</sub> Pb <sup>+</sup> (68-100); Me <sub>2</sub> Pb <sup>2+</sup> (54-85); Et <sub>2</sub> Pb <sup>2+</sup> (54-100)	Place 10 g sample in glass-stoppered tube, add 100 mL H <sub>2</sub> O + 5 g NaCl + 5 mL 0.5 mol/L NaDDTC + 5 mL n-hexane. Shake for 30 min and separate organic phase. If recovery is low, filter (0.45 µm) after shaking but without adding n-hexane. Then extract filtrate with n-hexane.	GC <sup>g</sup>	[99]
Sediment	R <sub>4</sub> Pb (85)	Vigorously shake 5 g wet sediment with 5 mL 0.1 mol/L EDTA + 5 mL hexane in a capped test tube for 2 h. Centrifuge for 10 min and separate hexane layer.	GC	[80]
Soil, street dust	R <sub>4</sub> Pb (no recovery data reported); Me <sub>3</sub> Pb <sup>+</sup> (61); Et <sub>3</sub> Pb <sup>+</sup> (67); Me <sub>2</sub> Pb <sup>2+</sup> (52); Et <sub>2</sub> Pb <sup>2+</sup> (66); Pb <sup>2+</sup> (no recovery data reported)	Add 15 mL 0.2 mol/L phosphate buffer (pH 9.0) to 5 g sample. Extract R <sub>4</sub> Pb with 3 mL hexane, wash twice with 2 mL H <sub>2</sub> O and dry over anhydrous Na <sub>2</sub> SO <sub>4</sub> . Extract ionic alkylleads three times with 5 mL hexane containing 10% 0.15 mol/L dithizone in THF. Reduce volume under a gentle flow of N <sub>2</sub> . Extract inorganic lead three times using 5 mL IBMK, back-extract combined organic phase with HNO <sub>3</sub> . <sup>e</sup>	GC <sup>g</sup>	[61]
Sediment, urban dust	R <sub>4</sub> Pb (not studied but assumed to be negligible) Me <sub>3</sub> Pb <sup>+</sup> (96); Et <sub>3</sub> Pb <sup>+</sup> (106); Et <sub>2</sub> Pb <sup>2+</sup> (80); Pb <sup>2+</sup> (0) (data for spiked sediment)	Sample (0.2-1.5 g) placed in extraction vessel over 0.2 g Cu powder (to avoid co-extraction of organosulfur species) with clean sand to fill void volume, and 50 µL methanol modifier. Static extraction for 4 min, then 4-20 min in dynamic mode using supercritical CO <sub>2</sub> at a flow rate of 0.85 mL/min, 80 °C and 45 MPa. Analytes collected in methanol.	GC <sup>g</sup>	[185]

<sup>a</sup> R = CH<sub>3</sub>(Me) or C<sub>2</sub>H<sub>5</sub>(Et)

<sup>b</sup> Discrepancies in recoveries for this method depending on source, [25] or [82]

<sup>c</sup> NaDDTC = sodium diethyldithiocarbamate, EDTA = ethylenediaminetetraacetic acid, NH<sub>4</sub>TMTDC = ammonium tetramethylenedithiocarbamate (pyrrolidinedithiocarbamate), THF = tetrahydrofuran, IBMK = isobutyl methyl ketone

<sup>d</sup> Aerosol particles collected on 0.45 µm filter positioned in sampling train used for collection of other vapour phase alkyllead species (see Table 6).

<sup>e</sup> Inorganic lead in HNO<sub>3</sub> back-extract is determined by electrothermal atomic absorption spectrometry

<sup>f</sup> HPLC = high performance liquid chromatography (see section 3.2.1), GC = gas chromatography with element specific detection (see section 3.2.2 and Table 10)

<sup>g</sup> Derivatisation of ionic alkyllead species prior to determination (see Table 2)

colloidal materials. In particular, this combination has the potential to determine trace element contaminants associated with colloidal suspended matter in environmental water quality and geochemical applications and to study the mechanisms related to adsorption and interaction with fine suspended particulates [158].

When the object of the investigation is to study alkyllead species, some form of extraction is required. Tetraalkyllead species are readily extracted into organic solvents, hexane or benzene being particularly suitable for this purpose [21,25,80,82]. Ionic alkyllead compounds can be forced into the organic solvent by salting-out and complexometric extraction procedures, and more details are given in Table 5 [61,79,80,82,97-99].

Recently, Witte et al. [182] determined trimethyllead in an urban dust sample circulated by the Commission of the European Communities Measurements and Testing Programme (formerly BCR) [183]. Treatment of the dust sample was performed according to a modified version of the procedure developed by Chakraborti et al. [97] (see entry 4 in Table 5), where sodium tetraethylborate was added to generate volatile ethyl-trimethyllead, which was extracted into hexane prior to quantification by GC with atomic emission detection (see section 3.2.2). It should be noted that this method can only yield unambiguous analytical data for one species, trimethyllead, as discussed above following Table 2.

Supercritical fluid extraction (SFE) is currently the subject of intense research activity regarding the isolation of organometallic compounds from solid samples. Advantages of SFE include [184]: (i) the use of non-aggressive chemicals for extraction minimising the risks for analyte decomposition; (ii) continuous removal of analyte from the extraction cell in the dynamic fluid phase, thus reducing redistribution and liquid-solid equilibrium limitations on the extraction efficiencies; and (iii) reduction in the use of organic solvents which would otherwise create waste handling problems. Johansson et al. [185] demonstrated the viability of SFE for recovering trimethyl-, triethyl- and diethyllead spikes from sediment. Optimal SFE conditions are included as the final entry in Table 5 [185]. The analytes were collected in methanol, extracted as diethyldithiocarbamate complexes into hexane, propylated (see Table 2 [60]) and determined by GC with mass-selective detection. Method validation was achieved using the urban dust sample distributed by the Measurements and Testing Programme, the result for trimethyllead ( $5.4 \pm 0.5$  ng/g as Pb) agreeing with the average value ( $5.4 \pm 1.1$  ng/g) obtained by five reporting laboratories in an intercalibration exercise [183].

### 2.3 Air samples

Most reported data concerning lead speciation in atmospheric samples are focussed on the concentrations of TAL compounds in the gas phase, and a variety of sampling techniques have been proposed [15,21,25,100-102]. The general applicability of most of these proposals is, however, undermined by the need to maintain the sampling device at extremely low temperatures, resulting in problems with water vapour freezing out in the traps, and the practical difficulties incumbent in employing cold-trapping techniques in the field [25]. Thus the use of solid adsorbents operated at ambient temperatures for the sampling of TAL species [23,38,103] is perhaps to be recommended (see Table 6).

The consensus of opinion also indicates that air samples should be filtered to remove particulate matter, although concern has been expressed that lead alkyls may be adsorbed onto the collected aerosol [104] leading to low recoveries. More recent results would indicate that such adsorption losses on filter-captured, particulate matter are insignificant [25]. Due to the possibilities for TAL decomposition (particularly of tetraethyllead) in the presence of ozone [23], a Teflon tubing pre-filter, packed with iron(II) sulfate crystals [103], should be included in the sampling train, as indicated in Table 6, second entry. This will effectively remove ozone from the filtered air sample and prevent degeneration of the sorbed TAL compounds.

Ionic alkyllead species can be trapped in two water-filled gas bubblers connected in series [102] after a pre-filter. This simple procedure (third entry in Table 6) is reported to allow reasonable recoveries of ionic alkyllead species, whilst TALs are only inefficiently ( $< 4\%$ ) absorbed in water [102].



**TABLE 6.** Sampling, storage and pretreatment of atmospheric lead compounds present in the vapour phase. The sampling train order given is that used in practise, e.g. filter, adsorbing medium, with a pump placed at the end in series. Storage conditions refer to the sample present in the sampling unit. Sample pretreatment is that required to release the lead compounds collected from the sampling unit, and if necessary to convert them to a form suitable for subsequent determination.

