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THE DETERMINATION OF MERCURY SPECIES IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

(Technical Report)

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The determination of mercury species in environmental and biological samples (Technical Report)

Abstract: Mercury is released into the hydrosphere, atmosphere and biosphere as a consequence of natural and anthropogenic processes. It is cycled in the environment and undergoes transformations of its chemical forms. Although the number of chemical species encountered in environmental and biological samples is not large, the range of matrices and the toxicological significance of mercury, with a consequent need for ultra-trace determination, have resulted in a considerable analytical literature. Sensitive and selective methods for mercury determination, micro methods for the determination of mercury species, the occurrence of mercury species in the various environmental compartments, and analytical considerations, including the availability of certified reference materials, are reviewed.

INTRODUCTION

Mercury occurs naturally as the metallic form and/or its sulfide ores such as cinnabar (HgS). A small concentration of mercury is found throughout the lithosphere, the atmosphere, the hydrosphere and the biosphere. The earth's crust contains 0.5 mg kg^{-1}, ambient air may contain 0.002–0.02 μg dm^{-3}, and sea water contains about 0.03 mg dm^{-3}. Mercury is also found in trace amounts in most animal and plant tissues. Recent estimates of global emissions of mercury to the atmosphere are highly variable, ranging from 2 000–3 000 t $year^{-1}$ to 6 000 t $year^{-1}$, because of the uncertainty about natural emission rates.

Elementary mercury finds extensive use industrially in lamps, batteries, thermometers, and as amalgams, and especially in the electrolytic manufacture of chlorine and sodium hydroxide.

Mercury compounds have been used as catalysts, fungicides, herbicides, disinfectants, pigments and for other purposes. The world production was about 10 000 t in 1973 [1] and about 6 500 t in 1980 [2]. In addition to the production of pure mercury by industrial processes, mercury is released into the environment by human activities such as the combustion of fossil fuels, waste disposal and by industry. Recent estimates of anthropogenic emissions are in the order of 2 000 to 3 000 t $year^{-1}$ [3–6].

Mercury cycles in the environment and undergoes transformations of its chemical forms. In the atmosphere mercury moves in its volatile forms such as elemental vapour or methylmercury, as well as in particulate-bound forms. In the marine and terrestrial environments, inorganic mercury is methylated to methylmercury species which are readily accumulated in marine organisms. A portion of environmental mercury becomes bound to sulfur, producing insoluble HgS which accumulates in sediments. On land, some plants are known to concentrate Hg as less-toxic chemical forms such as elemental Hg droplets or as HgS. To understand the environmental cycling of Hg, it is necessary to know the chemical forms of the element that exist in each compartment.

Mercury and its compounds are considered health hazards, and reports of Hg poisoning because of industrial, agricultural, and laboratory exposure as well as its suicidal use are numerous.

The toxicity of mercury is known to be highly dependent on its chemical form: organomercury is generally more toxic than inorganic mercury salts. Elementary mercury and insoluble HgS are the least toxic. Mercury accumulated in the tissues of fish is usually in the form of methylmercury, a highly toxic form.

The compounds and other physico-chemical forms of mercury may be classified in different ways, and the term 'speciation' has been used with different meanings by scientists who have different backgrounds and different interests in mercury studies. For toxicologists, for example, it is important to know whether mercury is in an organic form or an inorganic form. Massive human intoxication cases have been reported for methylmercury at Minamata, Japan and in Iraq. As well as toxic monoalkylmercury

compounds, dialkylmercury is also known to be toxic. New mercury pollution cases in developing countries have resulted from the use of amalgamation in gold mining and changes of chemical form in the environment have been reported. Thus, from this toxicological point of view, it is apparently necessary to 'speciate' mercury. Lindquist *et al.* [5] proposed speciation of mercury compounds into three categories, volatile species (Hg), reactive species (Hg^{2+}, HgO on aerosol particles, Hg^{2+} complexes with OH, Cl, Br, and organic acids) and non-reactive species (CH_3Hg^+, and other organomercurial moieties, $Hg(CN)_2$, HgS and Hg^{2+} bound to sulfur in fragments of humic matter).

For plant physiologists, mercury speciation is related to bioavailability which is related to solubility, stability and interaction with soil. Stable and insoluble forms such as HgS will have different consequences to other species of mercury. For industrial purposes, it is also necessary to determine specific mercury compounds in commercial products to guarantee product quality and safety.

Thus, the processes to identify and determine the individual chemical forms of the element are important. This approach may be called chemical speciation and involves differentiation of the various chemical forms of mercury which together make up its total concentration in a sample.

In this paper we will primarily follow that aspect of chemical speciation which classifies mercury compounds on the basis of their chemical structure, since we consider that the determination of each chemical species is important for the understanding of the biological function and physical transport of the element. The chemical forms that have been considered important for mercury in biological and environmental samples are listed in Table 1.

Table 1. Major mercury species in environmental and biological samples

Elemental mercury		Hg°
Inorganic mercury species	Mercuric ion	Hg^{2+}
	Mercurous ion	Hg^+
	Mercury sulfide	HgS
Organic mercury species	Methylmercury	CH_3Hg^+
	Ethylmercury	$C_2H_5Hg^+$
	Phenylmercury	$C_6H_5Hg^+$
	Dimethylmercury	$(CH_3)_2Hg$

In the geochemical cycling of the element, the physical properties of the mercury species together with any potential carrier are possibly of greatest importance. For example, gaseous mercury can be transferred for long distances in the atmosphere, while particulate-bound mercury is less mobile. Or dissolved mercury can be transported by sea currents while mercury bound to particulates is sedimented. In this respect, mercury may be classified into several species that are determined by physical properties.

Elementary mercury occurs naturally and is present in the atmosphere. The vapour pressure of Hg is 0.001201 mmHg at 20 °C, and the solubility of Hg in water is about 20 mg dm^{-3}. HgS (cinnabar) is the major mercury ore. This material is almost insoluble in water (10^{-5} g dm^{-3} at 18 °C [7]), but can be transformed under strong acidic conditions into a soluble form.

Mercury exhibits valences of +1 and +2 and forms stable salts: the divalent (mercuric) salts are more soluble in water than the monovalent (mercurous) salts. Hg forms covalent bonds with carbon and the resulting compounds show a variety of different physical and toxicological properties. Mono-substituted mercury compounds such as methylmercury (CH_3-HgX), ethylmercury (C_2H_5-HgX) and phenylmercury (C_6H_5-HgX) have been commercially synthesized and applied for biocidal use.

Because of the strong public concern about environmental mercury contamination, a large amount of analytical research has been done on the determination of mercury and its compounds. The number of published papers related to mercury determination reached to approximately 10 000 during the period 1967 to 1995 based on the records of the Chemical Abstracts Service (1995). Most of the papers were related to the determination of the total mercury concentrations in the samples and the literature

concerned with mercury speciation is still limited. Approximately ten percent of the published papers on mercury determination are related to a specific form of mercury, mainly methylmercury.

Because many review papers have been published on mercury determination, the present paper will focus on recent developments in mercury speciation. In Sections II and III we will describe determination methods for mercury and its chemical species. In each section, however, we will cite only a small number of selected representative works because this paper is not the appropriate place for an exhaustive bibliography on mercury determination. In Section IV, sample collection and sample treatment for real samples will be discussed. Information on the actual concentrations of mercury species are given as examples. In the last Section (V), analytical quality assurance will be discussed especially that based on certified reference materials.

The detection limits quoted in this review are taken from the original papers without adjustment or attempt at standardisation. Some of these papers use detection limit to mean the absolute amount of mercury detectable under defined conditions, others as the minimum detectable concentration. In addition, although detection limit is usually taken to be the concentration of analyte that produces a signal equal to 3× the SD of (usually) 10 readings of the same reagent blank, there was variation encountered in the definition in the papers cited herein. In most cases we feel that the use of 'detection limit' is clear but nevertheless refer the reader to the original literature if any doubt persists.

SENSITIVE AND SELECTIVE DETECTION METHODS FOR MERCURY DETERMINATION

A variety of detection methods have been used to detect mercury selectively and sensitively. Good review papers have been published for the determination of total mercury concentrations in environmental and biological samples. These methods can be listed as follows: gravimetry, micrometry, radiometry, titrimetry, colorimetry and fluorometry, atomic absorption spectrometry (AAS) (cold vapour, electrothermal etc.), atomic fluorescence spectrometry (AFS), atomic emission spectrometry (AES) [spectrography, inductively coupled plasma – atomic emission spectrometry (ICP-AES), microwave-induced plasma – atomic emission spectrometry (MIP-AES), direct current plasma – atomic emission spectrometry (DCP-AES) etc.], neutron activation analysis (NAA), X-ray fluorescence (XRF), electron probe micro-analysis (EPMA), proton induced X-ray emission (PIXE) etc.), mass spectrometry (MS), electrometry (polarography, amperometry, voltammetry etc.), chromatography, and other miscellaneous methods.

Micrometry Although no analysis is performed by micrometric methods today, it is useful to refer to the technique as the methods are historically interesting. Mercury was collected on copper dust from an acid solution, and was subsequently heated so that the distilled mercury was deposited on gold foil, which was then examined by eye with a hand lens [8]. Mercury has also been determined visually by reducing mercury compounds to the element in the form of a small sphere and measured by means of a microscope [9].

Colorimetry The popular method for mercury analysis in the 1960s, until the introduction of atomic absorption spectrometry in the late 1960s, was colorimetry using diphenylthiocarbazone or dithizone. The method is based on a spectrophotometric measurement of a coloured complex extracted into an organic solvent after all the mercury in the sample has been converted to Hg^{2+} by acid-digestion and complexed with dithizone. A number of variables influence the manner in which dithizone behaves towards mercury and interfering elements, and a large number of published papers have contributed to make these determinations even more specific, sensitive, accurate and rapid. The 1965 edition of the official method of analysis of the Association of Official Analytical Chemists (AOAC) employed a reversion procedure in which mercury dithionate in the chloroform layer was extracted into aqueous sodium thiosulfate solution and was again extracted with dithizone in chloroform after decomposing the mercury thiosulfate complex [10]. The limit of detection was of the order of 1μg mercury. However, there seems little need to review these procedures, since relatively few laboratories are continuing to perform analysis by means of dithizone procedures.

