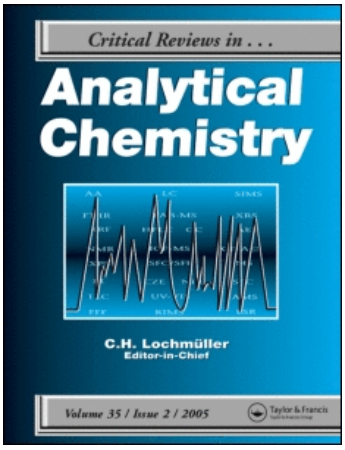

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

The Assessment of Reference Values for Elements in Human Biological Tissues and Fluids: A Systematic Review

S. Caroli[a]; A. Alimonti[a]; E. Coni[a]; F. Petrucci[a]; O. Senofonte[a]; N. Violante[a]
[a] Istituto Superiore di Sanità, Rome, Italy

To cite this Article Caroli, S. , Alimonti, A. , Coni, E. , Petrucci, F. , Senofonte, O. and Violante, N.(1994) 'The Assessment of Reference Values for Elements in Human Biological Tissues and Fluids: A Systematic Review', Critical Reviews in Analytical Chemistry, 24: 5, 363 — 398
To link to this Article: DOI: 10.1080/10408349408048824
URL: http://dx.doi.org/10.1080/10408349408048824

Critical Reviews in Analytical Chemistry, 24(5 & 6):363–398 (1994)

The Assessment of Reference Values for Elements in Human Biological Tissues and Fluids: A Systematic Review

S. Caroli, A. Alimonti, E. Coni, F. Petrucci, O. Senofonte, and N. Violante

Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

ABSTRACT: A survey is presented of the rationale of and the operative procedures for the assessment of reference values of minor and trace elements in human blood (whether entire, plasma, or serum), urine, milk, hair, kidneys, liver, and lungs. Literature sources available on this subject matter have been scanned over the past 30 years and reference values have been critically and selectively reported. The need for further harmonization of strategies and better comparability of experimental data is emphasized.

KEY WORDS: elements, biological tissues, biological fluids, blood, plasma, serum, urine, milk, hair, liver, kidneys, lung.

I. PRELIMINARY CONSIDERATIONS

A. Welfare and the Chemical World

Health for all is today one of the major issues as a consequence of the global concern raised in the early 1970s about the widespread use of chemicals of industrial origin. Thanks to the incisive actions promoted by international bodies, such as the Organization for Economic Cooperation and Development (OECD), the World Health Organization (WHO), and the European Economic Community (EEC), it was possible to set up over the past 2 decades overall consistent schemes for the effective control of chemical substances and their adverse effects on human health and the environment.[1-3]

To a certain extent, this framework also applies to elements, which pose quite specific problems in that they are naturally occurring and ubiquitous at the trace level. One of the most fundamental distinctions that can be made among elements is based on their being essential or not to human life. From this standpoint knowledge about the role played by some inorganics at major and minor concentrations in living organisms is well known. That vital biochemical functions can in turn be triggered by trace levels of several key elements is also not recent news, although novel evidence is periodically being produced about the essentiality of elements previously not suspected to qualify as such. One historical example would suffice for all: over the years Zn was considered first highly toxic and then indifferent to human beings, and it was only long afterwards that its indispensability in a number of enzymes (now > 100) was fully recognized.[4]

At present, besides the obvious constituents C, H, N, and O, the elements for which the importance to human survival is unquestionable are B, Ca, Cl, Co, Cr, Cu, F, Fe, I, K, Mg, Mn, Mo, Na, P, S, Se, Si, Sn, V, and Zn, whereas for a few others debate is

1040-8347/94/$.50

still open as to whether they exert any fundamental action at extremely low amounts (e.g., Cd).

On the other hand, anthropic activities since the advent of the Industrial Revolution have released into the environment increasingly huge quantities of organic and inorganic compounds, of which elements in general and heavy metals in particular definitely comprise a large fraction. Whether essential or not, all of them become noxious when organisms are exposed to exceedingly high concentrations. Actual catastrophic events such as the episode at Minamata and Niigata, Japan, where hundreds of people were killed by Hg-contaminated fish, testify to the seriousness of uncontrolled discharges of elements into ecosystems.[5] This adds to the natural mobilization of elements as a consequence of biogeochemical cycles. A case in point is the Hg belt that tranversely crosses the Mediterranean region, starting from the Idrija mines in Slovenia, moving down to the cinnabar deposits of Mount Amiata in central Italy, and finally heading to the open sea and the Iberian peninsula.[6] The metal discharged by chloralkali plants and other industrial activities accumulates with that of endogenous origin, thus creating local conditions of anomalously high Hg concentration, as reflected by levels of this element in the hair of individuals living in coastal zones.