Compounds collected <sup>a</sup> (collection efficiency, %)	Sampling rate	Sampling train <sup>b,c</sup>	Storage	Sample pretreatment	Ref.
R <sub>4</sub> Pb (100); R <sub>3</sub> Pb <sup>+</sup> (100); R <sub>2</sub> Pb <sup>2+</sup> (100)	—	0.45 µm filter; 20 cm long glass U-tube packed with 2 mm glass beads, immersed in liquid N <sub>2</sub> /ethanol slush bath (-130 °C)	—	Add 10 mL H <sub>2</sub> O + 2.5 mL n-hexane. Transfer with beads to glass tube, add 1.5 mL 0.5 mol/L NaDDTC + 1.5 g NaCl. Shake for 30 min, separate organic phase. <sup>e</sup>	[102]
Me <sub>4</sub> Pb (78); Me <sub>3</sub> EtPb (73); Me <sub>2</sub> Et <sub>2</sub> Pb (76); MeEt <sub>3</sub> Pb (56); Et <sub>4</sub> Pb (53)	100 mL/min	0.45 µm filter; Teflon tube (5 cm long, 5 mm i.d.) packed with 0.25 g Fe(II)SO <sub>4</sub> crystals; 8.3 cm long, 5 mm i.d. stainless steel adsorption tube packed with 0.5 g Porapak Q	14 days at -10 °C	Heat adsorption tube to 150 °C, sweep desorb TALs with carrier gas (140 mL/min) into glass-lined, stainless steel U-tube (15 cm long, 25 mm i.d.) packed with ground glass immersed in liquid N <sub>2</sub> bath (-196 °C). Flash-heat U-tube to 140 °C to desorb TALs. <sup>f</sup>	[38, 103]
Me <sub>3</sub> Pb <sup>+</sup> (78); Et <sub>3</sub> Pb <sup>+</sup> (70) Me <sub>2</sub> Pb <sup>2+</sup> (66); Et <sub>2</sub> Pb <sup>2+</sup> (46);	1 L/min	0.45 µm filter; two 125 mL gas bubblers (each containing 80 mL H <sub>2</sub> O) connected in series.	72 hours in dark at room temp.	Combine contents of bubblers, add 5 mL 0.5 mol/L NaDDTC + 5 g NaCl + 5 mL n-hexane. Shake for 30 min, separate organic phase. <sup>e</sup>	[102]
Total alkyllead (> 96)	< 3.6 L/min	0.45 µm filter; 60 mL 0.1 mol/L ICI in a darkened 125 mL gas bubbler. N.B. Additional water filled bubbler and activated charcoal containing U-tube required to protect pump from ICI vapour.	—	Note volume and transfer ICI solution to a separating funnel together with 5 mL H <sub>2</sub> O bubbler washing. Add buffer (0.9 % citric acid + 4 % hydrated NaNO <sub>3</sub> + 16 % conc. NH <sub>3</sub> ), 20 mL per 15 mL ICI, 5 drops 0.1 mol/L EDTA + 5 mL 2 % dithizone in CCl <sub>4</sub> . Separate organic layer, add further 5 mL CCl <sub>4</sub> and separate. Shake for 30 s with 2 mL (1 % H <sub>2</sub> O <sub>2</sub> + 1 % HNO <sub>3</sub> ) acid, discard organic phase. <sup>g</sup>	[102]

<sup>a</sup> R = CH<sub>3</sub> (Me) or C<sub>2</sub>H<sub>5</sub> (Et)

<sup>b</sup> H<sub>2</sub>O is assumed to be of sufficient purity for the intended purpose

<sup>c</sup> Iodine monochloride (ICI) stock solution (1 mol/L) made by dissolving 111 g KI in 400 mL H<sub>2</sub>O, adding 445 mL conc. HCl and slowly adding 75 g KIO<sub>3</sub> with stirring until dissolved (several hours). Cool and dilute to 1 L; dilute stock solution with H<sub>2</sub>O

<sup>d</sup> NaDDTC = sodium diethyldithiocarbamate, EDTA = ethylenediaminetetraacetic acid

<sup>e</sup> Ionic alkyllead species must be derivatised prior to gas chromatographic separation and lead-specific detection

<sup>f</sup> Determination of TAL species by gas chromatography with lead-specific detection (see section 3.2.2)

<sup>g</sup> Determination of total lead in relevant sample fraction by lead-specific detector (such as electrothermal atomic absorption spectrometry)

Prior to the determination of individual alkyllead compounds, using gas chromatographic separation and lead-specific detection (see section 3.2.2 below), the ionic species must be alkylated as shown in Table 2. Although all alkyllead species may then be determined simultaneously [25], the chromatogram is complex and several peaks overlap, leading to difficulties in quantification. For this reason it may be preferable to analyse for TALs and ionic alkyllead species separately, for example using the second and third procedures in Table 6, respectively.

The results for alkyllead speciation can be checked for accuracy using the classical iodine monochloride method [105,106] for total alkyllead (Table 6, final entry). This allows a quality control of the analytical speciation data to be made.

It should be pointed out that alkyllead species do not account for all the lead present in the gas phase (by definition unretained on a 0.45  $\mu\text{m}$  filter), indeed the largest fraction is inorganic [38]. Despite this fact, the gaseous inorganic lead fraction has received little attention, although it probably comprises lead containing particles ( $\text{PbBrCl}$ , not retained on 0.45  $\mu\text{m}$  filter) derived from the combustion of anti-knock additives in petrol.

## 2.4 Biological materials

The speciation of lead compounds in biological materials has received relatively little attention, as compared to environmental samples and the wealth of data concerning total lead concentrations [107,108]. This is probably related to the limited amount of material typically available for such studies (blood, biopsy samples), the complexity of sample matrices and analytical difficulties involved in speciation. On the other hand, sampling should not present any great difficulties as regards contamination, since inorganic lead concentrations are much higher in biological than water samples. The need for rapid analysis of biological materials may be particularly acute, however, since enzymatic activity and natural proteolysis and autolysis processes will continue after sampling and could alter the speciation.

**2.4.1. Urine.** Urine is a troublesome matrix because of the very variable composition and concentrations of constituents. For speciation studies, the urine should be freshly collected to avoid problems with precipitation, and filtered (0.45  $\mu\text{m}$  [109]). Alkyllead concentrations in urine are very low, trimethyl- (TML) and triethyllead (TEL) being below 0.43 and 0.45  $\mu\text{g/L}$ , respectively, in occupationally exposed workers [36]. The detection limit for TML was 0.15  $\mu\text{g/L}$  and 0.20  $\mu\text{g/L}$  for TEL using the analytical methodology described (see Table 7 and section 3.2.1). To date, only high performance liquid chromatography (HPLC) has been employed for the separation of alkyllead ions in urine [36], samples first being treated as described in Table 7 to mask inorganic lead and preconcentrate the species of interest. It is surprising that the extraction/derivatisation procedures used for the preparation of water, solids, tissues etc. (see Tables 4-7) for alkyllead determination by GC with an element specific detector (section 3.2.2) have not yet been applied to urine samples.

**2.4.2 Blood.** Nygren and co-workers have developed methods for the determination of tetraalkyl- [110] and ionic alkyllead [35] species in whole blood. The samples were hemolysed by freezing ( $-20\text{ }^\circ\text{C}$ ) for at least 24 hours; storage for extended periods under such conditions is possible. It was found essential to add fairly large amounts of buffer to obtain the desired pH for extraction (as blood has a considerable buffer capacity). Large excesses of EDTA and NaDDTC were also required to mask  $\text{Pb}^{2+}$  and to completely complex alkyllead ions, respectively, in the presence of competing metals. Details are given in Table 7. Nygren [186] has shown that the concentrations of trimethyl- and dimethyllead in blood are stable at  $4\text{ }^\circ\text{C}$  for one week, at  $-20\text{ }^\circ\text{C}$  for two months and at  $-70\text{ }^\circ\text{C}$  for one year. Conditions ensuring the stability of these species during transport have also been established [186].