Colorimetry using other colouring materials or molecular fluorimetry has also been carried out. However major advances in this approach may involve the use of high performance liquid chromatography (HPLC) with pre-column or post-column colouring. Dithizone, dithiocarbamate and their derivatives are complexed with mercury species and separated on reversed phase columns and detected by UV, visible and fluorescent means [11]. Capillary electrophoresis using water-soluble derivatives of dithizone has also been applied to the determination of mercury [12–14].

Atomic absorption spectrometry The most popular method for determining mercury in almost any type of sample has been based on cold vapour atomic absorption spectrometric measurement (CVAAS) and the technique is still widely used. Mercury is unique among metals in that it has a very high vapour pressure at relatively low temperatures and can be introduced quantitatively to the spectrometer as a vapour without difficulty. Absorption at 253.7 nm in the ultra-violet region has been measured with the use of mercury vapour lamps as well as hollow cathode lamps as the light source. Such methods are quite old; mercury vapour meters were first developed in the 1930s [15].

The way of liberating mercury from aqueous or digested samples is reduction, followed by volatilization and introduction of the mercury by aid of a gas stream. Sn^{2+} ions have been used as a reductant [16–19]. Recently, much work is done with $NaBH_4$ as the reductant. The use of Cr(II) as a reductant has also been reported [20]. Automatic apparatus utilizing the CVAAS method (Sn^{2+} reduction) has been constructed [19, 21–24].

Procedures to eliminate interference have been important in the determination of mercury in organic matrices. This is especially important for samples where complete decomposition is difficult even with the use of strong acids. Two approaches have been taken to remove interference by organic vapour: a gold amalgamation method to purify the mercury vapour and an optical background correction method for the spectrometry. The gold amalgamation method is based on the selective adsorption of mercury on a gold surface at room temperature. After the organic vapour is purged out, mercury is released from the gold fibre trap by heating and then introduced into the spectrometer. The method can work not only for mercury purification but also for the collection and accumulation of mercury into a small volume which makes it possible to achieve elevated sensitivity.

Optical background correction is employed to eliminate molecular absorption by organic vapours. Early work was carried out using a continuum light source [25] and later by using the Zeeman effect. These methods are necessary for electrothermal atomization, or flameless atomic absorption spectrometry especially in cases where samples are pyrolysed before introduction. Zeeman AAS is suitable for the direct analysis of solid samples, or samples without prior acid digestion, as spectral interference by the matrix is minimized by the Zeeman effect correction. Errors may come from the vaporisation and atomization processes which are often corrected by using a matrix modifier such as Pd [26]. The method has been applied to the analysis of sludge samples without their prior chemical decomposition [27].

Use of a quartz lamp and the 184.9 nm resonance line in the vacuum UV region provided increased sensitivity over the commonly employed 253.7 nm line. Improved accuracy for CVAAS was obtained by using the 184.9 nm resonance line [28,29]. It was found that the detection limit for Hg using the 184.9 nm is 30 times lower than that using the 253.7 nm line [30].

Atomic fluorescence spectrometry A number of articles have appeared on the development of atomic fluorescence spectrometry and its application to the determination of mercury [31–34]. Most of the early work used flame atomization. The limit of detection for mercury in aqueous solution, as determined by flame atomic fluorescence spectrometry, is 2 p.p.b. of mercury. Atomic fluorescence spectrometry was further developed by using an electrothermal atomization [35] or cold vapour atomization [36–42].

The fluorescence intensity in air is reduced because of quenching by oxygen and nitrogen [43]. The replacement of air by Ar gives a higher sensitivity (up to 86-fold). This is performed by using gold amalgamation or by phase separation with porous polytetrafluoroethylene tubing [44]. Atomic fluorescence spectrometric determination of picogram amounts of Hg in air and water has been reported [45]. Hg was liberated by reduction/aeration and was trapped on a Au filament. After replacement of air

with an Ar flow to minimize the quenching effect of N_2 and O_2, the Au filament was heated to 700 °C to liberate Hg which was introduced to a flow-type atomic fluorescence cell. The detection limit was 5 pg Hg with a relative standard deviation of 3% for 1 ng Hg. Flow injection analysis (FIA) by atomic fluorescence spectrometry involving on-line oxidation of organomercury species to Hg^{2+}, followed by reduction, gave a good sample throughput of 17 analyses per hour [46].

The use of an atomic fluorescence instrument that employs an inductively-coupled plasma (ICP) as an atomization cell and a pulsed Hg hollow cathode lamp produced a detection limit of 0.5 ng dm^{-3} [47]. Although the method was improved in sensitivity by the use of a Hg vapour lamp, it has no practical advantage over atomic absorption methods. The use of microwave argon plasma as an atomizer for atomic fluorescence spectrometry exhibits similar or even better sensitivity to ICP [48].

Atomic emission spectrometry Historically, emission spectrographic techniques have been very popular with geochemists. The method has sensitivity of about 1 μg. Atomic emission spectrometry has been developed in the past two decades by replacing the flame or arc emission source with a radio frequency plasma. Inductively coupled plasma, microwave induced plasma, and direct current plasma have also been used.

Hg in water has been determined by atmospheric pressure He microwave-induced plasma coupled with a cold-vapour technique (CV-MIP) [49]. The detection limit was 4 pg cm^{-3}. The use of a hollow-fibre membrane, by which hydrogen and water vapour were removed, for continuous introduction of Hg and other volatile metal hydrides to the plasma has been described [50]. The detection limit of Hg is 500 pg dm^{-3}. It was reported [51] that the CV-MIP detection limit was 50 pg cm^{-3} and that lowering of this limit was hampered by a residual blank signal equivalent to 0.5 ng. Several other papers also refer to atmospheric pressure helium microwave induced plasma atomic emission spectrometry coupled with CV. The detection limit is 16 pg Hg [52], and, by a flow injection method, detection limit of 0.1 pg Hg was reported [53].

Electrothermal vaporisation has been used for ICP-AES determination of mercury in drinking water [54]. Vaporisation was enhanced by post-injection of $(NH_4)_2S$, and the peak height was linear from 10–1 000 pg cm^{-3}.

Comparison of the analytical results for total Hg or organic Hg in whole blood and fish samples by cold-vapour AAS and by cold-vapour d.c. plasma atomic emission spectrometry (DCP) showed fair agreement [55]. The instrumental detection limits for inorganic Hg were about 4 mg dm^{-3} (CV-AAS) and 20 mg dm^{-3} (CV-DCP).

Neutron activation and X-ray fluorescence analysis The principal merits of the widely used neutron activation and X-ray fluorescence methods of analysis are the relatively short operator time, the ability to perform non-destructive analysis, and the good sensitivity and accuracy of the procedures. Neutron activation analysis measures the gamma radiation emitted by ^{197}Hg formed by irradiation with reactor neutrons. A number of variations have been published, but there are two main principles. On the one hand, there are instrumental techniques in which the intact irradiated sample is measured (INAA, non-destructive analysis), and, on the other hand, there are techniques involving different kinds of chemical separation by which the constituents of the sample are separated before measurement. Instrumental procedures have been described by a number of authors and their detection limits are in the order of 1 ng g^{-1}. The latter methods can achieve lower detection limits and high degrees of specificity.

Several textbooks and review papers list numerous applications of these methods to mostly solid environmental samples [56–60] and their application to atmospheric aerosols, particulates and sludge samples has been critically reviewed [61]. In neutron activation analysis, it is highly desirable to have the sample compacted and free from excessive amounts of moisture. Since biological samples are usually not dense and contain significant amounts of moisture, processes such as oven-drying, freeze-drying and ashing are often used to prepare samples for reactor irradiation and subsequent handling. There are reports that these procedures are often the cause of loss of the element [62,63]. Neutron activation analysis is mainly used for total mercury determination and its application to speciation analysis is limited.

X-ray fluorescence spectrometry measures total mercury in solid samples without acid digestion. Total-reflection X-ray fluorescence (TXRF) spectrometry is a relatively new multi-element technique with an elevated sensitivity.

Mass spectrometry Spark source mass spectrometry has been applied to the determination of mercury although it has not been used extensively. Early work used the method for the determination of mercury in apples [64]. However, by using a stable isotope as an internal standard, the method can be very accurate. Determination of total mercury in botanical and biological samples by isotope dilution spark-source mass spectrometry has been reported [65].

Inductively coupled plasma-mass spectrometry, coupled with a continuous reduction system using $NaBH_4$ was applied to the micro-determination of mercury in natural waters and a reference sediment. The absolute detection limit was 8 pg. The application combination of flow injection method to ICP-MS is reported [66]. Spark source MS, one of the most sensitive detection methods in the 1970s, is being replaced with ICP-MS.

The growing popularity of ICP-MS is extending applications which started with the analysis of geological samples. Mercury has been determined in various samples including petroleum and urine

Electrometry Polarographic techniques have been used to determine mercury species in dilute aqueous solutions [67]. The Redox potential of individual mercury species differs and thereby speciation is possible by polarography. Methylmercury was determined in a noncomplexing media by differential pulse anodic stripping voltammetry at a Au film electrode, with a detection limit of 2×10^{-8} mol dm^{-3} for 5 min plating time [68]. However, when it was applied to real biological and environmental samples, separation was necessary for which the procedure is not as simple and rapid as for the atomic absorption method. Even routine analysis of water samples previously done by polarography is now being done by CVAAS.

A fluorometric reaction-rate method for the determination of Hg(II) (0.03–0.3 p.p.m.), based on its catalytic effect on the autoxidation of 2,2′-dipyridyl ketone hydrazone, has been developed [69].

Thin gold film shows a proportional increase in electric resistance in the presence of Hg vapour. This phenomenon was utilized as an Hg detector and a commercial instrument is available as a gold film mercury analyzer. Good linearity was obtained between 5–40 μg Hg. The detection limit is 500 pg/Hg or 10 μg dm^{-3} for a 50 cm^3 sample after borohydride reduction.

Miscellaneous Sensitive detection of Hg by bioluminescence has been described [70]. Microorganisms were transformed with plasmid pGL4, which contains the Hg regulator from plasmid pDG106, the lux gene complex from *Vibrio haveyi*, and a Kanamaycin resistance gene. The minimum detection limit is ppb and the plasmid was specific for Hg in the presence of other heavy metals.