With this composite picture in mind it is quite obvious that two basically divergent requisites emerge as regards the presence of elements in the human body, i.e., the essential elements must be within given concentration intervals deemed optimal for the proper performance of vital processes, while potentially toxic elements should occur at the lowest possible levels. In the first case the ranges and their amplitudes are regulated by homeostatically controlled mechanisms that tend to restore the ideal balance whenever this is altered, either by resorting to the body's storage sites, if an insufficient uptake of a specific element takes place, or by excreting the excess if an overload occurs. Apparently, dietary habits, lifestyle, and the characteristics of the country of residence somehow influence such intervals; hence, they may vary to a certain extent from one population of healthy individuals to another.

These kinds of baselines are extremely useful to define "normality" for temporally and spatially identified groups of subjects, no matter how numerous, as any deviation outside the ideal limits will immediately highlight a possible pathological state and call for the appropriate action to be undertaken. As regards nonessential elements, the scope is totally different. Although their concentration in biological systems should be nil, a more pragmatic approach leads to the concept of tolerance values, i.e., threshold figures below which the deleterious consequences of their entry into the organism may be reasonably neglected.

B. Reference Approach

The rather qualitative considerations reported in the previous section have been systematically incorporated into a set of rigorous criteria, the development of which should be largely credited to the Expert Panel on Theory of Reference Values (EPTRV), established in 1970 by the Scientific Committee of the International Federation of Clinical Chemistry (IFCC), with the specific mandate of setting up an ad hoc nomenclature as well as recommended procedures for the production of reference values (RVs). It is also worth mentioning that since 1977 this group was flanked by the Standing Committee on Reference Values (SCRV) of the International Committee for Standardization in Haematology (ICSH).

The efforts of the EPTRV resulted in a series of guidelines, recently made available to the public at large, which deal with the meaning and implications of RVs, the rules to be followed to select and prepare suited individuals, the procedures to collect samples, the achievement of accuracy in the generation of RVs, stochastic aspects, and comparison of observed values (OVs) to RVs.[7-10] Several definitions were thus issued that were considered essential to avoid any ambiguity in describing the relation of data obtained for a specific subject (i.e., OVs) to RVs.

These terms, now generally accepted and currently used by the scientific community, are reported in Table 1. How the various concepts illustrated therein are interrelated is in turn set forth in Figure 1.

Obtaining reliable RVs is possible only when all conditions adopted are self-consistent and exhaustively described, from the rules of inclusion or exclusion used to form the reference population to the analytical methodology chosen to measure actual concentrations. These criteria are scrutinized with the necessary attention in Section II. Here, it is worth emphasizing that the mere assumption of health as the main parameter characterizing a reference population raises a first, unavoidable question: what is the meaning of health? It is defined by WHO as a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity. Even this assertion is not entirely satisfactory, as the notion itself is relative and depends on several competing parameters, such as the degree of development of the country of origin and aging.

In spite of this inherent lack of absolute validity, RVs are of prime importance for a variety of purposes, including the evaluation of health conditions for both single individuals and communities, the identification of groups at risk, and the decision-making process in regulatory activities, provided that the population under test is clearly defined and the targets of the specific investigation set in detail.

TABLE 1
Definitions Adopted in Connection with the Subject of Reference Values[7]

Reference individual: an individual selected for comparison using defined criteria.
Reference population: a population consisting of all possible reference individuals.
Reference sample group: an adequate number of reference individuals taken to represent the reference population.
Reference value: a value obtained by observation or measurement of a particular type of quantity of an individual belonging to a reference sample group.
Reference distribution: statistical distribution of reference values.
Reference limit: a quantity derived from reference distribution and used for descriptive purposes.
Reference interval: an interval between and including two reference limits.
Observed value: a value of a particular type of quantity, obtained by observation or measurement and produced to make a medical decision.