TABLE 7 Decomposition and pretreatment methods for the determination of lead species in biological samples following chromatographic separation (section 3.2)

Sample type(s)	Compounds determined <sup>a</sup> (extraction efficiency, %)	Sample pretreatment <sup>b</sup>	Separation technique <sup>c</sup>	Ref
Urine	$\text{Me}_3\text{Pb}^+$ ; $\text{Et}_3\text{Pb}^+$	Adjust pH of 50 mL urine to 10 with 2 mol/L NaOH (1.5–4 mL), add 37 mg EDTA to complex metal ions and remove precipitation by centrifugation. Pump (10 mL/min) through extraction column (8 mm i.d.) packed with 500 mg silica gel (40–63 $\mu\text{m}$ ). Desorb $\text{Me}_3\text{Pb}^+$ and $\text{Et}_3\text{Pb}^+$ using 2 mL acetate buffer (pH 3.7) containing 10 % methanol. Add 4 mL borate buffer (pH 10) to dilute and adjust pH to 8.	HPLC (Table 9, entry 1)	[36]
Blood	$\text{Me}_2\text{Pb}$ (92.5)	Hemolyse by freezing at $-20^\circ\text{C}$ for > 24 h, extract 4–8 mL blood with 2 mL n-heptane in an ultrasonic bath for 20 min, centrifuge (5000 rpm) to facilitate phase separation and withdraw organic layer.	GC	[110]
Blood	$\text{Me}_3\text{Pb}^+$ (93)	Hemolyse by freezing at $-20^\circ\text{C}$ for > 24 h, add 15 mL ammonium citrate buffer (pH 9) containing 7 g/L EDTA + 1 mL 10 % NaDDTC in $\text{H}_2\text{O}$ to 5 mL blood in a 50 mL screw-capped polyethylene centrifuge tube. Extract twice with 5 mL pentane, separate and combine organic layers (after centrifugation) and derivatise (Table 2, entry 2).	GC <sup>d</sup>	[35]
Bird tissue (egg, kidney, liver, brain)	$\text{Me}_3\text{Pb}^+$ (83–105); $\text{Et}_3\text{Pb}^+$ (74–85); $\text{Me}_2\text{Pb}^{2+}$ (25–94); $\text{Et}_2\text{Pb}^{2+}$ (59–95)	Homogenise mechanically and incubate 2.5 g homogenate in 20 mL of 5 % ethanol/0.5 mol/L $\text{NaH}_2\text{PO}_4$ (pH 7.5) containing 40 mg each of lipase (type VII) and protease (type XIV) for 24 h at $37^\circ\text{C}$ . Add 5 mL ammoniacal buffer (pH 10) to hydrolysate and extract three times with 10 mL 0.01 % dithizone in benzene/hexane. Centrifuge at 4400 rpm for 10 min, separate and combine organic extracts, and back-extract three times with 10 mL 0.15 mol/L $\text{HNO}_3$ . Neutralise with 4.5 mL 1 mol/L NaOH and add 5 mL ammoniacal buffer (pH 10) and re-extract ionic alkylleads three times using the dithizone solution. Derivatise according to Table 2, entry 1.	GC <sup>d</sup>	[34]
Fish tissue (intestine, flesh)	$\text{R}_2\text{Pb}$ ; $\text{Pb}^{2+}$ ; $\text{Me}_3\text{Pb}^+$ (86); $\text{Et}_3\text{Pb}^+$ (92); $\text{Me}_2\text{Pb}^{2+}$ (71); $\text{Et}_2\text{Pb}^{2+}$ (100)	Homogenise mechanically and digest 2 g homogenate in 5 mL 20 % TMAH in $\text{H}_2\text{O}$ at $60^\circ\text{C}$ in water bath for 1–2 h. Neutralise with 50 % HCl to pH 6–8, add 2 g NaCl + 3 mL 0.5 mol/L NaDDTC + 3 mL benzene and mix for 2 h on mechanical shaker. Separate a measured amount of benzene layer and derivatise (see Table 2, entry 1).	GC <sup>d</sup>	[98]
Grass, tree leaves	$\text{Me}_3\text{Pb}^+$ (104); $\text{Et}_3\text{Pb}^+$ (84); $\text{Me}_2\text{Pb}^{2+}$ (91); $\text{Et}_2\text{Pb}^{2+}$ (87)	Pulverise sample in liquid $\text{N}_2$ in a mortar. Add 30 mL 25 % TMAH in $\text{H}_2\text{O}$ to 1 g pulver in a beaker and swirl for 2 h on magnetic stirrer. Dilute unfiltered digest to 500 mL with $\text{H}_2\text{O}$ , adding citric acid monohydrate and ammonia to pH 9. Add citric acid and 5 g EDTA to mask $\text{Pb}^{2+}$ . Transfer to 1 L separating funnel, add 2 mL 0.25 mol/L NaDDTC + 20 mL pentane, shake for 2 min, collect organic layer in centrifuge tube together with 5 mL pentane used to rinse separating funnel. Add 1 mL ethanol and centrifuge (4500 rpm) for 5 min. Separate organic layer, and repeat pentane extraction and centrifugation steps twice, combine pentane phases and derivatise according to Table 2, entry 2.	GC <sup>d</sup>	[37]

<sup>a</sup> R =  $\text{CH}_3$  (Me) or  $\text{C}_2\text{H}_5$  (Et)<sup>b</sup> EDTA = ethylenediaminetetraacetic acid, NaDDTC = sodium diethyldithiocarbamate, TMAH = tetramethylammonium hydroxide<sup>c</sup> HPLC = high performance liquid chromatography (see section 3.2.1), GC = gas chromatography (section 3.2.2) with lead-specific detection (Table 10)<sup>d</sup> Derivatization of ionic alkyllead species prior to determination (see Table 2)

**2.4.3 Tissue samples.** For tissue samples, mechanical homogenisation is the primary stage. The tissue homogenate can then be digested in tetramethylammonium hydroxide (TMAH) [98] or, alternatively, enzymatic hydrolysis using lipase and protease can be applied [34]. In the latter procedure amino nitrogen is released from the sample during hydrolysis, and the recovery of methylated lead ions is observed to improve [34]. It is important that relatively mild tissue solubilisation conditions prevail to avoid complete destruction of alkyllead species, and the aforementioned procedures (see Table 7) appear to be most suitable in this context.

Forsyth [198] has developed a purge and trap system to isolate volatile TALs and butylated ionic alkyllead compounds from crude organic extracts prepared according to the fourth entry in Table 7. The extracts were ballistically heated to 165 °C or 180 °C for TAL or butylated ionic alkyllead compounds, respectively, over a 30 min period. The volatilised species were purged from the sample vessel by a flow of N<sub>2</sub> and collected in an impinger, containing glass beads, cooled in a methanol-dry ice bath. After cooling, 0.5 mL n-hexane was added to the sample vessel, heated and purged for a further 5 min to flush any residual lead species into the impinger. Analysis was then performed using GC with detection by atomic absorption spectrometry. This clean-up procedure was applied to the determination of organolead species in various lipid-rich matrices (animal and vegetable fats), providing recoveries in excess of 90 % for TALs and butylated ionic alkyllead compounds. Without purge and trap clean-up, such lipid-containing sample extracts rapidly contaminate the GC column, resulting in a loss in chromatographic resolution [198]. Sample clean-up is a frequently neglected aspect of lead speciation studies, despite its practical importance when employing chromatographic separation techniques, and the efforts made by Forsyth [198] are therefore commendable.