The inhibition of activity of horse liver alcohol dehydrogenase was applied to the determination of 80–300 mg cm^{-3} Hg (II) [71]. An enzymic method for the determination of Hg in natural waters was based on horseradish peroxidase in the oxidation of 3,3′,5,5′-tetramethylbenzidine or *o*-phenylenediamine by H_2O_2. The limits of detection were 3×10^{-7} and 8.3×10^{-7} mg cm^{-3} [72].

MICRO DETERMINATION METHODS FOR MERCURY SPECIES

The physico-chemical properties of the alkylmercury salts are dependent on the nature of the counter anion. For example, methyl-mercurycysteinate is soluble in water and insoluble in benzene while methylmercury chloride is insoluble in water and soluble in organic solvents. Differences of physico-chemical properties are utilized in sample clean-up processes prior to analysis. The affinity of CH_3Hg^+ for some ligands is high, especially toward the thiol group.

Dimethylmercury (Me_2Hg) is known to occur by methylation of methylmercury [73]. Dimethyl mercury is a volatile material with low solubility in water and is partially decomposed when strong acidic solutions are used for extractions. Me_2Hg can be lost during storage, homogenization, extraction, or other means of sample preparation. Although the material is observed in several environmental samples

[74], there is a possibility that it might be more often observed in the environment if an appropriate analytical method was used [74].

Analysis can be done by separating the individual mercury species in a sample, and measuring the quantity of each by selective and sensitive detection methods. The separation methods for mercury species may include the following: liquid chromatography [thin layer chromatography (TLC), HPLC, ion chromatography (IC), gel filtration chromatography, chelate exchange chromatography), gas chromatography (GC) (packed column GC, capillary column GC), partition, selective extraction, electrophoresis, dialysis, filtration, flow injection, co-precipitation, chemical reaction (selective reduction, thermal decomposition) and amalgamation.

More than one hundred methods are possible by combining the detection methods with such separation methods. However, only a few are widely used because of the cost of analysis, ease of operation, rate of sample throughput and instrument accessibility. Recent developments in separation techniques such as HPLC and capillary GC, and in detection systems such as plasma emission spectrometry, mass spectrometry and atomic absorption spectrometry have facilitated the sensitive and selective determination of mercury species.

Selective reduction A partial speciation into inorganic and organic mercury is possible by CVAAS [75–77]. The Magos method [75] is essentially based on the selective reduction of organic and inorganic mercury. Methylmercury in the presence of cysteine is reduced very slowly by tin(II) chloride in basic solution, whereas inorganic mercury is reduced immediately. Addition of cadmium chloride to the tin(II) chloride solution increases the rate of methylmercury reduction. Selective reduction can therefore be controlled in a basic solution with the use of a tin(II) chloride or tin(II) chloride-cadmium chloride. Modifications have been reported [78,79].

By changing the reducing conditions in an automated reaction/extraction system, three classes of species, inorganic mercury, arylmercury (such as phenylmercury), and alkylmercury (such as methylmercury) were differentiated using CVAAS detection [80]. The method has a detection limit of 1 ng Hg dm^{-3} and it is possible to apply it to natural waters.

Selective pre-reduction which employs sodium borohydride for total mercury and stannous chloride for inorganic mercury has been developed [81]. The volatilized elemental Hg was determined by CVAAS and the detection limits for inorganic and organic mercury species were in the 0.003–0.005 p.p.b. range. An interface has been described for the connection of a fused silica capillary GC system to a CVAAS instrument for the trace analysis of organomercury compounds. With the column coated with Superox 20M, the technique can be used to detect 100 pg Hg [82].

Simultaneous determination of monomethyl mercury, inorganic mercury, and total mercury in biological materials by GC/CVAAS after ethylation and room-temperature precollection is possible [83,84]. The $MeHg^+$ and Hg(II) present are converted to volatile ethyl derivatives, methylethyl mercury and diethyl mercury, by an aqueous-phase ethylation reaction with sodium tetraethylborate. The ethyl derivatives were precollected onto a trapping column at room temperature. Absorbance detection limits were 0.6 pg and 1.3 pg of Hg as $MeHg^+$ and Hg(II), respectively, corresponding to 0.3 ng and 0.6 ng g^{-1}(wet) of sample.

It has been rather simply assumed that elementary mercury is produced during aqueous reduction of methylmercury chloride by $NaBH_4$. However, methylmercury hydride (CH_3HgH), a volatile and unexpectedly stable species determined to have a half-life of approximately 2 h, has been identified as a product of the reaction [85]. The methylmercury hydride was concentrated in a purge and trap (PT) apparatus and separated by a wide-bore GC column. Identification was made by GC/FT-IR and GC/MS. The detection limit of PT/GC/FT-IR was 0.15 mg but probably can be improved by using GC/MS. Methylmercury hydride has also been identified by GC/AAS and GC/MS [86].

A method of detecting $MeHg^+$ and Me_2Hg using PT/GC/FT-IR has been further examined [87]. $MeHg^+$ was transferred prior to its detection as methylmercury hydride using $NaBH_4$. Two volatile forms of organic Hg were detected by PT/GC connected with a FT-IR and an AAS instrument. Detection limits were 100 pg for MeHg and 50 pg for dimethylmercury.

Picogram amounts of inorganic Hg(II) and methylmercury compounds were determined in seawater by using $NaBH_4$ reduction, cryogenically focusing the reduced mercury species onto a chromatographic stationary phase and AFS detection. Both Hg compounds are eluted according to their boiling points and were passed through a pyrolysis tube before entering the AF detector. For seawater, analytical detection limits of 0.1–1 ng dm^{-3} could be achieved [88].

Optimization details of a hydride-generation procedure for the analysis of mercury species, using a heated quartz furnace with atomic absorption spectrometry, have been discussed [89,90]. Absolute detection limits were 50 pg for MeHg and Et_2Hg and 110 pg for Hg(II).

A combination of electrolysis and CVAAS has been reported [91]. Inorganic mercury was deposited on the platinum electrode with an efficiency of 95% at –0.1 V, whereas the deposition of organic mercury started at voltage volumes more negative than –0.2 V. After electrolysis at –0.1 V, organic mercury was determined by sodium borohydride reduction and inorganic mercury was determined in the original solution by stannous chloride reduction.

Solvent Extraction The basic isolation procedure involves liberation of the methylmercury from the sample matrix by acidification and isolation by benzene or toluene extraction.

Determination of extracted mercury species in the benzene or toluene is usually done by atomic spectrometry after appropriate sample treatment. Continuous flow liquid-liquid extraction coupled with ICP-AES detection has been reported [92].

A rapid determination method for both organic and inorganic mercury in biological samples by graphite furnace atomic absorption spectrometry (GFAAS) has been described [93]. Organic mercury was extracted into benzene as a chloride derivative and then re-extracted into thiosulfate solution. Inorganic mercury was converted into a methylmercury derivative by methanolic tetramethyltin prior to extraction. A 20 cm^3 aliquot of the thiosulfate solution was injected into the GFAAS and the limit of detection of mercury was 0.04 ng.

Gas chromatography (GC) The most widely used determination method for methylmercury in biological and environmental samples is gas chromatography with electron capture detection (GC/ECD). Because electron capture detection is not sufficiently specific to methylmercury halides, a clean-up procedure is necessary.

Westöö reported [94] thin-layer and gas chromatographic separation of mercury compounds after their conversion to aryl or alkyl mercury chloride. A variety of organomercury compounds were separated and determined with GC techniques in relation to mercury contamination at Minamata [95].

Westöö developed [94,96] a back-extraction procedure whereby the methylmercury chloride was extracted into benzene and subsequently extracted to an aqueous cysteine solution as a mercury-cysteine complex (to eliminate interferences from benzene-soluble organic constituents) and then back-extracted again to benzene by acidification. With some modifications, Westöö's method has been employed as a recommended method by regulatory agencies world-wide.

Adsorption and decomposition of MeHgX in the chromatographic system has been demonstrated to present serious problems. It was observed in a gas chromatographic-mass spectrometric (GC/MS) study with packed columns [97] that, after injection of methylmercury chloride, substitution of the halides occurred with the formation of methylmercury iodide. The addition of mercuric chloride to the samples or repeated injections of MeHg caused improvements [98–101]. The use of glass capillary columns coated with OV-275 have been found to improve adsorption [102].

A gas chromatographic determination method for inorganic mercury and organomercury in biological material using packed-column GC/ECD has been developed [103]. Methyl-, ethyl-, and phenylmercury were first extracted as chloride derivatives and subjected to thiosulfate clean-up, and finally isolated as bromide derivatives. Organic mercury was isolated as methylmercury upon reaction with tetramethyltin. The recovery was assessed by a radiometric assay using ^{203}Hg labelled compounds. The method provides a detection capability of 1 ng g^{-1} or lower with a mean deviation of 3.2%.

Comparison of column efficiency using GC/ECD for methylmercury, ethylmercury and phenylmercury showed that of the packed columns, the stationary phase, AT-1000, yielded the best results [104]. Better results were obtained with two wide-bore thick-film fused-silica tubular columns FSLOT; Super ox-FA and RSL-300. With these FSOT columns, absolute detection limits at the sub-picogram level were achieved.

Development of new separation techniques using glass capillary column GC are needed as is the use of more specific detectors to obtain higher sensitivity and selectivity, and the use of appropriate recovery correction for improved accuracy.

Di-substituted mercury compounds show better characteristics on GC separation. A new derivatization procedure with diazomethane, resulting in the formation of methylmercury methylene bromide ($MeHgCH_2Br$), and its determination by GC/MS has been employed [105]. Propylmercury bromide (PrHgBr) was used as an internal standard.

Both elemental and inorganic forms (Hg^0 and Hg(II)) and methyl mercury are completely converted into dialkyl derivatives by treatment with butylmagnesium chloride. Dibutyl- and butylmethylmercury are determined by GC with element-specific detectors such as MIP/AES [106].

A new alkylation process was proposed using methylcobalamin. The produced volatile mercury species were analyzed with a purge-and-trap GC in line with a Fourier transform IR spectrometer (PT GC/FTIR) [107]. Because MeHg formation depended on different factors the practical applicability is not clear.

Aqueous phase ethylation using sodium tetraethylborate ($NaBEt_4$) as derivatizing reagent has been recently developed. Ethyl derivatives are collected at room temperature and introduced into a GC/CVAFS [83,108]. The method has been extensively used for the determination of mercury species in a variety of environmental and biological materials. Simultaneous derivatization of tin, lead and mercury compounds was possible [109].

