Review

Elements in environmental and occupational medicine

Pietro Apostoli*

Institute of Occupational Medicine and Industrial Hygiene, University of Brescia, Brescia, Italy

Abstract

Occupational and environmental medicine traditionally dealt with elements, particularly with heavy metals. The interest was justified by the wide exposure in the workplace and in the general environment and by the evidence of their specific biological and toxicological effects. During the last 2 decades of 20th century the availability of indicators of exposure or of internal dose has substantially increased thanks to improvement in AAS-ETAAS techniques and to the entrance of ICP-MS into the field of biological monitoring. There are now more and more demands for controlling pre-analytical and analytical factors, for analysing biological matrices in addition to blood and urine and for setting up methods for elements not yet extensively studied in respect to their possible biological or toxicological role. Finally, deeper knowledge has to be reached in order to evaluate the significance of elements and, possibly, of their species in biological fluids at current doses and in order to face their effects, especially those in the first portion of the dose–response curve, which is going to be the main field of interest of occupational and environmental toxicology for the next few years.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Heavy metals

Contents

*Tel.: +39-30-3700-604; fax: +39-30-394-902.
E-mail address: apostoli@master.cci.unibs.it (P. Apostoli).

1. Introduction to the theme

Metals (from Greek ''metallon'' mine and thus minerals) are elements with chemical and physical properties depending on their low number of electrons in the external orbits and thereby with the tendency to form positive ions. In non-metals, i.e., elements such as fluorine, iodine, selenium, phosphorous, few electrons are missing to complete the external layer, while semimetals (once called metalloids) are elements (boron, arsenic, antimony) with intermediate characteristics. Among the 103 elements that compose the periodic table (Fig. 1), 79 can be re-included into the group of metals, 17 into non-metals and seven into semimetals. On the basis of their specific mass (less or more than 5 g/cm^3) metals can also be classified as light and heavy.

Also in biological systems the elements tend to lose electrons and to form positive ions originating coordination compounds, named complexes or ion complexes, containing at least one atom of a metal that coordinates other specific atoms or molecules called ligands. The single ligands are defined as those which provide one duplet, such as carboxylic (–COOH), aminic (–NH$_2$), methylaminic (CH$_3$NH$_2$). Poly ligands are molecules or anions that can generate more than one dative bond and are important since they are found in complexes characterised by particular biological activity. Among these, we should recall the glycinates (OOC–CH$_2$–NH$_2$), oxalates (OOC–COO) and ethylendiamine (H$_2$N–CH$_2$–CH$_2$–NH$_2$).

Elements and, in particular, certain metals represent a traditional and extensive field of interest for occupational and environmental medicine. The reasons that call for this interest may be referred to extensive exposure, to the evidence of specific diseases and in more recent times to the possibility of studying more accurately their dose and effects.

Man began to extract and process metals almost 4000–5000 years ago (Fig. 2) but it was only with the dawn of fossil fuel in the 18th–19th centuries that the metals manufacturing progressively developed, up to first half of the 20th century.

Over the last few decades, in Europe there has been a drop in the production of some metals or alloys such as lead (Pb), mercury (Hg) and iron (Fe), whereas the production and process of aluminium (Al), copper (Cu) alloys or other elements such as molybdenum (Mo), tungsten (W), vanadium (V), cobalt (Co) have steadily been rising.

Mining, pouring,casting, processing and using metals have led to their dispersion into the general environment and impaired health of the general population by the ingestion of food and beverages containing or contaminated by metals, for instance fish contaminated by Hg, drinking water by As, rice by Cd [1]. In countries such as Italy, about 20–30% of blood lead concentration in adult males originates from metal in wine, in which Pb ranges between 50 and 100 μg/l, with peak levels at 500–700 μg/l [2].

Lead represents a good example of metal emitted into the atmosphere and absorbed by soil, plants, animals. Over the last 40–50 years the use of lead in gasoline has represented the main source of Pb absorption for the general population. For example, during the last 40–50 years it has been estimated that in the USA 100 000 to 200 000 tons per year of this metal have been discharged into the atmosphere [3].

Since the 1980s the industrialised countries have

Legend:

Atomic Number → | Symbol → | Atomic Weight →
50 +2
Sn +4
118.71
18·18·4
→ Oxidation States
→ Electron Configuration

Group IA	IIA	IIIB	IVB	VB	VIB	VIIB	VIII	VIII	VIII	IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA	Orbit
1 H +1 −1 1.00794 1																	2 He 4.00260 2 0	K
3 Li +1 6.941 2-1	4 Be +2 9.01218 2-2											5 B +3 10.81 2-3	6 C +2+4−4 12.011 2-4	7 N 14.0067 2-5	8 O −2 15.9994 2-6	9 F −1 18.9984 2-7	10 Ne 0 20.179 2-8	K-L
11 Na +1 22.9898 2-8-1	12 Mg +2 24.305 2-8-2											13 Al +3 26.9815 2-8-3	14 Si +2+4−4 28.0855 2-8-4	15 P 30.9738 2-8-5	16 S −2 32.06 2-8-6	17 Cl −1 35.453 2-8-7	18 Ar 0 39.948 2-8-8	K-L-M
19 K +1 39.0983 -8-8-1	20 Ca +2 40.08 -8-8-2	21 Sc +3 44.9559 -8-9-2	22 Ti +2+3+4 47.88 -8-10-2	23 V +2+3+4+5 50.9415 -8-11-2	24 Cr +2+3+6 51.996 -8-13-1	25 Mn +2+3+4+7 54.9380 -8-13-2	26 Fe +2+3 55.847 -8-14-2	27 Co +2+3 58.9332 -8-15-2	28 Ni +2 58.69 -8-16-2	29 Cu +1+2 63.546 -8-18-1	30 Zn +2 65.39 -8-18-2	31 Ga +3 69.72 -8-18-3	32 Ge +2+4 72.59 -8-18-4	33 As 74.9216 -8-18-5	34 Se +4+6−2 78.96 -8-18-6	35 Br +1+5−1 79.904 -8-18-7	36 Kr 0 83.80 -8-18-8	-L-M-N
37 Rb +1 85.4678 -18-8-1	38 Sr +2 87.62 -18-8-2	39 Y +3 88.9059 -18-9-2	40 Zr +4 91.224 -18-10-2	41 Nb +3+5 92.9064 -18-12-1	42 Mo +6 95.94 -18-13-1	43 Tc +4+6+7 (98) -18-13-2	44 Ru +3 101.07 -18-15-1	45 Rh +3 102.906 -19-16-1	46 Pd +2+4 106.42 -18-18-0	47 Ag +1 107.868 -18-18-1	48 Cd +2 112.41 -18-18-2	49 In +3 114.82 -18-18-3	50 Sn +2+4 118.71 -18-18-4	51 Sb +3+5−3 121.75 -18-18-5	52 Te +4+6−2 127.60 -18-18-6	53 I +1+5+7−1 126.905 -18-18-7	54 Xe 0 131.29 -18-18-8	-M-N-O
55 Cs +1 132.905 -18-8-1	56 Ba +2 137.33 -18-8-2	57* La +3 138.906 -18-9-2	72 Hf +4 178.49 -32-10-2	73 Ta +5 180.948 -32-11-2	74 W +6 183.85 -32-12-2	75 Re +4+6+7 186.207 -32-13-2	76 Os +3+4 190.2 -32-14-2	77 Ir +3+4 192.22 -32-15-2	78 Pt +2+4 195.08 -32-16-2	79 Au +1+3 196.967 -32-18-1	80 Hg +1+2 200.59 -32-18-2	81 Tl +1+3 204.383 -32-18-3	82 Pb +2+4 207.2 -32-18-4	83 Bi +3+5 208.980 -32-18-5	84 Po +2+4 (209) -32-18-6	85 At (210) -32-18-7	86 Rn 0 (222) -32-18-8	-N-O-P
87 Fr +1 (223) -18-8-1	88 Ra +2 226.025 -18-8-2	89** Ac +3 227.028 -18-9-2	104 Unq (261) -32-10-2	105 Unp (262) -32-11-2	106 Unh (263) -32-12-2	107 Uns (262) -32-13-2												O-P-Q

*Lanthanides

58 Ce +3+4 140.12 -20-8-2	59 Pr +3 140.908 -21-8-2	60 Nd +3 144.24 -22-8-2	61 Pm +3 (145) -23-8-2	62 Sm +2+3 150.36 -24-8-2	63 Eu +3 151.96 -25-8-2	64 Gd +3 157.25 -25-9-2	65 Tb +3 158.925 -27-8-2	66 Dy +3 162.50 -28-8-2	67 Ho +3 164.930 -29-8-2	68 Er +3 167.26 -30-8-2	69 Tm +3 168.934 -31-8-2	70 Yb +3 173.04 -32-8-2	71 Lu +3 174.967 -32-9-2

Orbit: N-O-P

**Actinides

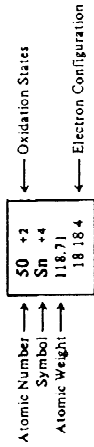| 90 Th +4 232.038 -18-10-2	91 Pa +5+4 231.036 -20-9-2	92 U +3+4+5+6 238.029 -21-9-2	93 Np +3+4+5+6 237.048 -22-9-2	94 Pu +3+4+5+6 (244) -24-8-2	95 Am +3+4+5+6 (243) -25-8-2	96 Cm +3+4+5+6 (247) -25-9-2	97 Bk +3+4 (247) -27-8-2	98 Cf +3+4 (251) -28-8-2	99 Es (252) -29-8-2	100 Fm (257) -30-8-2	101 Md +2+3 (258) -31-8-2	102 No +2+3 (259) -32-8-2	103 Lr +3 (260) -32-9-2

Orbit: O-P-Q

Fig. 1. Elements periodic table.

Fig. 2. Rock engraving of the late iron-age, probably depicting a blacksmith's house (Camonica Valley, Brescia, Northern Italy; by kind permission of the Camunic Centre of Prehistorical Studies).

issued regulations aiming to reduce Pb in gasoline, thus leading to drastic reductions of Pb in the air. From 1978 to 1988 approximately a threefold reduction of the content of Pb in gasoline yielded a decrease of the environmental lead concentrations to 0.5–1 $\mu g/m^3$ and consequently a clear reduction of lead in blood to 10 $\mu g/100$ ml or less [4–6].

Other traffic-related metals, are now under study for their possible environmental dispersion. They are palladium (Pd), platinum (Pt) and rhodium (Rh) widely used in car catalytic converters. A number of papers presented data about the presence of these metals in dust collected in the surroundings of roads [7–10]. The levels of these metals remained almost constant between 1970 and 1985, then a steep rise was demonstrated: an almost eightfold increase of concentrations around the year 1987 concentrations was demonstrated in 1992 [11]. Recently, Bergerow et al. [12] through a very sensitive analytical technique (magnetic sector field ICP-MS) have been able to detect Pd, Ir, Pt and gold (Au) in blood sample of individuals belonging to the general population. The determination of these pollutants in body fluids is of considerable importance for the investigation of possible correlations with health effects.

In some cases the environmental dispersion of toxic metals originated health problems to more restricted groups such as the well known cases of Minamata due to Hg, or those in Japan and Belgium due to Cd [13,14]. Less known, but very interesting as sentinel events, are some eco-dispersive phenomena, such as that described for thallium (Tl) among the population living in the surroundings of a plant emitting thallium containing dusts [15].

Moving to health effects, it is well known that since Greek and Roman times, some metals had been identified as possible cause of human diseases, and Ramazzini, the father of occupational medicine, in *De Morbis Artificum*, edited exactly 300 years ago, described some metal diseases in miners, gold platers, "decorators" dealing with Hg, chemists, potters, silver platers, and manufacturers of mirrors.

In modern occupational medicine, a metal, Pb, still occupies a prominent place, since after being the most important toxicant in the clinics of occupational diseases, it became the model on which occupational toxicology has been based. In fact, the indicators of dose and effect of this metal and, in general, the procedures for biological monitoring were firstly studied and then validated [16,17].

In recent years, thanks to technological innovations and preventive measures, risks related to high exposure–absorption of metals have drastically reduced. This conclusion is strengthened by the evidence of the reduction in acute or chronic intoxications and in occupational illnesses induced by metals and by the constant decrease of air concentrations of metals in workplaces or in biological media of exposed workers.

Specific occupational risks however still exist because of the persistence of metallurgical activities in the traditional areas of the European Union and because of the existence of working sites not yet adequately controlled, in particular the small factories. In these situations the health risk from high absorption of extensively studied metals such as Pb, Cr, Ni, Cd still exists. In addition, increasing importance has been assumed by the study of ''rare'' elements such as Be, Sb, V, Mo, W, arsenic, not so frequently used by themselves, but present in low or unpredictable percentage in fume or dust in ambient air of metallurgical plants.

Absorption of a certain toxicological relevance may also accidentally occur outside the occupational field for particular population groups such as children (ingestion of Hg- or B-based disinfectants) or in subjects drinking beverages kept in containers enamelled with lead.

In the past some elements (Hg and As) were used in the pharmacological therapy, others, such as the Pt coordination compounds, Au and Li salts, continue to be used.

Another medical source of toxic elements deeply investigated and still relevant is amalgam fillings by alloys containing Hg [18–21].

Metals may be present also in ''alternative'' drugs which are getting more and more popular in developed countries. Many people wrongly believe that all ''natural'' remedies are innocuous and can be taken without medical supervision. Such remedies may contain, or be contaminated by, toxic elements such as Pb, Hg, and As. Metal poisoning due to folk remedies is not so frequent in Europe, but some cases of Pb and arsenic poisoning due to folk remedies have been already reported [22–24].

The scenario of scientific and applicative interest regarding elements is wide, articulated and not only past-oriented and it is feasible to assume that metals will represent in the next years an issue of renewed efforts for research, mainly in analytical and toxicological fields.

2. The elements between essentiality and toxicity

2.1. Essentiality

For which elements does there exist an interest that calls for their biological determination? The answer to this question appears obvious: those that from time to time attract our attention or more simply those that can be measured. It is obvious however, that the greatest interest for the measurement in the biological media of elements lies in their possible biological and toxicological effects [25].

The elements of toxicological interest are definitely fewer compared to the organic compounds and their number is fixed (those of the periodic table). Some of them in respect to their biological outcome, may be compared to ''double-edged blades'', beneficial on one hand and toxic on the other. Chromium (Cr) is a good example of this ambivalence: in its inorganic trivalent species Cr is recognised as an essential element for carbohydrate and lipid metabolism; hexavalent species are genotoxic and carcinogenic [26].

Fig. 3 depicts the relationship between essentiality and toxicity: when an element is essential (I) it can
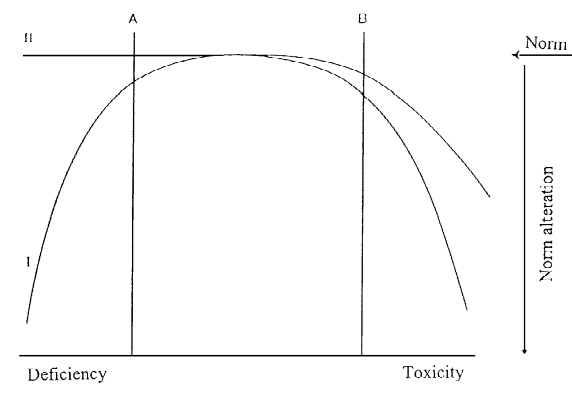

Fig. 3. Relationships between deficiency and toxicity for essential and toxic elements.

produce effects both when deficient and in excess; when it is toxic (II), the biological activity appears only when the concentration in critical sites exceeds certain levels. The A and B levels of absorption represent the points at which biological changes begin to appear both because of deficiency and excessive intake.

Several elements are physiologically present in the human body and for some of them an essential role in the biological systems has long been recognised. The criteria used to define essentiality of an element [27] can be summarised as follows: (a) the reduced ingestion must produce functional damage; (b) the deficiency inhibits growth or a vital cycle in the organism; (c) the influence on the metabolism of a given organism must be direct; (d) the reintroduction into the diet in right amounts must correct (prevent) deficiency symptoms; (e) the effect cannot be eliminated by substitution with another element.

Generally, even though essential elements are involved in different multiple functions and structures (enzymes, transport proteins, hormones, specific receptor sites), it could be stated that their main role is the co-enzymatic activity in approximately 25–30% of enzymes known to date. In this respect, Zn is a good paradigm being part of or activating several hundreds of enzymes.

