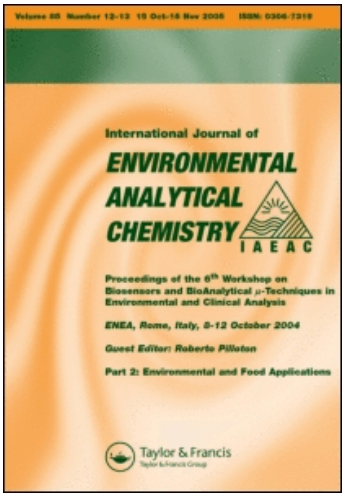

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Overview of Analysis of Carcinogenic and/or Mutagenic Metals in Biological and Environmental Samples I. Arsenic, Beryllium, Cadmium, Chromium and Selenium†

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One of the most dangerous and pernicious forms of pollution arises from the potential mobilization of a spectrum of toxic trace metals and metalloids in our environment. Among the most important elements in this regard are arsenic, beryllium, cadmium, chromium and selenium whose adverse toxic effects are now well recognized including their carcinogenicity and/or mutagenicity. These agents (and their derivatives) can be widely dispersed throughout the environment as a result of fossil fuel combustion, industrial and agricultural processes and natural processes. The trend for the immediate future appears to be of greater exposure to these metals not only as a result of generally increased usage patterns but also because of prospective enhanced use of fossil fuels for space heating and electricity generation.

In order to more readily evaluate trends of human exposure as well as the toxicity, bioavailability, bioaccumulation and transport of these elements, sensitive analytical procedures are required for the determination of their various oxidation states (as well as their organic derivatives) in complex matrices such as those found in both environmental and biological samples. Hence, the principal objective of this overview

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is to highlight the more recent trends and state-of-the-art methodologies for the determination of arsenic, beryllium, cadmium, chromium and selenium (in their various forms) in environmental compartments such as air, water, soil and in human tissues (primarily blood, urine, and milk). Techniques to be discussed primarily include atomic absorption spectrometry, neutron activation analysis, gas chromatography, differential pulse polarography and electrochemical analysis. The importance of quality control and differentiation according to speciation will also be stressed.

KEY WORDS: Analysis, biological, environmental sampler, carcinogenic mutagenic metals, exposure levels, speciation.

1. INTRODUCTION

We are becoming increasingly aware of both the ubiquity and toxicological aspects (particularly carcinogenicity and/or genotoxicity) of a number of important metals and metalloids and their compounds to which large segments of the population, both industrial workers and the general public, may have potential exposure. Additionally, we recognize the human essentiality of a number of trace elements such as chromium and selenium. It is of critical importance not only to know the nature of the myriad sources of exposure both from natural and anthropogenic sources but also their levels and valence states as well. This is so since it has been established that in a number of cases (e.g., arsenic, chromium and selenium) specific valence states of the metal and/or metalloid are crucial for the major toxicities exhibited as well as their bioavailability.

In order to more readily evaluate trends of human exposure as well as the toxicity, bioavailability, bioaccumulation, transport and transformation of these elements, sensitive analytical procedures are required for the determination of their various oxidation states (as well as their organic derivatives) in complex matrices such as those found in both environmental and biological samples.

Hence, the major objective of this overview is to highlight the more recent trends and state-of-the-art methodologies for the determination of arsenic, beryllium, cadmium, chromium and selenium (in their various forms) in environmental compartments, work environments and in human tissues (primarily blood, urine).

General aspects of exposure levels, sources, transport and alterations,[1,2] toxicity,[3-7] carcinogenicity and genotoxicity for arsenic,[4,5] beryllium,[4,5] cadmium,[5,6] chromium[4,5] and selenium[5,7] and their compounds have been reviewed.

2. ARSENIC

Arsenic is a ubiquitous element on earth, and the presence of inorganic and several methylated forms of arsenic as monomethyl-, dimethyl- and trimethyl arsenic compounds in the environment have been well documented.[8-15] Arsenic principally contributes to environmental pollution through emission from non-ferrous smelters, coal-fired and geothermal plants and application of arsenical pesticides. Human exposure to inorganic arsenic compounds occurs principally via inhalation of industrial dust and via ingestion of contaminated drinking water or food.[1-4,12-15] Estimated global man-made releases of the order of 24×10^6 kg/year may be compared to natural releases of about 8×10^6 kg/year. The major emissions of arsenic to air arise from smelting of metals, burning of coal, pesticide use and from volcanoes (Table I).[12,13]

A summary of representative concentrations of arsenic in the environment and of transfer rates to man is shown in Table II.[12] Arsenic in the air is present mainly in particulate form as arsenic trioxide with representative background levels of arsenic in air being 1 to 10 ng/m^3 in rural areas and 20 ng/m^3 in urban areas.

Table III summarizes the molecular forms of arsenic found in the environment and Figures 1 and 2 illustrates the environmental transformations of arsenic[14] respectively.

Estimates vary as to the daily intake and body burden of arsenic. It is widely acknowledged that ingestion of arsenic in the diet is quite variable, depending to a large extent on the amounts of seafood consumed. The daily intake of arsenic (as arsenic trioxide in the United States) was calculated to be 0.137–0.330 mg/person/day.[16] Other reports have suggested that arsenic is present in the diet at levels of 0.05–0.16 mg/kg (wet weight) which corresponds to an intake of 0.15–0.40 mg/person.[17-20] Based on measured absorption and retention of arsenic in man it has been estimated that an ingestion intake rate of arsenic in terrestrial foods of 1 mg/year

TABLE I

Global emissions of arsenic to the environment.[12,13]

Source		Emission rate (10^6 kg y^{-1})
Natural		
Volcanoes		7
Biological activity		0.26
Weathering		0.24
Forest fires		0.16
Sea spray		0.14
	Total	7.8
Anthropogenic		
Copper production		13
Iron and steel production		4.2
Lead and zinc production		2.2
Agricultural chemicals		1.9
Wood fuel		0.60
Agricultural burning		0.56
Coal combustion		0.55
Waste incineration		0.43
Chemicals production		0.20
Cotton ginning		0.023
Mineral ore mining		0.013
Residual fuels		0.004
	Total	23.7

contributes a concentration of arsenic in the body of 0.28 μg/kg and the relationship is 0.14 μg/kg per mg/year intake of organic arsenic in seafood.[13] The body burden of arsenic is estimated to be about 1 mg from reported tissue measurements and from representative intake estimates.[12,21] Other estimates of the arsenic body content are overestimates by comparison and range from <10 mg[22] and 3–4 mg.[12] It is generally acknowledged that more extensive data, especially in terms of geographic region and age of individuals are required to more realistically set a representative value of arsenic body content.[12] Table IV lists current levels of arsenic in air, soil, diet and man and utilizing this table, Bennett[12] estimated that the

TABLE II

Environmental arsenic; summary of representative values.[12]

Concentration		
Atmosphere		
rural	$6\,ng\,m^{-3}$	(0.2–40)[a]
urban	$20\,ng\,m^{-3}$	(10–750)
Lithosphere		
agricultural soil	$7\,\mu g\,g^{-1}$	(0.2–40)
Hydrosphere		
freshwater	$<10\,\mu g\,l^{-1}$	(variable)
marine	$<5\,\mu g\,l^{-1}$	
Biosphere		
terrestrial foods	$<0.3\,\mu g\,g^{-1}$	
fish	$3\,\mu g\,g^{-1}$	(1–10)
Transfer rates to man		
Intake		
ingestion	$120\,\mu g\,d^{-1}$	(30–370)
inhalation	$0.4\,\mu g\,d^{-1}$	(0.1–4)
Absorption		
gastro-intestinal tract	0.9	(0.8–1.0)
lungs	0.3	(0.2–0.4)

[a]Range of values in parentheses.

TABLE III

Environmental forms of arsenic.

As(III) arsenite ion, As(V) arsenate ion, $CH_3AsO(OH)_2$	Seawater Freshwater ponds, rivers, lakes
$(CH_3)AsO(OH)$	Seawater Freshwater
$(CH_3)_3As$ (or the oxide)	Freshwater
As(III), As(V) CH_3AsH_2, $(CH_3)_2AsH$, $(CH_3)_3As$,	Particulate over As-treated soil
As(III), As(V) $CH_3AsO(OH)_2$, $(CH_3)_2AsO(OH)$ $(CH_3)_3As$	Seaweed and epiphytes
As(III), As(V), $CH_3AsO(OH)_2$	Urine

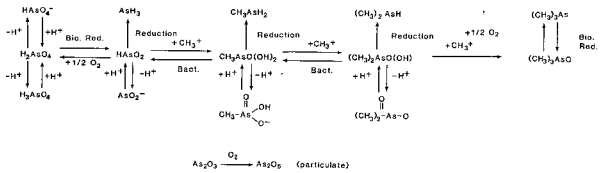


FIGURE 1 Summary of environmental chemistry of arsenic.

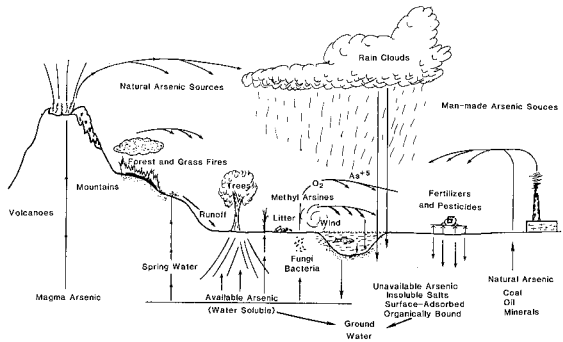


FIGURE 2 Environmental transfer of arsenic.[14]

TABLE IV

Current levels of arsenic in the background environment and in man.[12]

	Air	Soil	Diet	Body
Ingestion pathway				
Terrestrial foods	$6^{ng/m^3}$	$7^{\mu g/g}$	$15^{mg/y}$	$4^{\mu g/kg}$
Aquatic foods[a]			$33^{mg/y}$	$5^{\mu g/kg}$
Drinking water			$0.5^{mg/y}$	$0.1^{\mu g/kg}$
Inhalation pathway				
Urban	$20^{ng/m^3}$			$0.01^{\mu g/kg}$
Rural	$6^{ng/m^3}$			$0.004^{\mu g/kg}$
			Subtotal[b]	$4.1^{\mu g/kg}$
			Total	$9^{\mu g/kg}$
[Effects level $15–340\,\mu g\,kg^{-1}$][b]				

[a]Intake of organic forms of arsenic.
[b]Associated with intake of inorganic forms of arsenic.

concentration of arsenic in the body corresponds to a body burden of about 0.6 mg (9 μg/kg $\times$ 70 kg).