Three 'round-robin' exercises were performed in a collaborative investigation into detection problems and sources of error in the determination of methylmercury in biological samples. Sources of discrepancies were found for the matching calibrant, the most important area being the inadequacy of the packed chromatographic columns. The results obtained with Cp-Sil 8 capillary columns appeared in most cases better than those obtained using packed columns [110]. Extensive work was carried out to evaluate the Cp-Sil 8 capillary column [111].

The use of element-specific detectors for GC overcomes the difficulties of EC detection. AAS, AES, and AFS have been used for mercury-specific detection.

A method not requiring a clean-up step and involving separation of organic mercury compounds by GC and reduction of the organic mercury by flameless AAS has been reported [112] for the determination of alkylmercury in fish tissue. AAS has been employed as a specific detector for GC [113]. The detection limit was 0.3 p.p.m. of mercury for a 0.5 g fish sample.

Separation and determination of diethylmercury, methylmercury chloride, ethylmercury chloride and phenylmercury chloride using capillary GC with AAS detection has been achieved [114]. A OV-17 WCOT column (12 m × 0.3 mm) was used to separate the mercury compounds and the effluent from the column was led through a stainless steel pyrolyser kept at 700 °C and detected by AAS. The absolute detection limit was about 0.1 ng mercury. Sensitive instrumentation for mercury speciation employing a wide bore capillary GC column, on-line pyrolyser at 800 °C (to generate mercury atoms) and AAS detector in a quartz cuvette has been developed [115]. The use of the 184.9 nm line provided a more than five-fold increase in sensitivity, compared with the conventional 253.7 nm line, and an absolute detection limit of 0.5 pg of mercury.

A method for the determination of ethylmercury, at the picogram level, by aqueous-phase ethylation followed by cryogenic GC with cold vapour atomic fluorescence (CVAFS) detection has been reported [108,116]. The detection limit was 0.6 pg, or 0.003 ng dm^{-3}, for a 200 cm^3 sample. Further examination of ethylation/GC methods has been carried out for CVAFS [117].

Microwave emission detection (MED) for GC has been shown to be useful for the selective determination of several metallic elements including mercury [118–131].

Bache and Lisk first applied GC/MED to organomercury compounds [119]. Using packed columns (60/80 Chromosorb 101, 20% OV-17/QF-1(1:1)), five organomercury compounds (dimethylmercury, methylmercuric chloride, methylmercuric dicyanodiamide, phenylmercuric acetate and methylmercury dithizonate) were separated, The selectivity ratio of the 253.7 nm atomic emission line for mercury when comparing methylmercuric chloride to eicosane was at least 10 000 to 1.

A method reported in 1975 [120] specified a highly selective and sensitive microwave-induced plasma (MIP) as a GC detector for monitoring Hg emissions at 253.7 nm. Samples were homogenized, acidified with HCl and methylmercuric chloride was extracted into benzene for analysis. The method is very sensitive, selective and rapid, but several limitations have been suggested. For example, experimental manipulation is required to remove the solvent from the plasma since the injection of microliter amounts of solvents into the plasma can decouple the microwave resonance cavity, resulting in the total quenching of the discharge.

MIP detectors are commercially available and their good operational ability will find more applications to mercury analysis although they are expensive. Methylmercury chloride can be analyzed by a commercial MIP detector after separation by capillary GC [128]. The need for column conditioning with $HgCl_2$ was confirmed. A GC/AES technique was used after mercury species were butylated with a Grignard reagent [129,130].

MeHg, EtHg and inorganic mercury species in water were preconcentrated onto a sulfhydryl cotton microcolumn incorporated into a flow injection system. The retained mercury species were then eluted with 3 N HCl and subjected to phenylation before determination by GC/MIP/AES. The limits of detection for mercury species were 10 mg dm^{-3} for MeHg and EtHg and 16 ng dm^{-3} for inorganic mercury [131]. The use of solid-phase micro-extraction (SPME) or an on-line amalgamation trap was effective for removing carbon interference in the MIP/AES detection.

The use of capillary GC with atomic emission detection (GC-AED) for the simultaneous determination of organotin, organolead, and organomercury compounds in environmental samples has been studied. Pentylmagnesium bromide was used to pentylate ionic organotin, organolead, and organomercury compounds; the derivatives were then separated by GC and detected by AED. The calibration curves exhibited good linearity between 2.5 and 10 000 ng cm^{-3} for organomercury compounds [122].

Recently modified AED, which incorporated changes in the light source and the spectrometer improved sensitivity and selectivity. The detection limit for mercury was 0.1 pg g^{-1} using both the 184.9 nm and 253.7 nm lines [132].

Determination of methylmercury in biological samples by GC-MIP with a semi-automated headspace sampler has been described [121]. The methylmercury moiety was converted to methylmercuric iodide by using iodacetic acid as the liberating agent. The detection limit was 1.5 ng cm^{-3} of homogenate and the authors claimed that the principal merit of the method is that column degradation was no longer a problem.

Other atomic emission light sources include inductively coupled plasma (ICP) and direct current plasma (DCP). Atomic emission was observed axially in the discharge with an echelle grating spectrometer [133]. The system allowed the detection of 90 ng Hg as diphenylmercury. Capillary GC combined with axially viewed ICP-AES with an echelle grating has also been used [134]. By using a rather short capillary column and MeHgI as the derivatized form, very sensitive detection was achieved. The detection limit was 3 pg for methylmercury as Hg (S/N=2) and the linear dynamic range was 6 pg–6 ng.

A GC/DCP interface was optimized and the results obtained for methylmercury in fish compared with those obtained by GC-ECD and total Hg by CVAAS [135]. It was reported that in most cases, qualitative and quantitative results did not agree for GC-DCP and GC-ECD methods.

AFS has also been utilized as a GC detector. The method has high sensitivity to detect 0.6 pg methylmercury or 0.003 ng methylmercury for 200 cm^3 aqueous samples [108].

With the aid of a mass spectrometer attached to a conventional GC, methylmercury halides at the 50 pg level were determined [136].

A GC method with an atmospheric pressure active nitrogen (APAN) afterglow detector has been reported [137]. Methylmercury was isolated from samples as methylmercury chloride and extracted into methylene chloride. Dimethyl-, diethyl-, dipropyl-, dibutyl-, dihexyl-, and diphenylmercury were extracted from water with either methylene chloride or carbon disulfide. The extracts were concentrated to 1.0 cm^3 and subjected to gas chromatography with a 5% SE-30/6% SP2401 column. The detection limit of Hg in the APAN afterglow was about 10 pg and the method has the detection limit of 50 ng methylmercury in a 1 g fish sample.

The coupling of ICP-MS with capillary GC is a relatively new technique but its application is expanding [138–149]. The method is very sensitive and suitable for analysis of various metal species.

CH_3Hg^+ was isolated from sediments by distillation, converted to methylethylmercury by sodium tetraethylborate and analysed after purge-and-trap precollection on a Tenax adsorber and thermodesorption onto the GC column. Detection (ICP-MS) limits were found to be ≈1 pg (as Hg) absolute or 0.02 ng g^{-1} dry sediment. The precision was ≈4% relative standard deviation when 259 pg of methylmercury were processed [148].

A recently developed method for the simultaneous determination of methylmercury and inorganic mercury based on extraction, butylation, capillary GC separation and AE detection has been evaluated with respect to analytical quality. A number of reference and candidate reference materials have been analyzed with international inter-calibration exercises showing good agreement with respect to methylmercury and total mercury. Other small scale inter laboratory comparisons have also been made to assess the analytical performance.

Liquid Chromatography The use of high performance liquid chromatography (HPLC) for mercury speciation has the advantage of simplified sample preparation. In GC analysis, it is essential to form volatile, thermally stable derivatives, whereas this is not necessary for HPLC. The combination of HPLC with spectroscopic detection provides a simple and selective method of metal speciation.

Early investigations in HPLC separation of mercury species employed ordinary phase separation using silica gel [150]. Currently, reversed phase using C18 packings is the most commonly used HPLC separation mode for Hg speciation. The ordinary phase method was found to be disadvantageous because of poor retention reproducibility and diphenylmercury decomposition [151]. However, gradient conditions were necessary for the effective separation of dimethylmercury and diphenyl-mercury. Change of organic solvent concentration during gradient elution influenced AAS detection since the number of atoms in the ground or excited state was changed by the presence of organic materials.

Early HPLC investigations also used UV-Vis detection. The sensitivity for RHgX derivatives monitored at 205 nm or 210 nm was low (in the mg range). To achieve sufficient sensitivity, Hg compounds must be derivatized by complexation. The most common derivatization reagents are diethyldithiocarbamate [55,152–154], pyrrolidondithiocarbamate [152,155], dithizone [151,152], bis(2-hydroxylethyl)dithiocarbamate [156], and mercaptoethanol [157]. The complexations improved sensitivity to the sub-nanogram range but the detection limits were still high compared with GC-ECD and CVAAS methods.

Ammonium bis (2-hydroxyethyl) dithiocarbamate was used as a precolumn derivatization reagent for the reversed phase HPLC determination of Hg(II) which was detected at 405 nm [156]. By using an on-column preconcentration step prior to the separation, a detection limit of 20 ng dm^{-3} was obtained for 10 cm^3 sample.

Reversed-phase HPLC with UV detection was optimized for the simultaneous separation and quantification of nine organic mercury compounds: methyl-, ethyl-, phenyl-, methoxymethyl-, ethoxymethyl-, benzoic and toluylmercury, mersalylic acid and nitromersol [158]. The nine compounds were successfully separated on ODS columns by gradient elution with a methanol-water mixture ranging from 30% to 50% (v/v). The detection limits were in the range 70–95.1 ng dm^{-3}.

A reversed-phase HPLC separation of organic ionic mercury species was achieved by using pre- and on-column derivatization with Me thioglycolate. For the enrichment of the compounds from water samples, the complexed organometallic species were concentrated on a precolumn [159]. The use of Me

thioglucolate as the 2nd complexing reagent made it possible to detect all analytes, organolead and organomercury compounds, simultaneously [160].

Ion-chromatographic separation of mercury followed by post-column derivatization with meso-tetra(4-sulfonatephenyl)-porphyrin (TPPS4) was reported [161]. The detection limit for mercury was 5 ng.

Although these methods were shown to have rather high sensitivity, their selectivity was often not enough for the determination of the samples having complex matrices. Selectivity problems may be solved by using element-specific detectors such as AAS, AES and AFS.