For some elements such as Al, Bi, Ti a biological function cannot be wholly excluded and for this reason they are defined as "candidate elements", while others, such as Sn, are suspected to be essential and called "putative elements".

2.2. Toxicity

Once absorbed, the essential metals have few opportunities to produce toxic effects as a result of the presence of homeostatic mechanisms, such as intestinal control of absorption and presence of specific transport proteins. The competition between essential and toxic elements for protein binding sites lies at the basis of the toxicity of some of them.

The toxic effects of elements are partly due to direct inhibition of the enzymatic systems, partly due to the indirect alteration of the essential metal-ions equilibrium and to the consequent inhibition of their biological availability [25].

Metals form a vast variety of coordination compounds (organic binding metallic cation), whose stability is determined by specific equilibrium constants. The marked tendency to form compounds ensures that metals in vivo are invariably complexed with particular biological groups, such as sulfhydrylic (–SH), aminic (–NH$_2$), oxydrilic (–OH), disulfuric (–SS), carboxylic (–COOH). These groups also pertain to important molecules with catalytic, structural or transport functions.

Each metal has its own spectrum of affinity constants for organic binding; elevated values are observed for molecules rich in –SH groups, towards which metals such as Pb, As and Hg show particular reactivity. The reactivity for a wide range of biological ligands lies at the basis of the damaging action of the metal ion at the molecular level and determines the characteristic toxicity of the absorbed metal. The knowledge of mechanisms of action is relevant not only for identifying the possible targets and related possible biomarkers of effects, but also for improving the determination of elements, for example in selecting the most appropriate biological medium.

Effects induced by metals are various from irritant and acute or chronic systemic toxic effects to teratogenic, mutagenic and carcinogenic effects. They involve various organs and apparatus, as shown in Table 1, the effects have been described mainly for some metals (Pb, Hg, Cd, Cr) and specific system apparatus (respiratory, gastrointestinal, nervous).

2.3. Hormesis

Some authors suggested another possible action of toxic substances named hormesis, defined as the stimulatory action of sub-inhibitory amounts of a toxin. It occurs in a variety of species (microbes, plants and animals including humans), with a broad range of chemical classes and can involve many different endpoints such as growth, physiological or metabolic change, longevity, reproductive activity [28,29].

The scheme of mechanisms suggested for hormesis is reported in Fig. 4, divided into three parts: a stimulatory phase (A), antagonism of the stimulatory effect (phase B) and an inhibitory phase (C) which may be an extension of phase B. Each of these phases can be represented by a separate dose–re-

Table 1
Effects of the main elements on different organs and apparatus

	Nervous system	Cardiovascular apparatus	Gastroenteric apparatus	Endocrine system	Immune system	Kidney	Liver	Lung	Blood	Skin
Al	+							+		
As	+		+	+			+	+	+	+
Be								+		+
Bi						+	+			+
Cd	+	+	+			+		+		
Cr			+		+	+		+		+
Co	+	+	+	+	+			+		+
Fe	+		+				+	+		
Mn	+			+	+			+		
Hg	+		+			+		+		
Ni						+		+		+
Pb	+	+	+	+	+	+			+	+
Pt					+			+		
Cu	+		+						+	
Se			+			+				+
Sn	+		+							
Tl	+		+	+		+		+		
Zn			+						+	

sponse curve having different mechanisms of action. This type of dose–response interpretation establishes the link between hormesis and toxicity by assuming different mechanisms of action for the stimulatory and antagonistic phases of the response [30].

Davis and Svendsgaard [31] suggested that the effects of essential trace elements should not be included as examples of hormesis despite the fact that they are clearly examples of beneficial effects of subtoxic doses of chemicals. For toxic metals the designation of low dose effects as stimulatory or

Fig. 4. Graph depicting the dose–response curve of the chemical hormesis zone.

beneficial and high dose effects as inhibitory or adverse is somewhat arbitrary and does not provide a consistent definition for hormesis.

2.4. Element interactions

Increasing interest is being directed towards interactions among different elements, in particular in establishing whether combined exposure to metals, not toxic by themselves, may induce damage to health [32,33].

Information on the metals or alloys processed may sometimes be insufficient; an example may be drawn from the metallurgy of non-ferrous alloys where, in addition to well known components of the alloys (such as. Pb, Cu and Zn), unexpected exposure to other metals (such as Cr, Ni and Be) have been documented [34].

Following multiple exposure to metals, interactions during absorption and distribution have been demonstrated in animal experiments, even these studies cannot be easily extrapolated to humans. More data are available on toxicodynamic interactions, classifiable as antagonistic, additive and synergistic [35].The studies listed in Table 2, including those on animals, are focused on exposures which may occur in real working conditions, such as in
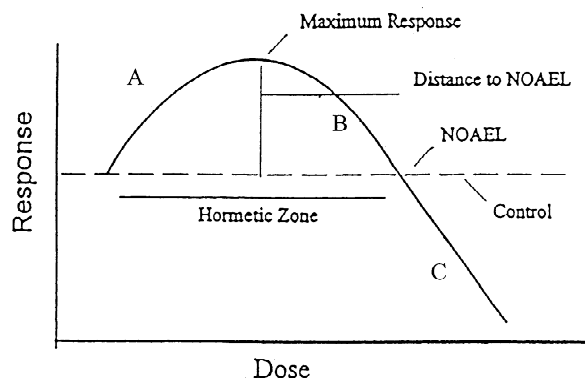
Table 2
Multiple exposures to metals: toxicodynamic interactions

Metal	Type of study			Effects
Antagonist effects				
Pb–Al	Rats	Pb 2 mg/kg Al 150 mg/kg	i.p. single administration	Liver ALAD inhibition<that induced by Pb alone
Pb–Zn	Rabbits	Pb 18 mg/kg Zn 50 mg/kg	s.c. single administration Pb pretreatment	RBC ALAD inhibition<that induced by Pb alone
Pb–Zn	Workers	PbB 40–100 µg/dl ZnS 102–308 µg/dl	Long-term	ALAU increase<that induced by Pb alone
	Rats	Zn 12 mg/kg Cd 4 mg/kg	Pretreatment, single administration, s.c. i.v.	Prevents lethal Cd hepat-toxicity
Cd–Pb	Rats	Pb 200 ppm Cd 50 ppm	Diet 10 weeks	ALAU decrease<that induced by Pb alone
Additive effects				
Pb–As	Rats	Pb 200 ppm Cd 50 ppm	Diet 10 weeks	CPU increase<expected both for As and Pb alone
Pb–MeHg	Human	PbB 10±1 µg/dl HgRBC 15–340 ng/g	Long-term	RBC ALAD inhibition. Correlated with both PbB and HgB
Synergistic effects				
Co–W	Rats	W 15 mg/100 g body mass Co 16 mg/100 g body mass	Intratracheal Short-term	Co+W causes lung fibrosis
Co–W	Workers	Co 125 µ/m^3 TWA	Long-term	Co alone does not cause lung fibrosis Co+W causes hard-metal disease
Independent effects				
Pb–Cd	Workers	PbB<62 µg/dl CdU<1 µg/dl	Long-term	Pb does not increase cd renal effects; Cd does not increase Pb effects on haem
Pb–Cu–Zn	Workers	PbB 57±15 µg/dl ZnS 106±12 µg/dl CuU 123±70 µg/ ZnU 1058±686 µg/l	Long-term	ALAU and ZPP levels as expected from exposure to Pb alone

i.p.=Intraperitoneal; s.c.=subcutaneous; i.v.=intravenous.

industries producing Cu alloys (Pb–Zn, Pb–Cu), glass (As–Pb), batteries (Pb–Cd) and hard metals (Co–W).

Of major interest were the studies carried out over the last few years on multiple exposures to Co and W carbide, studies firstly conducted in experimental animals and subsequently confirmed in humans. The investigations clearly demonstrated that the toxicity of Co–W carbide mixtures is quite different from that of Co metal powder [36,37].

In other cases, studies regarding multiple exposures to the same metals, carried out on animals and humans, have revealed different effects. For example, in the case of combined exposure to Pb and Cd in animals, an antagonistic effect was documented, whereas in workers employed in a factory producing battery independent effects were observed [38,39]. The differences could results from different degrees of exposure. This hypothesis seems to be confirmed in humans by the different results obtained from two groups of subjects with occupational exposure to Pb and Zn. The group of workers with higher exposure to Zn had urinary delta-aminolevulinic acid levels (U-ALA) much lower than those found in the other group [40]. In the group with lower exposure to Zn, the increase in the level of U-ALA was almost identical to that expected in subjects exposed only to Pb [41]. It is possible to classify the described phenomenon as a ''protective effect'' of Zn on the inhibition of delta-aminolevulinic acid dehydratase caused by Pb. This effect is well documented in in vitro and animal studies [42].

3. Biological media to be analysed

In recent years, different attention has been paid in setting up the analytical methods for metal determination, the first among the requisites for their admission to biological monitoring.

In the 45 monographs in the CEC series ''*Biological Indicators for the Assessment of Human Exposure to Industrial Chemicals*'' published between 1983 and 1991; the following metals were taken into consideration: Cd, Pb, Mn, Ti, Al, Cr, Cu, Zn, Mg, As, Co, V, Mi, Be, Se [43].

The American Industrial Hygienist Association

(ACGIH) suggested biological exposure limits (BEI) for the following elements As, Cd, Cr, Co, F, Pb, Hg, V [44], thus recognising that only for these elements the requisites in admitting them to biological monitoring had fully been respected.

Another Scientific Society, the Deutsche Forshungsgemeineschaft (DFG) suggested biological limit (BAT) values for Al, Pb, Hg, V, Cd and biological limit for carcinogenic elements (EKA) for Cr, As, Co, Ni [45].

One of the most interesting method to assess toxicological interest for an element is that adopted some years ago by the US Environmental Protection Agency (EPA – ATSDR). This method had been developed to identify substances having eco-toxicological interest which should be given priority [46].

Three factors are taken into account in classifying the substances: the intrinsic toxicity of the compounds, the frequency with which the compounds can be found in the environment, the probability of human exposure. On this basis, a ''*Priority Hazardous Substance List*'' was produced including 225 compounds subdivided into four groups. The following elements appear in this list: in the first group Mn, Sn, Sb, Ba, B, Co, Pb, Ni, As, Be, Cd, Cr; in the second V, Hg, Zn, Se; in the third Ag, Cu; in the fourth Sr, Ti, Mo.

The biological monitoring of these elements presupposes an analysis in biological matrices and thus the understanding about biochemistry and physiology of matrices is essential for the implementation of analytical methods and for an accurate interpretation of analytical data.

Limits in the analytical capability can also influence the choice of the biological media: for instance, the toxicologically ''active'' Pb in blood is most likely to be found in plasma, but, nevertheless, whole blood is used because the current analytical procedures are not sensitive enough to reliably determine Pb in plasma; likewise, the active fraction of Cd in blood is very likely the Cd–metallothionein complex, but a routine method for this complex is not available at present [47–49].

Exposure may take place via inhalation or ingestion, while absorption through the skin is very rare and regards some organic metal compounds and or impaired skin. Metabolic pathways of metals indicat-
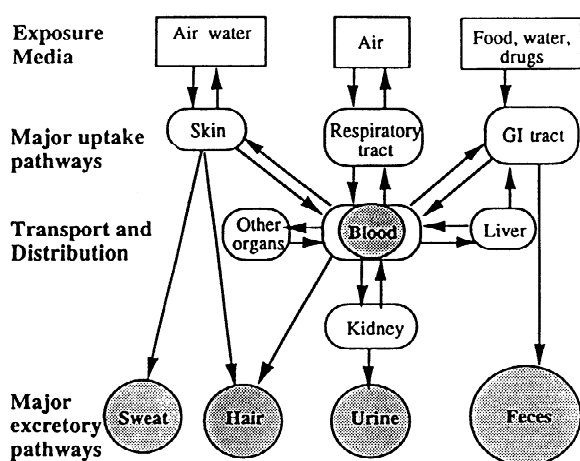
Fig. 5. Exposure and metabolic pathway for elements (from Friberg and Elinder [50]).

ing media for biological monitoring and critical organs are schematically shown in Fig. 5 [50].

In this figure, the arrows indicate how the elements are transported, the shaded circles indicate the possible media for biological monitoring and the other organs or systems in which elements are distributed, stored or active.

The most widely used media for spotted or repeated samples are urine and blood (whole blood, plasma or serum). Other media (hair, nails, teeth, bone) are used for specific purposes and for certain metals. Whatever media are used, adequate precautions must be taken to avoid contamination and when anticoagulant must be added (for example to measure the metal in plasma or whole blood) the metal content of the anticoagulant used should be determined [51].

3.1. Blood

The blood level of elements is influenced both by current exposure–absorption, by their body burden, by excretion rate.

Absorption of metals and metal compounds inhaled as particles is influenced by several processes including deposition, mucociliary and alveolar clearance, solubilization and chemical binding. After deposition in nasopharyngeal, tracheobronchial, or pulmonary compartments, the metal may be transported by mucociliary action to the gastrointestinal

tract. Metals may be also phagocyted by macrophages or taken up into the tracheobronchial or alveolar epithelium where the metal may remain for a long time or be transported into the interstitium. The kinetics of absorption and their distribution greatly vary among metals. The absorption of metals into blood is more (Hg vapour, Pb oxide) or less (Co and Cd oxide) rapid and complete [52,53].

The gastrointestinal absorption of inorganic salts of metals varies too, from less than 10% for Pb, Cd, In, Sn, U to almost complete absorption (90–100%) for water soluble inorganic salts of trivalent arsenic, germanium and thallium. The difference may be related to the presence of transport systems, particle size, solubility, dose, simultaneous administration of other substances, pH, rate of transit, host factors such as species, age, nutritional status and sex. High intestinal concentrations of Zn inhibit the absorption of Cu, whereas increase of Pb and Fe in the intestinal lumen enhance absorption of Mn. The complex interferences of Ca, Zn and Fe in intestinal absorption kinetics of Pb have been attributed to a competition for carrier proteins [54–56].

Metals in blood are bound to red cells or to plasma proteins. For metals bound almost completely to red blood cells (e.g., Pb and Cd), when haematocrit is low, for the same exposure concentration of metals in the blood will be lower [52,57].

The metals bound to plasma proteins represent the fraction available for transport into and out of the tissues. However, the concentrations for many toxic metals are so low that they are very difficult to measure [53].

Some differences can exist between measurements carried out in plasma or serum depending on the metal content of platelets and the inclusion of the metal in the clot mass.

The diffusible fraction plays an important role in determining the passage of metals from the blood into the interstitial fluid and intracellular compartment. For metals present in the ionised and unbound form, distribution is complete and is directly related to the concentration gradient in the different compartments.

Albumin is the plasma protein with a great capacity to bind several metals. For some metals, binding occurs with proteins which have a specific transport function such as transferrin, ceruloplasmin

and metallothionein. These are not only involved in the transport of essential elements such as Fe, Cu and Zn but also in those of toxicological interest such as Cd, Hg, Cr and Co. The binding at tissue level, which can be specific for a single metal, determines an increase of the concentration of the metal at the site of the toxic action or in organs and in soft tissues. These particular binding sites are important since they can neutralise the toxicity of the metal.

The metal bound to plasma proteins persists for a long time in the circulation and is distributed gradually and slowly over time. These interactions are generally reversible and the bound fraction keeps in dynamic equilibrium with the unbound fraction [58–61].

3.2. Urine

Urine is the most readily available biological medium. It is not simply an excretory medium, but also a product of kidney function, and thus kidney impairment could significantly alter the excretion of metals independently of changes in exposure or body burden of the metal. For example, urinary excretion of Cd and Hg is enhanced when tubular epithelium is damaged [62].

The mechanisms that firstly condition the presence of metal in urine are those related to glomerular filtration and renal clearances for several metals (such as Cr, Cu, Ni, Ti, U, Zn) have been determined.

The diffusible plasma fraction of the metal and that bound to proteins with molecular masses capable of crossing the glomerular membrane are excreted. The metals filtered can be excreted directly or after re-absorption (active or passive) at the proximal tubule as it occurs in the case of Pb. In specific cases (Cd, Hg) elimination can occur by exfoliation of the epithelial cells [63–65].