II. GENERAL GUIDELINES

A. A Consolidated Network

The main principles governing the theory and practice of RVs are acknowledged to be the same whatever the quantities taken into account may be, from enzymes to proteins to hormones to trace elements, etc. Instead, what certainly should be considered variable are the operative conditions chosen to carry out a specific task. Also important from this point of view is the mode of comparing OVs with a distribution of RVs, or with a defined fraction thereof, as this depends on the targets planned beforehand, such as ascertaining physiological changes, early detection of disease, differential diagnosis, monitoring of therapy response, or assessment of environmental effects.[7]

Thus, independent of the goals of the study, the selection of individuals for the assessment of RVs may follow one of two general approaches, the *a posteriori* or the *a priori* pattern. Both have their own merits, as witnessed by the abundant literature.[11-13] In the first instance individuals suited to the purpose at hand are selected retrospectively from a large population sample obtained randomly or nonrandomly. Consequently, partitioning and exclusion rules will be set up on an ad hoc basis according to the specific quantity considered.

This strategy is better tailored to the production of RVs from healthy individuals, and especially effective in the evaluation of partitioning and exclusion criteria in order to assess well-identified subgroups of the general population. These subgroups are characterized according to socioeconomic conditions, ethnic origin, and residence site, cross-linked or not with sex, age, and lifestyle. On the other hand, only a limited number of