To obtain high sensitivity, cold vapour generation and its introduction to a spectrometric detector is often used for mercury. Connection of the HPLC column and the AAS detector with a reduction system using sodium borohydride at the interface was devised for higher sensitivity [162–164]. The method was adapted for continuous flow analysis [165]. The ion chromatographic separation of MeHg, EtHg, and inorganic mercury as cysteine complexes was studied with CVAAS for detection using an online continuous flow cell coupled with a reduction system (sodium borohydride). The detection limits, evaluated on 100.0 cm^3 samples, were 2, 10, and 4 ng for inorganic mercury, methylmercury, and ethylmercury, respectively [166].

An LC method with online UV irradiation was developed [167] for AAS. Methyl-, ethyl- , phenyl- and inorganic Hg were separated on reversed phase C18 columns. A UV-irradiation lamp was used for the online destruction of the organomercury compounds. Samples and $NaBH_4$ solution were continuously fed to the reaction vessel where Hg was reduced, and the volatilized Hg was swept with nitrogen into the absorption cell of a CVAAS system. The detection limit for methylmercury was 80 pg absorbance (S/N=3). Further improvement was achieved with on-line RP C18 preconcentration [167]. Gradient elution has also been used for on-line HPLC/CVAA using reversed phase HPLC [11]. Prior to the determination of mercury, the organic ligands and the matrix were destroyed with $K_2Cr_3O_7$.

A computer controlled interface system was devised for directly coupling HPLC and AAS [168]. The eluent from the HPLC was collected on a series of 20 rotating platinum spirals, desolvated by an electrically heated furnace and finally transported into an atomizing air-acetylene flame. Detection limits for Hg^{2+}, CH_3Hg^+ and C_6H_5Hg were 3.5, 1.9 and 2.5 ng (as Hg), respectively.

A combined method of HPLC and ICP-AES was reported [151] but for more sensitivity, post-column cold vapour generation was included with combined HPLC and the ICP AES [169]. The separation was performed on a C18 stationary phase with a mobile phase consisting of 0.06 M ammonium acetate and 0.005% 2-mercaptoethanol with a gradient from 15 to 75% acetonitrile. Detection limits ranged from 32 to 62 p.p.b. of mercury for four mercury compounds, representing a three to four order of magnitude enhancement over detection limits obtained without cold vapour generation. Micro-HPLC and ICP-AES have been combined [170].

The introduction of mobile phases containing relatively high percentages of organic solvent into plasmas results, usually, in a decrease of sensitivity, a higher plasma background and decreased stability of the plasma. Therefore the use of alternative HPLC phases which contain surfactant-based phases is advantageous.

The direct coupling and application of MIPs to HPLC have proved problematic because of the low tolerance of conventional low power MIPs to the introduction of liquid aerosols. By using continuous cold vapour or hydride generation techniques as interfaces between the exit of the HPLC column and the MIP, inorganic mercury and MeHg were sensitively determined with detection limits of 0.15 ng Hg cm^{-3} and 0.35 ng Hg cm^{-3}, respectively [171].

Electrochemical detection has also been connected to HPLC for selective and sensitive detection of organomercury compounds. MacCrehan and colleagues [172–174] separated methyl, ethyl- and phenyl-mercury on an ODS column and detected them with differential polarography. The method offered excellent detection (40 pg) but required complete removal of reducible species including oxygen [172–175].

Electrochemical detection in the reductive mode at a gold amalgamated mercury electrode combined with reversed-phase HPLC to detect inorganic mercury(II) and three organomercurials in water samples

has been described [176]. Detection limits for the analytes varied from approximately 1 to 2 μg dm^{-3}. They later obtained very low detection limits for inorganic, methyl-, ethyl- and phenylmercury at 0.8–1.9 pg through careful elimination of oxygen diffusion by using stainless steel tubing and interface instead of PTFE tubing.

LC/ICP-MS was applied to the determination of methylmercury and thimerosal [177]. The separation was performed on a C18 column with a mobile phase consisting of 0.06 mol dm^{-3} ammonium acetate, 3% acetonitrile and 0.005% 2-mercaptoethanol. The detection limits were in the low ng cm^{-3} range and were improved further by post-column cold-vapour generation. The use of LC/ICP-MS for the speciation of mercury is expanding [178–181].

Miscellaneous Thin layer chromatography (TLC) is still valuable for *in situ* field examination because it requires no special apparatus. Mercury dithionate, methylmercury dithionate and phenylmercury dithionate were separated by TLC on aluminium oxide developed with light petroleum and diethyl ether (7:3). The detection limit was 0.2–0.02 mg [182]. The TLC behaviour of six organomercury compounds was examined [183]. RF values were presented for different solvent systems with both silica gel and alumina plates.

Polyurethane foam thin-layer spectrophotometry was used to determine mercury and phenylmercury which were adsorbed onto foam parallelepipeds loaded with diphenylthiocarbazone. The detection limits were 5 mg Hg dm^{-3} and 10 mg of phenylmercury dm^{-3} [184].

High performance thin layer chromatography (HPTLC) has been applied to the determination of inorganic and organic mercury. Inorganic Hg and some organo-Hg species were separated as dithionates by HPLC and detected *in situ* at the subnanogram level by densitometry [185].

Yeast, *Saccharomyces cervisiae*, was used to selectively separate methylmercury and Hg(II). Methylmercury was immediately bound to the yeast cells over a wide pH and temperature range. Hg(II), on the other hand, has less affinity for yeast cells and remains in solution. The method was applied to the selective determination of CH_3Hg^+ and Hg (II) in spiked water samples. In all cases good recoveries were obtained [186]. Adsorption to the external membrane of bacterial cells can be adapted to the speciation of Hg(II) and Hg(I) ions [187].

Inhibitor characteristics have been used for the determination of mercury, methylmercury and ethylmercury in aqueous solution. Using invertase with sucrose as its substrate, mercury species are detected in the 2–10 ng cm^{-3} range by monitoring with a glucose sensor [188].

MERCURY SPECIES IN ENVIRONMENTAL MEDIA

Mercury in the Atmosphere Mercury and some mercury compounds are volatile and, therefore, atmospheric Hg distribution is of importance in terms of global Hg cycling. Mercury is present in the atmosphere as particulate-bound forms and gaseous forms. For both species the scheme of analytical procedure is similar and composed of three steps: sampling by sorption in/on a medium, release of Hg from the medium, and detection.

Atmospheric particulates have been sampled onto a filter by means of an air sampler and total Hg, which is released as Hg vapour by acid digestion-reduction or pyrolysis, has been determined by various methods [189–193].

A non-destructive NAA method has also been reported [194]. It is generally accepted that particulate bound Hg comprises less than 10% of total atmospheric Hg, although it may occupy a significantly larger fraction in urban and/or industrialized environments [195]. Speciation methods for particulate inorganic Hg (such as $HgCl_2$ or HgS) have been tested using an evolved gas analysis (EGA)-He MIP system [196].

Gaseous total Hg is determined from filtered air samples. Absorption into solution, adsorption onto a solid sorbent, amalgamation onto noble metals and cryogenic trapping have all been used for sampling of total gaseous Hg. Amalgamation onto Au or Ag of various forms (wool, thin film, coated onto glass beads) is widely used [190–192,197–201] while solid sorbents e.g., cellulose or activated charcoal have been suggested [202,203]. Release of Hg from the collecting medium can be achieved by chemical reaction (such as reducing Hg with $SnCl_2$-used for the release of Hg collected in the acidic solutions),

and combustion and pyrolytic methods (for the release of Hg collected by adsorption/amalgamation). Various conventional methods, such as AAS, AFS, or AES, have been used for the Hg detection. Since Hg concentration in ambient air is as low as 1–10 ng m^{-3} and a low sampling flow rate (less than several dm^3 min^{-1}.) is inevitable for the efficient collection of Hg onto the sampling medium, the detection limit for total gaseous Hg varies considerably, but the lowest value reported is 0.02 ng Hg by AAS [199].

Determination of gaseous Hg species can be achieved by sequentially passing the filtered air sample through multiple collecting media with selective trapping characteristics for each Hg species. The basic design of this selective Hg species collection technique was first given by Braman & Johnson [204]. With this technique, five Hg species in the atmosphere, *viz.* particulate-bound Hg, $HgCl_2$ type compounds, CH_3HgCl type compounds, $(CH_3)_2Hg$ and HgO, can be fractionated. Firstly, particulate-bound Hg was collected onto a glass-wool filter. Gaseous Hg was then fractionated by passing the air stream through four sorbent-packed quartz tubes in sequence: first, through siliconized Chromosorb-W treated with HCl vapour for selective collection of $HgCl_2$; second, through Chromosorb-W treated with 0.05 M NaOH for CH_3HgCl; third, through silvered glass beads for HgO; and finally through Au-coated glass beads for $(CH_3)_2$ Hg. After sampling, each tube was heated and the released gaseous Hg was collected by amalgamation onto Au-coated beads. The amalgamated Hg was again released by heating and a DC discharge spectral emission system was used for detection. A detection limit of 0.01–0.05 ng Hg or 0.1–0.5 ng Hg m^{-3} for an air sample of 0.1 m^3 was achieved.

The speciation of Hg in the atmosphere by the technique of Braman & Johnson depends on the selectivity and efficiency of the collection of Hg species on the column packings. The selectivity and efficiency for each species was examined [204,205] and it was found that recovery was satisfactory for each species. This was partly supported by other work [206]. However, the selectivity of two of the sorbents has been questioned: Chromosorb-W treated with NaOH collected 46% of CH_3HgCl not 100% as reported; silvered glass beads collected 100% of both HgO and $(CH_3)_2Hg$ thus not allowing the selective collection of the two species [207]. Instead Tenax-GC and Carbosieve-B for selective collection of CH_3HgCl and $(CH_3)_2Hg$, respectively, were used [207].

More recently, GC has been employed for speciation of organic Hg in the atmosphere [114, 208–212]. In contrast to Hg species trapped on each tube being determined as Hg^0 in the selective adsorption method, the GC system gives a positive identification for each species. Chromosorb 106, Tenax, Carbotrap, etc. are used as Hg sampling media. After sampling, the collected Hg species were released upon heating and were trapped in an organic solvent such as toluene or benzene. A portion of this solvent was injected into the GC column. Otherwise, released Hg was directly introduced to a temperature-controlled GC column. Detection was by various methods including AAS, AFS, or AES. The detection limit was comparable to the selective collection method but 0.3 pg Hg for CH_3HgCl and $(CH_3)_2Hg$ were reported for a cryogenic GC-AFS system [212].