The rate excretion of a metal can be assessed rather accurately provided that urine samples are collected over a defined and sufficiently long period of time (e.g., 6 to 24 h). Because of the difficulty to achieve this type of collection spot samples are generally used. The variability over time and physiological status can be reduced by correcting metal concentration for specific gravity or creatinine, al-

though an agreement about this procedure is still lacking [66].

3.3. Other media

Table 3 reports the media in which elements have been determined in biological materials as resulting from articles reviewed by Taylor et al. in 2000 [67]. As can be observed, the biological matrices have different characteristics and therefore different analytical procedures may be required.

The analysis of elements in faeces is a problematic procedure, because of inter-individual variations in gastrointestinal excretion (hepatic, pancreatic, digestive tract) of elements and to their absorption, or of interactions with other elements and organic compounds. Element compounds such as methyl Hg, are for example secreted into the bile, re-absorbed in distal ileum, and re-excreted by the bile [68,69].

Hair, and to a lesser extent, nails are used to determine the concentration of trace elements as indicators of deficiency states in nutrition and to demonstrate poisoning states in forensic medicine [70]. Hair and nails are dead structures and literally "freeze" past exposures in time; thus recording the history of personal exposure to metals over time. Quantitative relationship between hair concentrations and blood or tissue concentrations has been shown for methyl Hg. A relationship also exists for inorganic arsenic or Pb, such as in hair of newborns from occupationally exposed mothers, but data for quantitative assessment are not currently available for metals in general [71,72].

Table 3
Biological matrices in which elements have been determined [67]

Blood and its fractions	Mucoses
Urine	Hair, nails
Faeces	Soft tissues
Milk	Liver biopsies
Bile	Kidney biopsies
Sweat	Muscle
Sputum saliva	Vessels
Tears	Lung biopsies
Cerebrospinal liquid	BAL
Seminal plasma	Dialisis liquid
Sinovial liquid	Gall and kidney stones
	Bone

The uptake and the passage of metals across the placenta depend on many factors such as blood levels and species of metals, gestational age, etc. Normal values are well-established for only a few metals (Cd, Pb and Hg) even if their relationship with the concentration in other biological media media are not always available [73,74].

Another classic media are teeth whose metal concentrations, have been used to assess long-term cumulative exposures. Deciduous teeth, extracted teeth or teeth collected at autopsy could, in theory, be used for measuring elements present in calcified tissues [75,76].

4. Atomic absorption spectrometry: the technique on which the modern toxicology of metals has been built

Walsh, starting from the classical and long-forgotten works by Kirchoff and Bunsen in the middle of 19th century and the experience gained on spectrochemical analysis of metals and molecular spectroscopy in the 1930s–1940s, conducted the first experiment of energy absorption emitted from a sodium lamp when a solution of sodium chloride is vaporized in a flame. It was on that occasion that he called a colleague and pronounced with great emotion the famous phrase ''Look, that's atomic absorption!'' [77].

These first results, to Walsh's great surprise, were totally ignored by the scientific world and it was only in 1956 during a conference in Lisbon and in a further meeting held at the Institute of Physics in London that the Australian research group were given credit for their discoveries.

The first application of the AAS technique in the biological field, dates back to 1956 and refers to the measurement of Mg in serum and milk.

Further progress was then achieved mainly on atomization systems (with the introduction of the gas mixture acetylene–air), optical pathways and monochromators.

It must be pointed out that the interest for biological determinations was one of the incentives of this development and the measurement of Ca in serum was the first objective that Perkin-Elmer proposed to Walsh.

The first attempts to identify new and more efficient atomization systems date back to the beginning of the 1960s which should obviate two limits already foreseen by Walsh: incomplete atomization and matrix effects.

The next, crucial step for biological matrices, was the electric atomization. The idea of L'vov was to electrically heat a pyrolytic graphite container until sufficient temperatures were reached to induce atomization. Insurmountable technical and organizational difficulties hampered the practical application of this principle [78].

It is significant, to confirm the strong link between the biological field with the development of AAS, that the only instrument constructed by the L'vov research group at the end of the 1960s was installed in the Moscow Institute of Medical and Biological research. The subsequent evolutions of the graphite furnace are marked by the names of West, Massmann, Woodriff, Robinson and Slavin whose fertile collaboration with L'vov led to the creation of the first prototype followed by the first commercially available model of the graphite furnace.

Following the introduction of this new instrument (April 1970) the fundamental work of Manning and Fernandez regarding the characterisation of elements in blood and serum, appeared in June 1970 [79].

As can be noticed by the small number of publications, the diffusion and scientific interest for the graphite furnace was less deep than expected, at least up to the middle of the 1970s. The reason depended on the high interferences due to the matrix.

In the context of the collaboration between Slavin and L'vov, the evolution of the atomization systems (pyrolytic platform) and the development of the analytical procedure named ''stabilized temperature platform furnace'' (STPF) system can be dated to the end of the 1970s [80].

Other innovations which led to the configuration of AAS instrumentation known to us today (such as autosampler, maximum power heating systems, control furnace temperature, development of electronic control systems, improvement of background correction systems) emerged between the end of the 1970s and the middle of the 1980s.

Selecting among still open questions in biological media analysis, I think it would be appropriate to recall something about atomization systems, back-

ground correction and matrix treatments. Further progress in these instrumental and analytical questions should make it possible to accurately determine more and more elements, minimising at the same time, the possible interferences caused by other substances present in the matrix.

4.1. Atomizers

It can be stated, without fear of being contradicted, that one of the critical aspects in biological analysis is represented by the system (atomizer) needed to produce the so-called atomization cloud. It enables to transfer the solution containing the metal to be analysed into a gaseous phase in which atoms are present in the ground state, so that they can absorb a luminous radiation emitted by a specific lamp.

Flame is the most classical atomizer and an air–acetylene flame (working temperature approximately at 2300°C) is normally used for metals with low excitation potentials. The sensitivity is in the range of ppm, although concentration might be enhanced by chelation extraction techniques.

The high speed of analyses permits one to perform a large number of determinations in the shortest possible time. The biological and toxicological interest are limited to metals such as Zn, Cu and Fe.

The hydride kit is an atomization system equally rapid but with a sensitivity in the range of ppb. The principle used in this case is based on the capability of some elements such as Hg, As, Se, Sb, Sn, Ge, Te and Bi to form volatile hydrides. Arsenic is reduced to As^{3+} and Se to the Se^{4+} form.

The solution containing the element reacts with sodium borohydride in the presence of an acid. The Hg or hydride thus formed is then driven from the cell by bubbling a gas carrier through the solution into a sampling cell placed in the light path of the AA spectrometer. In the cell, which can be heated by a flame or electrical device, decomposition of the hydride takes place with release of the free atoms of the element to be analysed.

The electric graphite furnace is the most advanced and widely used technique for analysis of metals in biological matrices since it allows one to reach an optimal compromise among simplification of the matrix, attenuation of interferences and generation of a good number (cloud) of atoms to be analysed. The

development of systems to control temperature and the optimisation of thermal programs has further improved the technique [81].

The tube, and the sample solution, are subjected to a multi-step temperature program which serves to generate the atom cloud. The thermal program consists of different steps: drying, ashing, atomization, clean out and cool down to room temperature. During the drying process 10–20 μl of solution is made to slowly evaporate until a solid residue is formed at the bottom of the graphite tube. Ashing (pyrolysis) is the most complex phase since the matrix must be completely removed without loss of metal to be analysed. Only by this way it is possible to obtain accurate and comparable signals for the different solutions under analysis. Atomization of the sample residue at an elevated temperature (>1000°C) leads to vaporization and subsequent generation of free atoms responsible for the absorption signal. Graphite furnace analyses are characterised by a sensitivity to the order of ppb (μg/l), i.e., sensitivities sufficient to measure the vast majority of metals in biological matrices. The reactions that occur during pyrolysis are very complex, not well known and not easy to describe. It must be pointed out, however, that since they are not combustion reactions, graphite furnace analyses are susceptible to matrix effects and more or less strong background effects [81,82].

The use of a pyrolytic platform inserted into the graphite tube enabled significant improvement in drying and ashing phases since the platform must be heated especially by radiation. It is precisely heating by radiation that causes atomization of the matrix in an elevated thermal environment. For conditions requiring less atomization temperatures, the platforms reveal their best capacity in the case of low boiling or easily atomized metals (Pb, Cd). Recently two kinds of platforms have become available on the market: the flat and the concave one, the latter platform allows one to determine either low boiling elements and the refractory ones without changing the tube [83].

4.2. Matrix effect correction

In the analysis of biological matrices, it appears necessary to correct non-specific absorbance signals

due to the matrix and arising during the atomization step, since the coexistence of atoms of the element and molecules of the matrix can lead to an equal absorption of energy. For this reason, the measured absorbance must be split into an ''atomic'' and a ''background'' signal.

The classic deuterium background correction system, with its non-specific spectrum, has two important limitations: it can only read instant situations and can originate erroneous corrections.

The Zeeman effect background correction was introduced later, in two configurations, with a magnet positioned longitudinally or transversally across the graphite tube. It is applicable only for graphite furnace analyses and enables the correction of structured backgrounds and spectral interferences usually not corrected by the deuterium system.

As already mentioned the development of the AAS technique in biological analysis can be considered a continuous attempt to minimize the interferences originated by the matrix and the background correction systems currently available are not always able to compensate for the matrix interferences on the signal of the element. For this reason in order to reduce the matrix effect within acceptable values it is necessary to treat the sample [84].

Acid digestion wet ashing is probably the most diffuse technique to treat biological samples for trace element analysis. Oxidation is carried out by acids (HNO_3, $HClO_4$ and H_2SO_4 in various combinations and sometimes also with H_2O_2 or HF) at temperatures of up to about 200°C. Heating is achieved on a hot plate.

Another simple method able to completely remove the matrix can be obtained through processes of chelation extraction with solvents or resins or ion-exchange columns. The first method is the best known and most widely used which enables one to separate the metals from the biological matrix and if necessary to pre-concentrate them (for instance, extracting the chelated complex in the biological liquid with a reduced volume of solvent).

The factors that greatly influence complexation are dilution (an increase in dilution also increases the dissociation of the complex), excess of reagent (an excess of complex, displaces the equilibrium towards the formation of a complex hindering at the same time its dissociation), pH (high pH increases non-

dissociated forms). The pH is the most important factor in the case of formation of amino complexes considering the basic nature of NH_3, a strong acceptor of protons.

The main complexes (binding) are halogen compounds, oxygen, sulfur and nitrogen. The latter are partly anionic and partly neutral and the relative coordination compounds can in turn be anions, cations or neutral compounds. Among the anionic compounds are APDC and NaDDC. Although not a specific chelating agent (it forms complexes with 30 metals) APDC permits to operate in a wide range of pH and whenever necessary it is possible to extract several elements at the same time. The ideal extraction solvent must be poorly soluble in water, burn completely, neither cause background absorption nor give rise to toxic combustible products. For this reason, it is advisable not to use aromatic solvents (benzene or toluene) since they produce a ''smoky flame'' which reduces instrumental precision.

IBMK is used since it burns completely and does not cause non-specific absorptions. With respect to other ketones it is moderately soluble in water which causes emulsions and slowness in the separation of the phases. To eliminate this inconvenience the addition of ammonium sulfate to the sample was proposed before addition of the complex or the use of cooled centrifuges.

The other method which enables one to minimize the matrix effect by a decrease in its quantity-concentration consists in dilution of the samples (with water supplemented if necessary with tensioactive agents). It must obviously take into account the concentration of the metal in the sample and the sensitivity of the method.

Also atomization systems, especially STPF and thermal programs can be considered as methods able to partly eliminate the matrix. STPF and adequate thermal programs (using oxygen or air) can prevent the build-up of residual carbon favouring oxidation in situ of the biological matrices [83].

Chemical matrix modifiers, however, must be dealt with separately.

They act permitting the development of most appropriate thermal programs giving rise to the formation of stable compounds with the metal/metals of interest. These compounds permit one to

increase the ashing temperature and thereby facilitate the removal of the interferences present in the matrix [84,85].

The significance and the function of matrix modifiers can be explained analysing the behaviour of Mg $(NO_3)_2 \cdot 6H_2O$ added to the biological samples. This compound at 300–400°C is reduced to an oxide which reacts with elements such as Al, Mn, Be, Cr, Co, Ni, "trapping" and making them more volatile during the ashing step.

Matrix modifiers can be divided into: (a) ammonium and magnesium salts; (b) acids; (c) metallic salts.

Ammonium phosphate has been used for a long time as a modifier, especially in the measurement of Cd and Pb. For this modifier and for magnesium nitrate, which turns out to be the most widely used non-metallic modifier, great attention is paid to their purity degree, to avoid sample contamination.

Acid modifiers include nitric acid, sulfuric and phosphoric acid which exploit the original capacity shown for ammonium nitrate (NH_4NO_3) of reducing the volatility of the halogens and hence the risk that some elements get lost. In fact, the addition of an acid in excess gives rise to the formation of highly stable compounds.

Drawbacks of the open digestion method include the use of large volumes (several ml) of one or, in most cases, several acids, which introduces high blank levels, losses by volatilisation of elements such as Se, Cd, Pb, As, Hg and is time consuming.

A useful alternative to the technique of acid digestion is afforded by microwave heating. The digestion takes place in a closed PTFE (polytetrafluoroethylene) bomb by heating in a microwave oven. The high internal pressure developed ensures rapid digestion without any loss of analyte. No acid is lost during digestion so acid consumption can be limited and the blank keeps minimal. In addition, contamination from the air is eliminated. In several cases HNO_3 is sufficient for the dissolution. The combination of microwave digestion and analysis by ICP-MS has been applied by several research laboratories,determining elements such as Rb, Sr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, Hg and Pb. Generally the sample preparation consisted of a two-step microwave digestion with 2.5 ml sub-boiled HNO_3. The final solution was then diluted with pure water.

The most widely employed metallic salts are those composed of Ni, Pd: the use of Pd has been extensively studied enabling to considerably increase the ashing temperature [86,87].

The use of a mixture of Pd and ammonium or magnesium nitrate, for determining low boiling metals such as Cd, Pb, Mn, Sb and Se, has been suggested [88,89]. It allows to reach temperatures of 800–850°C and markedly reduces the background absorption. The mixture is at least equivalent to the previously recommended ammonium phosphate modifiers, but avoids the interference found with these latter compounds.

It must be pointed out that the prolonged use of metallic modifiers may contaminate not only the graphite tube and platform but may also get in contact with the graphite furnace: this must be considered when the elements used as modifiers have to be measured. Furthermore the risk of dispersion of metals (some of which, such as Ni, are highly toxic) in the laboratory environment should not be underestimated.

Two other methods may be used to simplify the matrix: enzymatic digestion and UV irradiation. Non-specific protease enzymes are capable of breaking down a wide range of protein into their amino acid components and may be used, for example, for digesting serum proteins.

Christensen and Pedersen [90] and Shakra et al. [91] suggested the use of the enzyme pronase, which is a protease with wide specificity, isolated from *Streptomyces griseus*. The use of the enzyme for sample digestion allows one to decrease the level of intact proteins in serum, is cheap, reproducible and does not require to handle corrosive materials. It however does not break down all the proteins present and therefore a residual background effect may exist.

Another technique found to be advantageous over the conventional acid digestion is UV photolysis [92,93]. It requires a minimal addition of reagents and the blank values can be kept extremely low. Among the others, it has been experimented for human blood by Pisch et al. [94], with irradiation of 0.5 ml samples for 6 h, after the addition of H_2O_2 and HCl.

To date, UV photolysis gave the best results when fairly simple matrices with low organic content were treated.

4.3. Flow injection

The flow injection system (FIAS) allows stable and highly efficient nebulisation, constant flame conditions, control of dispersion phenomena; possibility of analysing highly viscous solutions or solutions with a high content of salts. It also offers other advantages: automatic analysis of hydrides, the possibility of automatic addition of reagents, the possibility of measuring Hg and elements which form hydrides directly in the graphite tube treated with an appropriate modifier, improving by 500 times the detection limits (this technique is called FIFU, flow injection furnace).

Another interesting feature of FIAS is the possibility of concentrating the metals complexed prior to the extraction procedure on the C_{18} columns and to guarantee subsequent elution with an adequate solvent in a shorter period of time. Among recent applications of this technique to biological media, those for Hg, Pb, Cd, Bi and Pt have been reported [95–100].