At this point it should be mentioned that Szpunar-Lobinska et al. [176] determined trimethyl- and triethyllead in protease and lipase at concentrations between 8 and 123 ng/g, which represent a considerable source of contamination in sample preparation by enzymatic hydrolysis. Trimethyllead was also detected (at about 5 ng/g) in an older bottle of TMAH.

**2.4.4 Tree leaves and grass.** In the aftermath of the controversy concerning the hypothesis that ionic alkyllead compounds were responsible for the decline of wooded areas in the Black Forest, Germany [21,83,111-114], van Cleuvenbergen et al. [37] developed a method to extract such species from grass and tree leaves. (Note that the analytical data presented are summarised in Table 1). This appears to be the most reliable procedure currently available for this important matrix type, and is outlined in Table 7. Following extraction, the ionic alkyllead species are butylated (see Table 2) and determined by the techniques discussed in section 3.2.2.

### 3 ANALYTICAL TECHNIQUES AND SPECIATION SCHEMES

In this section, the most important analytical techniques for lead speciation in environmental and biological samples are considered. Except in those cases where a chromatographic separation is combined with a lead-selective detector, specific species are not usually identified. (One exception is the use of X-ray spectrometry for the determination of inorganic lead species in solid samples - see section 3.3). Instead, groups of species are identified on the basis of gross behavioural differences and physicochemical properties. Such speciation schemes are operationally defined, and their limitations should be clearly understood. Speciation schemes for natural waters are discussed in sections 3.1 and 3.4, and those for solid samples (sediment, soil and particulate matter) in sections 3.3 and 3.4.

#### 3.1 Electrochemical techniques

Electroanalytical methods have been widely employed in the study of bioavailability and toxicity of the various physicochemical forms of trace metals present in natural waters [6,8,11,17,30,40,115]. In the scope of this review, only anodic stripping voltammetry (ASV) will be considered, as this is most

commonly used and, because of the preliminary concentration step, provides lowest detection limits (1-40 ng/L depending on the electrode configuration and modulation waveform).

In order to determine the various physicochemical forms of lead in a water sample, ASV must be combined with a speciation scheme. Several such schemes have been devised and one recent example is given in Table 8 [30]. One important advantage with this procedure is that the labile fraction 2 in seawater is obtained without any reagent addition, and with minimum sample handling. The labile fraction is regarded as being bioavailable, and in some cases has been shown to be highly correlated with metal toxicity. In the case of lead, the lipid-soluble fraction may also be significant in terms of toxicity, due to the presence of alkyllead species.

**TABLE 8.** Speciation scheme for lead in natural water samples using anodic stripping voltammetry. Adapted from Ref. [30]

Subsample <sup>a</sup>	Pretreatment	Physicochemical form of metal
1.	Add HNO <sub>3</sub> to 0.05 mol/L + 0.1 % H <sub>2</sub> O <sub>2</sub> . UV irradiate for 8 h, adjust pH to 4.7 with acetate buffer	Total
2.	For fresh waters, buffer with 0.025 mol/L acetate to pH 4.7. Seawater untreated	ASV-labile
3.	UV irradiation in presence of 0.1 % H <sub>2</sub> O <sub>2</sub> at natural pH	Organically bound (by difference, 3-2)
4.	Pass through ion exchange column containing Chelex 100 resin	Very strongly bound
5.	Extract with hexane - 20 % n-butanol. Retain aqueous phase and treat as in 1	Lipid-soluble (by difference, 1-5)

a Each subsample 10 - 20 mL, previously filtered

As seen from Table 8, three of the subsamples are subjected to UV radiation, which can be performed *in situ* in the measurement cell, which is covered with a quartz beaker to minimise contamination risks. Procedures for the UV radiation of water samples, in the context of ASV speciation schemes, have been given [116,117].

It is important to note that electroanalytical speciation schemes are operationally defined, which means that the parameters determining the reduction of lead species at the electrode must be well-controlled and documented. For example, the labile lead fraction will be affected by the deposition potential, electrode surface area, pulse frequency, stirring rate, pH, temperature and buffer composition [17,30]. When measuring the ASV-labile fraction, it is important to use an ionic metal peak calibration curve rather than standard additions. In many natural waters, part of the added metal ions can be complexed and the increase in the signal will not correspond to the change in the concentration. Furthermore, the addition of lead ions might cause the equilibrium distribution in the samples to be disturbed. It should also be realised that in the very act of measurement, the current through the solution disturbs the ionic equilibria, although the same is equally true for most other separation/speciation techniques.

Another important point to consider is that the deposition potential should not be more negative than required - otherwise there is a risk that directly reducible lead complexes may contribute to the signal. This means that multielement determinations are not to be recommended in speciation schemes.

A frequently observed problem with ASV is caused by the adsorption of organic matter on the mercury electrode, resulting in a reduced diffusion current. This problem can be minimised by covering the electrode with a permselective coating of Nafion, cellulose acetate or other suitable materials [118-120].

### 3.2 Chromatographic separation

**3.2.1 Liquid chromatography.** The separation of lead species by high performance liquid chromatography (HPLC) offers a number of potential benefits. These include minimal preparation of liquid samples and separation at ambient or slightly elevated temperatures avoiding thermal decomposition risks for unstable lead species. Even involatile and inorganic lead compounds should be amenable to separation by HPLC. However, to date, relatively little use of HPLC has been made in lead speciation studies, which is not surprising, given that most instruments are equipped with electrochemical or spectrophotometric on-line detectors, which are not ideal in several respects for lead detection [121-123]. For electrochemical detection, lead species must be reduced at the electrode, which limits the choice of electrode materials to mercury or gold. The presence of dissolved oxygen in the mobile phase may also present problems, as  $O_2$  is electroactive under reducing conditions [124]. Thus oxygen must be excluded from the effluent or specialised electrochemical detection techniques be used [124] to enable lead speciation.

To render lead compounds detectable by spectrophotometry, a post-column chemical reactor is required. Blaszkewicz et al. [125] spectrophotometrically determined trialkyllead species at 515 nm in HPLC effluent after decomposition into dialkyllead by iodine and formation of 4-(2-pyridylazo)resorcinol (PAR) complexes. For application to urine [36] and natural water [78] samples (instrumental conditions given in Table 9), a preliminarily, off-line, solid-phase enrichment was necessary (see Tables 4 and 7), together with an on-line, pre-column concentration step. These procedures are necessary, since detection limits are in the nanogram range, and the sample must be cleaned up to eliminate potentially interfering concomitants which also form PAR complexes [125]. The accuracy of the overall procedure was verified by comparison with a mass spectrometric detector [126], and the detection limits [36] for trialkyllead species were in the range 150-200 ng/L for an initial 50 mL urine sample ( $3\sigma$  background criterion).

There is an obvious need for highly sensitive and selective, on-line HPLC detectors, and numerous attempts have been made to employ atomic spectrometric devices, see for example [14,127]. Direct coupling of HPLC to detectors using either flame atomic absorption spectrometry or inductively (or direct) coupled plasma (ICP) atomic emission spectrometry is fairly straightforward due to the continuous flow operational characteristics. However, the sensitivities of these detectors (particularly the former) are not sufficiently good for lead speciation in most sample types of interest, although some improvements as regards ICP-based detectors have recently been made [128,129]. Although preconcentration procedures could be included, such as that used by Blaszkewicz et al. [36,78], the speciation scheme would become more complicated and risks for contamination and analyte losses would increase. Furthermore, the use of gradient elution HPLC conditions will alter the nebulisation efficiency (and hence sensitivity) during the chromatographic run, and certain mobile phases are incompatible with such detectors [14].