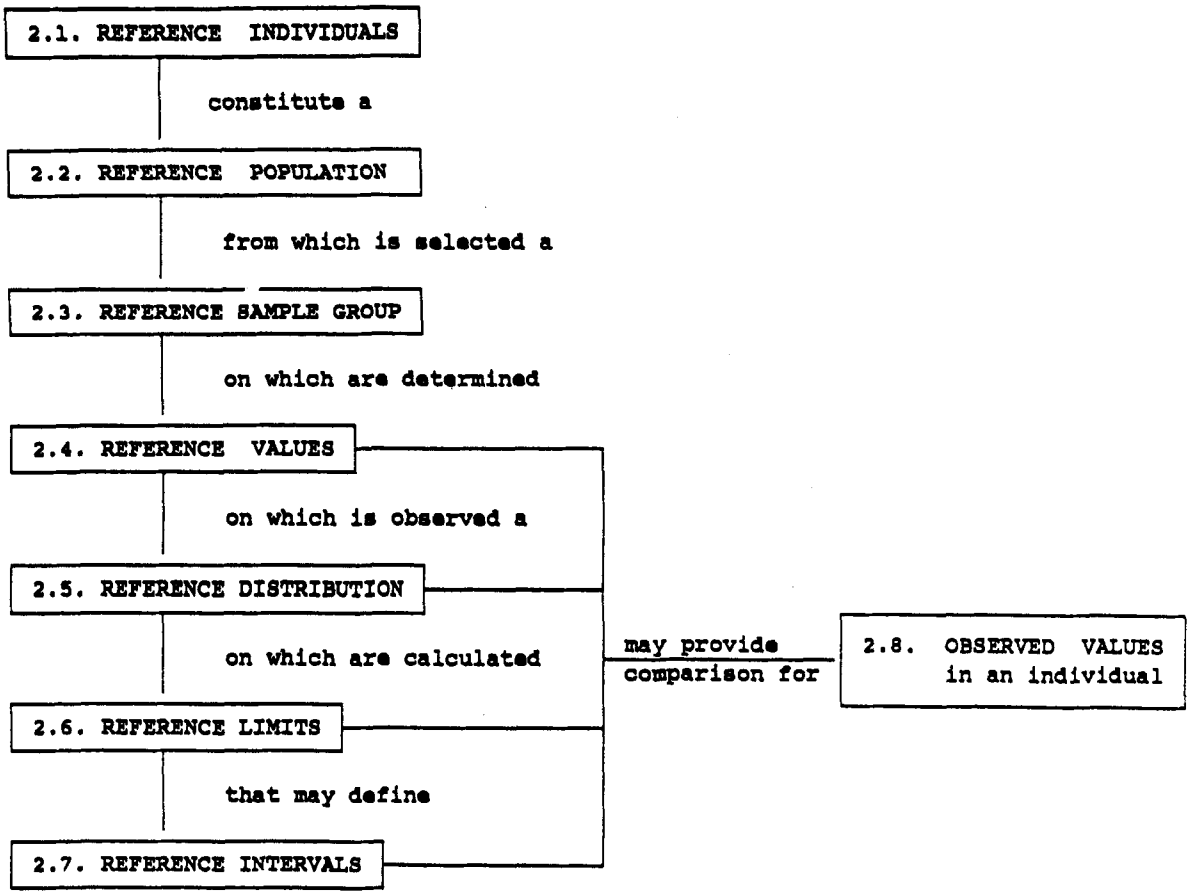

FIGURE 1. Relationships among the various reference terms. (Reproduced from Reference 7, with permission.)

centers have the facilities and financial resources to entail in their studies large population groups. As regards the latter approach, the *a priori* selection is definitely more manageable in terms of investment required, even though the partitioning and exclusion criteria must be taken for granted before the study is begun, be these already known from previous similar investigations or set through partly arbitrary assumptions.

The trend today is clearly in favor of this last option, which also offers the advantage of being applicable to all instances. The two alternatives are graphically shown in Figure 2. As already mentioned, crucial to both procedures is the adoption of exclusion and partitioning criteria adequate to the use for which the RVs are intended. Along this line of thought, whenever RVs from healthy subjects are needed, individuals suffering from clearly ascertained pathophysiological states —receiving pharmacologically active agents, consuming alcohol or tobacco, or being characterized by a modified physiological state in consequence, e.g., of pregnancy, physical training, and psychological and mental disorders—should be excluded.[8,14]

The partitioning process, in turn, categorizes individuals who passed the exclusion step according to parameters such as age, sex, genetic fingerprint, socioeconomic status, environmental conditions, and particular biological features. It goes without saying that each criterion is required to be dealt with specifically and carefully. As one example of this need, it is often more expedient to subdivide age not into equal ranges but into intervals that may better reflect the pattern followed by an essential element during the various life stages (perinatal period, childhood, puberty, etc.).

Once RVs for a given set of individuals have been produced, they must be organized into suitable reference intervals designed to