4.4. Multielemental analysis by atomic absorption spectrometry

The impossibility of measuring several elements simultaneously represented one of the main limits of AAS analysis, which is by definition a monoelemental technique.

AAS has however some surprises to offer. In fact a spectophotometer (Perkin-Elmer SIMAA 6000), able to determine up to six elements simultaneously, has been launched on the market. This instrument is dedicated to graphite furnace analysis with Zeeman background correction. The technology which has enabled this jump in quality is due to the use of special optics, a polychromator and to the use of a newly devised solid state detector. The graphite tube is heated transversally, thus increasing thermal stability and eliminating memory effects. The results obtained offer reproducibility, accuracy, detection limits similar or superior to those obtained with more advanced spectrometers.

Once again opinions advocated by illustrious scientists with regards to AAS have been confirmed: the success and the development of good ideas and principles depend on the ability of implementing and improving their technical and applicative aspects [77,101].

5. Inductively coupled plasma mass spectrometry: the perspective technique for biological analysis?

Inductively coupled plasma mass spectrometry (ICP-MS) is one of the most sensitive analytical techniques for single element measurement in biological matrices and enables rapid multi-element determinations, combining the multielement capability and broad linear working range of ICP emission with the detection limits of GF-AAS. In addition it is one of the few analytical technique for isotopic analysis of elements [102,103].

ICP-MS is the combination of an inductively coupled plasma, to generate singly charged ions from the elements within a sample, with a quadrupole mass spectrometer, which, similarly to monochromator in AAS or ICP-AES systems, separates ions according to their mass-to-charge ratio.

Normally ICP uses an argon plasma, obtaining atomisation and ionisation of the element at a temperature around 7000 K. At this temperature more than 40 elements are ionised to 90% or more. To a lesser extent (10–40%) also a number of non-metals having a high first ionisation energy (e.g., P, S, As, Se) are ionized. A full mass spectrum can be obtained with the possibility of multi-element analysis with excellent detection limits (ng–pg/ml) [104,105].

ICP-MS attracted the attention of researchers involved in the analysis of biological materials, since it was ideal for measuring the absorption of single or multiple elements in groups of the general population or of occupationally exposed workers, ideal for correlating biological data to effects or specific diseases for epidemiological studies. This technique may also be of interest for clinical or forensic purposes to demonstrate acute poisoning by unknown element(s) [106–109].

When examining (biological) samples, however, ICP-MS suffers from important interferences. The most studied are isobaric interferences, in which the analyte ions are not the only species observed, but elements with a second low ionisation energy would

be produced. In addition, other interferences may be created by polyatomic ions originating from the plasma (Ar), from the matrix (O, H, N, P, S, Cl) and from the air surrounding the plasma (C, N, O). Deeply investigated are interferences of $^{35}Cl\ ^{16}O$ with ^{51}V, those of $^{40}Ar\ ^{35}Cl$ with ^{75}As and those of $^{40}Ar\ ^{23}Na$ with ^{63}Cu.

Sample preparation plays an important role for ICP MS analysis and therefore all the procedures of sample treatment seen before (see Section 4.2 in particular for UV photolysis, concentration and extraction) could be considered. Special care has to be taken during sample preparation to minimize contamination or loss of analytes. For the analysis of biological materials, whenever possible only HNO_3 should be used in sample preparation, because H, N and O do not increase the spectral interferences [110–112].

An attempt to quantify the possible interference in biological matrices was carried out by Vanhoe et al. [113], who used for the determination of Fe, Co, Cu and Zn a simulated solution containing the same concentration of Na, S, Cl and Ca, similar to a fivefold diluted serum solution. The interferences (and therefore the necessary corrections) varied from 4% for Cu to 55% for Co. Most biological fluids contain large concentrations of proteins, which can originate blockage of the pneumatic nebulizer, of the central quartz tube of the torch and of the sampling orifice. For reducing this phenomenon a dilution of the sample may be adopted, and in the analysis of serum, blood, protein solutions, urine it consists merely of five- or 10-fold dilution with distilled water and 0.14 M HNO_3, or with 0.28 M HNO_3. Mulligan et al. [107] recalled that the application of this approach is restricted by the presence of a number of spectral interferences in the range of 10–80 u.

Among the other methods adopted to overcome this problem, gel filtration was used to desalt the analysed protein solutions [119], ion chromatography to eliminate the interference from $^{40}Ar,\ ^{35}Cl$ on the determination of As in urine [120], an anion-exchange resin column (DOWEX-1) to separate chlorine and sulfur from the trace metals in serum and urine [121].

Besides separation techniques electrothermal vaporisation (ETV) can be used as a system for treatment-introduction of biological samples to reduce some spectral interferences. ETV significantly reduces the levels of polyatomic interferences, thanks to the absence of any solvent.

The use of an internal standard as close as possible to that of the analyte is a well-known procedure. The interferences are mass number-dependent, and when a multi-element analysis is carried out several internal standards have to be adopted [114]. The two other approaches are the standard addition (in which the spiked elements undergo the same suppression or enhancement as the analytes) and the external calibration [115,116].

A last remedy is the isotope dilution technique based on the measurement of isotope ratios in the un-spiked and spiked sample, as well as in the spike itself. A disadvantage of this method, besides a relatively complicated sample preparation, is that it cannot be applied to all elements since two isotopes have to be available (e.g., Al, Mn, Co, As, I and Cs are monoisotopic). In general, isotope dilution gives more precise results than external calibration or standard addition [117,118].

New perspectives in ICP-MS technique have been reached by the introduction of sector field (SF) ICP-MS. The technique is also known as high resolution ICP-MS and double-focusing magnetic sector field ICP-MS. By this technique, many of the isobaric interferences seen in Q-ICP-MS can be solved by using the higher resolution available with a sector field ICP mass spectrometer. Townsend [122] showed that, in medium resolution mode, the first row transition elements (Co, Cr, Cu, Fe, Mn, Ni, Sc, V, Zn), could be accurately determined in a range of reference materials including oyster tissue and human urine. Indium was used as an internal standard. The improvement in accuracy for Zn was confirmed by Sturup, who measured Zn isotope ratios and total Zn in human faeces, urine and serum [123].

This freedom from interferences and the excellent sensitivity available with SF-ICP-MS allows the multi-element determination of a wide range of elements. Rodushkin et al. [124] developed a method for 60 elements in whole blood. Samples were pressure-digested with HNO_3 using microwave heating. Three elements (As, Ge Se), were determined in high-resolution mode; 13 elements in medium res-

olution and the remainder in low resolution. The importance of contamination-free sampling was stressed. Begerow et al. [125] examined the potential of SF-ICP-MS for monitoring environmentally and occupationally relevant metals and compared it with ETAAS. Thirteen elements were studied in urine and serum. Sample treatment was simply UV photolysis with HNO_3–H_2O_2 and subsequent dilution. They concluded that the technique was valuable for multi-element screening where the type of exposure is not known and for determination at low concentrations for which ETAAS had insufficient sensitivity (Au, Pd, Pt, Th and U).

6. Anodic stripping voltammetry

Voltammetry is based on Faraday's law, for which an amount of a compound transformed in an electrode process is equivalent to a precise electric charge. It requires that metals to be determined are completely dissolved in the solution and that interfering analytes are absent, since even traces of organic matter can severely interfere and lead to erroneous results. It is particularly true for the biological materials and therefore strict decomposition procedures must be adopted prior to voltammetric determinations.

When metal levels are lower, an in-situ enrichment is necessary using the so-called stripping technique [126]. Metals that form an amalgam with Hg such as Cd, Cu, Pb, Zn, are accumulated by cathodic deposition in a (drop or film) Hg electrode. Commonly the solution is agitated or the electrode (film) rotated at this stage. Subsequently the amalgam is anodically oxidised in the determination stage. The Hg drop electrode can be applied down to approximately 0.1 μg/l.

Mercury(II) nitrate may be added to the analyte and the Hg electrode is formed by simultaneous in-situ deposition of a few hundred Å thick Hg film during the cathodic enrichment step for the metals to be determined. Alternatively metals such as Hg, bismuth and arsenic are deposited as an elemental monomolecular film at a solid gold of graphite electrode. Subsequently the film is also anodically

oxidised. This approach is termed anodic stripping voltammetry (ASV).

For ultratrace determinations, however, the use of the Hg film electrode (MFE) is necessary to achieve detection limits at the ng/l level. In a study by Horng [127] differential-pulse anodic stripping voltammetry on a hanging Hg drop electrode was used for the simultaneous determination of Zn, Cd, Pb and Cu in the urine of workers employed in a steel production plant and their matched normal controls. The results indicated that the urinary Zn, Cd, Pb and Cu levels of workers are significantly higher than those of the controls, thus demonstrating the possibility to use ASV in biological monitoring practice in occupational medicine.

Voltammetric stripping achieves very low detection limits compared to other techniques for a considerable number of toxicologically and environmentally significant elements. For body fluids as well as for solid biological and environmental materials the complete digestion necessary prior to voltammetric determination is the limiting factor for detection power. Compared to GFAAS, which allows the direct analysis of trace metals in body fluids ASV is somewhat inferior.

ASV, provided that a proper digestion is applied, is suited for single element analysis with appreciable accuracy and precision in routine and analytical quality assessment [128,129]. It has to be mentioned, however, that the potential range over which most metals are oxidised is relatively narrow.

Anodic voltammetry was of great importance for the determination of Pt levels in urine of subjects environmentally or occupationally exposed to very low levels of the metal, like health care workers handling cytostatic drugs. In a study by Ensslin et al. [130] the urinary Pt levels were determined after UV photolysis by voltammetry enabling the determination of Pt concentration of 4 ng/l. The results showed an elevated level of urinary Pt in one pharmacist (22.3 ng/g creatinine) in comparison with a non-exposed control group. It must be recalled that the current levels of Pt in urine of this kind of workers cannot be determined by ETA-AAS and ICP-MS.

A further promising property of voltammetry is that it is specific for distinct compounds and thus it can be successfully used in the speciation of metals.

7. The element speciation

7.1. The speciation in biological matrices

The element speciation related to the analysis of biological matrices can provide better knowledge about absorption, distribution reactivity to binding sites, bioavailability, toxicity and excretion of elements. The determination of different oxidation states of arsenic [As(III), As(V)] as well as of methylated species (monomethylarsonic, MMA; dimethylarsinic, DMA, acids) is the correct way to evaluate occupational arsenic exposure [131]. Speciation can play an important role also in order to assess the element toxicity. The toxicity of the three-oxidation states of Hg differs considerably: metallic Hg is associated with damage to the central nervous system, while mercurous compounds give a limited number of salts with mild, local irritant properties [132].

Speciation has been defined as an analytical challenge and the possibility to speciate elements in biological matrices depends mainly on the result of this challenge, in other words on the availability of analytical methods suitable for that purpose.

The analytical difficulties are related to the generally low (very low) species concentrations, to the change in species induced by sample treatment; to the interference of biological matrix. An ideal analytical procedure must therefore enable the identification of species, the preservation of the original status

of species and the determination of species in matrix (and we must add in a matrix easily and ethically sampled).

The preservation of species represents the most limiting condition, since any treatment before analysis, even the simple dilution, may induce chemical variation. No single method nowadays exists which currently enables the separation and measurement of species in a biological matrix. Speciation is therefore achieved by combining two different techniques, one to separate species, the other to determine the element in a different way associated to the separated fraction.

We can mention many separation techniques based on physical (ultrafiltration, pyrolysis), chemical (acid–solvent extraction) or physico–chemical principles (mainly chromatography–liquid chromatography). Among analytical techniques AAS and ICP-AES or MS in various combinations are the most used (Table 4).

Assuming that separated fractions and elements should indeed be associated to each other, speciation is currently carried out by an indirect way, quite different from that guaranteed by methods such as voltammetry or electron paramagnetic resonance. Since most of separation procedures are based, as stated before, on physical–chemical treatments, all pre-analytical and analytical variables have to be carefully checked to avoid contamination or losses.

The use of radiotracers could also be useful to study processes like accumulation, distribution,

Table 4
Analytical techniques for elements speciation

Separation	Analysis	Elements and compounds
Acidification–basification	Colorimetry	Organo-Sn
Solvent extraction	AAS (F, Hy, Gf)	
Volatilisation	ICP (AES, OES, MS)	As, Hg, Se methylated forms
Pyrolysis	Voltammetry	
Dialysis	ISE	Cr(III), Cr(IV)
Ultrafiltration	NAA	
Centrifugation		
Chromatography		Different oxidation states
Gas		and organic compounds
Liquid		
Supercritical fluid		
Ion		
Chelation-solvent extraction		Organo As, Sn, Pb
Electrophoresis		Protein complexes

metabolisation by demonstrating the specific activity, the stability of fractions and the activity/volume ratio.

Among separation techniques, the major interest is nowadays focused on chromatography. This technique enables one to identify both oxidation state and some organic compounds. In some cases it may be coupled directly with the instrument which measures the element. Our experience in speciation has been carried out mainly by using this type of separation and analysis coupling HPLC with ICP-MS, a method which fulfils among the other advantages that of being an on-line real-time speciation system.

The key areas in assessing species significance are the relationship with exposure (dose); the biological activity; the variety and stability of species. To this purpose the classification by Cornelis [133] helps to clarify some aspects by separating the species into: (i) small organo metallic molecules, food contaminants, water or hair which remains unchanged and the body (e.g., organotin or organoPb compounds); (ii) biomarkers of exposure for which the biological mechanisms change the species of the element (e.g., arsenic inorganic species metabolised mono-methylarsonic acid and dimethylarsinic acid); (iii) elements with different valence states, affecting their biological action [e.g., Cr (III)/(VI), Fe (II)/(III)]; element "building blocks" of the bio molecule (e.g.,

Zn in several metalloenzymes or Cu in caeruloplasmin); and (iv) elements forming a metal ligand complex with a compound of different molecular mass (e.g., Al with citrate or Cr to transferrin or albumin).

From a practical point of view we might consider on one side the oxidation state and on the other the organic compounds (Table 5). In comparison with the number of theoretical oxidation states, the oxidation states of proven biological interest are very few. Mn provides an interesting example: five oxidation levels ($+2$ to $+7$), one ($+2$) of definite and one ($+3$) of possible biological interest. The group of organic compounds includes numerous species, such as those from methylated to arylated compounds and from amino acid–peptide complexes to protein or hormone chelates. It is noteworthy that just for the components of this group, the demand for speciation is rapidly increasing.

Elements may undergo conversion from elemental form to cation by the loss of one or more electrons (oxidation). The opposite process (reduction) may also occur in tissues in a limited number of cases since each metal has its characteristic oxidation–reduction or redox potential. A change in the oxidation state may be produced either by chemical action, depending upon the local redox potential in the cell or biological fluid, or by enzymatic action. In

Table 5
Element species of biological interest

Element	Oxidation state	Organic compounds
Ag	I	Metallothionein
Al	III	Transferrin
As	III, V	Mono-tetramethylated, alkylated
Cd	II	Metallothionein
Co	II, III	Cyanocobalamin
Cr	II, III, VI	Serum proteins, hemoglobin
Cu	I, II	Amino acid and peptide complexes, caeruloplasmin
Hg	I, II	Methylated, arylated, alkylated
Mn	II, III, IV, VI, VII	Macroglobulin, globulin, transferrin
Mo	VI	
Ni	II, III	
Pb	II, IV	Ethylated, methylated, albumin
Sb	III, V	Mono-dimethylated
Se	II, IV, VI	Methylated, selenomethionin, selenocystein
Sn	II, IV	Ethylated, alkylated
Te	II, IV, VI	
V	V	
Zn	II	Metallothionein

general, the removal or addition of electrons to the metal atom influences the chemical activity and therefore the ability of the metal to interact with tissue ligands. Although, there is no general rule relating the toxicokinetics and toxicodynamics of elements with their oxidation state, it is generally accepted that the toxicity depends, among others, on the oxidation state.

Cr provides a well-known example of the role of different oxidation states in the toxicokinetics of an element Cr(VI) is absorbed much more readily than Cr(III): approximately 10% of the orally administered Cr(VI) is absorbed in comparison to less than 0.5% of Cr(III). Once absorbed, Cr(VI) can be reduced to Cr(III) in the gastrointestinal tract, thereby reducing the total uptake. Cellular uptake of Cr(III) is very poor, whereas Cr(VI) probably crosses the membrane by simple diffusion: 10% of the cellular Cr(VI) content is associated with the nucleus, whereas Cr(III) is nuclear. Metabolism of Cr involves cellular reduction of Cr(VI) to reactive non-stable intermediates which are able to react with DNA [134,135].