Attempts to couple HPLC to electrothermal atomic absorption spectrometry (ETAAS), and thus exploit the latter's very high sensitivity and specificity for lead, are generally considered to be fraught with difficulty due to the discrete sampling mode of ETAAS. Typically, a fraction collector is used to assemble discrete samples of column effluent (50-500  $\mu$ L) prior to subsequent ETAAS determination [130]. Alternatively, the HPLC effluent can be continuously passed through a small volume "well sampler", with aliquots being withdrawn and injected into the graphite tube periodically [131]. The major drawback with such approaches is that only well-separated species can be distinguished. Judging from a chromatogram shown in Ref. [131], this particular HPLC-ETAAS approach leaves much to be desired, as inadequate resolution of the five tetraalkyllead species is clearly evident under the conditions used, and is thus not to be recommended.

Weber [109] utilised the first-mentioned approach in a preliminary investigation of inorganic lead species in urine by HPLC-ETAAS. Both chloride and sulfate forms were detected in samples spiked with  $Pb^{2+}$  using the conditions given in Table 9.

The first real on-line coupling of HPLC to a continuously heated graphite tube atomiser was described by Nygren et al. [132], who employed a thermospray interface for the determination of tin species. This system should also be suitable for lead speciation, although some limitations concerning the relatively low (up to 0.2 mL/min) effluent flow rate and choice of mobile phase constituents must be borne in mind. A

fundamental drawback with the on-line coupling of HPLC-ETAAS results from the massive expansion of effluent on being heated from near ambient to temperatures in excess of 1000 °C, which drastically reduces the residence times of analyte atoms in the measurement zone [132]. Consequently, interfaces which can facilitate effective desolvation and deliver a dry aerosol to the graphite tube atomiser would be beneficial. Several such interfaces have been described [133-135] and their evaluation for lead speciation by coupled HPLC-ETAAS should be of interest.

Blais and Marshall [79] have utilised quartz T-tube atomic absorption spectrometry (QTAAS) as an ionic alkyllead specific detector for HPLC using a thermospray-microatomiser interface. The methanol (70-100 %) effluent was introduced via a fused silica capillary transfer line through a heated (700-1000 °C) side arm on the stem of the quartz T-tube atomiser, and combusted in the presence of oxygen. See Table 9 for further details and chromatographic conditions. In a later modification [136], post-column ethylation was used to convert the ionic alkyllead species into their volatile tetraalkyllead derivatives. These were then purged from the reaction mixture by hydrogen in a gas-liquid separator and transferred to an electrically heated QT atomiser. Absolute detection limits in the range 0.1-0.2 ng (expressed as Pb) for ionic alkyllead compounds [136] were reported, roughly an order of magnitude improvement over the aforementioned thermospray interfaced system [79].

Production of volatile lead derivatives in a post-column reactor is a conceptually more attractive approach to coupling HPLC to atomic absorption-based detectors. This is because the aforementioned problems with effluent expansion during heating to atomisation temperatures are avoided. With the fairly high gas flow used in the gas-liquid separator (50 mL/min H<sub>2</sub>), a quartz T-tube is also preferable to a graphite tube atomiser due to the longer path length and hence higher sensitivity, providing that sufficient temperatures can be reached in the former to completely atomise the analyte species, and that the band-broadening contribution of the detector does not seriously impair the resolution of the lead species.

The introduction of inductively coupled plasma - mass spectrometry (ICP-MS) has presented new possibilities for element-specific HPLC detection [169]. Most work reported to date has been focussed on optimising the separation mode (reversed-phase, ion-pairing or ion-exchange) and conditions [159-161]. Al-Rashdan et al. [159] observed that ICP-MS gave absolute detection limits (in the range 25-87 pg for inorganic-, trimethyl- and triethyllead) that were three orders of magnitude improved over ICP-AES utilising conventional nebulisation of the HPLC effluent. Using a direct injection nebuliser (DIN) to interface a narrow-bore HPLC column to ICP-MS, Shum et al. [161] obtained absolute detection limits for the same three lead forms of about 0.2 pg in urine. Advantages of the HPLC-DIN-ICP-MS combination include [161]: (i) Complete transfer of the HPLC effluent to the ICP owing to the high efficiency of the DIN interface; (ii) Low dead volume of the DIN (< 2 µL) and absence of a spray chamber minimises post-column band broadening which could otherwise compromise the resolution achieved; (iii) Compatibility with narrow-bore HPLC columns and eluent flow rates (0.03 - 0.1 mL/min) which are most effective in terms of chromatographic separation characteristics. More details are given in Table 9, entry 5.

Although analytical applications of coupled HPLC-ICP-MS systems has so far been rather limited [159,161], it is anticipated that this instrumental combination will soon find more widespread utility in speciation studies.

Another interesting development for lead-specific detection in HPLC comprises the use of laser-enhanced ionisation (LEI) spectrometry as demonstrated by Epler et al. [187]. In the LEI configuration used, a pair of dye lasers induced two-step photoexcitation of the lead atoms produced in an air-acetylene flame, prior to collisional ionisation. By applying a potential across electrodes in the flame, the current resulting from the lead ions was measured. The HPLC-LEI instrumentation was applied to the determination of lead compounds in NIST SRM 1566a "Oyster tissue" following a sample preparation procedure similar to that reported for fish tissue in Table 7. Triethyllead was identified in several samples at the low to sub-ng/g level, although there was a high degree of within-bottle variation. No alkyllead species at all were detected in a second bottle of "Oyster tissue". These variations could be accounted for in terms of inhomogeneity or differences in storage conditions which could have affected the stability of the triethyllead [187]. It was also noted that the recoveries of alkyllead spikes were below 50 % (i.e. much poorer than those for fish tissue reported in Table 7) and the need to improve the sample preparation methodology was highlighted.

TABLE 9 Chromatographic conditions and detection systems used for the speciation of lead by high performance liquid chromatography (HPLC)