A sensitive determination method for methylmercury by GC/ICP-AES after collection into an impinger containing toluene/HCl has been reported [134]. By using a short length capillary fused silica column for GC and an axially viewed ICP for detection, a low detection limit of 3 pg as Hg was obtained for methylmercury. When air containing CH_3HgX is bubbled through pure water, part of the airborne CH_3HgX, depending on the distribution constant (air/water), will be retained in the water phase. The resulting concentration can, in many cases, be determined by GC-AFS [213].

Application of these techniques to real samples has been reported and the results are summarised in Table 2. Both Braman & Johnson [204] and Takizawa *et al.* [206] demonstrated that $HgCl_2$ was the main species in atmospheric gaseous Hg, and that CH_3HgCl and HgO composed a significant fraction. In both of the studies the selective adsorption method [204] was employed, although AAS was used as an Hg detector by Takizawa *et al.* [205]. On the other hand, it was demonstrated that HgO was the predominant species in gaseous Hg in air samples from metropolitan Toronto, and this was followed in significance by CH_3HgCl, $(CH_3)_2Hg$ and $HgCl_2$ in that order [214]. The modified selective adsorption method mentioned above and AFS as a detector were employed.

The inconsistency between these results in the proportion that each Hg species contributes to total gaseous Hg remains unresolved. This may be due to different Hg species collection methods, or to geographical, meteorological, or some other factors [215]. Analysis of air samples from Long Island

Sound, USA, by a highly sensitive GC-AFS system [212] revealed that HgO composed 95–100% of the total atmospheric Hg with the remainder being monomethyl Hg. Dimethyl Hg species were not detected in the samples from outside the laboratory. The authors cast doubt on the reliability of selective collection of HgO and $(CH_3)_2Hg$ by Ag for field sampling.

Table 2. Examples of total and organic mercury concentrations in natural waters.

	T-Hg (ng dm^{-3})	O-Hg (ng dm^{-3})	O/T (%)	References
River Water				
R. Ottawa	4.6–9.8	1.6–2.8*	26–46	[294]
R. Nagara	19.3	5.8*	30	[294]
R. Suimon	25.9	7.0*	27	[294]
R. Kurobe	0.7	<0.005	–	[233]
Ground water				
(Kobe)	1.3	<0.005	–	[233]
Lake water				
Lake waters	22–189	3–28**	6–20	[80]
Wisconsin Lakes	0.15–4.8	0.04–2.2	[295]	
Lake Baikal	0.14–0.77	0.002–0.0038	1–5	[296]
Sea water				
Japanese coastal	6.3–16.7	0.04–0.156	0.6–1.26	[233]
Off Hachijoh Island	0.877	0.080	9.14	[297]
Minamata Bay	8.640	0.582	6.74	[297]
Rain water (snow)				
	8–60	0.1–0.25	–	[296]

* as CH_3Hg^+

** as $CH_3HgCl + C_2H_5HgCl$

By GC methods, $(C_2H_5)HgCl$, in addition to CH_3HgCl and $(CH_3)_2Hg$, can be determined. It was, however, not detected in the air sample from downtown Toronto (detection limit was 0.5 ng Hg m^{-3}) [201]. C_2H_5HgCl was detected at a concentration of 9.8 ng m^{-3} in laboratory air and concentrations of 12.5 and 271 ng m^{-3} were found in air from a room where Hg samples were stored, but it was not detected in air samples from other sites (detection limit was 0.5 ng Hg for 240–600 dm^3 air samples) [114].

In summary, several methods have been put forward for Hg speciation in the atmosphere, but the analytical results obtained by these methods are not consistent. Development of sensitive and reliable speciation methods, especially in relation to the sorption characteristics of various sorbents for Hg species determination in the atmosphere, is necessary.

A mobile lidar system was established equipped with a narrow band tunable laser transmitter able to generate pulses of adequate power at the mercury resonance line of around 254 nm [216]. Mercury measurements were made with a sensitivity down to typical background levels, 2 ng m^{-3}. Three dimensional mapping of atmospheric atomic mercury was thus performed for the first time.

Amalgam tooth fillings constitute the main source of exposure to mercury for the general population. Elemental mercury released in oral air has been measured with a gold film Hg analyser [217–220].

A mercury speciation adsorption method was reported for a variety of combustion flue gas matrices [221–230]. Oxidized Hg species [Hg(II) and MeHg] in flue gas were adsorbed by a potassium chloride impregnated soda lime sorbent, elemental Hg was collected by an iodated carbon sorbent after passing through the KCl/soda lime sorbent. CVAFS was used for detection of Hg collected on the solid sorbents, after sample digestion and preparation. The presence of gaseous methylmercury reported by Chow *et al.* [227] may be an artefact of their digestion procedure [221]. There is a need to further improve collection

procedures [231]. Several on-line mercury analysis instruments are being developed based on well-established techniques [228,229].

Overall mean speciation results suggested that Hg was present mainly in the form of Hg(II) (67%) and Hg^0 (33%) in coal combustion flue gas at the inlet to pollution control devices. Measurement of water-soluble Hg(II) was performed by using a refluxing mist chamber for sample collection [231]. Hg(II), perhaps a gas-phase $HgCl_2$ or $Hg(OH)_2$ was found in combustion sources, its presence was also suspected in ambient air [213].

Mercury in Water Mercury in the aquatic environment is of particular importance in terms of global Hg cycling as well as to human exposure by fish consumption.

Mercury present at ultratrace levels in natural waters and the determination of Hg, even that of total Hg, has been a challenge to analytical chemistry. In particular, the concentration of each contributing species will be lower than total mercury concentrations. Partly owing to the inadequacy of the current analytical methods, a computer-modelling approach for speciation has been performed [e.g. 232] but is considered outside of the scope of this article.

An old but still used method is solvent extraction. Methods that involve the analysis of individual species of mercury include the following. Extraction from an acidified water sample of large volume (2–3 dm^3) into benzene followed by the detection of CH_3Hg-dithizonate with AAS [233]. The detection limit was 5 pg Hg for CH_3Hg^+ in coastal seawater from Japan, corresponding to <1% of total Hg. An automated selective reduction system for Hg^{2+}, phenyl Hg^+ and alkyl Hg^+ species using three reagent mixtures, *viz.*, EDTA + water, EDTA + $SnCl_2$ and $SnCl_2$ + $CdCl_2$ has been developed [80]. It was found that CH_3Hg species were the only organomercurials detected in seawater samples and that their proportion of total Hg was up to 20%. A more specific method for CH_3HgCl determination was the application of the dithizone-benzene extract to a TLC plate with the CH_3HgCl band being subjected to AAS detection [234]. By this method, CH_3Hg^+, Hg^{2+} and other Hg forms could be separated, and the concentrations of the species were 1.6–7.0 ng dm^{-3} (26–46% of total Hg), 2.1–16.8 ng dm^{-3} (43–61%) and 0.6–2.1ng dm^{-3} (9–25%), respectively, in river water from Japan and Canada. Dialkyl Hg compounds were separated using GC with a detection limit of 0.02 ng Hg for $(CH_3)_2Hg$ and possible application to water samples was indicated [235].

A field method for the preconcentration on to Au of traces of Hg^{2+} methylmercury and phenylmercury compounds by reduction with sodium borohydride has been reported [236]. Hg was determined using He DC plasma emission spectrometry with a low detection limit of 0.5p.p.m. for a 20cm^3 sample.

For the measurement of mercury species in environmental waters, the ability to measure at the ng dm^{-3} and sub ng dm^{-3} level is crucial. It is necessary to include a preconcentration step in the analysis. Preconcentration on resins [237–248], by amalgamation on silver or gold [249–253] or by liquid-liquid extraction [233,254,255] has been employed.

Results of the international aqueous mercury speciation inter-comparison exercise for total Hg and methyl Hg in pristine lake water showed good convergence between the participating laboratories for both total and MeHg [116]. The use of distillation in the extraction step gave a slightly higher value (0.420 ± 0.055 ng dm^{-3}) than that of solvent extraction (0.296 ± 0.043 ng dm^{-3}). The distillation procedure was developed to overcome low recovery in complex media, such as brown waters [256].

A sampling technique suitable for the preconcentration of mercury species from seawater was developed using solid-phase extraction in a microcolumn with a sumichelate Q-10 commercially available resin containing sulfhydryl groups [257]. This chelating resin showed a very high affinity for both inorganic and organic mercury and released mercury species into slightly acidic 5% thiourea. Subsequent determination was done by CVAAS, GC-ECD or GC-MIP [124].

The preconcentration of mercury species in waters was also developed by using a column containing a resin with immobilized dithiocarbonate (DTC) groups [258], or a sulfhydryl cotton [259]. Off-line preconcentration using a dithiocarbamate resin gave detection limits of 16 ng dm^{-3} for methylmercury and 17 ng dm^{-3} for mercuric chloride [181].

The storage stability of the analyte species on the column was found to be poor, indicating that elution from the DTC resin and further sample processing should commence as soon as possible after loading. A similar sampling method was evaluated for the simultaneous determination of mercury species at sub-mg dm^{-3} levels in humic-rich natural waters [130].

A recently developed solid-phase microextraction (SPME) which involves the extraction of volatile or semi-volatile organic compounds directly from aqueous or gaseous samples onto a fused-silica fibre coated with an appropriate stationary phase was applied to an analytical procedure for the determination of CH_3Hg^+ and labile Hg^{2+} using *in situ* aqueous ethylation with $NaBEt_4$ subsequent SPME sampling and then GC-MS determination. The procedure gave detection limits of 10 pg (as Hg) and 13 pg (as Hg), respectively for CH_3Hg and Hg^{2+} [260].

Analysis of mercury species in water may be done on the basis of different concepts; reactive and non-reactive, or cationic and anionic forms, or particulate-bound and dissolved form. This aspect is especially important for understanding the environmental fate of the element.

Dissolved mercury may be operationally defined as mercury which can pass a 0.45 μm membrane filter. This includes inorganic and organic metal complexes and metal adsorbed onto inorganic and organic colloids [261]. Total particulate Hg can be determined by conventional methods. Seritti *et al.* found 0.3–80 ng dm^{-3} of Hg on particles and 1.7–12.2 ng dm^{-3} in dissolved form in coastal seawater from Italy [262].