Separation may be useful for assessing the source of elements.

In occupationally exposed subjects, the urinary excretion of arsenic metabolites varies in accordance to the dose absorbed from the working environment but it may also be influenced by other sources among which food and water can play an important part.

The results of a study carried out in our Institute [136] indicate that ingestion of drinking water in which the concentration of inorganic arsenic was around 45 μg/l, even for a short period,can rapidly induce excretion of high amounts of element, with a progressive increase of methylated species of arsenic (MMA and DMA). The percentage of total arsenic excreted was around 50% of ingested arsenic and the increase from basal values was higher for inorganic arsenic and MMA (from 0.06 to 1.6 and from 0.4 to 5.2 μg arsenic/l, respectively), even though DMA remains the most excreted species (50% of total arsenic). From our investigation, when the exposure concentrations are 10 μg arsenic/m^3, the following concentrations of urinary arsenic species are to be expected: As(III), 4.3 μg/l; As(III)+As(V), 5.3 μg/l; MMA, 7.5 μg/l; DMA, 26.9 μg/l; sum of inorganic As, MMA, DMA, 43.7 μg/l.

Hakala and Pyy [137] suggested a biological monitoring method involving only the measurement of inorganic arsenic: they found that a urinary excretion of 5 μg inorganic arsenic/l could be expected after exposure to 10 μg arsenic/m^3. Our data are in good agreement with this suggestion.

Methylation reactions allow As(III) binding sites to be cleared by formation of MMA and DMA with lower affinity for binding sites. Another point worth stressing is that differences in methylating capacity are probably due to methyltransferase gene polymorphism, as suggested by Vahter et al. [138].

As(III) and As(V) could, therefore, be more closely related to the most critical effect (cancer) than other biological indicators of the element, although the use of the sole inorganic arsenic as indicator for routine biological monitoring of occupational exposure to arsenic is up to date restricted by the analytical method (more difficult because of the lower concentration of inorganic arsenic to be measured) and by the complexity of procedures to separate it.

In the last few years, as reported in Table 6 about 15 elements, 20 oxidation states, 25 organic compounds have been involved in the speciation for biological studies. From data provided in this table it can be argued that: (i) arsenic and Hg are the most speciated elements, followed by Cr, Se, Pb, Cd; (ii) several biological matrices have been considered but blood, serum and urine were the most analysed. Tissue was relevant for Cu, Cd, Zn; (iii) for the 15 elements considered, about 20 different oxidation states represented the object of investigation; (iv) organic compounds such as ethyl, methyl and aryl, have been demonstrated for four elements (As, Hg, Pb, Pt, Se); other organic compounds (complexes with amino acids, proteins, macromolecules) have been demonstrated for seven other elements (Ag, Cd, Cu, Cr, Pb, Se, Zn); and (v) among the procedures for the separation–identification of species, various chromatographic techniques are the most frequently used, followed by chemical chelation.

7.2. The hyphenated approach

Separation and analysis techniques may be combined for speciating elements. Gas chromatography (GC) has been combined with FAAS or ICP, while

Table 6
Elements speciation in biological matrices

Element	Matrix	Separation	Analysis	Species
Al	Serum Brain	Ion-exchange chromatography; SEC separation; reversed-phase HPLC; chelation with desferrioxamine	ETA-AA ICP-OES	Complexes Al protein; complexes between Al and dopamine-noradrenaline
As	Urine, hair blood, plasma	pH modification; ion-exchange resins; microwave heating; cation-exchange; solid phase HPLC; ion chromatography; acid digestion; chelation; solvent extraction; photoreaction	AA-Hy AAHy-ETA ICP-MS-OES ETA-AA EDXRF	As(III), As(V), methylated forms arsenobetaine arsenocholine As species As binding compounds
Co	Urine, serum	Chromatography HPLC	NAA AA-ETA	Co bond to protein Co species
Au	Liver blood, urine	Pyrolysis chromatography	NAA ICP-MS	Au complexes
Cd	Intestine, liver tissues, kidney	HPLC Electrophoresis; gel chromatography permeation; HPLC acid digestion	AA ICP-OES F-AA	Cd binding protein, Cd bond to different mass material, Cd metallothionein
Cr	Urine, plasma, red cells	Filtration; acid separation; chelation agents; volatilisation TFAA HPLC	AA-ETA ICP-MS	Cr(III), Cr(VI) protein complex
Cu	Tissues, serum, blood liver	HPLC; chromatography SEC separation membrane; solvent extraction; acid extraction; agarose electrophoresis	FAA ICP-MS ETA-AA	Isoforms of metallothionein, Cu bond protein, glycine-Cu, Cu(II), Cu complexes, Cu caerulopasmin
Hg	Urine, blood, liver, hair	Volatilisation; iodo-acetic acid digestion; acidification; SEC separation; GC HPLC derivative; solid-phase column; solvent extraction; ion chromatography; distillation	AA-Hy ETA-AA AA-AE ICP-MS	Organomercury compounds Hg(II), methylmercury, Hg species, inorganic Hg, protein complex
Pb	Urine, blood, serum, tissues	HPLC; acid digestion; purge-trap; size-exclusion chromatography; SEC separation LC coupled	ICP-MS ETA-AA F-AA	Methylated species, Pb containing proteins Pb species, Pb-macromol.
Pt	Urine, blood, serum, tissues	HPLC; chelation; acid digestion; reversed-phase ion pairing; LC	ICP-OES ICP-MS ETA-AA	Pt ultrafiltrable Pt species Pt bound to protein
Sb	Tissue	Acid digestion and extraction	ETA-AA	Sb species
Se	Serum, blood, hair, tissues Urine, blood	Chemical extraction; column extraction modifiers Chemical extraction; GC; HPLC	ETA-Hy AF-Hy ICP-MS AA-Hy ICP-MS	Se(IV), Se(VI), selenocholine, Se methionine, cysteine, purine, trimethyl selenium Organotin compounds
Te	Urine	Acid digestion ion-exchange	ETA-AA-Hy	Te(IV), Te(VI)
V	Urine, blood	 Anion-exchange resins; liquid chelation and extraction	F-AA ETA-AA	VO^{2+}, V species
Zn	Serum, liver, tissues	SEC; HPLC ultrafiltration	ICP-MS ETA-AA, AA	Zn macroglobulin, Zn prot. comp., isoforms of metalloth.

See Nomenclature.

the combination of GC with ETAAS is more problematic due to its non-continuous mode of action. The most common way to overcome this problem is to introduce the sample into the graphite cuvette, which is held at the atomisation temperature for the entire time of analysis. Coupling liquid chromatographic (LC) techniques to AAS instruments results in a reduced sensitivity [139–141].

The reduction in sensitivity is evident also when combining LC or HPLC to ICP. The eluent from HPLC may be rich in organic constituents which can adversely affect the stability of the plasma and deposit on the skimmer, resulting in increased noise and varying response.

A review for speciation analysis with atomic spectrometry techniques has been written by Sanz-Medel [142], while Vela and Caruso dealt whit coupling liquid chromatographic equipment to IPC-MS [143]. Another review discussing deeply the interfacing different techniques is that by Chau [144].

8. Use of inductively coupled plasma mass spectrometry for element multiple analysis in an unusual matrix

The process of multiple absorption of elements is likely to cause metabolic interactions between essential and toxic elements with important biological consequences, such as the possible effects on male fertility. Several investigations attempted to evaluate human sperm quality (number and motility of spermatozoa) in relation to the concentration in blood or in seminal fluid of essential elements such as Zn, Ca, Mg, Se, Cu [145–147] or toxic metals such as Pb, Hg and Cd [148–150].

We used an ICP-MS method in order to measure several essential and toxic elements, in 15 controls (mean age 31.3 years, range 21–44) and in seven Pb exposed workers (mean age 34.1 years, range 27–51) focusing on some aspects of the relationship between elements in seminal plasma and in sperm cells [151].

In controls five elements were determined in the order of mg/l and the mean value for Mg, Ca and Zn ranged from 75 to 158 mg/l. The mean values of Al, Mn, Ni, Cu, Se, Sr, Cs, Ba, Pb, Cr, and Cd varied from 1 to 87 μg/l. In some individuals Pb, Cd and

Ba concentrations were below the detection limit. In Pb exposed workers, two metals (Sb and Sn) were detected in addition to the others; Pb and Cu were found in higher amount. On the contrary, Se, Zn and Ca were measured in lower concentrations. When number and concentrations of elements in seminal plasma are considered, our results are in good agreement with the literature data.

The identification of the most appropriate matrix (seminal plasma and/or sperm cells) seems to be important in investigating essential and toxic elements in sperm, since the sources and the significance of elements in the two matrices are different. In fact, in seminal plasma elemental concentrations are influenced by several gland secretion, whereas spermatozoa, nucleated cells, may provide better information as a possible target for toxic agents as well as for essential elements. However it must be pointed out that elemental exchange between plasma and sperm cells might be possible.

9. Lead in plasma: at long last an accessible biomarker

The current challenge for biological monitoring of an ancient toxic metal like Pb regards first of all the availability of methods able to detect blood Pb concentrations in the order of few micrograms per litre. Secondly it concerns the accessibility to analytical methods finally able to (routinely) determine Pb in plasma, i.e., the biomarker of internal dose best correlated with effects.

For the first aspect, an acceptable level of sensitivity may be gained adapting the well experimented AAS methods by lowering the sample dilution, using matrix modifiers and setting up adequate temperature programs.

For the Pb determination in plasma, a sort of chimera for toxicologists and occupational physicians, during the last years interesting perspectives seem to be opened.

As is known, more than 95–98% of Pb in blood is contained in red blood cells (Pb-B), but the plasma lead (Pb-P) is more relevant from a toxicological point of view. It is in fact the active fraction of the body Pb pool, although in absolute terms its concentration is very low. Pb-P is in equilibrium with

extra-cellular pool and is directly involved in all the movements of Pb among the different biological compartments [152].

There are studies which did not show any association between Pb-B and Pb-P [153,154], while some other clearly indicated a curvilinear relation between Pb-B and Pb-P [48,155]. Non-linear relations with Pb-B have also been found for urinary Pb excretion and health effects [153–156]. Hirata et al. [157] found linear relation among Pb-P, Pb-B and U-Pb using AAS. The Pb-P level corresponding to 400 $\mu g/l$ of Pb-B was 5.7 $\mu g/l$.

In last decade attempts were made to introduce Pb-P into the routine biological monitoring of Pb, despite the analytical difficulties, with progress since the ICP-MS technique has been introduced for the measurement of Pb in plasma [48,158–160].

We are carrying out a survey on the determination of Pb-P, and examined 109 male Pb workers employed in a battery plant. The blood Pb was examined in ETAAS and plasma Pb by ICP-MS using the modified methods of Schultz et al. [48]. Plasma (500 μl) was mixed with 2 ml of nitric acid (0.15 M) and 50 μl of bismuth nitrate (50 $\mu g/l$) was added to each sample as internal standard.

Based on the correlation equation in our study (Fig. 6), the Pb-P level corresponding to Pb-B levels of 400 $\mu g/l$ was calculated to be about 2.6 $\mu g/l$, similar to the 2.2 $\mu g/l$ reported by Schultz et al. [48]. The ratio of Pb-P/Pb-B increased with increasing Pb-B, with a logarithmic relationship.

Bergdahl [161] studied the relationship among Pb-P, Pb-B and bone Pb, and reported again positive correlation only between the ratio of Pb-P/Pb-B and bone Pb. By the correlation equation from Cake et al. study [162], the Pb-P level corresponding to Pb-B level of 400 $\mu g/l$ was around 6.7 $\mu g/l$, three times higher than our value.

Our study, in accordance with others [48,161], shows that Pb-P can be accurately measured by ICP-MS method, using a simple dilution of the samples and internal standard addition for correcting interferences. The correlation between Pb-B and Pb-P is logarithmic probably because of the saturation of Pb binding sites into the erythrocytes, for example proteins, present with Pb-B higher than 40 $\mu g/100$ ml [163].

10. How to correctly interpret the results of biological analysis

The importance of reference values (RVs) in order to interpret the values obtained from biological

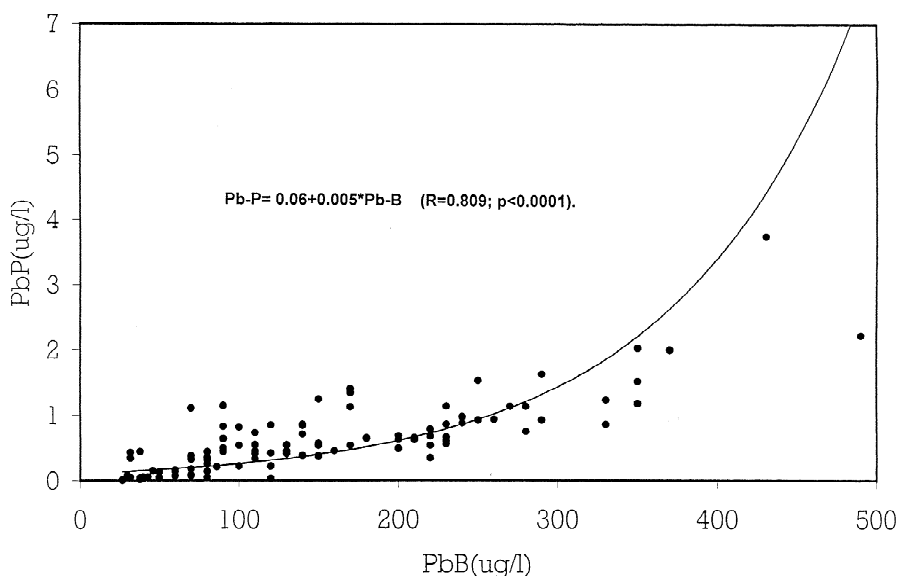Pb-P= 0.06+0.005*Pb-B   (R=0.809; p<0.0001).

Fig. 6. Correlation between blood lead (Pb-B) and plasma lead (Pb-P).

monitoring is widely recognised both in environmental and in occupational medicine. It is, however, necessary to control the sources of undue biological and analytical variability, as we tried to do in the investigation about RVs for urinary Cr [164].

The choice of U-Cr was justified by considerations regarding its nature and possibilities for measuring it. Cr is an essential and widespread metal of both natural and industrial origin; its toxicological role, mainly related to its immunological or carcinogenic activities, is relevant at the lowest concentrations. Cr in urine is determined in several laboratories and over past years standardised and validated methods and procedures for assuring analytical quality have been proposed. Finally, it is possible to compare the data regarding reference values for U-Cr with other reports in the scientific literature. For example, Cr has been included in the TRACY project for defining reference values through meta-analysis and the authors [165] dealt with a "normal concentration" of Cr in serum and urine reviewing the results of 11 studies.

Our polycentric study was planned for the assessment of RVs of U-Cr in the Italian population, overcoming the drawbacks from the analytical procedures and the selection of subjects to be examined.

Eight-hundred and ninety subjects were selected on the basis of precise criteria and the number of subjects established considering the number of stratification variables and morning spot samples of urine were collected following standardised procedures. The U-Cr was determined by ETAAS with a detection limit of 0.05 μg/l.

Considering the high proportion of undetectable urine samples, the geometric mean (GM) and geometric standard deviation (GSD) were estimated using the procedure of linear interpolation [166]. Because of skewness of the distribution, statistical analysis for evaluating the influence of the different covariates on U-Cr was performed after logarithmic transformation of the original values, using one-half of the detection limit in the undetectable cases (=0.025 μg/l). The influence of sex, age, region, residence, smoking and drinking habits were also evaluated using multiple regression analysis.