Human exposure to inorganic arsenic compounds occurs mainly via inhalation of industrial dust and via ingestion of contaminated food or drinking water. Exposure to organic arsenicals occurs mainly through compounds biosynthesized across the food chain. These compounds are characterized by a high absorption rate and exhibit a metabolic behavior and degree of toxicity different from that of inorganic arsenic. Information on the behavior of arsenic in the human body has often been conflicting in regard to the accumulation of As^{+3} in the body[23] whether As^{+3} is oxidized to As^{+5} are methylated in the body.[8,25-27] However, the more current consensus is that biotransformation occurs in the body from trivalent to pentavalent form and from inorganic to methylated forms which may serve as a detoxifying mechanism.[12,27]

(a) Determination

As noted above, methylated forms and inorganic forms of arsenic can co-exist in the environment. Speciation is of particular importance since the toxicity and ecological fate of an element is determined by its chemical form. Hence, the determination of arsenic and its speciation in the occupational and the general environment as well as in biological samples is of critical importance and justifies the continued development of improved techniques for the speciation analysis of environmental samples. Methods of analysis for arsenic and its compounds in environmental samples have been reviewed.[4,10,14,28-34] As with other metals and metalloids, the method of choice for a particular application is dependent on a number of factors including: equipment available, sample matrix, specificity, detection limits, accuracy, etc. It should also be noted that variations in sensitivity, precision and accuracy occur not only among different methods but also among various models of equipment as well as among different operators. Many methods have been developed for the determination of total arsenic and for the various inorganic and organic forms of arsenic. Aspects of pre-treatment and dissolution of the sample by wet ashing, dry ashing, oxygen combustion and fusion, stability of solutions during storage, preconcentration and isolation of arsenic for eventual determination

of total (inorganic) arsenic by procedures involving coprecipitation and adsorption, liquid–liquid extraction have been reviewed by Talmi and Bostick[28] and Talmi and Feldman.[29] The methods most frequently used for the determination of arsenic include: molecular absorption-spectrophotometry, neutron activation analysis (NAA), polarography, atomic absorption spectrometry (AAS), flameless atomic absorption spectrometry (FAAS), X-ray fluorescence spectroscopy and gas–liquid chromatography. Table V lists a number of analytical methods for arsenic and its compounds in environmental samples.[4]

It is well established, as noted earlier, that methylated forms and inorganic forms exist in the environment. In the past their determination and speciation at low levels normally found presented considerable problems which now have been largely overcome.[8, 10, 30, 45–48.]

The various arsenic forms may be separated by reduction of the arsenic compounds to the corresponding arsines. Since there is evidence that only the undissociated forms of the arsenic acids are capable of being reduced to the corresponding arsines, some measure of selectivity can be achieved by controlling the pH of the medium. The difference in their boiling points permit the separation of the arsines either by gas chromatography[45, 46] or by fractional volatilization.[47] Various techniques including microwave emission spectrometry,[45] electron capture and flame ionization detection[46] and d.c.-discharge emission,[47] hydrogen–air flame atomic absorption[46] and graphite furnace atomic absorption spectrometry[48] have been employed for the determination of the arsines. DC-emission spectrometry provides high sensitivity and element selectivity and is considerably less prone to interferences than is atomic absorption.[30] Additionally, arsine can be determined colorimetrically using either a hexamethylene tetramine chloroform (or ephedrine–chloroform) solution of silver diethyldithiocarbamate (AgDDC) or a pyridine solution of AgDDC which produces a red color. Gas liquid chromatography with a nitrogen–phosphorus detector has also been used for the determination of the arsines. The absolute limit of detection is around 0.2 μg for the colorimetric technique using silver diethyldithiocarbamate, 0.1 μg for the flame atomic absorption technique and the gas–liquid chromatographic technique, 0.01 μg for the flameless atomic absorption technique and 1.0 ng for the emission spectroscopic technique.[28]

TABLE V

Analytical methods for arsenic and arsenic compounds.[4]

Sample matrix (Reference)	Sample preparation	Assay procedure	Sensitivity or limit of detection
Air[35]	Collect on filter; wet ash with nitric and perchloric acids; solubilize in nitric acid; pipette into graphite tube	FAAS	Working range: 0.1–1.3 mg/m^3 air
Air[36]	Collect on filter; wet ash with nitric and sulphuric acids; convert to trivalent arsenic with potessium iodide and stannous chloride; reduce to arsine with zinc in an arsine generator	Visible spectro-photometry	0.0004 mg/m^3
Arsenic trioxide[37]	Sample at 3 l/min for 3 min; dissolve filter paper in sodium hydroxide	UV	$>$0.0006 mg/m^3
Water[38] As(III)	Dilute; purge with nitrogen; treat with hydrochloric acid; add zinc; reduce to arsine	Visible spectrophotometry photometry	0.001 mg/l
Water[39]	Digest with nitric acid and hydrogen peroxide; stabilize with nickel nitrate	FAAS	0.078 mg/l
Water[40]	Use the enzyme glyceraldehyde-3-phosphate dehydrogenase	Fluorescence	0.02 mg/l
Water[41]	Adjust pH to 3; add sodium hydrogen sulphate; boil; neutralize and boil	Differential pulse polarography	0.007–0.02 μg/l

TABLE V (continued)

Sample matrix (Reference)	Sample preparation	Assay procedure	Sensitivity or limit of detection
Soils and plants[42]	Homogenize; irradiate; dissolve in perchloric and nitric acids; add hydrogen fluoride; add perchloric acid; pass through stannous dioxide column	NAA	
Soils and plants[36]	Wet ash with nitric and perchloric acids; add hydrochloric acid, potessium iodide and stannous chloride; add pyridine solution of silver diethyldithiocarbamate; generate arsine	Spectrophotometry (535 nm)	0.01 mg/kg
Soils and plants[43]	Irradiate; wash with sulphuric, nitric, hydrochloric and hydrobromic acids; distill; precipitate arsenic sulphides with acetamide	NAA	
Wines, fruit juices,[44] raisins, rice, shellfish, beef liver)	Dry ash with magnesium nitrate; rinse with hydrochloric acid; reduce to arsine with sodium borohydride in an arsine generator	AAS	0.02 mg/l (beverages) (0.02 mg/kg) (foods)

The determination of arsenic and its speciation in the aquatic environment has engendered increasing interest in recent years particularly since the presence of arsenic in drinking water supplies is considered to represent a significant health hazard.[49] Arsenic contamination in potable water has been linked with cancer and neurological as well as skin pigmentation and keratoses.[4, 50, 51] The most predominant oxidation states of arsenic in natural waters including drinking waters appears to be +3 and +5.[52] Except in instances of high arsenic pollution, total arsenic in water is generally in the 1–10 ppb range (and in air in the 0–10 ng/m^3 range).[10]

Electrothermal atomic absorption spectrometry (ETA) is one of the useful techniques employed for measuring ng/ml levels of arsenic in water samples. The ETA technique coupled with hydride evolution[53] or ammonium pyrrolidine dithiocarbamate-methyl-isobutylketone (APDC-MIBK) extraction[54] has been used to differentiate between As^{+3} and As^{+5}. Another potential analytical technique that can be used to distinguish As^{+3} and As^{+5} is linear scan anodic stripping voltammetry (LSASV).[55, 56] Subramanian *et al.*[56] recently determined As^{+3} and As^{+5} and total arsenic in polluted waters utilizing the APDC-MIBK-ETA procedure and a toluene extraction anodic stripping voltammetric (ASV) procedure. The total arsenic content of these water samples was also determined using the direct electrothermal atomic absorption spectrometry (ETA) and the nickel-matrix modification-ETZ methods. There was good agreement among the various methods on the total arsenic content of water samples. Table VI illustrates the analytical parameters for arsenic using some electrothermal atomic absorption spectrophotometric methods.[56] It should be noted that the detection limit and sensitivity of all methods used in the above study were sufficiently low to permit the determination of arsenic in polluted waters.

In the ASV method, the detection limit (3 SD of blank) and linear working range were 1.0 ng As/ml and 10–500 ng As/ml respectively. The precision expressed as the percent coefficient variation at the 95% confidence interval for 4 replicate measurements was 5.2 and 2.9 at 10 ng As/ml and 50 ng As/ml respectively.

Species specific analysis for nanogram quantities of arsenic in natural waters by arsine generation followed by graphite furnace atomic absorption spectrometry was described by Shaikh and

TABLE VI

Analytical parameters for arsenic using some electrothermal atomic absorption spectrophotometric methods.[56]

Parameters[a]	Direct ETA	Ni-matrix modification ETA	APDC-MIBK-ETA
Sensitivity, $ng\,ml^{-1}$	2.8	2.3	1.3
Detection limit, $ng\,ml^{-1}$	4.0	1.5	0.7
Linear range, $ng\,ml^{-1}$	0–100	0–60	0–90
Precision (%) at $5 \times$ d.l.	21	14	17
$10 \times$ d.l.	12	11	9

[a]Sensitivity is concentration in $ng\,m^{-1}$ for 0.0044 absorbance units. Detection limit is twice the standard deviation of the blank. Precision refers to coefficient of variation at the 95% confidence level for 100 determinations.

Tallman.[48] The various arsenic species in a water sample were reduced with a 5% solution of sodium borohydride in 0.1 M NaOH to the corresponding hydrides, collected in a liquid nitrogen cold trap from which they were selectively vaporized and then swept direct through the sample port into a graphite furnace for detection. Figure 3 illustrates the arsine generation trapping and volatilization apparatus.[48] The reproducibility of the method is quite good. At the 100 nanogram level corresponding to arsine generation from 50 ml of a 2 ppb water sample, 10 replicate determinations of inorganic arsenic in reference samples yielded a r.s.d. of 2%. Somewhat poorer

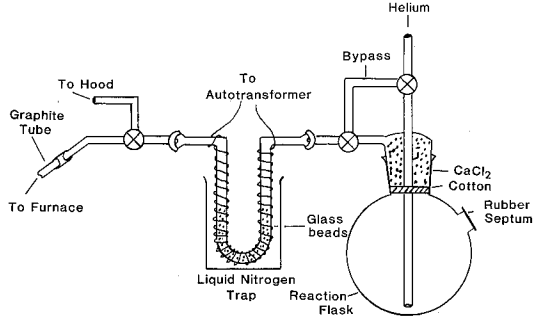


FIGURE 3 Arsine generation, trapping and volatilization apparatus.

precision was obtained for methylarsenic acid (MAA) (r.s.d. 5%) and dimethyl arsenic acid (DMAA) (r.s.d. 5%). Detection limits in terms of nanograms arsenic were 1, 15 and 10 ng for inorganic arsenic, MAA and DMAA respectively.

Andreae[46] described a method for the determination of arsenate, arsenite, mono-, di- and trimethylarsine, monomethylarsinic and dimethylarsinic acid and trimethylarsine oxide in natural waters with detection limits of several ng/liter. The arsines were volatilized from the sample by gas stripping. The other species were then selectively reduced to the corresponding arsines by $NaBH_4$ and volatilized. The arsines were collected in a cold trap cooled with liquid nitrogen and separated by slow warming of the trap or by gas chromatography and measured with atomic absorption, electron capture and/or flame ionization detectors. The modular approach of Andreae[46] was found to be highly efficient in the analysis of natural waters with a high variability of arsenic species composition. Whereas the inorganic forms of arsenic could always be determined by the relatively rapid boiling point–separation–atomic absorption detection technique (about 20 min per sample for As^{+3} and As^{+5}), the slower and somewhat cumbersome GC separation with electron capture detection allows the determination of the organic arsenic species in the low parts per trillion range often found in natural waters.

The procedure of Braman *et al.*[47] for the separation of nanogram amounts of inorganic arsenic and methyl arsenic compounds involves the initial reduction of arsenate and arsenite ions, methylarsonic acid and dimethylarsinic acid in aqueous solutions to arsine and the corresponding methylarsines respectively at pH 1–2 by sodium borohydride in a reaction chamber. Entrained by helium carrier gas the arsines are frozen out in a liquid nitrogen cooled U-trap and their separation accomplished upon warming the trap. Arsines carried out of the trap by the carrier gas are passed through a direct current electrical discharge and the arsenic atomic emission liver produced are detected by a recording, scanning monochromator system. Limits of detection for arsenic are approximately 1 ng for each of the arsines.