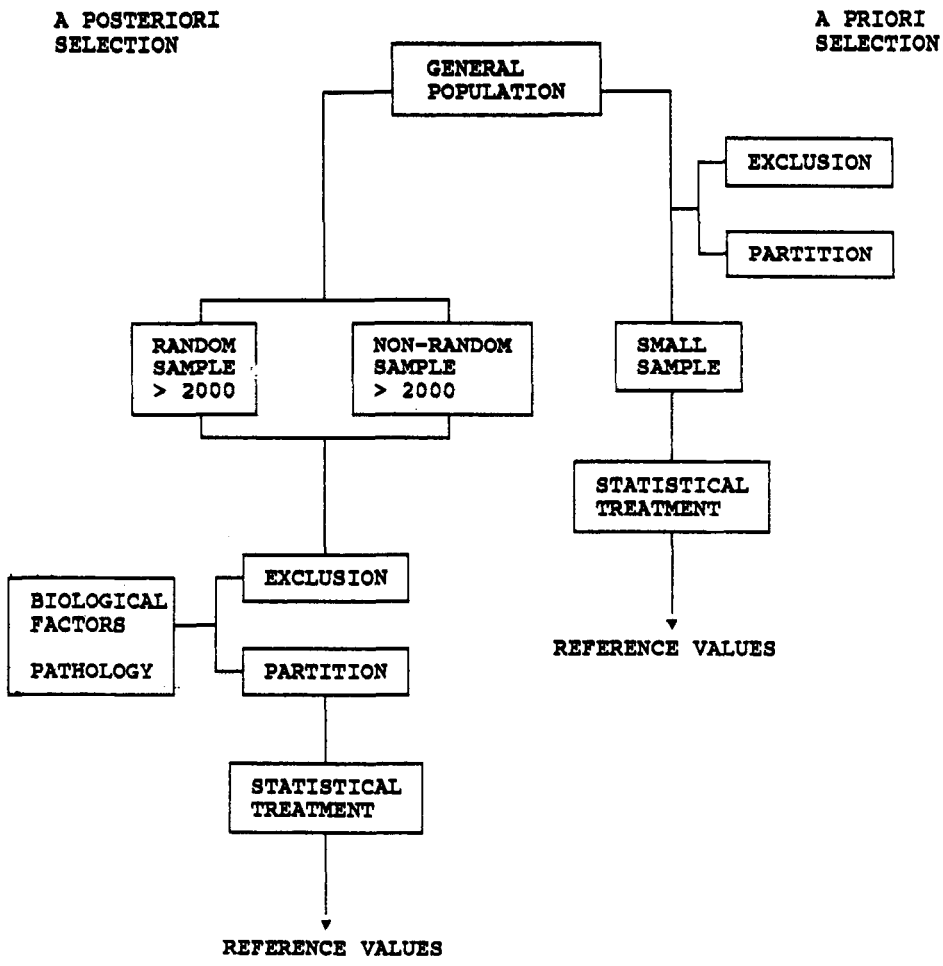

FIGURE 2. A sequential scheme for the *a posteriori* and *a priori* selections. (Reproduced from Reference 8, with permission.)

meet the requirements of different statistical problems. Those currently used are called the interfractile, tolerance, and prediction ranges, respectively. It must be considered, however, that from the usual clinical point of view the reference interval simply helps to assess in a rather intuitive manner the biological information conveyed by OVs.[15,16] In practice, all three approaches display modest relative numerical variations. However, references ranges of the fractile type are preferred in this context, because they have the additional advantage that they permit the use both of parametric and nonparametric statistical methods.[17] By definition, an α-fractile is a quantity value limiting upward a specified fraction of the cumulative distribution. When 100 is taken as the basis, the fractile is called percentile. Conventionally, the central 95% fraction of the reference distribution is chosen, thus leaving two equivalent 2.5% aliquots on both ends. Under particular conditions an asymmetric location also may be adopted. A correct approach to the estimation of fractiles also demands that α be well above $1/N$, where N is the number of persons in the reference sample group.

As a rule, the nonparametric method should be resorted to unless the distribution form (gaussian or not) has been reasonably ascertained or can be achieved through a proper transforming function applied to the original data, e.g., by means of their logarithms, square roots, or any other type of power or exponential function.[18,19] This is an extremely important aspect, as very often ar-

bitrary assumptions are made about the normality (the conformity to the gaussian model) of the distribution of values for a given quantity measured in different subjects, what is instead certainly valid for repeated measurements of said quantity in the same individual.[20]

The far-reaching consequences of erroneous assumptions when dealing with distributions of biological data cannot be disregarded, as this will result in the assessment of unreliable borderlines between health and situations of clinical relevance, with the attendant gross misclassifications. In other words, this means that the distribution of values in healthy subjects cannot be assumed to be gaussian in the vast majority of the cases, unless strong and unequivocal evidence is provided to the contrary. Not rarely, in fact, wrong limits for clinical evaluations are established that are then hard to remove and lead to invalid decisions, with all the involved social costs. Hence, the ability of a laboratory to generate accurate information on RV distributions largely depends both on carefully selected reference sample groups and the awareness to resort to nonparametric methods whenever indicated.

As regards the gaussian mode, various procedures have been designed by statisticians to verify the normality of a distribution. A preliminary check is obviously the possibility to partition the collected RVs in subclasses when the superposition of two or more distributions becomes apparent. Bartlett's and Fisher's tests are examples of this statistical approach. Once this has been accomplished, the plain visual inspection of the distribution through obtaining the pertaining histogram is helpful in ascertaining the occurrence of outliers or aberrant values, as well as of peculiarities such as skewness and kurtosis. In particular, the latter two characteristics are statistical measures of the asymmetry and peakedness of the distribution, respectively. More precisely, skewness may be negative or positive depending on whether there is a too-long left or right tail, whereas negative kurtosis stands for a too-flat-topped, short-tailed distribution and positive kurtosis for a too-peaked, long-tailed distribution. All these anomalies can be corrected for through sound application of statistical methods.[16,18,19]

After all these confounding aspects have been clarified, tests for normal distribution can be safely employed, which go from simple graphical procedures (not very precise, but useful for a first, rough idea of whether the gaussian bell-shaped function is obeyed) to the more sophisticated Kolmogorov-Smirnov or Anderson-Darling tests.[21] Should this be the case, then fractiles can be estimated parametrically by means of the expression $x_m \pm c_{1-\alpha} s_x$, where x_m is the arithmetic mean of N values, s_x their standard deviation, and $C_{1-\alpha}$ a constant obtained from statistical tables (e.g., for $\alpha = 0.025$ and $1 - \alpha = 0.975$, $c_{1-\alpha} = 1.960$). Other formulas also have been developed to estimate the confidence intervals of the reference limits.[22]