The distribution of U-Cr values in males (519) and in females (371) living in six Italian regions are reported in Fig. 7. In 248 cases (27.9%), 114
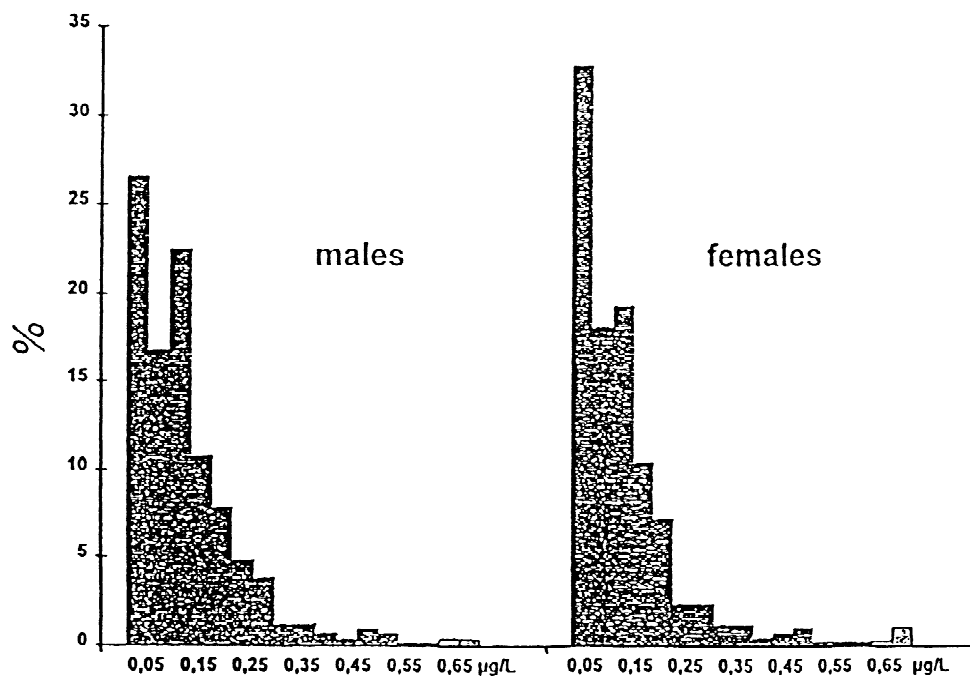

Fig. 7. Percentage distribution of urinary chromium values in males and females [164].

(30.7%) females and 134 (25.8%) males U-Cr was undetectable (less than 0.05 μg/l).

The estimated GM of U-Cr in the population was 0.08 μg/l in males, 0.07 μg/l in females and 0.024 μg/l was the 95th centile (95% C.I.: 0.20–0.31).

On the basis of multiple regression analysis, only geographical area and sex significantly influenced the U-Cr levels. The values of U-Cr observed in our sample of the Italian population are similar to the lowest values reported in the literature of the last years and summarized by Brune et al. [165]. Kilunen et al. [167], for example, in non-exposed subjects observed an arithmetic mean value of 0.12 μg Cr/l, with a GM of 0.078 μg/l, even though the detection limit of their analytical method (0.01 μg/l) was perhaps lower than ours (0.05 μg/l).

The observed values are lower than those obtained in the past in our country [168]: we believe that better procedures to control pre-analytical and analytical factors, but above all, the precise criteria for the selection of subjects (in particular those for excluding them), can explain the difference. Also the prevalence of samples without detectable concentrations of Cr above our detection limit (0.05 μg/l) seems to be a result of strictly controlled procedures.

This study confirms the gradual reduction of a "normal" U-Cr concentration: over 20 years U-Cr values in general population groups have dropped from values greater than 1 μg/l to values between 0.5 and 0.2 μg/l. The reasons of this progressive decline cannot be attributed to a reduced intake (never demonstrated to our knowledge), but it is primarily due to improvements in analytical instrumentation and methods. A further decrease may be definitely ascribed to a careful control of pre-analytical factors and to a more accurate definition of the characteristics of the subjects to be selected.

11. Metals to be validated for biological monitoring: the examples of manganese and beryllium

11.1. Manganese

Interest in biological and toxicological aspects of Mn has grown in the past few years for many reasons: the industrial use of the metal is expanding in many fields, from ferroalloy to the iron industry and the use of Mn based alloys during welding. In agriculture Mn based pesticides are widely used, especially in developing countries. Environmental exposure to Mn can also occur when the organic compound of Mn known as MMT (methylcyclopentadienyl Mn tricarbonyl, $C_9H_7MnO_3$) is used in some countries, as Pb substitute in gasoline [169]. The extensive exposure to this metal and the demonstration of early effects after prolonged exposure are causing wide spread concern about possible human health implications.

For several years, whole blood (Mn-B) and urinary (U-Mn) Mn and less frequently serum and hair Mn have been suggested as biomarkers of exposure. However, both Mn-B and U-Mn are generally considered unsuitable to assess Mn exposure on an individual basis, although they can discriminate between groups of occupationally exposed and control subjects [170].

We carried out an investigation in two ferroalloy plants (94 workers subdivided into three levels of exposure) and in a control group (89 subjects) in order to assess the significance and reliability of biological monitoring for Mn [171].

Mn-B and U-Mn were determined by ETAAS Zeeman background correction, according to a method previously published [172]. Mn-B levels ranged from 4 to 27 μg/l in the exposed subjects and from 2 to 10 μg/l in the controls. U-Mn levels ranged from 0.4 to 21 μg/l in the exposed subjects and from 0.1 to 7 μg/l in the controls. Arithmetic and geometric mean values for U-Mn were, respectively, 4.9±3.7 and 3.8 μg/l in the exposed, and 1.2±1.42 and 0.7 μg/l in the controls.

Age, smoking habits and alcohol consumption did not show any association with Mn-B and U-Mn.

The relationship between external and internal parameters of exposure was assessed by linear relationship. In this case, the simple regression between log values of Mn in air (Mn-A) and Mn-B (Mn-B=8.829+0.007×Mn-A; r=0.34; r^2=0.112) was statistically significant (P=0.001) although with a limited explanation of variance. A similar result was observed between Mn-A and U-Mn (r=0.36, P=0.009, r^2=0.12).

A significant relationship was observed between the two biological indicators Mn-B and U-Mn, in the

exposed group (U-Mn$=0.379+0.438\times$Mn-B; $r=$ 0.47, $r^2=0.22$, $P<0.0001$), whereas it was not significant in the control group.

In occupational settings, Mn is mainly absorbed through inhalation and, to a lesser extent, through the gastrointestinal tract (via contamination). After absorption Mn travels into the blood bound to transferrin in the trivalent state and to a α_2-macroglobulin in the divalent state [173]. Once in the bloodstream, Mn is rapidly distributed and excreted. Being an essential element, this metal is under constant control of very efficient homeostatic mechanisms, that by regulating absorption and excretion rates, are able to maintain Mn internal doses within a specific range of normality. In particular, dose-dependent biliary excretion of divalent Mn serves to regulate the percentage of absorbed Mn in systemic tissues [174]. Although the urinary excretion is low and about 0.01–1% of the absorbed dose, Mn can be measured in urine. Since Mn-B and U-Mn have a short half-life (likewise the concentration of organic solvents in blood), the internal dose may represent the amount of chemical absorbed during the sampling time or shortly before [175].

According to the most recent literature, our results confirm that Mn-B and U-Mn represent indicators of exposure on a group basis. Given the high variability of the results, they cannot be considered as suitable biomarkers of exposure on an individual basis.

As the currently available tests (Mn-B and U-Mn) are not suitable for biological monitoring, attention should be focused on other indicators, such as Mn in plasma and serum. Due to the low concentrations of Mn in serum and its fractions, the accuracy and sensitivity of analytical techniques are crucial.

11.2. Beryllium

The pulmonary chronic Be disease (an immunologically mediated syndrome) and lung cancer are well known effects of occupational exposure to Be. Be is classified as an A human carcinogen by the ACGIH and a carcinogen of group 1 by the IARC. When we consider immunological diseases and cancer, exposure to low and very low concentrations may be of great interest, and the ACGIH is going to propose a ''notice of intended changes'' for Be, with a TLV-TWA of 0.2 μg/m^3 as inhalable particulate,

instead of the current TLV-TWA of 2 μg/m^3 [176]. For the biological monitoring of subjects with low exposure to Be, the sensitivity and specificity of the analytical method determines the accuracy with which the amount of Be in biological matrices is measured. The determination of Be has generally been performed using GFAAS and more recently by ICP-OES/MS. Direct analysis by GFAAS is, however, the most frequently used technique for biological samples and when correctly performed it allows to reach adequate detection limits for determining Be concentrations in urine, for example in the range 0.1–1 μg/l [177–179].

To assess the feasibility of the biological monitoring of occupational exposure to Be, we investigated [180] four groups of metallurgical workers exposed to Be employed in two electric steel plants and in two Cu-alloy foundries. Possible sources of Be are the presence of the element in dust and smoke from row materials or from steel and Cu alloys during and after the melting process.

As a control group, we examined workers employed in mechanical activities (assembling, finishing trucks) know not to be exposed to metals. The urinary Be analysis was carried out by ICP-MS adapting the method of Schramel et al. [181]. The detection limit was 0.03 μg/l and the precision among series for concentration of 0.5 μg/l ranged between 9.5 and 12%.

The ambient Be concentrations exceeded the proposed TLV-TWA of 0.2 μg/m^3 only in the Cu alloy foundries in which the values were in the range from 0.4 to 0.8 μg/m^3, three for casting from 0.3 to 0.9 μg/m^3.

The urinary Be concentrations for the occupationally exposed subjects ranged from <0.03 to 0.45 μg/l (median 0.09–0.2 μg/l) and different to the values in the general population, which, according to our experience, are below 0.03 μg/l urine.

Analysis of the relationship between external and internal Be exposure revealed a significant correlation: for the recommended TLV-TWA of 0.2 μg/m^3, urinary Be excretion corresponded to about 0.15 μg/l.

The Be levels usually encountered in past years at workplaces were in the order of μg/m^3, while presently it ranges between some nanograms to some hundreds ng/m^3 [182–184]. Also under these expo-

sure conditions, the use of biological monitoring for the assessment of internal exposure may be interesting.

It is generally accepted that the urinary Be levels of groups of exposed subjects differ significantly from those observed in the general population. Considering the reference values defined in the 1990s, we can observe that the urinary Be levels measured in the general population range from undetectable values (0.03–0.06 μg/l) to around 0.5 μg/l.

In groups examined in Italy at the beginning of 1990s, urinary Be was measured in a population not occupationally exposed and a mean value of 0.2 μg/l (SD 0.16; range <0.03–0.8) was found. 15–20% of the individuals were found to have urinary Be levels below the detection limit (<0.03 μg/l). This is in agreement with the experience of the laboratories of the Institute of Occupational, Social and Environmental Medicine of the University of Erlangen-Nurenberg and with the study of Wegner et al. [184], which reports for the general population urinary Be concentrations below the analytical detection limit of 0.06 μg/l urine. In the cross-sectional study by Wegner et al. [184] on 57 gemstone cutters, Be could be detected in 27 cutters. The median for the pre-shift urine samples was 0.09 μg/l, the values were in the range from <0.06 to 0.56 μg/l. The corresponding median for the post-shift urine samples was <0.06 μg/l (detection limit), the range <0.06 to 0.029 μg/l urine.

In our study the Be concentrations in the air samples exceeded the proposed TLV-TWA of 0.2 μg/m^3 only in some samples from the Cu alloy foundries, where the medians of 0.27 μg/m^3 (furnaces) and 0.31 μg/m^3 (casting) were above this limit. Much lower values were determined in steel plants, probably because of the different amounts of Be present in the raw materials. Regression analysis was performed with the media values from four work areas and the corresponding median urinary Be concentrations. A significant correlation (r=0.971) was found for the relationship between external and internal exposure. However, it must be taken into consideration that 19 values for the urinary Be concentrations were below the analytical detection limit. The confidence limits for the regression line show the wide range of scatter of the values. For the

recommended TLV-TWA of 0.2 μg/m^3 for inhalable dust, the corresponding Be levels in urine were between 0.12 and 0.15 μg/l. The aspects to be discussed when evaluating a biological limit value for urinary Be may be summarised as follows.

(i) The ''normal'' urinary Be concentrations reported earlier in the literature are too high, mainly as a result of the poor specificity and sensitivity of the analytical methods previously adopted. Using adequate GF-AAS or ICP-MS techniques, the Be concentrations in urine samples of individuals not occupationally exposed are below the detection limits of 0.03–0.06 μg/l.

(ii) Assuming exposure at the level of the latter threshold limit value, ventilation of 10 m^3 per shift and 100% absorption, the intake of the metal might be around 2 μg/day and the urinary excretion of the same order or lower.

(iii) The significance in preventive and diagnostic terms of the availability of ways of measuring toxicologically relevant concentration of the metal is important for the clinical and epidemiological feature of Be, i.e., the immunologically mediated diseases and cancer. Wegner et al. [184] showed that for one subject the immunological test was positive and stimulation indices were significantly higher in subjects with detectable Be in urine than in those with urinary Be below the detection limit.

The determination of Be in urine may be therefore useful, together with other laboratory tests, also on individual basis to improve health surveillance programs or diagnostic pathways.

12. Some closing remarks

Goyer, in his conclusion of the conference held in Stockholm on biological monitoring of metals in May 1993 [185], identified the following needs for research.

(i) To differentiate the biological end points that reflect toxicological or adverse health effects from changes that reflect physiological adaptations.

(ii) To identify markers of subclinical toxicity, that is, markers that can be recognised at a level of exposure below which it produces overt or clinical toxicity.

(iii) To validate the markers of toxicity identified in experimental models in humans.

(iv) To expand the knowledge of the biokinetics or toxicokinetics of essential trace metals to lower levels of exposure.

(v) The biokinetics of elements would be determined in highly susceptible members of the general population, including the very young and elderly and those with particular biological requirements (pregnancy) and those with genetic abnormalities or pathological states that enhance susceptibility.

During the 1990s has significant progress been made in those directions? Even if focusing our attention only on dose assessment, the answers are, as expected, articulated.

The present qualitative level of analysis in biological matrices is in fact the result of the development in three fields of research: instrumentation, analytical methods and quality assurance procedures.

The progress in instrumentation has already been mentioned: from AAS atomization and background correction systems to ICP-MS entrance in the field of biological analysis.

Since atomic spectroscopy techniques complement each other, we have to bear in mind some criteria to compare and select them. White [186] compared ICP-MS and ETAAS methods for the determination of Pb and Cd in whole blood and Al, Cd, Co and Ni in urine. Simple sample pretreatments were used for both instrumental techniques. The correlation between results obtained by the two techniques was good for elements measured, ranging from $r=0.88$ for Co in urine to $r=0.99$ for both Pb and Cd in blood. The criteria for comparing the two techniques include detection limits, analytical working range, sample throughput and interferences. The detection limit achievable represents an important requisite in selecting an analytical technique, making it possible (easy) to perform the determination of elements. In Table 7 the detection limits for most measured elements in biological matrices by different spectroscopy techniques are reported. Generally the best detection limits are attained using ICP-MS, even if, for some elements, GFAAS or cold vapour hydride offer better analytical performance.

An adequate analytical working range (i.e., the concentration range of analyte for which results can

be obtained without having to recalibrate the system) minimises analysis time, reduces sample handling, thus minimising the potential preanalytical and analytical errors. ICP-MS is characterised by the widest working ranges reaching 7–8 orders of magnitude of the signal intensity. Moving to the sample throughput (number of samples which can be analysed or elements which can be determined per unit time) FAAS, provides exceptional results when large number of samples for limited number of elements have to be analysed (a typical analysis requires 5–10 s). GFAAS, due to the need of thermal program, has relatively high (2–3 min) sample throughput. ICP-MS multi-elemental technique has exceptional throughput (20–30 elements per minute) depending on concentration levels, required precision and equilibration of the plasma (15–30 s) with each new sample.

As seen before the matrix interferences are of particular importance when analysing biological samples. In this respect we can affirm that no analytical techniques are free of interferences: those of atomic spectroscopy techniques, reported in Table 8, have been however well studied and documented and various methods exist to overcome them.

Significant improvements have also been achieved in the knowledge of pre analytical and analytical factors, which solution can be considered a real pre-requisite to accurately measure smaller and smaller amounts of elements in biological matrices.

Important advances have been made in the treatment of the biological matrix in its widest definition, beginning from the use of chemical modifiers, which solved some analytical questions such as those about Se or enabled to determine metals (Cd, Pb) in highly concentrated matrices, otherwise not measurable in highly diluted samples.