Mercury phase speciation in colloidal forms by use of an ultrafiltration technique which further subdivided the fraction passing a 0.45 μm filter into 0.45 μm–1 kDa and <1 kDa fractions has been reported [263]. Those fraction sizes include macromolecules and microparticles which, it is suggested, are passing through the filter associated with most of the Hg.

The reactive and non-reactive Hg differentiation method can be summarised as follows: reactive Hg is reduced to Hg^0 by $SnCl_2$ in acid solution, whereas non-reactive Hg is reduced by $NaBH_4$ or it is converted to a reactive form by HNO_3 [264]. The reactive forms include Hg halides, $Hg(OH)_2$ and Hg(II) complexes with organic acids, while non-reactive forms include CH_3Hg halides, HgS and S-containing organic Hg [265]. Using this method, it was demonstrated that >80% of total Hg was present in reactive forms in the North Atlantic (total Hg 2–3 ng dm^{-3}) [266]. This agrees with the observations that alkyl Hg species occupy a minor portion (<1–20% of total Hg) in seawater obtained in studies that determined chemical species.

Analysis of cationic and anionic forms, which approximately correspond to inorganic Hg and organically-bound Hg, was done for soil leachates by ion-exchange chromatography followed by a Hg detection system [267]. It was found that organically-bound Hg was predominant. Nakayama *et al.* further separated organically-bound Hg, adsorbed onto pm XAD-2 resin, into lipid-, protein- and carbohydrate-bound Hg [268]. Lipid-bound Hg was eluted with $CHCl_3$. Protein-bound Hg was salted out with $MgCl_2$ and separated by filtration, and the filtrate was regarded as containing carbohydrate-bound Hg. They found that 50% of total Hg in seawater was organically-bound, 69% of which was protein-bound and the remainder was lipid-bound.

The nature of mercury in water at very low concentrations is not yet well understood. Decreased concentrations of inorganic mercury in water stored in PE bottle is often encountered when initial concentrations are low. Scholz and Mayer identified by differential pulse anodic stripping voltammetry on a glassy carbon electrode that almost all mercury in tap water was atomic [269]. In solutions which did not contain thiocyanate, ionic mercury diminished within hours when the concentration was below 10^{-9} mol dm^{-3}.

Mercury in sediment and soil Sediment is another major compartment in the aquatic Hg cycling process. Biomethylation of inorganic Hg by microorganisms was recognized as occurring in the sediment [136]. Photochemical and humic substance-mediated methylation have also been recognized to play important roles in the natural formation of CH_3Hg^+ in the aquatic environment.

Work on chemical species of Hg in sediments has focused on the distinction between inorganic and organic Hg. For total Hg determination, the following conventional method is most common: acid

digestion (HNO_3/H_2SO_4 or *aqua regia*) of wet/dry sediment, reduction by $SnCl_2$ and AAS detection [270–273]. NAA [274] and solid sample introduction-Zeeman AAS system [275] have also been used.

The GC/ECD method, which Westöö [276] originally developed for the determination of CH_3Hg^+ in fish [277–282] will likely be the most popular method for the determination of organic mercury species in sediment samples. In all of these procedures the sediment sample is first treated with HCl to extract organic Hg as organic Hg chloride.

Analysis of inorganic and organic Hg with a steam distillation-AAS method has been reported [283]. Steam distillation using KCl and H_2SO_4 is used for CH_3Hg^+ in sediment with detection of CH_3Hg^+ by GC-ICP-MS [137, 261].

Alkali (1 N KOH-ethanol) digestion for sediment CH_3Hg^+ determination has been reported [284].

Use of alkali for the digestion prevents the formation of an emulsion, and produces homogeneous digests and uniform sample distribution [285]. Kanno *et al.* compared the analytical results for CH_3Hg^+ concentration in Hg treated sediment samples obtained by the alkali digestion-dithizone-benzene extraction method with those by the HCl treatment-benzene extraction method [284]. Systematically higher extraction efficiency and the absence of interfering peaks on the chromatogram were achieved by their alkali digest method. The use of supercritical fluid extraction has also been examined [115].

Interference by sulfide in the determination of sediment organic Hg was reported but elimination of sulfide as H_2S proved to be effective [282]. The presence of clay or humic acid did not interfere with Hg measurement.

The influence of the sample storage method has been evaluated for total Hg. Storage at -20 °C did not result in significant change [270].

Conversion of Hg species may be possible during storage since natural sediments contain various microorganisms and humic substances. Fulvic acids have also been found to have methylating properties, at least at elevated temperatures [282]. Evidence has been presented for spurious formation of methylmercury during supercritical fluid extraction of sediment [283].

Analytical results for total Hg and CH_3Hg^+ in sediments, along with % CH_3Hg^+, are summarised in Table 3. These results agreed that up to 1.5% of total Hg exist in sediments as organic Hg, although the concentrations of HgS are fairly variable according to location (river sediments seem to contain higher CH_3Hg^+ than do marine sediments).

Table 3. Total and organic mercury concentrations in sediments

Location	T-Hg (mg g^{-1})	O-Hg (ng g^{-1})	O/T (%)	References
R. Rhein	5–17	10–110	0.2–1.0	[278]
R. Yssel	12	70	0.6	[278]
R. Mersey	1.2–11.3	1.6–60.6	0.05–1.4	[280]
R. Clyde	0.4–4.4	0.3–5.4	0.01–0.4	[280]
R. Clyde	<0.05–5.31	<0.3–7.8	0.81	[298]
R. Suimon*	9.4–16.9	3–24	0.02–0.14	[282]
San Francisco Bay	0.1–1.3	0.4–1.9	0.03–1.0	[278]
Irish Sea	<0.01–0.2	<0.3–2.7	1.35	[280]
Kastela Bay* (w) (Yugoslavia)	0.5–6.13	<2–20	1.37	[281]

* samples from Hg contaminated area. 'w' indicates that concentration is reported on a wet weight basis and others are on a dry weight basis.

In most Hg-contaminated soils, the major portion of Hg is present in inorganic form, Hg^0 or mercuric sulfide. Since several researchers have shown that mercuric sulfide is an insoluble and non-bioavailable form of Hg, determination of mercury species in contaminated soils is significant in terms of the ultimate

fate of Hg. Sequential extraction methods have been used for the speciation of inorganic Hg [286–288]. Wang *et al.* reported a method for the determination of total and 'available' Hg in field soils and Hg in rice [229]. 'Available' Hg was defined as an extractable fraction when a 3g soil sample was treated with 20 volumes of 0.03% thioglycolic acid-0.067 M Na_2HPO_4 (pH 8). The correlation of 'available' Hg in soil to brown rice Hg concentration was good.

A differential determination method for mercury, mercury (II) oxide, and mercury (II) sulfide in sediments has been reported [289] and was based on successive extraction followed by CVAAS detection. Methylmercury was extracted with chloroform, mercury oxide being extracted with 0.05 M sulfuric acid and finally HgS was extracted with 1M HCl containing 3% NaCl and a small amount of copper (I) chloride. A selective determination of mercury sulfide in soils and river sediment has been used [290]. HgS was extracted with saturated Na_2S solution and determined by CVAFS.

Comparison of three different sequential extraction methods on five test soils indicated that Hg in the sample was predominantly the relatively insoluble mercuric sulfide or metallic Hg, but the relative proportions of each did not agree well between procedures. Therefore further development of speciation methods is necessary [291].

The thermal evaporation of mercury from soils and sediments with high concentrations of organic matter indicate that a qualitative conclusion can be derived about the content of metallic mercury as well as mercury associated with organic matter or sulfide [292]. Thermal release analysis of mercury species in contaminated soils was carried out by continuous heating of samples in a furnace coupled to an AAS [293]. The method can be used as a tool for observing mercury oxidation states and/or reactions of mercury (Hg, Hg_2Cl_2, $HgCl_2$, HgO, HgS).

Mercury in biological samples Human methylmercury poisoning resulting from ingestion of contaminated seafood has been well-documented by Japanese epidemics at Minamata and Niigata. Consequently, many countries have established maximum permissible mercury concentration in fish used for human consumption, usually in the range of 0.4 to 1.0 mg Hg g^{-1} tissue (wet weight).

Certain species of commercial marine fish, usually top predators, e.g. tuna, shark, bonito, swordfish, are known to concentrate environmental mercury, and are therefore, a major human dietary source of the element. Tuna has been the most frequently surveyed species because of its extensive world-wide consumption. Most published analytical reports generally confirm that methylmercury exceeds 75% of the total mercury content in the edible fillet of tuna.

The most commonly used method for the determination of inorganic mercury and total mercury in fish and other food samples is the selective reduction cold vapour atomic absorption technique. [17,75,299,300]. Other methods which are cited in section III are also applied to biological samples. Recently, it was suggested that the method might in some cases overestimate the concentrations of inorganic mercury in biological materials [301]. The error is caused by a demethylation of methylmercury to inorganic mercury in the analytical procedure.

The complex biological matrix causes considerable interferences in the extraction process and makes calibration difficult. Results from standard addition will only be correct if the calibrant added and the determinant are in the same chemical form and homogeneously distributed in the sample to be analyzed. This presupposition will be fulfilled if the sample is digested into a homogeneous solution by alkaline hydrolysis.

When the methods were compared, the alkali digestion method gave a higher methylmercury concentration than was given by conventional benzene extraction, suggesting the higher efficiency of the alkali digestion method [297]. The methylmercury value obtained by the alkali digestion was up to more than 95% of the total mercury in the fish samples examined.

To avoid uncontrolled losses of methylmercury at high pH, cysteine is added at the beginning of the pretreatment procedure [302]. Methylmercury can be lost from blood when it is freeze-dried [303].

Sulfuric acid was used to release all methylmercury from biological (fish) tissue, in conjunction with iodoacetic acid to convert the methylmercury into the iodide form. Finally the head space gas was introduced semi-automatically to a GC equipped with MIP. Use of a semi-automated head space for

methylmercury compounds in biological samples involved such compounds being converted into methylmercury iodide which has the highest vapour pressure among methylmercury halides, and analyzed by gas chromatography with microwave induced plasma emission spectrometry [121]. The detection limit for methylmercury was 1.5 mg g^{-1} of homogenate. The sample matrix effect was examined and then applied to the determination of MeHg in cod caught in the North Sea [125].