Brown and Button[31] described a relatively simple method of arsenic speciation which permitted the determination of low concentrations of As^{+5} and As^{+3} in water, soil, sediment and biological samples. The method is based on the fact that arsenate

like phosphate and silicate, forms an alcohol extractable molybdate complex (blue colored when reduced) while arsenite does not.[57] This procedure used prior to total arsenic analysis enables the subsequent ppb determination of As^{+3} and As^{+5} by atomic absorption.

The high enrichment, relative to the earth's crust of certain elements associated with atmospheric particulates, has long been recognized.[58] Arsenic is one of the elements commonly found to have an enrichment factor of $10\times$ to $1000\times$.[59,60] While volatile arsenic compounds are known to be produced by biological action, both aerobically[61] and anaerobically,[62] there is a paucity of analyses reported on the speciation of arsenic in atmospheric samples.[10,63] Air analyses for arsenic have proved difficult[10] since arsenic is present as fine particulate and is enriched as noted above. The presence of trimethylarsine, dimethylarsine, methylarsine, arsine and their oxidation products as well as inorganic, non-volatile As^{+3} and As^{+5} could be present in air and have to be specifically identified.[10] Ambient volatile arsenic in air may be well below $5\,ng/m^3$ in the absence of a soil contamination with arsenic.[10] Johnson and Braman[63] described initial studies of alkyl- and inorganic arsenic in air samples. Arsenic samples were taken by drawing air through a two compartment collector at a rate of 4.2 l/min. The The first stage was a 25 mm glass fiber filter which retained particles of size $>0.3\,\mu$. The second stage was a 14 mm (i.d.) pyrex tube containing a 3 cm bed of 60/80 mesh silver plated pyrex beads. Tests showed that volatile arsines (AsH_3, CH_3AsH_2, $(CH_3)_2AsH$ and $(CH_3)_3As$ were quantitatively collected on this stage under the conditions of sampling,[10] as is arsenic trioxide vapor and/or five particulate.

Quantitative removal of the arsines without dealkylation or disproportionation was obtained by a mild, warm, alkaline wash followed by a hot water wash. The aqueous solutions resulting from the above treatment were analyzed for arsenic by the d.c. discharge–emission spectroscopy technique of Braman *et al.*[64] The sensitivity of the analytical method was on the order of 0.5×10^{-9} g of As. The small sampler sizes used, 4.5 to 18 m^3, and the inorganic As blank for the glass fiber filters, limited the analytical significant arsenic results to $>0.2\times10^{-9}$ g As/m^3 for the inorganic fraction. Much lower alkyl-As blanks gave a limit of detection for alkyl-arsenic of $<0.1\,ng/m^3$. The analytical precision was estimated at $\pm10\%$ for one standard deviation.

One of the major sources of environmental exposures to arsenic is the smelting of nonferrous metal ores, especially copper. Epidemiologic evidence links arsenic exposure among smelter workers with increased incidence of lung cancer.[4] Numerous investigations have revealed elevated levels of arsenic in air, water, soil and vegetation in the vicinity of smelters. These levels have been shown to persist in the soil years after smelting activities ceased.[65]

Although the most ubiquitous of the arsenic compounds found in industrial environments appears to be arsenic trioxide,[37] traditional air sampling methods and analysis by atomic absorption spectrometry do not distinguish the inorganic species collected.[66] Traditional filter samples have low collection efficiencies for arsenic trioxide vapor and that when this substance is present quantitative results which neglect filter collection efficiencies are meaningless.[67]

Inorganic arsenic compounds in particulate form, arsenic trioxide vapor and arsine are possible workplace contaminants in a number of industrial processes such as the lead–acid battery manufacturing process and may indeed coexist.[66] While multiple species of inorganic arsenic can coexist in industrial environments, traditional air sampling methods and analysis by atomic absorption spectrometry quantify only total inorganic arsenic. Costello *et al.*[66] recently described the measurement of multiple inorganic species in a lead–acid battery manufacturing plant and showed that in the presence of heated arsenic sources, monitoring solely with *conventional* filters will underestimate arsenic exposure since arsenic trioxide is not efficiently collected. If particulate inorganic arsenic and arsine coexist in air samples, previously validated NIOSH sampling methods,[68,69] which utilize 37 mm cellulose ester filters for particulate arsenic collection and charcoal tubes for arsine, could produce erroneous results since charcoal tubes can collect small amounts of particulate arsenic which would be interpreted as arsine. An alternative method for measurement of arsenic in air is the sampling train patterned after the design of Hill and Arnold,[70] who developed it for pesticide sampling. This sampling train consists of a 1.2 mm inlet orifice, a 13 mm cellulose ester filter without backup pad and a standard 150 mg charcoal tube. Air is drawn through the inlet, the filter and the charcoal tube at 0.2 liter/min. This system was selected for segregation of particulate arsenic and arsine. The CARB and 37 mm cellulose ester membrane filters were found to be

essentially 0% efficient collectors of arsine in this work and quantitative collectors of particulate arsenic.[68] The treated filters ("CARB" filters) were prepared by adding 0.3 ml of 9:1 M Na_2CO_3:glycerol solution to a conventional cellulose ester filter. Used by itself the CARB filter[71,72] is a more efficient collector of arsenic trioxide vapor than the classical filter method[68] and is an equivalent collector of particulate arsenic. As noted above, when heated sources of arsenic are present, previously reported arsenic values, derived solely from filter samples, have underestimated arsenic exposure. When there is no arsenic trioxide vapor, the NIOSH Method S309[68] gives reliable results. When concurrent CARB and untreated 37 mm filter samples are collected and the backup pad of the 37 mm filter is analyzed, particulate arsenic and arsenic trioxide could be distinguished.[66]

One NIOSH procedure[73] for the determination of arsenic in air involves the initial collection of particulate material containing arsenic on a membrane filter. The filter and trapped particulate material are wet-ashed with a mixture of nitric, perchloric and sulfuric acids. The resulting As^{+5} is reduced to As^{+3} with an acidic solution of sodium bisulfite and ferrous perchlorate. An acid solution of hydrazine dihydrochloride is added to prevent air oxidation of Fe^{+2} ions. The chemically reduced sample is then analyzed by anodic stripping voltammetry (ASV) with a composite gold-graphite electrode (CGGE). The linear range of the electrode response extends from the detection limit 0.0005 mg/m^3 to an upper limit of about 500 ng arsenic or 0.005 mg/m^3 in a 100 liter sample of air. The precision (CVT) is 0.08 over the above range.

A rapid and inexpensive method for the assay of arsenic trioxide dust was developed by Snyder and Isota.[37] Air was sampled at 3 liters/min for 3 min and the filter paper dissolved in 1 N sodium hydroxide solution. The resultant solution was determined by UV spectrophotometry at 222 nm. The limit of detection was >0.006 mg/m^3.

Exposure to arsenic or its derivatives and ingestion or absorption by the skin and respiratory tract results in the major portion of arsenic being excreted in the urine, a small portion in the stools and through the skin, hair and nails and possibly a trace through the lungs.[74-76] In humans, methylation of ingested arsenic occurs and the primary forms of arsenic in urine being dimethylarsinic acid

(cacodylic acid) and methylarsonic acid with lower levels of pentavalent and trivalent inorganic arsenic. The average levels of arsenic in urine found in adults without known exposures to arsenic were 13 ppb[77] and 23 ppb.[8] The total arsenic levels found in urine in asymptomatic chemical workers and non-fatal poisoning cases were reported as 820 ppb or more and as high as 4000 to 6000 ppb respectively.[78] Urinary arsenic levels greater than 200 ppb are considered abnormal and strongly suggestive of arsenic poisoning. Several factors influence the rate of arsenic excretion including arsenic valence state. Pentavalent arsenic as arsenate is rapidly excreted whereas trivalent arsenic (as arsenite) tends to accumulate in the body.[76, 80]

Although the literature regarding the determination of arsenic in biological material is abundant, it is nevertheless at times conflicting and confusing mainly because arsenic may exist in different chemical forms *in vivo*. The forms studied most frequently are inorganic trivalent and pentavalent arsenic with their well known different toxicities. Their mono- and dimethyl derivatives represent the main metabolites *in vivo*.[81]

After oral ingestion of As_2O_3 by man, the urinary excretion of inorganic arsenic and its metabolites is important and rapid (approximately 60% are eliminated by the oral route with a half-life of 30 hours).[26] While the excretion occurs in the form of inorganic species during the first hours following the ingestion, a methylating process is rapidly triggered and leads to a preponderant excretion of dimethylarsinic acid one day after ingestion. In the case of ingestion of seafood containing arsenic, the urinary excretion occurs at a higher rate (half-life 18 hours) apparently without transformation.[26, 82] That importance of estimating separately the inorganic and organic arsenicals present in human urine has been stressed,[28, 82–86] since it is very useful to distinguish between occupational exposure to arsenic mainly via exposure to arsenic oxide and less frequently to some organic pesticides and exposure to arsenicals present in marine organisms.

Lauwerys[86] reviewed aspects of biological monitoring for industrial exposures to arsenic and Lauwerys *et al.*[82] and Buchet *et al.*[84, 85] recently reviewed the techniques proposed for measuring the concentration of total arsenic and that of some specific arsenic species (As^{+5}, As^{+3}, aromatic and aliphatic arsenic derivatives in

biological materials mainly in urine). Additionally, a comparison was first made of various sample pre-treatments of various inorganic as well as aliphatic and aromatic arsenic derivatives in water samples for the possible application for the measurement of the arsenicals present in urine. Several methods proposed to measure total arsenic concentration in urine do not necessarily do so since quite often the mineralization procedure is not drastic enough to completely release arsenic from its covalent bonds.[82] Several arsenic species (inorganic tri- or pentavalent arsenic, mono- and dimethylated arsonic acids) can be determined in water samples by electrothermal atomic absorption spectrometry (ET-AAS) after appropriate acidification procedures (concentrated HCl or a mixture of $HCl/HClO_4/HBr$) and extraction by toluene in the presence or absence of KI. The determination of aromatic derivatives and of arsenic thiol complexes requires a wet or dry ashing step. The procedures for water analysis are not directly applicable to urine analyses since under the best conditions a total of inorganic plus 85% on the average of the methylated arsenicals present in urine are measured after acidification with concentrated HCl and extraction by toluene in the presence of KI. Total arsenic content including arsenic from marine origin, is measured only after a drastic mineralization step (e.g., MgO treatment at 600°C). The results obtained by the ET-AAS technique and those obtained by neutron activation analysis were found to be in excellent agreement.[84]

When the presence of arsenic of marine origin is suspected in the urine, it was suggested that the analysis of inorganic arsenic and its metabolites be performed by an arsine generation (AG) technique. The sum of inorganic arsenic and of its mono- and dimethylated derivatives determined by such a technique is identical with the results obtained by electrothermal atomic absorption spectrometry after complete mineralization of the sample as long as no arsenic from marine origin is present.[84]

A modification (AG-AAS) of the arsine selective volatilization technique of Braman and Foreback[8] was developed by Buchet *et al.*[84, 85] allowing the use of an atomic absorption spectrophotometer as detection device instead of the plasma excitation emission spectrometer used by the authors.[8] This method compared the classical ET-AAS preceded by dry ashing of the sample with MgO and proved to be quite satisfactory when urine from subjects who

had not eaten fish were compared. The determination of urinary arsenic by AG-AAS appears to be the most suitable method for biological monitoring of workers exposed to inorganic arsenic. It is not influenced by the eventual excretion of arsenic from seafood origin. Additionally, the relative proportion of the inorganic form versus the dimethylated form could yield information concerning the length of time between exposure and urine sampling.[84]