Considering the metals traditionally analysed it could be seen that in addition to easily foreseeable media like blood and urine, many other biological matrices such as soft tissues, hair, nails have to be taken into consideration. There are thus new demands for developing methods for the analysis of an increased number of matrices and for a larger number of elements. At this purpose it must be pointed out that for some metals AAS no longer represents the most accurate technique, both for sensitivity and possibility to determine them. The

Table 7
AAS and ICP-MS detection limits for elements determined in our laboratory in biological matrices (μg/l)

Element	Flame AA			Hydride			GFAA			ICP-MS		
	U	S	B	U	S	B	U	S	B	U	S	B
Ag	–	–	–	–	–	–	0.1	0.5	–	0.01	0.05	–
Al	–	–	–	–	–	–	2.0	2.0	1.5	0.5	0.1	–
As	–	–	–	0.05	–	–	1.0	–	–	0.1	–	–
Au	–	–	–	–	–	–	1.0	1.5	–	0.5	–	–
B	–	–	–	–	–	–	–	–	–	0.1	–	–
Ba	–	–	–	–	–	–	–	–	–	0.02	–	–
Be	–	–	–	–	–	–	0.05	–	–	0.03	–	–
Bi	–	–	–	0.05	0.03	–	0.5	–	–	0.06	–	–
Cd	–	–	–	–	–	–	0.01	–	0.05	0.01	–	0.05
Co	–	–	–	–	–	–	0.5	–	1	0.1	–	0.1
Cr	–	–	–	–	–	–	0.05	0.1	–	–	–	–
Cu	10	10	–	–	–	–	3	5	–	0.5	0.5	–
Fe	15	10	–	–	–	–	0.5	1	–	–	–	–
Hg	–	–	–	0.5	–	0.5	–	–	–	0.01	–	0.05
I	–	–	–	–	–	–	–	–	–	0.5	–	–
Mn	–	–	–	–	–	–	0.5	0.1	0.2	0.05	0.05	0.05
Mo	–	–	–	–	–	–	0.1	0.1	–	0.05	–	–
Ni	–	–	–	–	–	–	0.2	0.1	1	0.05	–	–
Pb	–	–	–	–	–	–	3	–	5	1	0.5	0.5
Pt	–	–	–	–	–	–	25	–	–	0.01	–	–
Sb	–	–	–	0.05	–	–	–	–	–	0.01	–	–
Se	–	–	–	0.05	0.05	–	5	1	1	0.5	–	0.1
Sn	–	–	–	–	–	–	0.5	–	–	0.02	–	–
Ti	–	–	–	–	–	–	0.5	–	–	0.05	–	–
Tl	–	–	–	–	–	–	0.01	–	–	0.05	–	–
V	–	–	–	–	–	–	0.1	–	–	0.05	–	–
Zn	10	10	–	–	–	–	–	–	–	–	–	–
W	–	–	–	–	–	–	–	–	–	0.01	–	–

U, Urine; S, serum; B, blood.

Table 8
Atomic spectroscopy interferences

Technique	Type of interference	Method of compensation
Flame AA	Ionization	Ionization buffer
	Chemical	Releasing agent
	Physical	Dilution, method of additions
GFAA	Physical and chemical	STPF conditions
	Molecular absorption	Zeeman background correction
	Spectral	
ICP emission	Spectral	Background correction or the use of alternate
	Matrix	Internal standardisation
ICP-MS	Mass overlap	Interelement correction,
		Cell technology, higher mass resolution
	Matrix	Internal standardisation

increasing need for higher analytical sensitivity is justified by the decrease in occupational exposure to metals, by the need of assessing exposure of groups of general population exposed to low levels of metals and by the attention to be paid in the measure of elements, as close as possible to the site of biological or toxicological action. The ICP-MS methods present several advantages: simultaneous multielemental determinations; excellent detection limits; wide linear dynamic range; high sample throughput. Studies are underway to overcome the interferences in ICP-MS analysis by using FI, electrothermal volatilisation, special nebulizers or different detectors (secondary ion mass spectrometry, spark source mass spectrometry, thermal ionization mass spectrometry). Experience has been gained in the use of high resolution ICP-MS for multielement determination and the range of elements that can be determined is impressive, as results from the traditional annual update about the element analysis in biological media presented in the *Journal of Analytical Atomic Spectroscopy* [67].

Regarding speciation, combinations of HPLC with ICP-MS have been investigated mainly for elements in urine. It is, however, the association of elements with proteins in serum that is proving more difficult to be studied, mainly for the difficulty in achieving their separation and identification. Techniques based on electrophoresis seem however to promise that result.

The essence of biological monitoring also for metals, is the accurate measurement of their amount in biological media, measurement generally complicated by their low concentration. The production of an analytical value includes a number of sources of variability which condition the final result and quality assurance procedures are taken to ensure the overcoming of the undue variability.

Quality assurance is not an abstract concept, but it must be adapted to the different situations. If the test is carried out in the context of risk assessment (e.g., to demonstrate that a substance causes a given effect), quality assurance requisites must be emphasized surely more strictly than during routine biological monitoring practices. Different needs of quality may also arise from the analytical methods adopted (e.g., if new experimental of routine). Another level of need of quality might be associated with the ability to interpret the obtained data, which depends, for example, on the availability of correct reference values [187].

Only systematic improvements in analytical and toxicological knowledge could enable one to tackle the problems associated with exposure to small doses of single metals, to those connected with simultaneous exposure to several elements (multiple exposure) and to those regarding the biological significance of the different chemical species of metals.

Through a correct measurement of the doses and the interpretation of their physiological and toxicological meaning, it will finally be possible to cope with the problem of effects of metals, especially with regards to toxic effects, to those occupying the first portion of the dose–response effect curve which represents the main field of interest in preventative disciplines.

13. Nomenclature

AA	Atomic absorption
AAS	Atomic absorption spectrometry
AE	Atomic emission
AES	Atomic emission spectrometry
AF	Atomic fluorescence
AFS	Atomic fluorescence spectrometry
APDC	Ammonium pyrrolidinedithiocarbamate (ammonium pyrrolidin-1-yl dithioformate
CRM	Certified reference material
CV	Cold vapour
DMF	*N,N*-Dimethylformamide
ECD	Electron-capture detection
EDL	Electrodeless discharge lamp
EDTA	Ethylenediaminetetraacetic acid
ETA	Electrothermal atomization
ETAAS	Electrothermal atomic absorption spectrometry
ETV	Electrothermal vaporization
FAAS	Flame AAS
FI	Flow injection
GC	Gas chromatography
GD	Glow discharge
HCL	Hollow cathode lamp
hf	High frequency

HG	Hydride generation
HPLC	High-performance liquid chromatography
IBMK	Isobutyl methyl ketone (4-methylpentan-2-one)
IC	Ion chromatography
ICP	Inductively coupled plasma
ICP-MS	Inductively coupled plasma mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LA	Laser ablation
LC	Liquid chromatography
LMMS	Laser-microprobe mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
NAA	Neutron activation analysis
NaDDC	Sodium diethyldithiocarbamate
NIES	National Institute for Environmental studies
NIST	National Institute of Standards and Technology
NTA	Nitrilotriacetic acid
OES	Optical emission spectrometry
PIXE	Particle-induced X-ray emission
PMT	Photomultiplier tube
ppb	Parts per billion
ppm	Parts per million
PTFE	Poly(tetrafluoroethylene)
QC	Quality control
rf	Radio frequency
RM	Reference material
RSD	Relative standard deviation
S/B	Signal-to-background
SEC	Size-exclusion chromatography
SEM	Scanning electron microscopy
SFC	Supercritical fluid chromatography
SIMS	Secondary ion mass spectrometry
SRM	Standard reference material
SSMS	Spark source mass spectrometry
STPF	Stabilized temperature platform furnace
TCA	Trichloroacetic acid
TIMS	Thermal ionization mass spectrometry
TLC	Thin-layer chromatography
TMAH	Tetramethylammonium hydroxide
UV	Ultraviolet
UV–Vis	Ultraviolet–visible
XRF	X-Ray fluorescence

References

[1] R.W. Dabeka, A.D. McKenzie, G.M.A. Lacroix, Food Addit. Contam. 4 (1987) 89.
[2] P. Apostoli, Ann. Inst. Super. Sanità 34 (1998) 121.
[3] J.E. Fergusson, Sci. Total Environ. 50 (1986) 1.
[4] G. Ducroffe, F. Claeys, P. Bruaux, Environ. Res. 51 (1990) 25.
[5] J.L. Annest, in: M. Rutter, R.R. Jones (Eds.), Lead Versus Health, Wiley, New York, 1983, p. 35.
[6] A. Schutz, R. Attewell, S. Skerfing, Arch. Environ. Health 44 (1989) 391.
[7] R. Lobinsky, H. Chassaigne, J. Szpunar, Talanta 46 (1998) 271.
[8] Y. Tan, P. Ager, W.D. Marshall, H.M. Chan, J. Anal. Atom. Spectrom. 11 (1996) 183.
[9] H. Goenaga Infante, M.L. Fernandez Sanchez, A. Sanz-Medel, J. Anal. Atom. Spectrom. 14 (1999) 1343.
[10] M.P. Richards, J.H. Beattie, J. Chromatogr. 648 (1993) 459.
[11] A.Z. Mason, S.D. Storms, K.D. Jenkins, Anal. Biochem. 186 (1990) 187.
[12] J. Bergerow, M. Turgeld, L. Dunermann, J. Anal. Atom. Spectrom. 12 (1997) 1095.
[13] H.A. Roels, R.R. Lauwerys, J.P. Buchet, A. Bernard, Environ. Res. 24 (1981) 117.
[14] C. Toyama, Z.A. Shaikh, K. Nogawa, E. Koyashi, R. Honda, Arch. Toxicol. 50 (1982) 159.
[15] A. Brockhaus, R. Bolgner, U. Ewers, H. Wiegand, Int. Arch. Occup. Environ. Health 48 (1981) 375.
[16] R.F. Henderson, W.E. Bechtold, J.A. Bond, J.D. Sun, Crit. Rev. Toxicol. 20 (1989) 65.
[17] M.J. Mercier, A.E. Robinson, Int. Arch. Occup. Environ. Health 65 (1993) S7.
[18] K.R. Snapp, D.B. Bayer, C.W. Svare, J. Dent. Res. 68 (1989) 780.
[19] G. Sallesten, L. Barregard, O. Vesterberg, Appl. Occup. Environ. Hyg. 7 (1992) 434.
[20] J. Akesson, A. Schutz, P.O. Glantz, Arch. Environ. Health 46 (1991) 102.
[21] L. Soleo, G. Elia, P. Apostoli, G. Ital. Med. Lav. Erg. 20 (1998) 75.
[22] M.G. Van Vonderen, E.C. Klinkenberg-Knoll, M.E. Craanen, D.J. Touw, S.G. Meuwissen, P.A. De Rel Smet, Am. J. Gastroeneterol. 95 (6) (2000) 1591.
[23] B.M. Spriewald, A. Rascu, K.H. Schaller, J. Angerer, J.R. Kalden, T. Harrer, Occup. Environ. Med. 56 (4) (1999) 282.
[24] C.A. Mitchell-Heggs, M. Conway, J. Cassar, Hum. Exp. Toxicol. 9 (3) (1990) 195.
[25] C. Abernathy, R. Cantilli, O. Levander, in: J. Saxena (Ed.), Hazard Assessment of Chemicals, Hemisphere, New York, 1992, p. 81.
[26] J. Alexander, Scand. J. Work Environ. Health 19 (1993) 126.
[27] B. Venugopal, T.D. Luckey, Chemical Toxicity of Metals and Metalloids, Plenum Press, New York, London, 1979.
[28] L. Sagan, Bell Newslett. 2 (1993) 13.
[29] E.J. Calabrese, L.A. Baldwin, Chemical Hormesis: Scientific Foundations, Final Report, School of Public Health, University of Massachusetts, Amherst, MA, 1998.

[30] K.K. Rozman, Med. Hypothesis 51 (1998) 175.

[31] J.M. Davis, D.J. Svendsgaard, J. Toxicol. Environ. Health 30 (1990) 71.

[32] R. Tardif, R. Goyal, J. Brodeur, Toxicol. Ind. Health 8 (1992) 37.

[33] M. Ikeda, Toxicol. Lett. 77 (1995) 9.

[34] M. Crippa, P. Apostoli, C. Quarta, L. Alessio, Med. Lav. 82 (1991) 261.

[35] B. Ballantyne, J. Occup. Med. 27 (1985) 85.

[36] G. Lasfargues, D. Lison, P. Maldague, R. Lauwerys, Toxicol. Appl. Pharmacol. 112 (1992) 41.

[37] B. Swennen, J.P. Buchet, D. Stanescu, D. Lison, R. Lauwerys, Br. J. Ind. Med. 50 (1993) 835.

[38] B.A. Fowler, K.R. Mahaffey, Environ. Health Perspect. 25 (1978) 87.

[39] J.P. Buchet, H. Roels, A. Bernard, R. Lauwerys, J. Occup. Med. 23 (1981) 348.

[40] B. Dutkiewicz, T. Dutkiewicz, G. Milkowska, Int. Arch. Occup. Environ. Health 42 (1979) 341.

[41] F. D'Andrea, P. Apostoli, F. Brugnone, G. Piemonte, P. Mozzo, Ann. Inst. Super. Sanità 17 (1981) 475.

[42] E.A. Border, A.C. Cantrell, T.A. Kilroe-Smith, Br. J. Ind. Med. 33 (1976) 85.

[43] L. Alessio, A. Berlin, R. Roi (Eds.), Biological Indicators of Human Exposure to Industrial Chemicals, CEC, ECSC–EAEC, Brussels, Luxembourg, 1983–1991.

[44] ACGIH, Threshold Limit Values For Chemical and Physical Agents and Biological Exposure Indices, ACGIH, Cincinnati, OH, 2000.

[45] DFG, List of MAK and BAT Values 2000, Commission for the Investigation of Health Hazard of Chemical Compounds in the Work Area, Wiley–VCH, Weinheim, 1999.

[46] Agency for Toxic Substances and Disease Registry [ATSDR-11] Decision Guide for Identifying Substance Specific Data Needs Related to Toxicological Profiles, USA EPA Federal Register, Vol. 54, 1989.

[47] P.E. Desilva, Br. J. Ind. Med. 38 (1981) 209.

[48] A. Schutz, I.A. Bergdahl, A. Ekholm, S. Skerfving, Occup. Environ. Med. 53 (1996) 736.

[49] H. Roels, R. Lauwereys, J.P. Buchet, A. Bernard, J.S. Garvey, H.J. Linton, Int. Arch. Occup. Environ. Health 52 (1983) 159.

[50] L. Friberg, C.G. Elinder, Scand. J. Work Environ. Health 19 (1993) 7.

[51] A. Aitio, J. Jarvisalo, Ann. Clin. Lab. Sci. 15 (1985) 121.

[52] L. Friberg, Am. Ind. Hyg. Assoc. J. 46 (1985) 633.

[53] C.G. Elinder, L. Gerhardsson, G. Oberdorster, in: T.W. Clarkson, L. Friberg, G.F. Nordberg, P.R. Sager (Eds.), Biological Monitoring of Toxic Metals, Plenum Press, New York, London, 1988, p. 1.

[54] R.L. Bornschein, P. Succop, K.N. Dietrich, Environ. Res. 38 (1985) 108.

[55] J. Quaterman, J.N. Morrison, Br. J. Nutr. 34 (1975) 351.

[56] C.S. Fullmer, J.F. Rosen, Environ. Res. 51 (1990) 91.

[57] E. Hassler, B. Lind, M. Piscator, Br. J. Ind. Med. 40 (1983) 420.

[58] L. Friberg, in: L. Friberg, G.F. Nordberg, V.B. Vouk (Eds.), Handbook on the Toxicology of Metals, Elsevier, Amsterdam, 1986, p. 269.

[59] D.H. Hamer, Annu. Rev. Biochem. 55 (1986) 913.

[60] S.H. Oh, J.T. Deagen, P.D. Whanger, P.H. Weswig, Am. J. Physiol. 234 (1978) E282.

[61] R.D. Palmiter, G. Norstedt, R.E. Gelinas, R.E. Hammer, R.L. Brinster, Science 222 (1983) 809.

[62] B.G. Armstrong, G. Kazantzis, Br. J. Ind. Med. 42 (1985) 70.

[63] F.Y. Falck, J.L. Fine, R.G. Smith, K.D. McClatchey, T. Annesley, B. England, A.M. Schork, Am. J. Ind. Med. 4 (1983) 541.