Sample type(s)	Sample preparation <sup>a</sup>	Compounds detected <sup>b</sup> (absolute detection limit)	Chromatographic conditions <sup>c</sup>	Detection <sup>a,d</sup>	Ref.
1A Rain, snow, natural waters	See Table 4 [78]	Me <sub>3</sub> Pb <sup>+</sup> (7.5 ng); Me <sub>2</sub> EtPb <sup>+</sup> ; MeEt <sub>2</sub> Pb <sup>+</sup>	5 mL sample injected at 2 mL/min onto a pre-column (20 x 4 mm i.d.) packed with Nucleosil 10-C <sub>18</sub> . Retained compounds eluted in back-flush mode using 20 % (v/v) methanol in 0.1 mol/L acetate buffer (pH 4.6) onto analytical column (250 x 4.6 mm i.d.) packed with Partisil ODS 3, 5 µm, at 2 mL/min	Trialkyllead species reduced to dialkyllead forms by I <sub>2</sub> and Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> at 50 °C. Dialkylleads complexed by 4-(2-pyridylazo)-resorcinol at 1-5 °C, pH 9-10, and detected spectrophotometrically at 515 nm	[36] [78] [125]
1B Urine	See Table 7 [36]	Et <sub>3</sub> Pb <sup>+</sup> (10 ng)			
2 Urine (spiked)	Filtration (0.45 µm)	PbCl <sub>2</sub> ; PbSO <sub>4</sub>	50, 100 or 200 µL sample injected onto pre-column (30 x 4 mm i.d.) and flushed onto analytical column (250 x 4 mm i.d.) at 1 mL/min. Both columns packed with Spherisorb S5 ODS 1. Mobile phase 100 % 0.04 mol/L H <sub>3</sub> PO <sub>4</sub> in H <sub>2</sub> O for 5 min, linear gradient to 95 % 0.04 mol/L H <sub>3</sub> PO <sub>4</sub> in methanol after 50 min	Lead determined in collected fractions (20 per min) by electrothermal atomic absorption spectrometry. Instrumental conditions not reported	[109]
3 Water, soil, sediment	See Tables 4, 5	Me <sub>3</sub> Pb <sup>+</sup> (1.0 ng); Et <sub>3</sub> Pb <sup>+</sup> (1.7 ng); Me <sub>2</sub> Pb <sup>2+</sup> (1.5 ng); Et <sub>2</sub> Pb <sup>2+</sup> (1.8 ng); Pb <sup>2+</sup> (3.4 ng)	25-75 µL sample injected onto analytical column (150 x 4.6 mm i.d.) packed with 5 µm Nucleosil C <sub>18</sub> via a pre-column filter with Nucleosil C <sub>18</sub> insert. Mobile phase methanol-water (3 + 1 v/v) containing 0.6 g/L NH <sub>4</sub> TMDTC, flow rate 1 mL/min	Lead absorbance monitored in a 100 x 7 mm i.d. quartz T-tube (heated to 800 °C) at 283.3 nm. Column effluent (fuel) introduced via heated side-arm on stem of T, and combusted lead species atomised with O <sub>2</sub> (200 mL/min) in 1400-1600 °C flame, prior to detection	[79]
4 Standard solutions	—	Me <sub>3</sub> Pb <sup>+</sup> (0.12 ng); Et <sub>3</sub> Pb <sup>+</sup> (0.15 ng); Me <sub>2</sub> Pb <sup>2+</sup> (0.10 ng); Et <sub>2</sub> Pb <sup>2+</sup> (0.13 ng);	As in 3 above, except 300 x 4.6 mm i.d. analytical column, and mobile phase methanol-water (4 + 1 v/v) containing 0.6 g/L NH <sub>4</sub> TMDTC	Effluent mixed with 0.1 % m/V NaBEt <sub>4</sub> (flow rate 1 mL/min) in post-column reactor at 70 °C. Volatile ethylated species purged from solution by 50 mL/min H <sub>2</sub> in gas-liquid separator and transferred to 100 x 7 mm i.d. quartz T-tube atomiser at 1000 °C. Lead absorbance monitored at 283.3 nm	[136]
5 Urine (NIST SRM 2670)	EDTA added at 10 mg/L	Me <sub>3</sub> Pb <sup>+</sup> (0.2 pg); Et <sub>3</sub> Pb <sup>+</sup> (0.2 pg); Pb <sup>2+</sup> (0.2 pg)	2 µL sample injected at 0.1 mL/min onto analytical column (50 x 1.6 mm i.d.) packed with CETAC C <sub>18</sub> material. Mobile phase 5 mmol/L ammonium pentane-sulfonate in acetonitrile-water (1 + 4 v/v), pH 3.4, saturated with silica by passage through an Adsorbil filled pre-column	Lead monitored in column effluent at <i>m/z</i> = 208 using ICP-MS interfaced to the HPLC system via a direct injection nebuliser	[161]

<sup>a</sup> EDTA = ethylenediaminetetraacetic acid<sup>b</sup> Me = CH<sub>3</sub>, Et = C<sub>2</sub>H<sub>5</sub><sup>c</sup> NH<sub>4</sub>TMDTC = ammonium tetramethylethiobarbamate (pyrrolidinedithiocarbamate)<sup>d</sup> ICP-MS = inductively-coupled plasma - mass spectrometry



As the absolute detection limits obtained were, at best, 20 pg for tetraethyllead (about two orders of magnitude poorer than for HPLC-DIN-ICP-MS [161]), and due to the expensive equipment employed, HPLC-LEI is likely to remain a specialised research tool rather than a routine technique for speciation studies.

**3.2.2. Gas chromatography.** As only volatile, thermally stable compounds are amenable to separation by gas chromatography (GC), ionic alkyllead and  $\text{Pb}^{2+}$  species must be derivatised to their tetraalkyl (or alkyl/phenyl [62]) analogues (see Table 2). Ionic lead compounds exhibit very poor chromatographic characteristics [57,137], hence the desire to derivatise.

Both packed and capillary columns have been used for the separation of organic lead species by GC, with the former being most popular so far, possibly due to convenience in re-packing, ease of handling and large sample capacity. On the other hand, capillary columns provide much better resolution and have been used together with an electron capture detector (ECD) [138], where the better separation efficiency reduces the risks for co-elution of potentially interfering compounds with the alkyllead species of interest. However, the ECD and other standard GC detectors generally lack the necessary selectivity for the determination of lead species. Therefore a variety of atomic and mass spectrometric detectors have been coupled to GC systems. Here, only the most sensitive and widely used detectors will be considered (see Table 10).

Microwave-induced plasma-optical emission spectrometry (MIP-OES) is inherently a multi-element detection system, useful for determining essentially all elements in the periodic table when helium is employed as plasma gas [139]. This allows, in principle, the empirical elemental ratios to be determined for the species eluting from the GC system, permitting greater certainty in compound identification [140]. Absolute detection limits for lead using MIP-OES are also very good, typically below 1 pg [57]. Various MIP cavity designs have been described and are discussed in an excellent review by Zander and Hieftje [139], the most applied, until recently, being the so-called Beenakker  $\text{TM}_{010}$  configuration [141] which is operated at atmospheric pressure. For coupling to a capillary column with its low helium carrier gas flow-rate, it is necessary to separately control an additional plasma gas flow to provide a stable discharge. Further performance benefits are obtained by introducing a tangential, shielding gas flow between two concentrically arranged tubes, which helps to centre the plasma and hinders deposition of sample residues in the discharge tube [142]. The MIP discharge can not, however, tolerate large sample loads, and a suitable venting system is necessary to avoid it being extinguished by the solvent [57,137,143]. Other disadvantages include the complex background emission which arises in the presence of sample and the potential risk for spectral interferences [144], and so some form of background correction is to be recommended. Several successful applications of MIP-OES as a GC detector in earlier lead speciation studies have been described [57,137,145,146].

Undoubtedly the most exciting development in the field of GC-MIP-OES has been the introduction of a new commercial instrument [143,144] featuring numerous technical innovations. The plasma is generated within a water-cooled discharge tube installed in a novel re-entrant microwave cavity [143]. A computer-controlled, moveable photodiode array is mounted at the spectrometer focal plane, permitting the simultaneous background corrected detection of up to four elements in a spectral window of about 30 nm [144]. Using this system, Lobinski and Adams [165,166] obtained absolute detection limits for organolead species in the range 0.02 to 0.1 pg (as Pb), which represent the best values ever reported. A method for the comprehensive speciation of organolead compounds in polar snow at levels down to fg/g (as Pb) has been developed [167] and used to measure tri- and diethyllead in Greenland ice cores. In an extension of this work [188,189], the determination of ionic methyl- and ethyllead species in Greenland snow samples was used to identify pollution sources in combination with backward air mass trajectory data.

The development [190] and application of analytical methodology based on GC-MIP-OES to elucidate the origins of alkyllead contamination in wines [191] has also been reported.