Lauwerys *et al.*[82] note that whether the relative concentrations of the inorganic, aliphatic and aromatic forms of arsenic found in human urine represent their *true* distribution in the urine secreted by the kidney remains to be further investigated since possible changes can occur in the valence state in the bladder mainly at alkaline pH, as well as breakdown of arsenical derivatives during urine pretreatment. It was also noted that no available system has been found that will digest tissue without changing the original valence state of arsenic, hence only total arsenic can be measured with accuracy in tissues.[82, 84]

With the exception of neutron activation analysis, the usual techniques available (e.g., spectrophotometry, atomic absorption, polarography, gas chromatography) for determining *total* arsenic concentration in biological materials require a pretreatment of the samples. Additionally, it is noted that different samples pretreatments permit the specific determination of some arsenic derivatives.[82, 84]

Although neutron activation analysis is recognized to be one of the most sensitive methods for the determination of *total* arsenic concentration in biological samples with a detection limit of 0.1 ng using a thermal neutron flux of 10^{-12} neutrons/cm^2 sec, the cost of the analysis and access to a reactor limits its utility. In the procedure of Buchet *et al.*[84, 85] for measuring total arsenic concentration in urine, the digest is acidified with a mixture of concentrated $HCl/HBr/HClO_4$ (10:3:3 by volume) and trivalent arsenic is extracted with toluene in the presence of KI. A back-extraction of the organic phase with an aqueous solution of cobalt nitrate yields an As–Co complex which can be measured by flameless atomic absorption spectrophotometry. The absolute limit of detection is 0.1 ng and thus by using 5 ml of urine the relative limit of detection of the technique is 2 μg/l.

The biological monitoring of workers chronically exposed to inorganic arsenic in industry has frequently been carried out by

measuring the total amount of arsenic present in urine collected at the end of the shift or at the beginning of the next shift.[87] It should be noted that when the workers have not been instructed to refrain from eating fish or shellfish for 2 to 3 days before urine collection, misleading results can be obtained by measuring total arsenic.[86] According to Lauwerys[86] the determination of inorganic arsenic, monomethyl arsonic acid and dimethylarsenic acid (cacodylic acid) in urine appears to be the method of choice for the biological monitoring of workers exposed to inorganic arsenic since these determinations are not influenced by the presence of organo-arsenicals from marine origin. It has been estimated by Buchet *et al.*[85] that a time-weighted average exposure to 50 μg inorganic As/m^3 would lead to an average urinary excretion of 220 μg As (sum of inorganic arsenic, monomethyl arsonic acid and cacodylic acid) per gram creatinine (urine collected at the end of the shift after a few days of exposure). In contrast, in non-occupationally exposed individuals exposed to arsenic, the sum of these three arsenical species does not usually exceed 20 μg/g urinary creatinine,[86] although high values can be found in geographical areas where the drinking water contains significant amounts of arsenic.

A NIOSH recommended method (P&CAM140)[88] for determining arsenic in urine is an arsine generation–colorimetric technique employing silver diethyldithiocarbamate. The range of this method for 25 ml of urine is from 0.01 mg/l to 1.0 mg/l. An additional NIOSH procedure[89] for the determination of arsenic in urine involves anodic stripping voltammetry (ASV) with a composite gold graphite electrode. The principle of the method involves the initial wet-ashing of the urine with a mixture of nitric, perchloric and sulfuric acids. The resulting pentavalent arsenic is reduced to trivalent arsenic with an acidic solution of sodium bisulfite and ferrous perchlorate. An acid solution of hydrazine dihydrochloride is added to prevent air oxidation of Fe^{+2} ions and the chemically reduced sample then analyzed by anodic stripping voltammetry. The detection limit of the method is estimated to be about 16 ng/ml of arsenic in a 1.0 ml sample of urine. The linear range of the electrode response is estimated to extent from the detection limit to 600 ng of arsenic. This upper limit corresponds to 600 μg/l of arsenic in a 1.0 ml sample of urine. The precision of the method (CV_T) is 0.08 over the above range.[89]

An arsine evolution–electrothermal atomic absorption method for the determination of nanogram levels of total arsenic in urine and water was described by Cox.[90] The procedure involves wet digestion with nitric, sulfuric and perchloric acids to ensure decomposition of organo-arsenic compounds, especially dimethyl arsinic acid (cacodylic acid) which accounts for 50–65% of the arsenic in urine and is highly resistant to decomposition. Sodium borohydride, a redesigned hydride generator and an electric-heated absorption tube, are used for arsine evolution (from As^{+3}) and its conversion to atomic arsenic. The method has a detection limit of 6 ng/ml, a sensitivity of 1 ng/ml and is linear from 0 to 110 ng/ml of arsenic.

It has also been suggested that the determination of arsenic concentrations in workers' blood specimens may also serve as a monitor of arsenic exposure.[91,92] The arsenic concentration in blood (as in urine) reflects mainly recent exposure. The biological half-life of arsenic in blood is about 60 hours.[93] Lauwerys[86] has stated that "there is not sufficient information in the literature to establish the relationship between the intensity of arsenic exposure and its concentration in blood nor to judge the value of measuring the different arsenic metabolites in blood".

The NIOSH (P&CAM192) procedure[92] for the determination of arsenic in blood is based on anodic stripping voltammetry (ASV) with a composite gold-graphite electrode (CGGE). The detection limit was estimated to be about 16 ng/ml in a 1 ml sample of blood and the linear range extends from the detection limit to 600 ng of arsenic (0.016 to 0.6 μg/ml) in 1 ml of blood. The range can be extended by analyzing a smaller blood sample or by diluting the sample digestate with the acid solution of hydrazine dihydrochloride and taking an aliquot of the diluted solution for the ASV determination.

Davis *et al.*[91] described the analysis of total arsenic in urine and blood at the ppb level by high speed anodic stripping voltammetry. The method requires only 2 ml of blood and 1 ml urine. The samples were wet ashed with a mixture of nitric, perchloric and sulfuric acids and the ashed material was subjected to a procedure involving reduction and distillation to reduce As^{+5} to As^{+3} and to separate arsenic from the sample matrix. Collected arsenic was then quantitated by anodic stripping voltammetry (ASV) at a gold film electrode. The ASV analysis time was only 2 minutes. The method

was as accurate, precise and reliable at the nanogram level as the more universally accepted colorimetry techniques are at the microgram and milligram levels. Method precision ranged from ± 1.4 ppb at the 5 ppb level to ± 0.96 ppb at the 25 ppb level and accuracy was estimated at $\pm 6\%$ over the range 5 to 500 ppb arsenic.

Arsenic determination in hair is generally considered to be unreliable for monitoring workers' exposure since it is difficult to distinguish between externally deposited arsenic and that systematically incorporated into hair.[85,94,95] However, the determination of arsenic in hair may be more useful for evaluating the environmental exposure of the general population to inorganic arsenic. The arsenic levels in hair of non-occupationally exposed adults are usually below 2 mg/kg.[96]

3. BERYLLIUM

Germane aspects to the environmental[1,2,11,97] and toxicological effects[3-5,97,98] of beryllium have been published. Limited epidemiological studies were considered by IARC[4,5] to provide limited evidence that exposure to beryllium may lead to human lung cancer. A number of beryllium compounds are carcinogenic in rats, rabbits and monkeys and this evidence has been judged sufficient by IARC.[4,5]

Although beryllium is a moderately rare element, it is used widely in industry in three principal forms: as the metal (33%), Be–Cu alloys (50%) and other alloys (10%) and as BeO (5%). The world production of beryllium in 1979 was 3000 metric tons and the U.S. used about 10% of the total.[11] The principal uses for beryllium and its compounds are in the manufacturing of electrical components, nuclear reactors, aerospace applications, chemicals, ceramics and X-ray tubes.[1,4,11,97]

The major sources of beryllium exposure to the general population is through the combustion of coal. World coals contain 0.1 to 1000 ppm Be[97] and the U.S. Environmental Protection Agency estimates that 0.26 kg of Be is released for every 907 metric tons (1000 tons) of coal burned.[1,97] Although the form in which beryllium occurs in the effluent from coal burning utilities is not known, it is generally believed that they are oxides, mostly of the

high fuel and refractory type rather than beryl (a beryllium aluminum silicate, $3BeOAl_2O_3.6SiO_2$).[99]

The combustion of oil also contributes to the release of beryllium. Oil used in 1968 contained 0.08 ppb Be providing an emission of 7.3 metric tons (8 tons) of Be upon combustion in the United States.[1]

The following sources are the principal occupational sources of beryllium exposure principally through inhalation of beryllium dusts or fumes: ore extraction, alloy manufacture and fabricating (foundries, machining facilities), ceramic and phosphor manufacturing, nuclear reactor workers, electric and electronic equipment workers, incinerators and waste-disposal sites.[1,97,98] In 1970 OSHA estimated that approximately 30,000 workers potentially had exposure to the dust or fumes of beryllium of which 2500 workers were employed in its production. Actual worker exposure to beryllium varies considerably.[4,99,100]

Unpolluted air contains less than 0.0001 $\mu g/m^3$ beryllium with generally higher levels in urban than rural air. The average daily atmospheric concentration of Be in the U.S. is less than 0.0005 $\mu g/m^3$.[1,97] In the past, Be concentrations have been found in air near Be processing plants. However, it should be noted that pollution control equipment is available to meet U.S. air standards (average of 0.01 $\mu g/m^3$ Be over a 30-day period).[97]

Beryllium is almost non-existent in natural waters and fresh water averages less than 0.001 ppm. Finished U.S. waters averaged about 2×10^{-4} ppm and range from 1×10^{-5} to 1.2×10^{-3} ppm.[97,101] Beryllium concentrations in effluents from metal industries, power plants and from industrial and municipal waters ranged from 18–21 $\mu g/l$.[4]

Data concerning the mobility and persistence of beryllium in soils, water and air are meagre as are data concerning beryllium content in foods. In limited studies of Be in Australian and German foods, the level in the former were generally low and ranged from 0.01 to 0.10 ppm while those in German foods were slightly higher.[4] There is no evidence of beryllium being transformed or biomagnified within food chains.