Hair samples were digested in alkali solution/toluene in an ultrasonic bath at approximately 50°C. After cooling and treatment with HCl (6 mol dm^{-3}) and addition of a saturated solution of copper sulfate, the organic phase was extended in toluene by GC-ECD using a DB17 capillary column. The practical detection limit for the method for methylmercury was 50 ng g^{-1} using a 100 mg sample of hair [304].

Automated analytical methods are important for routine analysis. Automated continuous flow vapour generation coupled to atomic fluorescence spectrometry was used for the determination of mercury in urine [305] and the method was applied to water analysis [306]. A wide variety of on-line manipulations of samples has been carried out by flow injection (FI) techniques providing a safe, contamination-free enclosed sample handling system. An automated determination method has been devised based on CVAAS for blood [24]. A system for on-line treatment of liquid samples in a microwave oven and determination of mercury by CVAAS was designed and applied to water and urine [307] and to blood samples [308].

A simple and rapid determination method of chemical forms of mercury in human hair was described [309] using acid leaching and CVAAS. Hair samples were also analyzed by an alkali-digestion method and results were similar. LC/ICP-MS has been used for qualitative and quantitative determination of mercury species in human hair [310]. Methylmercury was back extracted into the HPLC mobile phase (0.02 mol dm^{-3} cysteine in 0.1 mol dm^{-3} acetic acid; pH 2.2).

Urine has been analysed for mercury species by CVAAS and GC/AAS [311–313]. An electrothermal AAS procedure has been employed for the determination of organic and inorganic mercury in various biological materials including blood. Inorganic mercury was converted to a methyl chloride derivative by methanolic tetramethyltin prior to extraction [314].

Selenium has been shown to counteract both inorganic mercury and methylmercury toxicity in several animal species. Several marine mammal species which feed on fish, accumulate mercury to high concentrations with a molar mercury/selenium ratio of 1:1 without showing any evidence of methylmercury poisoning [315]. To assess the nature of possible Hg-Se compounds, which may be involved in mercury accumulation and detoxification in seafood, it is necessary to identify the specific forms of mercury and selenium present in the edible tissue.

In most instances, mercury and selenium in the edible tissues are present as water-extractable species. In the aqueous fraction, mercury and selenium may be bound to amino acids, peptide, low molecular weight proteins and high molecular weight proteins. A low molecular weight methylmercury complex having properties similar to methylmercury glutathione was isolated from rat cerebrum [316], whereas the demonstration of the presence of a glutathione mixture complex Hg^{2+}, selenite and glutathione suggested the possible intermediate formation of a high molecular weight mercury selenium complex [317]. It was shown that methylmercury is associated with haemoglobin-containing and low molecular weight tissue components [318]. A Hg-Se protein complex was isolated from rat plasma which contained the elements in a 1:1 atomic ratio, and it was postulated that selenium was attached to the SH-groups on the protein and that mercury was attached to the selenium [319]. A soluble low molecular weight Hg-Se complex has been partially purified from dolphin liver [320], but, on the other hand, the mercury-selenium complex in dolphin liver was reported to be mercuric selenide [321]. These studies, however, have not fully defined the chemical form of mercury and more work is required.

It was demonstrated that added selenite can release methylmercury from protein linkage in various animal tissues (blood, liver, kidney and tuna muscle) and thereby influence its tissue distribution [322]. The non-protein bound complex containing methylmercury and selenium in a 2:1 ratio was isolated from rat erythrocytes and rabbit blood which had been incubated with methylmercury and selenite [317,323]. The complex, extractable by benzene, was identified as bis (methylmercuric) selenide, $(CH_3Hg)_2Se$. There was a small fraction (<10%) of the total tissue mercury that was benzene-extractable in tuna, but whether it is actually $(CH_3)_2Hg$ or $(CH_3Hg)_2Se$ remains to be established.

ANALYTICAL CONSIDERATIONS

Sampling and Sample storage Sampling and sample storage methods are, in general, critical aspects for trace element determination/speciation in natural waters. Attention has been given to post-sampling loss. For example, it was shown that mercury concentrations in river water, which was sampled into polyethylene (PE) bottles, decreased even after a few minutes following sampling and this decrease continued over time [324]. The applicability of PE bottles to water storage for mercury analysis has been tested and it was found that 95% of the initial mercury (5 ng cm^{-3}) was lost during 21 days when samples were stored without preservatives [325]. Of the 95% loss, 77% was attributed to the adsorption onto the bottle wall and 18% to vaporisation This significant loss could be reduced to 2% by addition of 0.05% $K_2Cr_2O_7$ + HNO_3 or Au + HNO_3 at pH 0.5.

Storage of water samples in PE bottles cannot be recommended because it was found that an increase of mercury concentration occurred in water, which was being stored in a PE bottle after the pH was adjusted to 1.5 with HCl [326]. This was attributed to the addition of atmospheric mercury which was permeable through the PE wall. Storage of water samples for Hg determination in Pyrex containers with 1% H_2SO_4 and 0.05% $K_2Cr_2O_7$ as preservative has been recommended [327]. Addition of Au or $K_2Cr_2O_7$ to acidified samples maintained the initial mercury level at least for 5 months [328].

In speciation studies, addition of a preservative prior to storage might result in a change of the species present. Goulden and Anthony found that a significant portion of added alkyl-, phenyl- and diphenyl-Hg (200 ng Hg dm^{-3} each) was converted to Hg^{2+} during the storage period (30 days) under the recommended conditions [80]. They recommended H_2SO_4 alone for storage, by which negligible conversion was observed for these Hg species. However, it should be further tested whether conversion/loss/adsorption takes place for lower and real Hg species concentration in natural waters. Freezing should be avoided for freshwater samples, although it may be acceptable for seawater samples. Storage at 4°C has been recommended for heavy metal determination [261]. Prompt analysis is, more than anything, most desirable.

Certified Reference Materials In contrast to precision, the accuracy of an analytical method is difficult to evaluate. Biased data may result from poor methodology, improper instrumentation, poor calibration, faulty experimental techniques, lack of a proper control, or a combination of these factors. Many analytical chemists are publishing new analytical methodologies and techniques for environmental and biological samples. To validate these analytical methods, and to link those methods to more established methods, accuracy examination is required by use of appropriate certified reference materials (CRM). The increased awareness and concern for public health has led to a much increased demand for routine methods that are fast and economic. These methods require validation and quality control.

Many environmental and biological reference materials certified for total mercury content are now available from NBS, IAEA, BCR, NRC Canada, NIES and other agencies. Although the number of CRMs available for individual mercury species was small, it is now increasing (Table 4).

For determining a certified value, it is necessary to have analytical results obtained by at least two different analytical methods which differ in principle. Methods that were used for BCR reference materials included the Westöö extraction/capillary EC-ECD, HCl/toluene extraction/GC-ECD, acetic-sulfuric acid extraction/AFS, $NaBH_4$/capillary GC-FTIR, butylation/GC-MIP, distillation/$NaBEt_4$ /GC-AFS and others [331,332].

Although certified values for methylmercury or organomercury are not given for the rest of the currently available CRMs for total Hg, the reported values for MeHg in CRMs are informative. For example, Bushee determined methyl-mercury in NBS RM-50 Albacore tuna by LC/ICP-MS. The samples were prepared by toluene extraction of the chloride form. The value obtained was 873 ± 60 ng cm^{-3} which compared favourably with the values reported (760 to 890 mg) [177]. The MeHg concentrations in six biological CRMs have been reported [329].

Most of the CRMs are stored at room temperature and undoubtedly it is important to investigate the effect of storage conditions on the stability of methyl Hg and other organic Hg in these CRMs. This will be the subject of further research.

TABLE 4. Concentration of mercury species in Certified Reference Materials (mg g^{-1} dry unless stated)

		Me-Hg	Total-Hg
Sediments			
IAEA 356	Harbour sediment	0.00546 ± 0.00039	
IAEA 142	Harbour sediment	0.00477 ± 0.0043	
Tissues			
LUTS-1	Fresh lobster	0.0094 ± 0.0006	0.0167 ± 0.0022(wet)
	hepatopancreas	0.063 ± 0.004	0.112 ± 0.015
TORT-1		0.128 ± 0.014	0.33 ± 0.06
TORT-2		0.152 ± 0.013	0.27 ± 0.06
DOLT-1	Dogfish liver		0.225 ± 0.037
DOLT-2		0.693 ± 0.053	1.99 ± 0.10
DORM-1	Dogfish muscle	0.731 ± 0.060	0.798 ± 0.074
DORM-2		4.47 ± 0.32	4.46 ± 0.26
NIST			
SRM 1974a*	Mussel	0.0772 ± 0.0038	0.176 ± 0.013
SRM 2974	Mussel	0.0772 ± 0.0038	0.176 ± 0.013
SRM 2976	Mussel	0.0277 ± 0.002	0.061 ± 0.0035
NIES No.13	Human hair	3.8 ± 0.4	4.4 ± 0.4
Sea Plant			
IAEA 140/TM†	Sea plant homogenate		
NIES No.9	Sargasso	5.7 ± 0.1*	0.04†

* Information only.Ref. 329, †Ref. 330

The Canadian reference materials (DORM-1, TORT-1 and DOLT-1) have recently been given values for methyl mercury.

CONCLUSIONS

Speciation analysis is important for the understanding of biogeochemical cycling of the elements and the toxicological implications for humans and for ecosystems. With recent developments in chromatography and atomic spectrometry, the analytical methods for total mercury and methylmercury seem well established for samples in solution at the p.p.m.–p.p.b. level. Reliable sensitive methods are necessary for determinations at the ppt level of these and other less stable species. In solid samples, however, extraction steps for each mercury species from major matrices can be sources of error, especially in sediments and biological samples. An adequate correction method for recovery is important. For accuracy control, including recovery correction in extraction procedures, the use of certified reference materials is crucial. Numbers of CRMs available for mercury species are still small but increasing; further preparation of such materials is needed.

Some mercury species are known to be lost during storage. This is important for the measurement as well as long-term storage of samples such as in specimen banking. Further research is also necessary in this area. Interaction of mercury species with other components in organisms, both in animal experiments and in environmental field observations, is not well established. Selenium, for example, is known to modify mercury toxicity through chemical interactions but the mechanism is not understood. Further research is necessary in this field and new analytical methods may be required for such a purpose.

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