Atomic absorption spectrometry (AAS) is a sensitive and highly selective technique, relatively free from spectral interference effects, but which lacks the inherent multi-element capability of emission

**TABLE 10** Examples of typical gas chromatograph and specific detector components and operating conditions used for the determination of lead species in environmental and biological samples<sup>a</sup>

A. Gas chromatography (GC)				Ref.	
Column <sup>b</sup>	Column temperature <sup>c</sup>	Carrier gas (mL/min)	Injection conditions (volume)	Detector used <sup>a,d</sup>	
50 m x 0.32 mm i.d.; OV-101, 0.2 µm film thickness	30 °C for 1 min, then to 220 °C at 16 °C/min with 5 min hold	H <sub>2</sub> (3.5)	On-column at ambient temperature (2 µL)	ETAAS	[35]
25 m x 0.32 mm i.d.; HP-1, HP-1, 0.17 µm film thickness	45 °C for 3 min, then to 200 °C at 20 °C/min with 0.5 min hold	He	Temperature-programmed cool injection system (25 µL)	MIP-OES	[165-167,176] [182,188-191]
30 m x 0.25 mm i.d.; SPB-1, 0.25 µm film thickness	50 °C for 5 min, then to 175 °C at 15 °C/min with 5 min hold	He	Splitless injector, 250 °C (2 µL)	MS, single ion monitoring mode	[196,197]
2.1 m x 2 mm i.d.; 3 % OV-73 on Chromosorb WHP 100/120 mesh	40 °C for 1 min, then to 140 °C at 15 °C/min, to 170 °C at 10 °C/min, to 200 °C at 15 °C/min with 5 min hold	He (30)	Splitless injector, 200 °C	QTAAS	[199]
B. Specific detectors				Ref.	
Detector system <sup>d</sup>	Conditions	Auxiliary gas flows (mL/min)	Detector dimensions	Detection limits	
ETAAS	Graphite tube atomiser operated at 1000-1100 °C	—	9 mm x 3 mm i.d.	8-16 pg	[35]
MIP-OES	Microwave cavity temperature 250 °C	He (300) + H <sub>2</sub> + O <sub>2</sub>	—	0.025-0.1 pg	[165]
MS	Detector temperature 280 °C, electron impact ionisation	—	—	0.66-3.4 pg	[196,197]
QTAAS	Electrically heated quartz tube operated at 900 °C [138]	H <sub>2</sub> (50)	100 mm x 7 mm i.d.	1.6-2.3 pg	[199]

<sup>a</sup> Note that, with a suitable interface (usually operated at or above the maximum column temperature) and due consideration of the gas flow requirements of the GC and detector systems, the components listed may be interchangeable

<sup>b</sup> In general, non-polar, i.e. dimethyl polysiloxane stationary phases can be recommended for the separation of TALs and derivatised ionic alkyllead species

<sup>c</sup> Temperature programming is advisable to obtain good peak shapes for late eluting compounds and to minimise total analysis time

<sup>d</sup> ETAAS = electrothermal atomic absorption spectrometry, MIP-OES = microwave-induced plasma - optical emission spectrometry, MS = mass spectrometry, QTAAS = quartz (or alumina or silica) tube atomic absorption spectrometry

spectrometry. For lead speciation studies involving GC separation, AAS is used most extensively. Atomisers capable of providing a temperature of around 1000 °C are sufficient to completely decompose alkyllead species in the presence of H<sub>2</sub> [21,25,147]. Thus, graphite tube atomisers [35,100,101,110,148] and electrically- and flame-heated quartz tubes [12,15,21,25,58-63] are all suitable atom cells for lead determination in GC effluents by AAS, and a variety of simple interfaces have been described (see Table 10 and cited literature). So far, however, no systematic studies have been performed to optimise the dimensions of the atom cell with respect to detection limits and peak resolution for lead species (or any other analyte for that matter). It should be realised that a number of factors are important for achieving optimum performance from the GC-AAS combination, such as carrier gas flow, optical configuration, length and diameter of the atomiser, dead volume etc. Some compromise will obviously be necessary, and interesting observations on this subject have been made by Andersson et al. [110]. These authors inserted the end of the fused silica column directly into the graphite tube atomiser, and to avoid rapid destruction of the quartz, used a fairly high hydrogen carrier gas flow rate (5 mL/min) to cool the interface. This effectively reduced the column efficiency and residence times of lead atoms in the graphite tube. These undesirable effects could have been minimised by using a glassy carbon interface [132] where additional cooling would be unnecessary.

Detection limits obtained by GC-AAS systems for organolead species vary widely depending on the type of atomiser used and the nature of the column. Comparisons of such figures of merit have been compiled by van Cleuvenbergen et al. [162] and by Lobinski and Adams [165]. From such compilations, the following recommendations can be made for coupling GC and AAS instruments in such a way as to yield the best possible detection limits: (i) Open tubular (capillary) columns are to be preferred, as these provide higher resolution and sharper, more concentrated solute bands than packed columns [110]. Thus capillary columns give greater peak heights per analyte mass injected. In addition, operation at lower carrier gas flow rates is possible, increasing the residence time of analyte in the atomisation cell; (ii) Atomisers of the quartz tube type generally result in higher sensitivity than graphite tube systems (owing to the greater cell lengths typically employed), which often improves the signal-to-noise ratio for a given analyte mass [163].

Mass spectrometry (MS), despite being a commercially available GC detector, has seldom been used in lead speciation studies. This is probably the result of a combination of factors including relatively high cost and no better detection limits in comparison with the best of the AAS based detectors. What GC-MS does offer is the ability to determine the exact identity of detected species in many cases, not just the presence of lead in the molecule [23]. In this respect, GC-MS is particularly useful in confirming the structure of derivatised lead compounds [35]. The use of isotope dilution MS also facilitates the monitoring and correction for potential decomposition of lead species during either the sample collection step or the subsequent analytical procedure [23]. Thus GC-MS is a valuable reference tool during method development. Recently, interest in GC-MS has been revived, with application to the separation and detection of atmospheric TALs collected on a solid sorbent consisting of a mixture of Tenax and Porapak (35 + 65) [196] and to the determination of trimethyllead in the urban dust [197] and synthetic rainwater samples discussed in section 2.2 [183].

The use of ICP-MS for detection in GC has now gained momentum, and suitable interface designs have been described [172,173]. Kim et al. [173] obtained detection limits for TALs at the picogram level, but no applications of GC-ICP-MS to environmental or biological samples have yet been reported.

**3.2.3 Supercritical fluid chromatography.** In recent years, supercritical fluid chromatography (SFC) has rapidly gained in popularity as an alternative separation technique to HPLC and GC [170]. It is possible to separate thermally labile, non-volatile and high molecular weight compounds not readily amenable to GC with shorter analysis times and less solvent use than required for HPLC [169]. In SFC, temperature, pressure and mobile phase composition can be controlled to effect the separation. Most frequently CO<sub>2</sub> is used as the mobile phase, to which a variety of modifier components may be added to optimise the solvating strength for the species under consideration [170].

The analyte species are chromatographed with the mobile phase in the supercritical state (above 7 MPa and 40 °C for CO<sub>2</sub>). As the mobile phase makes the transition from supercritical conditions to atmospheric pressure prior to the detector, net cooling takes place and so the interface must be heated to temperatures in excess of 150 °C to prevent condensation of the analyte [169,170]. Otherwise, interfacing SFC to ICP-MS is more straightforward than for HPLC, as the column effluents are introduced in the gas phase. Nevertheless, both CO<sub>2</sub> and the sample solvent cause some quenching of the plasma and a corresponding signal reduction. This may be particularly problematic when using pressure programming to separate the analyte species. It has been recommended that the stationary and mobile phases used for SFC should be selected so that the analytical peaks do not elute at higher pressures [171], to avoid sensitivity losses. Thus, despite the advantages of ICP-MS detection in terms of analyte selectivity and low limits of determination, some constraints are placed on the chromatographic conditions. In the single-ion monitoring mode, SFC-ICP-MS detection limits for organolead species in the range 0.5 to 10 pg (as Pb) have been reported and further application to environmental samples in the near future is to be expected [171].