(a) Determination

Methods for sampling beryllium-containing materials and for the

separation, concentration and analysis of beryllium and comparisons of analytical procedures have been reviewed by Drury *et al.*[97] and Hurlbut.[102]

Prior to the 1960's, beryllium in environmental and biologic samples were largely determined by spectroscopic, fluorimetric and spectrophotometric methods. These techniques were generally acknowledged to be either time consuming, required expensive equipment, were additionally imprecise, not sufficiently sensitive and subject to many variables and interferences.[97] For example, although many colored reagents were employed as well as reagents such as acetylacetone salicylic and sulfosalicylic acids whose compounds with beryllium display an intense absorption, spectrophotometric methods are only sufficiently sensitive for the determination of micro amounts of the element. At one time widely used reagents were naphthazarim, naphthochrome green G and chrom azurol S. The limit of detection with these methods was 100 ng Be/sample but cumbersome preparatory procedures have rendered them obsolete as well as a method based on the fluorescent dye morin, which had a limit of detection of 0.02 ng Be/sample.[3] Spectrographic methods include direct current arc,[103] alternating current arc[104] and alternating current spark.[105] These have limits of detection in the range of 0.5–5.0 ng Be/sample.[3,97]

Since 1965 both flame and flameless atomic spectroscopy (the latter is more sensitive) have become more attractive for environmental monitoring requiring only moderate sensitivity.[97,102,106] Newer developments have made atomic absorption spectrophotometry the most convenient and useful technique except where very great sensitivity is required (e.g., less than about 2 ppb).[97]

Even greater sensitivity and specificity are available with gas chromatography which is now the method of choice. The use of GC for the determination of beryllium requires that the metal be converted to a volatile form such as a halide, beta-diketone, or fluorinated beta-diketone (e.g., trifluoroacetylacetone) which appears to be the most popular derivative.[107–110] When beryllium is chelated with trifluoroacetylacetone and an electron-capture detector is used, as little as 0.08 picograms of beryllium could be detected in biological samples by this technique.[107] When gas chromatography has been coupled with mass spectrometry for the analysis of

beryllium, sensitivities in the 0.04–10 pg Be/sample range have been obtained.[111] The chelation-gas chromatographic method of analysis for beryllium in the environment has two major advantages over the atomic absorption method, e.g., greater sensitivity and virtually no interference from other metals.[97, 110]

Other analytical methods such as polarography, enzyme inhibition and various types of activation techniques also appear attractive for specific *limited* apllications, but it is generally conceded that currently it would seem unlikely that they would be used extensively in the analysis of beryllium in a variety of environmental and biologic samples.[97]

The current NIOSH procedures (P&CAM288)[112] for the determination of beryllium in occupational air environments involves filter collection, acid digestion and graphite furnace atomic absorption. The working analytical range of this method is 0.05–1.0 μg/sample or 0.5–10.0 $\mu g/m^3$ of air, based on a 90 liter sample. The sensitivity of this method using the 10 ml final solution volume is 2.4 pg of Be for a 1% absorption and the detection limit is 5 pg of beryllium.

The current NIOSH procedure (P&CAM279)[113] for the determination of Be in biological tissue involves mixed acid washing, dissolution in dilute sulfuric acid, and furnace atomic absorption spectrophotometry. The linear range is 5–250 ng Be/g fresh tissue. The sensitivity of the method is dependent on graphite furnace parameters and can vary with different instruments. Under the furnace conditions described it is 2.5 pg/1% absorbance and the estimated detection limit for the conditions given is 2.5 pg/aliquot.

The determination of nanogram amounts of beryllium in a variety of biological samples (urine, feces, hair, fingernail) by flameless atomic absorption spectroscopy was described by Hurlbut.[106, 114] For example, in urine samples containing beryllium at a concentration of 5 ng/g, the element was directly determined with a sensitivity (1% absorption) of 0.2 ng/g, a detection limit (twice background) of about 0.1 ng/g, a relative standard deviation of 8% and a relative error of 2%. The rapidity and convenience of the flameless atomic absorption technique was indicated by the authors reporting the possibility of analyzing up to 200 urine samples per day by this method.[114]

In regard to procedures for biological monitoring of workers for

beryllium exposure, although Be can be determined in blood and urine, presently these analyses can only be used as *qualitative* tests to confirm exposure to the metal. It is not known to what extent the concentrations of beryllium in blood and urine may be influenced by recent exposure and by the amount already stored.[86] Additionally, it is difficult to interpret the limited published data on the excretion of Be in exposed workers because generally the external exposure has not been adequately characterized.[86]

4. CHROMIUM

The environmental,[1,2,4,115–117] toxicological,[3–5,118–120] carcinogenic,[3–5,116,121,122] and genotoxic effects[4,5,121,123–125] of chromium have been reported. Although chromium is an abundant element in the earth's crust and occurs in oxidation states ranging from Cr^{+2} to Cr^{+6}, only the trivalent and hexavalent forms are of apparent significance with the trivalent the more common form. Chromium is a ubiquitous metal found in varying concentrations in air, water, soil and essentially all biological tissues that have been examined. Trivalent chromium is an essential metal in man and in animals and plays an important role in insulin metabolism as the glucose tolerance factor.[3,115,126]

Exposure to chromium compounds constitutes a health hazard to workers in a number of different occupations. Ulcerations, dermatitis and respiratory cancers have been reported and the damage appears to depend on the oxidation state of the chromium and the solubility of the particular compound involved. For example, there is sufficient evidence for the increased incidence of lung cancer in the chromate (hexavalent chromium) producing industry and possibly also among chromium platers and chromium alloy workers. There is also a suggestion of increased incidence of cancers at other sites.[3–5,115–120]

In 1980 the aggregate domestic production in the U.S. of chromium and 10 important chromium compounds was over 752 million pounds. Sodium chromate is the highest volume chemical of all chromium compounds with production of 554 million pounds in 1980. Sodium chromate is the principal commercial product from which other chromium compounds are made. Its primary use is in

the production of pigments (which are widely used in inks, paints, paper, rubber and composition floor coverings); secondary uses include production of chrome salt used as a tanning agent for leather, mordant in dyeing, wood preservatives, fungicides, and anticorrosive in cooling systems, boilers, and oil drilling muds. Chromium is also widely used in metal alloys such as stainless steel, as protective coatings on metal and on magnetic tapes.

Chromium in ambient air occurs from natural sources, industrial and product uses as well as burning of fossil fuels and wood. In rural areas the concentration of Cr seldom reaches 0.01 $\mu g/m^3$ while that in the ambient air of large industrial cities of the U.S. usually ranges from 0.01 to 0.03 $\mu g/m^3$.[115] About 68% of the chromium emitted to the atmosphere is estimated to be derived from ferrochrome production, while ore refining, chemical and refractory processing and indirect sources also contribute to the atmospheric burden. Of the indirect sources, the combustion of coal is by far the largest[117] with additional significant contributions emanating from cement-producing plants and the wearing of brake linings containing asbestos (since asbestos can contain approximately 1,500 ppm of chromium).[127]

Relatively large amounts of chromium are discharged in the form of wastewaters from the plating and finishing industries (only about 10–20% of the Cr used in chrome-plating processes ends up in the product). Chromium in industrial wastes occurs predominantly as the hexavalent form in chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$). Hexavalent chromium treatment frequently involves reduction to the trivalent form prior to removing the chromium from industrial waste.

The concentration of chromium in rivers has been reported to range between 1 and 10 $\mu g/kg$ (1–10 ppb),[115] however concentrations of 35 ppb in municipal drinking water supplies have been recorded.

The chromium content in most foods is considered to be extremely low with vegetables (20–50 ppb), fruits (20 ppb) and grains and cereals (excluding fats, 40 ppb) as illustrative examples.[115] Estimates of the daily intake of chromium in man vary. Schroeder[127] estimated the mean daily intake of chromium from food, water and air to be 280, 4 and 0.28 μg respectively, while Hammond and Belites[128] estimated the daily intake in man at 60 μg (30 to 100 μg) with 10 μg contributed by water. It should also be noted that tobacco

has been reported to contain up to 30 mg/kg of Cr with most values being below 5 mg/kg.[129]

(a) Determination

Analytical methodologies for the determination of chromium in environmental and biologic samples have been reviewed.[3,115,126,130] Although a wide variety of techniques is now available for the analysis of trace elements, in general chromium remains an extremely difficult element on which to obtain reliable data. The most common methods for chromium analysis are atomic absorption spectroscopy (AAS), spectrophotometry. Methods for the analysis of total chromium have either not been clearly defined nor generally accepted. It is generally conceded that absolute values for chromium in biological samples vary considerably from laboratory to laboratory and even within the same laboratory.[126,130] For example, values for serum chromium range from several hundred ppb to the presently accepted value of 1 ppb or less.[126] The urine and serum values obtained in the late 1970's indicating a chromium content of 1 ppb or less for the urine and serum of normal individuals from the U.S. and other westernized countries appear by present standards to be accurate. The variations illustrated by the range of figures available for serum chromium illustrate that great care must be exercised in this field to eliminate to the greatest degree possible, sampling errors, wrong sample handling, poor analysis and sometimes poor choice of technique.[130] The analysis of chromium is complicated by extreme matrix effects, low concentration in biological samples, possible volatility of some chromium complexes and the inherent property of Cr complexes to bind non-specifically to reaction vessels. Chromium may exist in a variety of forms in the circulation. Serum chromium exists mainly as Cr^{3+} and bound to plasma proteins, most is found in the beta-globulin fraction attached to transferrin (siderophilin).[130] Anderson[126] has admonished that absolute values for chromium in urine, blood and tissues be accepted with extreme caution. This is true whether inter- or intra-laboratory comparisons are made, different analytical techniques yield disparate results.[131]

A variety of methods to determine chromium in biological samples has been described[3,115,126,130] and include: atomic absorption

spectrophotometry (AAS),[131-137] neutron activation analysis (NAA),[138-142] photometric methods using diphenylcarbazide,[143-145] coulometry,[134] polarography,[143-148] arc emission spectrography,[147,148] spark source mass spectrography,[149] and gas chromatography.[150-152].

In gas chromatography, the chromium is converted to a volatile chelate, usually a fluorinated acetylacetonate and the detection is conveniently done by electron capture,[152-154] flame photometry,[155,156] microwave plasma emission[157] or mass spectrometry.[158]

Flameless atomic absorption spectrophotometry is claimed to have a sensitivity of Cr of 1 to 10 pg which corresponds to 5 to 50 μl of a solution containing 0.1 to 1.0 μg/l. This range is only really achievable with aqueous samples which have no complex matrices.[130] It is generally acknowledged that the major difficulty with atomic measurements has been the need for accurate background correction during atomization, especially with serum or urine samples.[135] The traditional colorimetric method employing the violet complex of 1,5-diphenylcarbazide (DPC) is still widely used for analyzing chromium. The detection limit is about 3.5 ng but the method is subject to interference by other ions.[143,144]

Flame photometry, X-ray emission spectrography and polarography are less sensitive than the diphenylcarbazide method. Although polarographic methods with a detection limit as low as 1 ng have been described, they are also subject to interference by other ions.[143,144] Although neutron activation has been used for the determination of Cr in urine,[141] at present it does not seem to offer special advantages for chromium analysis in biological materials that outweigh its disadvantages.[130]

The utility of gas chromatographic chromatographic procedures for the determination of chromium in biological samples has been cited. However, it has been cautioned that analyses at the picogram range is difficult unless proper precautions are taken.[152] If these are taken (e.g., use of non-metal syringes, buffering of samples to about pH 6.0, removal of traces of trifluoroacetylacetone (Htfa), then the GC analysis of $Cr(tfa)_3$ can be used to determine not only elevated levels arising from occupational exposure but also the extremely low, normal levels of chromium in human urine. The electron capture detector has a detectable amount of 0.1 pg chromium injected as the $Cr(tfa)_3$ chelate in the determination of Cr in urine.[152]

Table VII summarizes a number of instrumental methods for the determination of chromium both in biologic and environmental samples.[126]

The toxicity of chromium is attributed to hexavalent compounds that can be absorbed by the lung and the gastrointestinal tract and even to a certain extent by the intact skin.[159] In the trivalent state, chromium is very poorly absorbed (e.g., about 0.5–0.7%);[160] and, once absorbed, chromium is mainly rapidly excreted via urine.[161] The half-life of chromium in urine has been estimated to range from 15–41 hours.[162]

In the procedure of Tossavainen *et al.*[162] in which the biological half-times of urinary chromium were calculated for 4 welders and 4 electroplaters, urinary chromium analyses were performed with atomic absorption spectrophotometry (AAS) and the results were calculated per gram of excreted creatinine. The water-soluble fraction of the welding fume was analyzed by both the AAS and s-diphenylcarbazide method which gave identical results within the analytical error of the two procedures indicating that the water-soluble chromium compounds were mostly hexavalent.