[64] K. Lauwerys, M. Roels, J.P. Buchet, A. Goret, Environ. Res. 20 (1979) 375.

[65] R.G. Price, Toxicology 23 (1982) 99.

[66] L. Alessio, A. Berlin, A. Dell'Orto, F. Toffoletto, I. Grezzi, Int. Arch. Occup. Environ. Health 55 (1985) 99.

[67] A. Taylor, S. Branch, D.J. Halls, M. White, J. Anal. Spectrom. 15 (2000) 451.

[68] E. Adamsson, M. Piscator, K. Nogawa, Environ. Health Perspect. 29 (1979) 219.

[69] J.B. Wyman, K.W. Heaton, A.P. Manning, A.C.B. Wicks, Gut 19 (1978) 146.

[70] K.M. Hambidge, Am. J. Clin. Nutr. 36 (1982) 943.

[71] M. Laker, Lancet 283 (1982) 260.

[72] D. Airey, Sci. Total Environ. 31 (1983) 157.

[73] J. Chisolm, C.R. Handorf, Clin. Exp. Hypertens. Pregn. B4 (1985) 117.

[74] R. Capelli, V. Minganti, G. Semino, W. Bertarini, Sci. Total Environ. 48 (1986) 79.

[75] P. Saenger, J.F. Rosen, M.E. Markowitz, Am. J. Dis. Child. 136 (1982) 312.

[76] L.J. Somervaille, D.R. Chettle, M.C. Scott, Phys. Med. Biol. 30 (1985) 929.

[77] A. Walsh, Anal. Chem. 63 (1991) 933.

[78] B.V. L'vov, Anal. Chem. 63 (1991) 924.

[79] K.S. Subramanian, Prog. Anal. Spectrosc. 9 (1986) 237.

[80] W. Slavin, R. Carnrick, C. Manning, E. Pruszkowska, Atom. Spectrosc. 4 (1983) 69.

[81] S. Abbey, C.L. Chakrabarti, P.C. Bartels, Anal. Chem. 54 (1982) 136.

[82] S.R. Lawson, R. Woodriff, Spectrochim. Acta 35B (1980) 753.

[83] M. Hoenig, P. Van Hoeywegen, A.M. De Kersabiec, Analusis 16 (1988) 18.

[84] L.M. Voth Beach, D.E. Shrader, Spectroscopy 1 (1986) 49.

[85] G. Schlemmer, B. Weltz, Spectrochim. Acta 41B (1986) 1157.

[86] L. Ping, K. Fuwa, K. Mutsumoto, Anal. Chim. Acta 171 (1985) 279.

[87] L.M. Voth Beach, V.E. Sharder, J. Anal. Atom. Spectrom. 2 (1987) 45.

[88] X. Yen, G. Schlemmer, B. Weltz, Anal. Chem. 59 (1987) 1462.

[89] B. Weltz, G. Schlemmer, J.R. Mudakavit, J. Anal. Atom. Spectrom. 3 (1988) 695.

[90] J.M. Christensen, L.M. Pedersen, Acta Pharmacol. Toxicol. 59 (1986) 399.

[91] F.A. Shakra, M. Raymari, N. Ward, G. Bastiani, J. Anal. Atom. Spectrom. 12 (1997) 429.

[92] J. Begerow, M. Turfeld, L. Dunemann, J. Anal. Spectrom. 11 (1996) 913.

[93] A.S. Ennslin, A. Pethan, R. Schierl, J. Int. Arch. Occup. Environ. Health 65 (1994) 339.

[94] J. Pisch, J. Schafer, D. Frahne, GIT Fachz Lab. 6 (1993) 50.

[95] H.P. Chen, D.C. Paschal, D.T. Miller, J.C. Morrow, Atom. Spectrosc. 19 (1998) 176.

[96] S. Rio Segade, C. Bendicho, Spectrochim. Acta 54B (1999) 1129.

[97] X.P. Yan, M. Sperling, B. Welz, J. Anal. Atom. Spectrom. 14 (1999) 1625.

[98] J.P. Valles Mota, M.R. Fernandez de la Campa, J.I. Garcia Alonso, A. Sanz Medel, J. Anal. Atom. Spectrom. 14 (1999) 113.

[99] J.L. Burguera, M. Burguera, C. Rivas, C. Rondon, P. Carrero, M. Gallignani, Talanta 48 (4) (1999) 885.

[100] E. Ivanova, F. Adams, Fresenius J. Anal. Chem. 361 (5) (1998) 445.

[101] J.W. Robinson, Anal. Chem. 65 (1994) 472.

[102] K.E. Murphy, E.S. Beary, M.S. Rearik, R.D. Vocke, Fresenius J. Anal. Chem. 368 (4) (2000) 362.

[103] J.S. Becker, H.J. Dietze, Fresenius J. Anal. Chem. 368 (1) (2000) 23.

[104] A. Montaser, D.W. Golightly, in: Inductively Coupled Plasma in Analytical Atomic Spectrometry, VCH, New York, 1987, p. 361.

[105] A.R. Date, A.L. Gray, in: Applications of Inductively Coupled Plasma Mass Spectrometry, Blakie, Glasgow, 1989, p. 24.

[106] M. Krachler, K.J. Irgolic, J. Trace Elem. Med. Biol. 13 (3) (1999) 157.

[107] K.J. Mulligan, T.M. Davidson, J.A. Caruso, J. Anal. Atom. Spectrom. 5 (1990) 301.

[108] M.A. White, J. Trace Elem. Med. Biol. 13 (1–2) (1999) 93.

[109] C.J. Park, D.C. Smith, J.C. Vanloon, Trace Elem. Med. 7 (1990) 103.

[110] S.H. Tan, G. Horlick, Appl. Spectrosc. 40 (1986) 445.

[111] G.R. Gillson, D.J. Douglas, J.E. Fullford, K.W. Halligan, S.D. Tanner, Anal. Chem. 60 (1988) 1472.

[112] H. Kawabuchi, T. Tanaka, T. Nakamura, M. Morishita, A. Mizuike, Anal. Sci. 3 (1987) 305.

[113] H. Vanhoe, C. Vandecasteele, J. Versieck, R. Dams, Anal. Chem. 61 (1989) 1851.

[114] F. Vanhaecke, H. Vanhoe, R. Dams, C. Vandecasteele, Talanta 39 (1992) 737.

[115] D.R. Wiederin, R.E. Smyczek, R.S. Houk, Anal. Chem. 63 (1991) 1626.

[116] D. Beauchemin, J.W. McLaren, S.S. Berman, J. Anal. Atom. Spectrom. 3 (1988) 775.

[117] H.T. Delves, M.J. Campbel, Anal. Atom. Spectrom. 3 (1988) 343.

[118] M.J. Campbel, H.T. Delves, J. Anal. Atom. Spectrom. 4 (1989) 235.

[119] T.D.B. Lyon, G.S. Fell, R.C. Hutton, A.N. Eaton, J. Anal. Atom. Spectrom. 3 (1988) 601.

[120] B.S. Sheppard, J.A. Caruso, D.T. Heitkemper, K.A. Wolnik, Analyst 117 (6) (1992) 971.

[121] J. Goossens, R. Dams, J. Anal. Atom. Spectrom. 7 (1992) 1167.

[122] T. Townsend, J. Anal. Atom. Spectrom. 15 (4) (2000) 307.

[123] S. Sturup, J. Anal. Atom. Spectrom. 15 (4) (2000) 315.

[124] I. Rodushkin, F. Odman, R. Olofsson, M.D. Axelsson, J. Anal. Atom. Spectrom. 15 (8) (2000) 937.

[125] J. Begerow, M. Turfeld, L. Dunemann, J. Anal. Atom. Spectrom. 15 (4) (2000) 347.

[126] J. Wang, Stripping Analysis, Principles, Instrumentation and Applications, VCH Verlagsgesellschaft, Weinheim, Deerfield Beach, FL, Basel, 1985.

[127] C.J. Horng, Analyst 121 (10) (1996) 1511.

[128] S.B. Adeloju, M. Bond, M.H. Briggs, Anal. Chem. 57 (1985) 1286.

[129] P. Ostapczuk, M. Stoeppler, H.W. Durbeck, Fresenius Z. Anal. Chem. 322 (1988) 662.

[130] A.S. Ensslin, R. Huber, A. Pethran, H. Rommelt, R. Schierl, U. Kulka, G. Fruhmann, Int. Arch. Environ. Health 70 (1997) 205.

[131] J.P. Buchet, R. Lauwerys, Arch. Toxicol. 57 (1985) 125.

[132] T.W. Clarkson, J.B. Hursh, P.R. Sager, in: T.W. Clarkson, L. Friberg, P.R. Sager (Eds.), Biological Monitoring of Toxic Metals, Plenum Press, New York, 1988, p. 199.

[133] R. Cornelis, Ann. Clin. Lab. 26 (1996) 252.

[134] V. Ducros, Biol. Trace Elem. Res. 32 (1992) 65.

[135] V. Bianchi, A.G. Levis, Total Environ. 71 (1988) 351.

[136] P. Apostoli, D. Bartoli, L. Alessio, J.P. Buchet, Occup. Environ. Med. 56 (1999) 825.

[137] E. Hakala, L. Pyy, Toxicol. Lett. 77 (1995) 249.

[138] M. Vahter, G. Concha, B. Nermell, Eur. J. Pharmacol. 293 (1995) 455.

[139] L. Ebdon, S. Hill, R. Ward, Analyst 112 (1987) 1.

[140] Y. Cai, S. Rapsomanikis, M.O. Andreae, J. Anal. Atom. Spectrom. 8 (1993) 119.

[141] Y.S. Fung, W.C. Sham, Analyst 119 (1994) 1029.

[142] A. Sanz-Medel, Analyst 120 (1995) 799.

[143] N.P. Vela, J.A. Caruso, J. Anal. Atom. Spectrom. 8 (1993) 787.

[144] Y.K. Chau, Analyst 117 (1992) 571.

[145] F.R. Abou-Shakra, N.I. Ward, D.M. Everard, Fertil. Steril. 52 (1989) 307.

[146] J.L. Lafond, B. Sele, A. Favier, J. Trace Elem. Electrolytes Health Dis. 2 (1988) 19.

[147] J. Alvarado, R. Moreno, A.R. Cristiano, J. Trace Elem. Electrolytes Dis. 5 (1991) 173.

[148] H.J. Donat, J. Matthies, I. Schwartz, Andrologia 22 (1990) 401.

[149] J.P. Bonde, A. Giwercman, Reprod. Med. Rev. 4 (1995) 59.

[150] B. Xu, S.E. Chia, M. Tsakok, C.N. Ong, Reprod. Toxicol. 7 (1993) 613.

[151] P. Apostoli, S. Porru, C. Morandi, A. Menditto, J. Trace Elem. Med. 11 (1997) 182.

[152] R.W. Baloh, Arch. Environ. Health 28 (1974) 198.

[153] J.F. Rosen, C. Zarate, E.E. Trinidad, J. Pediatr. 84 (1974) 45.

[154] C.N. Ong, W.O. Phoon, B.L. Lee, L.H. Chua, Ann. Occup. Hyg. 30 (1986) 219.

[155] P.E. Desilva, Br. J. Ind. Med. 38 (1981) 209.

[156] Y. Ikeya, T. Sakai, I. Araki, K. Ushio, Traumatol. Occup. 35 (1987) 834.

[157] M. Hirata, T. Yoshida, H. Kosaka, T. Tabuchi, Int. Arch. Occup. Environ. Health 68 (1995) 58.

[158] Y. Muras, A. Premel, S. Berre, P. Allain, Clin. Chim. Acta 218 (1993) 201.

[159] D.R. Smith, R.P. Ilustre, J.D. Osteloh, Am. J. Ind. Med. 33 (1998) 430.

[160] B. Gerken, R.M. Barnes, Anal. Chem. 63 (1991) 283.

[161] I.A. Bergdahl, Am. J. Ind. Med. 32 (1997) 317.

[162] K.M. Cake, R.J. Bowins, C.L. Gordon, R.H. McNutt, D.R. Chettle, Am. J. Ind. Med. 29 (1996) 440.

[163] Y. lolin, P. O'Gorman, Ann. Clin. Biochem. 25 (1988) 688.

[164] P. Apostoli, G. Maranelli, P.G. Duca, P. Bavazzano, C. Soave, Int. Arch. Occup. Environ. Health 70 (1997) 173.

[165] D. Brune, A. Aitio, G. Norberg, O. Vesterberg, Scand. J. Work Environ. Health 19 (1993) 39.

[166] R.W. Hornug, L.D. Reed, Appl. Occup. Environ. Hyg. 5 (1990) 46.

[167] M. Kilunen, J. Javirsaalo, O. Matkie, A. Aitio, Int. Arch. Occup. Environ. Health 59 (1987) 67.

[168] C. Minoia, E. Sabbioni, P. Apostoli, M. Gallorini, R. Pietra, E. Capodaglio, Sci. Total Environ. 95 (1990) 89.

[169] L. Wallace, T. Slonecker, J. Air Waste Manage. Assoc. 47 (1997) 642.

[170] H. Roels, R. Lauwerys, P. Genet, J.P. Buchet, Am. J. Ind. Med. 11 (1987) 297.

[171] P. Apostoli, R. Lucchini, L. Alessio, Am. J. Ind. Med. 37 (2000) 283.

[172] P. Apostoli, C. Minoia, A. Ronchi, in: C. Minoia, S. Caroli (Eds.), Application of Zeeman ETAAS in the Chemical Laboratory and in Toxicology, Pergamon Press, London, 1992, p. 409.

[173] R.A. Gibbons, S.N. Dixon, H.W. Symonds, Biochim. Biophys. Acta 444 (1976) 1.

[174] M.E. Andersen, J.M. Gearhart, H.J. Clewell, Neurotoxicology 20 (1999) 161.

[175] A.M. Bernard, Toxicology 101 (1995) 65.

[176] ACGIH Symposium on Beryllium, Cincinnati, OH, 23 September 2000, ACGIH Booklet, ACGIH, Cincinnati, OH, 2000.

[177] J. Angerer, K.H. Shaller (Eds.), Beryllium. Analysis of Hazardous Substances in Biological Materials, Vol. 5, Wiley–VCH, Weinheim, 2000.

[178] P. Apostoli, S. Porru, L. Alessio, Med. Lav. 80 (1989) 390.

[179] D.C. Paschal, G.C. Bailey, Atom. Spectrosc. 7 (1986) 1.

[180] P. Apostoli, K.H. Shaller, Int. Arch. Occup. Environ. Health 74 (2001) 162.

[181] P. Schramel, I. Wendler, J. Angerer, Int. Arch. Occup. Environ. Health 69 (1997) 219.

[182] M.R. Cullen, Am. Rev. Resp. Dis. 135 (1987) 201.

[183] P.K. Ridenour, O.P. Preuss, in: M.D. Rossman, O.P. Preuss, M.B. Powereds (Eds.), Acute Pulmonary of Beryllium, Williams and Wilkins, Baltimore, MD, 1991, p. 103.

[184] R. Wegner, R. Heinrich-Ramm, D. Nowak, Occup. Environ. Med. 57 (2000) 133.

[185] R.A. Goyer, Scand. J. Work Environ. Health 19 (1993) 139.

[186] M.A. White, J. Trace Elem. Med. Biol. 13 (1–2) (1999) 93.

[187] A. Aitio, P. Apostoli, Toxicol. Lett. 77 (1995) 195.

Pietro Apostoli was born in Brescia, Italy in 1948 and studied medicine at the University of Padova. He is specialised in occupational medicine, in hygiene and preventive medicine.

Fellow and researcher at the Institute of Occupational Health, University of Verona, he moved in 1986 to the Institute of Occupational Health of the Brescia University firstly as researcher then as associate professor. Since 1987 is the Director of the Laboratory of Industrial Hygiene and Toxicology of the Institute. From 2000 he is full professor of Industrial Hygiene at the University of Brescia.

He is the president of Italian Society of Reference Values (SIVR), member of the National Council of the Italian Society of Occupational Medicine and Industrial Hygiene, member of Scientific Committee on Metal Toxicology of the ICOH. His main fields of interest are the environmental and biological monitoring of xenobiotics, in particular metals and organic solvents, the study of biomarkers of exposure and early effect, the risk assessment, the quality assurance in occupational medicine and industrial toxicology, and published on these themes in more than 450 papers in national and international journals.