### 3.3 Non-destructive spectroscopic techniques

By studying the interaction of X-ray radiation with solid samples, it is possible to identify the elemental composition independent of chemical combination because the transitions observed involve non-bonding electrons (for all but the lightest elements). Such techniques therefore permit lead determinations to be made on solid samples with the minimum of prior manipulation, which is ideal for speciation. The relative detection limits are, however, insufficient to determine lead at ultra-trace levels. On the other hand, lead tends to be accumulated on suspended solid matter and sediments in aqueous systems, or on airborne particles, topsoils and street dusts. For these types of samples, speciation of inorganic lead compounds has been performed using X-ray powder diffraction (XRD). Biggins and Harrison [149] found PbSO<sub>4</sub>, Pb<sup>0</sup>, PbSO<sub>4</sub>·(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, Pb<sub>3</sub>O<sub>4</sub>, PbO·PbSO<sub>4</sub> and 2 PbCO<sub>3</sub>·Pb(OH)<sub>2</sub> in street dust analysed by XRD, with the first mentioned compound being most frequently observed. With the same technique, O'Connor and Jaklevic [150] observed, among other compounds, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>·PbSO<sub>4</sub> in aerosol particulate samples. The lead salt was suggested to be associated with source activity, although this compound may also have been formed by interaction of automotive PbBrCl with atmospheric (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

Using scanning electron microscopy/energy dispersive X-ray spectrometry (SEM/EDX), the qualitative elemental composition and morphology of individual urban dust particles has been assessed [151]. Such information is useful, particularly when coupled with size fractionation techniques [149] since adsorption of pollutants on the surface of airborne (and waterborne) particles is an important factor in determining the origin, transport and deposition of lead compounds.

Doern and Wotton [174] collected airborne particulates from an urban industrial environment. Using an automatic SEM/EDX technique it was possible to identify various lead sources from the specific shape and composition of the particulates. For example, automotive exhaust was characterised by a Pb-Br or Pb-Br-Cl spectral pattern. However, because of special sampling requirements and restrictions inherent in the technique of analysing individual particles, such methods must be regarded as complementary to conventional chemical analyses [174].

### 3.4 Other spectroscopic techniques

Another technique capable of studying the distribution of lead in small sample cross sections is laser microprobe mass analysis (LAMMA). Lorch and Schäfer [152] localised lead in algal filaments using LAMMA, an approach which would appear to have great potential in investigating the mechanisms of lead accumulation in biological tissues. However, LAMMA is not species specific and thus limited in the present context.

Atomic absorption spectrometry using either a flame (FAAS) or electrothermal graphite tube (ETAAS) atomiser has proven to be extremely useful for total lead determinations in a variety of sample matrices of environmental [38,61,98,106,153] and biological [107,108] interest. For speciation studies, however, AAS must be combined with chromatography for separation (see section 3.2, above) or with some operationally defined speciation scheme, as discussed in the following paragraphs.

In Donnan dialysis, water samples are separated from receiver electrolytes by an ion exchange membrane. Cox et al. [154] used two electrolytes, 0 or 0.1 mol/L HCl (both containing 0.6 mol/L Na<sub>2</sub>SO<sub>4</sub>), to enrich free metal and labile complexes or total soluble metal, respectively. The lead concentrations were then determined in the two electrolytes by FAAS. Donnan dialysis was used for lead speciation in a lake water sample and results were compared to those of an electroanalytical scheme [155,156]. The "Donnan dialysis labile" fraction was found to lie between the very-labile and very- plus moderately-labile fractions as determined by anodic stripping voltammetry according to Figura and McDuffie [155,156]. This observation illustrates the difficulty in assigning different operationally defined fractions to any specific group of lead compounds, and emphasises the need for complementary speciation schemes to avoid drawing incorrect or irrelevant conclusions. One important disadvantage with Donnan dialysis is that it cannot be applied to saline samples, other limitations also being noted by Cox et al. [154].

The speciation of lead in soils and sediments is usually achieved by the sequential application of a series of chemical extractants. After treatment with a particular reagent, the sample is filtered, lead determined in the filtrate by FAAS or ETAAS, and then the next chemical extraction step commences. The success of the scheme depends largely on the selectivity of the individual reagents in extracting or dissolving each target phase. Numerous sequential extraction schemes have been presented and discussed in various reviews [7,86-93,157].

Up to six fractions may be separated by sequential extraction, termed (i) exchangeable; (ii) carbonate bound; (iii) easily reducible - manganese oxides and hydroxides; (iv) moderately reducible - iron oxides and hydroxides; (v) organic and (vi) residual [90]. The number of steps and the order of reagent application tends to vary considerably depending on the object of the study [7,157]. Difficulties with this type of approach may arise due to differences in the bulk composition of samples, for example the carbonate bound fraction obtained is highly dependent on the acidity and buffer capacity of the soil or sediment. As emphasised by Ure et al. [157], there is an acute requirement for interlaboratory comparisons and reference materials to aid in normalising this type of speciation work.

Efforts are also being made within the Commission of the European Communities Measurements and Testing Programme to normalise procedures used in the sequential extraction of elements, including lead, from soils and sediments. This has led to the preparation of several RMs certified for both total and aqua regia soluble concentrations [192], and the adoption of a three-stage sequential extraction protocol proposed by a European working group coordinated by the Measurements and Testing Programme [193]. This procedure involves the following three sequential extraction steps: (1) 0.11 mol/L acetic acid to extract the exchangeable, water and acid soluble fractions; (2) 0.1 mol/L hydroxylammonium chloride at pH 2 to dissolve reducible iron/manganese oxides and the associated metals; (3) 8.8 mol/L hydrogen peroxide followed by 1 mol/L ammonium acetate at pH 2 to extract metals from the oxidisable components such as organic matter and sulfides.

#### 4 CONCLUDING REMARKS

Many of the speciation procedures described rely heavily on the sampling strategy and on the extraction of the compounds of interest from the sample matrix. For speciation studies it has been recommended to employ fractionation *in situ* by dialysis or ultrafiltration, or immediately after sampling to avoid storage effects [175]. Evaluation of the efficiency of the extraction process is based on the recovery of standard species 'spikes' from the matrix under study. However, as has been frequently emphasised [37], a spiked standard species might react differently with the matrix compared to the intrinsic compound. Thus the recovery test might not accurately reflect the extraction of analyte species originally present in the sample.

This drawback can only be alleviated by the comparison of several radically different extraction procedures.

At present, the analytical methodologies for the determination of alkyllead species in essentially all sample matrices of environmental and biological interest are available. What remains to be done here is to perform interlaboratory comparisons to verify the accuracy of the diverse sample preparation and analytical techniques currently available for alkyllead determinations. Ideally, this type of work should lead to the production of certified reference materials (CRMs), and thus enable all workers in the field to perform quality control checks of their procedures. Some progress in this area is now being made under the auspices of the Measurements and Testing Programme. An interlaboratory exercise has been initiated to evaluate the performance of analytical techniques used for the determination of trimethyllead in simulated rainwater and urban dust [183]. It is anticipated that a certification campaign will commence during 1995, ultimately leading to the preparation of CRMs for the aforementioned sample types.

A clear trend in recent work on lead speciation has been the coupling of ICP-MS with the various chromatographic techniques. The instrumental combination HPLC-ICP-MS appears to be a particularly attractive alternative to GC-atomic spectrometric techniques and will become of increasing importance in the future. As a variety of suitable detection systems are now available, more attention must be diverted towards improving and verifying sample preparation methodologies.

Although current legislation has greatly curtailed the levels of TAL additives in petrol, and various incentives have led to the increased use of unleaded fuel in many parts of the world, recent data indicates that pollution problems will continue for some time [188,194]. Indeed, the concentrations of alkyllead compounds in rainwater have not been reduced as dramatically as inorganic lead levels [194]. There is also evidence to suggest that alkyllead compounds may be more persistent in the atmosphere [188] than expected on the basis of laboratory experiments. Therefore it is anticipated that the need to speciate lead in environmental and biological samples will remain as acute for the next 10-20 years [195].

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