A number of investigators have suggested that chromium concentration in urine can be used as an index of recent exposure to hexavalent soluble chromium compounds.[163–165] The normal urinary chromium concentration is usually less than 5 μg Cr/g creatinine.[161] An exposure of 0.05 mg/m^3 hexavalent chromium for 8 hours has been reported to lead to a urinary concentration of chromium of approximately 30 μg Cr/g creatinine at the end of the work period.[163] This estimate appears to be confirmed by the work of Berode and Guillemen[164] and Gilseth *et al.*[165] In regard to utility of blood chromium measurements for biological monitoring of exposures, currently too few data are available to enable an evaluation. Hence, for the present, it would appear to be of little or no value to biological exposure testing.[86, 163]

A number of procedures have been described for the determination of chromium in air in occupational environments. In the NIOSH procedure (S323),[166] chromium, soluble chromic and chromous salts as Cr are collected on a cellulose membrane filter, then following nitric acid digestion, determined by atomic absorption spectrophotometry. The working range of the method was estimated to be 0.05–2.5 mg/m^3 for a 901 sample size. The sensitivity of this

TABLE VII

Instrumental methods for the determination of chromium.[126]

Analytical method	Important application	Detection limit	Selectivity
Atomic absorption spectroscopy	Biologic solids and fluids: tissue, blood, urine; industrial waste-waters.	0.2 μg/l	Total chromium is measured.
Atomic absorption spectroscopy (flame)	Fresh and saline waters, industrial waste fluids, dust and sediments, biological solids and liquids, alloys.	0.05 μg/l	All of the extracted chromium is measured, but only Cr(VI) is extracted from the original sample unless oxidative pretreatment is used.
Neutron activation analysis	Air pollution particulates, fresh and saline waters, biologic liquids and solids, sediments, metal, foods.	Sensitivity varies with sample and processing conditions. Typical sensitivities are: 0.2 ng/g (petroleum), 30 ng/g (environmental samples) 0.2 g/g (biologic material).	Total chromium is measured.
Spectrophotometric	Natural water and industrial waste solutions having 5 to 400 μg/l hexavalent chromium may be analyzed. Higher concentrations must be reduced by dilution.	3 μg/l	This method determines only the hexavalent chromium in solution.

TABLE VII (continued)

Analytical method	Important application	Detection limit	Selectivity
X-ray fluorescence	Atmospheric particulates, geologic materials.	2 to 10 μg/g (liver) 1.5 μg/g (coal).	Total chromium is determined.
Gas chromatography (electron-capture detection)	Blood, serum, urine, natural water samples.	0.03 pg	Only chromium that is chelated and extracted is measured; other electro-negative substances may elute from the column and be detected at the same time as the Cr chelate.
Gas chromatography (atomic spectroscopic detection)	Blood, serum, orchard leaves	1 ng	Only chromium that is chelated and extracted is detected. Atomic spectroscopic methods of detection are inherently more selective for Cr in complex samples however.
Gas chromatography (mass spectrometric detection)	Blood plasmas, serum.	0.5 pg	Only chromium that is chelated and extracted is capable of being detected.
Emission spectroscopy (arc)	A wide variety of environmental samples.	0.5 ng	Total chromium is determined.
Emission spectroscopy inductively coupled plasma source	A wide variety of biological and environmental samples.	0.0003, 0.001 μg/ml	Total chromium is determined.

method for a 90 liter air sample using the 20 ml final solution volume is 0.01 mg/m^3 chromium. The method can be extended to higher values by dilution of the sample. The precision (CV_T) of the procedure was 0.085. A NIOSH procedure (P&CAM182)[162] for the determination of hexavalent chromium in air involves the initial collection of a PVC filter, extraction with water and reaction kinetic analysis. In the catalytic reaction, Cr^{+6} catalyzes the oxidation by hydrogen peroxide of o-dianisidine to its semiquinone in an aqueous–ethanolic solution near pH 4. The rate of production of the semiquinone is proportional to the concentration of Cr^{+6} and is determined by the spectrophotometric measurement of absorbance at 450 nm as a function of time. The detection limit of the method excluding the collection step, is estimated to be 1.0 μg in a single sample which corresponds to a Cr^{+6} concentration of 0.01 mg/m^3 in a 100 liter sample of air. The rate of the catalyzed reaction is proportional to amounts of hexavalent chromium in the range of 0.5 to 25 μg in the recommended analysis procedure. In a 100 liter air sample this corresponds to a range of 0.01 to 0.25 mg/m^3. The precision (CV) of the procedure was 0.03 at 0.05 mg/m^3.

Hexavalent chromium in aerosols produced by metallurgical and engineering processes such as steel-making, cutting, grinding and welding are of global concern because of the toxicity and possible carcinogenic activity.[4,5,119] The usual procedure of workplace monitoring for this contaminant is to collect an aerosol sample on a filter for subsequent extraction and analysis. Gray *et al.*[168] reported that fumes generated from stainless steel by high temperature processes can continue to evolve chemically for several minutes. In some cases their hexavalent chromium content appears to rise to a maximum sometime after formation of the aerosol and then partly decay again. Hence, this finding appears to have important implications for the monitoring of metallic aerosols produced during the manufacture, cutting, grinding and welding of chromium alloy steels.[168]

Bohgard *et al.*[169] described an analytical procedure for determining chromium in samples of airborne dust which is capable of separating the different oxidation states of chromium and also soluble from less soluble chromium. The method for assessing occupational exposures to chromium aerosols involves four procedures. The first, proton induced X-ray emission (PIXE)

measures total chromium. The second, electron spectroscopy for chemical analysis (ESCA) measures the oxidation state of the particle surfaces. The third procedure, which uses sym diphenylcarbazide (DPC), measures the proportion of water-soluble hexavalent chromium. The fourth procedure, transmission electron microscopy (TEM), provides information concerning the size and shape of the particles and guides the interpretation of the ESCA-data. These methods were applied to the analysis of samples of fume generated in welding operations with stainless steel.

5. CADMIUM

Environmental cadmium pollution is a problem of increasing concern. While trace heavy metals and metalloids may be considered inherently toxic, some such as chromium or selenium have nutritional significance. However, in the case of cadmium there appears to be no biologic value, but a very serious health hazard.

The environmental,[1,2,170-178] ecotoxicological[179-181] and health effects[3,170-172,179,183-189] of cadmium have been extensively reviewed. Studies have suggested that human exposure to cadmium, primarily as the oxide, is associated with increased risks of prostatic, respiratory and genito-urinary cancers, although in some cases, the excess risk was not statistically significant.[5,190,191] The evidence for the carcinogenicity of cadmium and certain of its compounds (e.g., chloride, oxide, sulfate, and sulphide) in experimental animals is considered by IARC[5] to be sufficient.

The world (western countries only) cadmium metal production over the last decade has been between 12,000 and 14,000 tons/year.[175] Cadmium production in the European Economic Communities (EEC) increased from 2,084 tons in 1968 to 2,160 tons in 1978 showing an average annual increase of about 2%.[181] In 1979, Europe consumed about half of the world's cadmium (some 7,000 tons), the U.S. took about one-third (4,800 tons) and Japan about one-tenth (1,100 tons). While the rate of growth in consumption appears to be slowing down in industrialized countries, there has been strong growth in developing countries although the quantities used are still quite small in comparison.[175]

Cadmium and certain compounds are widely employed

commercially in electro-plating, alloys, soldering, plastic stabilizers, batteries, fungicides, and in pigments for inks, glass ceramics, textiles and paper and in phosphors for television tubes. The 1980 production in the U.S. of cadmium and eight high-volume cadmium compounds was approximately 17 million pounds with cadmium oxide accounting for about 10 million pounds (60%). Plastic stabilizers (e.g., cadmium stearate or some other organic salt) utilize 2 million pounds/year for primarily PVC.[99]

Of the 6,000 tons/year of cadmium consumed by the EEC for the period 1976–1978, the estimated share uses were as follows: pigments, 30%; stabilizers, 15%; batteries, electroplating and alloys, 10% each; and surface treatment, 25%. Cadmium based pigments and stabilizers employed in plastics during this period was 1,500 and 600 tons/year respectively.[181] At present, the rough breakdown of worldwide cadmium consumption is plating, 40%; pigments, 25%; batteries, 20%; stabilizers, 10%; and alloys, 5%.[175]

Cadmium is generally acknowledged to be truly one of the critical environmental chemicals since it enters into many biologic and chemical processes. Cadmium is not destroyed by any environmental process and any cadmium discharged into the environment may move from one compartment to another at varying rates which can hence result in an accumulation in compartments such as soils and some biota. Most of all discharges of Cd into the environment and about 90% of global Cd emissions directly into the air are considered to be anthropogenic in origin.[180]

Table VIII summarizes current cadmium inputs into the EEC environment.[174] Due to its various applications, cadmium is found in every environmental compartment, e.g., soil, groundwaters, surface waters and air. Concentrations of cadmium in air normally range from 0.001 to 0.05 $\mu g/m^3$ but may be as high as 5 $\mu g/m^3$ near a point source of emission such as a smelter.[192] Rural areas usually have air levels below 0.005 $\mu g/m^3$.[170] Air pollution by Cd is mainly caused by industrial emissions, although combustion of coal (mean Cd content 1 $\mu g/g$) and fuels may contribute to elevated levels.[1,170] Rural soils are considered to be sinks for Cd generated from most U.S. coal-fired generating plants.[193] Soil concentrations of Cd are usually less than 1 ppm but may rise to approximately 100 ppm as a result of aerial deposition, irrigation with contaminated water, the application of sewage-based fertilizers, superphosphate fertilizers, pesticides

TABLE VIII

Summary of current cadmium inputs to the EEC environment (t year^{-1}).[174]

Source	Compartment		
	Air	Land	Water
Volcanic action	20	ND	BD
Nonferrous metal production			
Zinc and cadmium	20	200	50
Copper	6	15	ND
Lead	7	40	20
Production of cadmium-containing materials	3	90	108
Iron and steel production	34	349	ND
Fuel combustion			
Coal and lignite	8	390	ND
Oil and gas	0.5	14.5	—
Waste disposal	31	1434	ND
Sewage sludge disposal	2	130	33
Phosphate fertilizers	—	346	62
Totals	132	3009	273

Note. ND—not determined.

containing cadmium or by the discharge of liquid or solid Cd containing wastes from mining, metallurgical, industrial or urban activities.[1,180]

Cadmium concentrations in non-polluted fresh waters are usually lower than 1 μg/l.[1,170] (In drinking water the WHO has recommended that Cd levels should not exceed 5 μg/l.)

It has been suggested that increased utilization of compounds containing Cd has accelerated the rates of mobilization and transport of Cd which far exceed the rates of the natural, abiotic cycling processes.[194] As noted earlier, these rates have led to increased deposition of Cd in atmospheric, aquatic and terrestrial environments with subsequent increased uptake of Cd by accumulation in the biota.[194]

The major non-occupational routes of human cadmium exposure are through food[170,171,181] and tobacco smoke.[170,171,181,195,196] As noted earlier, the cadmium contents of ground water and

drinking water are normally very low, at 0.1–0.2 μg/liter and is extremely low compared to the amount of Cd derived from food.

National estimates of the average oral intake of Cd vary from the lower levels of 10–20 to the higher levels of 60–70 μg/day. Comparing these values with the FAO/WHO proposal maximum tolerable weekly intake of 400–500 μg, it can be seen that this limit has already been reached in some countries.[181]

Table IX illustrates the estimated daily cadmium intake from food[171] and shows that some of the high values for average Cd intakes (from 40 μg/day/person and upwards) are from countries with very intensive industrial activity (e.g., Japan, United States, Federal Republic of Germany) in contrast to more moderate intakes of Sweden, New Zealand, and the United Kingdom.

In man, the average absorption of Cd has been estimated as 5% of that ingested and the amount absorbed from food has been estimated between 0.24 and 5.04 μg/day.[187] Tobacco in all its forms contains appreciable amounts of cadmium. American cigarettes have been found to contain 1.5 to 2.0 μg/cigarette[197] and about 70% of this passes into the smoke.[198] Since the absorption of Cd from the lung occurs at a substantially higher rate than from the G.I. tract, smoking contributes significantly to total body burdens.[171] Most data agree that 0.1 to 0.2 μg Cd are inhaled for each cigarette smoked hence smoking 20 cigarettes/day can result in the inhalation of about 3 μg/day. Since inhaled Cd is absorbed more readily, this is equivalent to ingesting 25 μg from food.[171]

TABLE IX

Daily intake of cadmium from food.[171]

Country	μg/day
United States	38
Canada	52
Federal Republic of Germany	48
Rumania	38–64
Czechoslovakia	60
Japan (unpolluted areas)	59
Sweden	16–19
Australia	30–50
New Zealand	21–27
United Kingdom	15–35

A number of populations appear to be at special risk for adverse effects of cadmium exposure. For example, NIOSH has estimated that 100,000 Americans have potential occupational exposure to cadmium and its compounds.[202] As noted earlier, smokers appear to comprise a group at special, if not greatest, risk.[171] Persons with diets which include more than normal amounts of leafy vegetables, visceral meats, fish, and shellfish may also be at increased risk since these food groups can contain rather large amounts of cadmium. Additionally, persons with severe nutritional deficiency, Fe, Ca, Zn, protein, Vitamin D, etc., which may be aggravated by Cd are conceivably at special risk.[171]

(a) Determination

Significant health problems have occurred following either acute high level exposures or chronic low-level exposures to cadmium. Extensive reviews of the toxicological effects of long term, low-level exposure to cadmium indicate that the kidney is the critical organ.[170,171,183–187,191,199] General aspects of biological monitoring of Cd have been reviewed by Lauwerys.[86] Cadmium has been shown to be a very cumulative toxin with a biological half-life of about 20 years in man. In non-occupationally exposed adults, Cd levels in blood are usually below 1 μg/100 ml whole blood (median value about 0.15). Over 70% of the Cd is bound to red cells in blood. Cadmium accumulates mainly in the kidney and liver with about 50% of the body burden found in these organs. Cd is bound in all tissues to metallothionein. Cadmium is principally excreted via the urine and in adults not occupationally exposed to Cd, the level in urine is usually below 2 μg Cd/g creatinine, and the extent of urinary excretion increases with age.[86]

In urine, Cd is present mainly bound to metallothionein, thus the determination of the level of this protein in urine may provide the same information as the determination of cadmium. Additionally, metallothionein analysis presents an advantage over Cd analysis since it is not subject to external contamination[200] and a radioimmunoassay has been developed for its assay in urine.[201] The prevalence of renal dysfunction can also be related to the level of Cd in urine and its was suggested by Lauwerys[86] that to prevent renal dysfunction in workers exposed to Cd, the concentration of Cd in urine should not exceed 10 μg/g creatinine.

Cadmium in blood has been shown to reflect mainly the last few months exposure in workers and a value of 1 μg Cd/100 ml whole blood was proposed as a tentative no effect level for long-term exposure.[86]

A survey of the analytical methods utilized for cadmium detection has been described by Smiley and Kessler[203] and Stoeppler.[204] The NIOSH procedure (P&CAM224)[205] for the determination of cadmium in urine involves the initial wet ashing of a sample of urine with a mixture of nitric, perchloric and sulfuric acids, dissolving the residue in an acetate buffer solution then analysis of this solution by anodic stripping voltammetry with a composite mercury–graphite electrode. The detection limit is 1 ng which corresponds to 1 μg/l in a 1 ml sample of urine. The limit range is estimated to extend from 1 to 1000 ng Cd which corresponds to 1000 μg/l in a 1 ml sample of urine.

Several electrothermal atomic absorption methods have been published for cadmium in urine.[203, 204, 206, 207] Most previously published methods require extraction of cadmium from the biological matrix,[208] and only a few direct determinations of low levels of cadmium in blood and urine have been reported.[209, 210] Sample contamination and matrix effects appear to cause significant problems in the accurate determination of submicrogram levels of cadmium in biological matrices.[211] Because of the ultra trace levels of Cd, less than 1 μg/l in urine, and less than 3 μg/l in blood found in the normal population (non-occupationally exposed to cadmium) it is desirable to have a direct *in situ* method for AAS analysis of cadmium. Subramanian *et al.*[212] recently reported graphite furnace atomic absorption spectrometry with matrix modification for determining nanogram per milliliter levels of Cd in human urine. The procedure utilized a diammonium hydrogen phosphate–nitric acid, an ammonium nitrate–nitric acid matrix modifier in addition to the urine sample prior to determination. It was suggested that the detection limits were sufficiently low for screening programs.

Stoeppler and Brandt[213] recently described simple, rapid methods for the determination of cadmium in whole blood and urine by means of electrothermal atomic absorption spectrophotometry (ETAAS). Fifty to 200 μl aliquots of whole blood were treated with 1 M HNO_3 for deproteinization and matrix modification. After centrifuging, the supernatant is analyzed by automated ETAAS.

Precision expressed as day-to-day precision varied from 30% at 0.4 μg Cd/l to 3.8% at 9.3 μg Cd/l whole blood. If 25 μl of a 1+3 diluted blood are injected, a detection limit of ≦0.2 μg Cd/l was achieved.

Direct automated Cd determinations in urine are feasible after dilution from about 0.2 μg Cd/l. Solvent extraction using sodium diethyldithiocarbamate (NaDDC)/methyl-isobutylketone (MIBK) is possible to ≦0.1 μg Cd/l. The day-to-day reproducibility varies from 26% at about 0.6 μg to 7.1% at about 27 μg Cd/l urine. The accuracy of these methods was checked by differential-pulse-anodic-stripping voltammetry (DPASV) and independent AAS procedures and found to be acceptable with an average total error of ≦30%. For routine analysis, up to 200 Cd measurements are possible in whole blood and up to 160 direct Cd measurements in urine per day (10 hours) and hence the above procedure of Stoeppler and Brandt[213] would appear feasible for screening programs.

A semi-automated procedure was developed by Watanabe *et al.*[214] for the determination of cadmium in blood by utilizing the combination of block-digestion-autosampler-flameless atomic absorption spectrophotometry. For block-digestion which can mineralize 144 samples in 10 hours, a special micro kjeldahl tube of special shape was utilized. When applied to the mass analyses of cadmium in 1761 blood samples from non-exposed subjects, the system as a whole possessed sufficient sensitivity with the lowest detection limit of 0.2 ng Cd/ml blood. Fifty samples per day could be analyzed by this procedure.

The NIOSH procedure (P&CAM233)[215] for the determination of cadmium in blood involves an initial wet-ashing of a sample of whole blood with a mixture of nitric, perchloric and sulfuric acid, dissolving the residue in an acetate buffer solution and analyzing the buffered sample by anodic stripping voltametry with a composite mercury–graphite electrode. The detection limit is 2 ng which corresponds to a 0.002 μg/ml in a 1 ml sample. The linear range is 0.002 to 1 μg/ml in 1 ml of blood. The precision (CV) of the method is 0.05 at 0.05 μg/ml.

As noted earlier, a large part of the blood cadmium is related to recent exposure. Under long term low-level exposure, the concentration in blood is thus a useful indicator of the exposure of cadmium in recent months. The half-time of Cd in blood is

estimated to be 2–3 months,[216] if the exposure levels do not undergo major changes.[217]

A number of studies have been published over the last two decades presenting data on average or "normal" levels of Cd in blood samples from people in the general population in various countries.[218,219] When comparing such data substantial differences in the results were noted which indicate principal shortcomings in sampling and analysis. Additionally, different laboratories can also obtain quite disparate results when analyzing the sample. Hence, most of the results from published results cannot readily be compared.[219]

Elinder *et al.*[219] recently determined cadmium and blood levels in whole blood samples from 473 non-occupationally exposed adults from the general population of Sweden. Analyses were performed using atomic absorption spectrometry equipped with an electrothermal atomization unit. Accuracy of the analysis was confirmed by the analysis of quality control samples. Blood Cd levels were very strongly influenced by smoking habits and a significant correlation existed between the number of cigarettes consumed daily and blood Cd concentration. The median blood Cd levels for non-smoking males was 0.2 μg Cd/liter ($\leq$0.2, detection limit) and for females 0.3 μg Cd/liter. About 90% of all non-smokers had Cd concentrations in blood below 0.6 ng Cd/liter whereas about 90% of the current male and female smokers had Cd concentrations in blood of 0.6 μg Cd/liter or more.

An assessment of exposure to cadmium and lead through analysis of blood and kidneys was recently reported by Friberg and Vahter in a report of the results of a UNEP/WHO global study.[216] The following 10 countries participated: Belgium, India, Israel, Japan, Mexico, Peoples Republic of China, Peru, Sweden, United States and Yugoslavia. No laboratory started the monitoring before achieving satisfactory results of quality control (QC) analysis (samples of cow blood spiked with cadmium and lead and freeze-dried horse kidney cortex for cadmium analysis). QC samples were analyzed in parallel with the monitoring samples to assure validity of the obtained results. The quality assurance program also included preanalytical quality control. There was considerable variation in metal exposure between areas. Geometric means for Cd in blood ranged from 0.5 μg Cd/liter in Stockholm and Jerusalem to 1.2 μg Cd/liter in Brussels

and Tokyo. Cadmium levels were considerably higher among smokers than non-smokers.

Body burden of cadmium in man at low levels of exposure from specimens of tissues from 86 traumatic accidents in Finland[220] as well as an investigation on the concentration of Cd in human tissue in pathological-anatomical specimens in the Federal Republic of Germany[221] have been reported. The concentration of Cd in the samples was determined by atomic absorption spectrophotometry using the flame and electrothermal AAS methods. Since the concentrations of Cd in renal cortex and the liver permit an evaluation of total body burdens, it is possible to estimate the cadmium body burden in historical times from the concentrations of Cd in old kidneys and liver specimens (e.g., from the period 1897–1939) in the study described by Drasch.[221] The concentration of Cd in the kidney increased nearly 50 fold in this period while the concentration in the liver remained more or less the same. An increase in the total body burden of cadmium by a factor of 4 to 5 in West Germany since the first part of the century was also noted.[221]

Ellis *et al.*[222] recently evaluated the relationship between the body burden of cadmium and indirect biological indices of exposure, e.g., urine, blood and hair concentrations employing graphite furnace atomic absorption spectrometry. The detection limits were 1 μg/l for urine, 0.1 μg/dl for blood and 0.5 μg/g for hair. It was found that in general, blood and urine Cd levels are useful only as indices of *significant* exposure. At present, direct *in vivo* measurements of kidney and liver cadmium were suggested to provide the most accurate data on body burden of cadmium as well as the best indices of accumulative exposure. The *in vivo* determinations of kidney and liver cadmium were performed by neutron capture gamma-ray analysis.

6. SELENIUM

There is increasing recognition that selenium is an important metalloid with industrial, environmental, biological and toxicological significance. Selenium is an essential element in many species, including humans.[223] Deficiency of this element in animal diets has

led to serious diseases. The role of Se in the mammalian systems, in the enzyme glutathione peroxidase, has been fully documented;[224] although the chemical form in which it occurs in selenoprotein is unknown. The toxicology of selenium and its compounds is often conflicting and controversial. While the carcinogenicity of selenium has been reported,[225-229] its prevention (as sodium selenite) of several chemically induced cancers in animals has also been described.[230-236] Additionally, sodium selenite is an anticarcinogenic, antimutagenic, anticlastogenic agent that under certain conditions induces sister-chromatid exchanges, unscheduled DNA synthesis and chromosome aberrations.[237]

General reviews on selenium which are particularly germane include: chemical and physical properties,[238] occurrence and significance in the environment[239-244] and toxicology.[245-246] Selenium levels in ground and surface waters normally may range between 0.1 to about 400 μg/l, although it should be noted that depending on geological factors, ground water may reach concentrations up to 6000 μg/l.[246] Selenium is generally a minor constituent in potable water with concentrations ranging from 0.1 to 100 μg/l.[244]

Although atmospheric concentrations of Se usually are of the order of a few nanograms/m^3,[244,247] point source emissions primarily from the combustion of fossil fuels may contribute to the local air pollution by selenium.[244,246] Other possible sources of Se in the atmosphere include the incineration of paper and rubber tires, open trash burning, etc.[244]

Recent total diet studies in the U.S. reported the following selenium levels: 0.07 ppm for dairy products group; 0.20 (0.10–0.40) ppm for meats, fish and poultry group; 0.24 (0.10–0.40) ppm for grains and cereal products group and trace amounts in the other food groups.[239] The estimated normal dietary daily intake of Se for humans in most parts of the world ranges from 4–35 μg/person in infants to 60–300 μg/person in adults.[239,248] Based on lack of definitive toxic effects on man of Se in food origin and considering the normal levels in foods, Lo and Sandi[239] estimated that 500 μg/man/day may be regarded as a maximum tolerable level. Drinking water contributes only 1–6% of selenium uptake for typical concentrations and a whole consumption of 3 l/day.

Selenium is obtained primarily as a by-product of copper refining

with more than 90% of the U.S. output and more than 80% of the world's production derived from the anode mud deposited during electrolytic refining of copper. Significant tonnages of selenium are produced in Canada, United States and Japan and smaller quantities by Belgium, Chile, Finland, Peru, Sweden, Federal Republic of Germany, Yugoslavia and Zambia.[238]

Selenium is widely employed in industrial products and processes[241, 244, 246] in principally the glass industry (27%), electronic uses (23%) in areas related to primarily its semiconductor and photoelectric characteristics; approximately 23% of the total demand for selenium is in duplicating machines; and inorganic pigments (about 14%) (principally cadmium sulfoselenide used in plastics, paints, enamels, inks, rubber and ceramics). The remainder of the selenium (approximately 13%) is used in a broad spectrum of applications including: as accelerators and vulcanizing agents in rubber production, in stainless steels and as selenides of refractory metals for use in lubricants. Table X lists a number of selenium compounds and their uses.

There is evidence that selenium can be microbially transformed in the environment to volatile methylated forms.[241, 249]

(a) Determination

Soluble selenium compounds appear to be readily absorbed via the lungs and the G.I. tract and thence methylated to trimethylselenonium ions and eliminated via the kidney. Urinary excretion is rapid and represents the most important route of selenium elimination. It should be noted that only under conditions of high exposure is the volatile metabolite dimethylselenide eliminated via the lungs.

Lauwerys[86] has reviewed aspects of biological monitoring of selenium in industrial exposures. Selenium concentrations in blood and urine appear to reflect mainly recent exposure. The normal concentrations in blood and urine are known to vary considerably depending on the nature of the dietary intake. The concentrations of Se in serum or plasma have been reported to range from 5 to 18 μg/100 ml.[250] The mean urinary concentrations of selenium found in non-occupationally exposed persons by Glover,[251] Young and Christian,[252] Valentine *et al.*[253] and Lauwerys[86] were 34, 7, 79 and

TABLE X
Some selenium compounds and their uses.

Compound	Use
Selenium	Rectifiers, photoelectric cells, blasting caps, in xerography, stainless steel; dehydrogeneation-catalyst.
Sodium Selenate Na_2SeO_4	As insecticide; in glass manufacture; in medicinals to control animal diseases.
Sodium Selenite Na_2SeO_4	In glass manufacture; as soil additive for selenium deficient areas.
Selenium Diethyldithiocarbamate	Fungicide; vulcanizing agent.
Selenium Disulfide SeS_2	In veterinary medicine.
Selenium Dioxide SeO_2	Catalyst for oxidation, hydrogenation or dehydrogenation of organic compounds.
Selenium Monosulfide SeS	In veterinary medicine.
Selenium Hexafluoride SeF_6	As gaseous electric insulator.
Selenium Oxychloride $SeOCl_2$	Solvent for sulfur, selenium, tellurium rubber, bakelite, gums, resins, glue, asphalt and other materials.
Aluminum Selenide Al_2Se_3	Preparation of hydrogen selenide for semiconductors.
Ammonium Selenite $(NH_4)_2SeO_3$	Manufacture of red glass.
Cadmium Selenide	Photoconductors, photoelectric cells, rectifiers.
Cupric Selenate $CuSeO_4$	In coloring copper and copper alloys.
Tungsten Diselenide WSe_2	In lubricants.

25 μg/l respectively. Although a biological threshold limit value for selenium in urine of 100 μg/l has been proposed, it was noted that the epidemiological data supporting this proposal are very limited.[86]

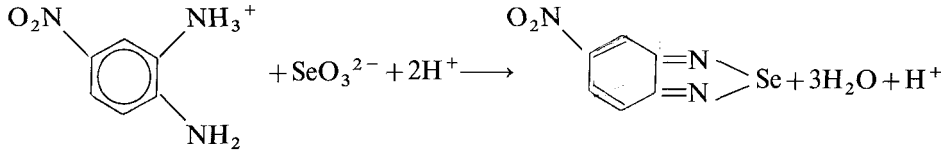
The determination of selenium in environmental and biological samples has been reviewed by Cooper,[254] Olsen *et al.*,[255] Shendrikar[256] and Robberecht and Van Grieken.[257] The methods

generally employed for analyzing selenium in biological materials initially involve the destruction of organic constituents with concurrent oxidation of the element to the tetravalent or hexavalent state and its subsequent determination by a variety of techniques. The most commonly employed techniques are atomic absorption spectrometry, fluorimetry, spectrophotometry, neutron-activation analysis, gas chromatography and to a lesser extent X-ray fluorescence and polarography.

Electron-capture gas chromatography has evolved as a more sensitive and reliable analytical method for selenium. The procedure is based on the reaction of tetravalent selenium and various aromatic o-diamines to form piazselenols[258] which can be extracted into organic solvents and measured by GC and detection by electron capture or microwave emission spectrometry. The o-diamines which have been used include 2,3-diaminonaphthalene[252] and the 4-chloro-,[259] 4,5-dichloro-,[260] 4-nitro-,[261,262] and 3,5-dibromo-[263] derivatives of 1,2-diaminobenzene. The above methods are specific for tetravalent selenium (selenite) and only the total selenium content is measured. Hexavalent selenium (selenate) is also present in biological materials. However, relatively few methods for its determination have been published. These include spectrophotometry,[264] fluorimetry[265] and gas chromatography.[266]

Cappon and Smith[267] described a simple method for determining specific forms of selenium in biological samples. The procedure is based on the selective chelation of tetravalent selenium with 4-nitro-o-phenylenediamine to form 5-nitropiazselenol (Equation 1) which is measured by electron-capture GC. Organoselenium and selenite (SeIV) are determined by digesting the sample in concentrated nitric acid and the total selenium is determined by further treatment of the digest with hydrochloric acid. The difference between the two values obtained represents the selenate (SeIV) content. Selenium recovery ranges from 75–90% and was assessed by using a ^{75}Se-labeled tracer for liquid scintillation spectrometric assay. Gas-chromatographic conditions allowed the detection of selenium concentrations below 1 ppb and mean deviation and relative accuracy averaged 2.3 and 3.4%. This method has been used in human population studies to assess selenium–mercury correlations and to examine the Se content and form in specific protein fractions of fish muscle. The formation of 5-nitropiazselanol from selenium and 4-nitro-o-phenylenediamine

is as follows: Equation 1:



4-Nitro-o-phenylenediamine 5-Nitropiazselenol

The determination of selenium in environmental samples (biological and plant tissues, coal, fly ash and scrubber solutions) using gas chromatography with a microwave emission spectrometric (MES) detection system was described by Talmi and Andren.[268] The analysis is based on chelating tetravalent selenium with 5-nitro-o-phenylenediamine (as above) to form the thermally stable and volatile piazselonol complex. This was followed by extraction into toluene, separation by GC and finally determination by the MES via monitoring the emission intensity at the 204 nm selenium line. The detection limit is 40 pg and the relative sensitivity is 0.1 μg/liter for water samples and 15 ppb for solid samples. The relative error ranged from 0–17.5% and the relative standard deviation from 2.2–9.5%.

Routine methods of selenium determination which are applicable to the analysis of plant materials include colorimetry, fluometry, atomic fluorescence using hydride generation,[269,270] flame atomic absorption and hydride generation[271–275] and flameless atomic absorption.[276] Selenium is present in the ng/g range in unprocessed foods. Brooks *et al.*[274] recently described optimum conditions for hydride generation of selenium and its determination by atomic absorption spectrophotometry that permitted a limit of detection of about 2 ng selenium.

A procedure utilizing wet digestion followed by hydride generation/condensation-flame atomic absorption has been developed by Hahn *et al.*[275] for the routine analysis of selenium in different varieties of vegetables and grains. The lowest quantifiable level, based on 2 g of sample was 1 ng/g (dry weight) for all crop types studies. The precision for the total analysis was 3.7% relative standard deviation (RSD) at a mean concentration of 100 ng/g and 13% RSD at a mean concentration of 1 ng/g.

The utility of hydride generation/condensation system with an inductively coupled argon plasma polychromater (HGC/ICP) for the simultaneous determination of Se, As, Bi, Ge, and Sn in food has recently been described.[277,278] Detection limits for selenium and arsenic was 0.1 and 0.02 ng/ml respectively.[277]

Since Challenger[279] proved that selenium was susceptible to biomethylation, methylated selenium compounds were shown to be produced by plants,[280,281] soils,[249,282–285] lake sediments,[249] and sewage sludge.[284,286,287] It has been noted earlier that under conditions of high exposure, the volatile metabolite dimethylselenide is eliminated via the lungs by man. Jiang *et al.*[288] established a gas chromatographic–graphite furnace atomic absorption (GC–GFAAS) combination for the determination of dimethylselenide $[(CH_3)_2Se]$, dimethyldiselenide $[(CH_3)_2Se_2]$ and diethylselenide $[(C_2H_5)_2Se]$. A combination of a cryogenic sampling procedure with this identification and detection system permitted the determination of the above methylated Se derivatives in environmental air down to 0.2 ng/m^3.[288]

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