In the nonparametric mode the N reference values must be sorted following an increasing pattern from which the rank number of the α and $(1 - \alpha)$ fractiles is calculated according to the expressions $\alpha\ (N + 1)$ and $(1 - \alpha)(N + 1)$, respectively. The corresponding confidence intervals are then deduced from ad hoc tables. The proper production of dependable reference ranges and reference limits with their confidence intervals is essential to obtain meaningful information from OVs. However, in a strict sense the comparison is not entirely valid, as OVs are mostly generated at a later stage than the corresponding RVs, i.e., from populations which do not coincide.

The relation between OVs and RVs can be investigated with different strategies. The straightest approach is the classification of OVs as unusually low, usual, or unusually high with dependence on their being situated below, within, or above the reference interval as defined by its reference limits. Another type of transformation, to be applied only with gaussian distribution and preferably with a number of subjects higher than 500, is based on the comparison of the term $(OV - x_m)/s_x$ with the set of RVs. In the fractile procedure the OVs are coupled with the corresponding ordinate value on the cumulative number fraction reference distribution. Further methods display a relatively higher

degree of complexity and are exhaustively described in the literature.[23,24] Which methods should be selected mainly depends on the final target of the study.

B. Operative Aspects

What has been detailed in the previous section guarantees the most effective and consistent exploitation of experimental information, although the validity of this information remains an independent question. The risk inherent in applying a highly harmonized set of rules and tenets to data which might be questionable is self-evident, eventually leading to a ruinous waste of time and efforts, not to mention the deleterious consequences for the individuals under test. The strictest precautions are thus to be incorporated into all steps of study planning, sample collection, and actual analytical measurements, in accordance with the principles of total quality control and assurance.[25]

Beyond the general code of conduct that must inspire obtaining trustable experimental data, each specific type of biological tissue and fluid requires its own dedicated approach; neither should it be overlooked that the determinand itself greatly influences the overall procedure set up for a given kind of specimens. By universal acknowledgment, a major source of error in the analytical process can be ascribed to an inadequate sampling strategy, both from the viewpoint of a real representativeness of the system under examination and of the occurrence of contamination or loss phenomena. If this phase is carried out erroneously, all subsequent operations become irrelevant when not dangerous for clinical assessment.

In this context a clear distinction should be made between the sampling of liquids and that of tissues, as in the former instance this can always be made in the living subject, while the latter does demand obtaining autoptic specimens for inner organs such as brain, heart, or liver due to the quite obvious impracticability of conducting biopsies on the large scale necessary to generate a set of RVs. For a more convenient illustration of the features of these two opposite cases, the discussion of each item is dealt with separately.

1. Biological Fluids

In order to eliminate or minimize the contribution of confounding factors, detailed and operatively rigorous protocols must be prepared beforehand and carefully adhered to in the course of the actual sampling process. Among the more significant sources of variability one should annoverate both biological and methodological causes, the latter being traceable back to two main classes of factors, the phase of specimen collection and the subsequent procedures for sample manipulation.[26,27] Biological factors consist of two types: those characterizing the reference population (age, sex, and geographical area of origin), which understandably cannot be removed, but can be exploited to form subgroups (Section II.A); and those affecting the subject as a single person. The second category encompasses factors influencing the individual metabolism, from the assumption of drugs and alcohol to the smoking habit and the like.

No universal guidelines beyond these simple criteria can be elaborated because of the diverse nature of organic liquids, each deserving its own set of rules. Whether blood, urine, milk, saliva, sperm, tears, spinal fluid, or any other body liquid, it is mandatory to judiciously estimate the specific influence exerted by relevant parameters on the balance of carbohydrates, amino acids, proteins, lipids, enzymes, and any other substance which governs the distribution of elements in the organism. A general recommendation, however, arises from the need not to exceedingly deviate from the conventional clinical practice, as it is just after this that OVs will be produced.

With respect to methodological aspects, again the greatest care should be devoted to the identification of those factors that are likely to arouse uncontrolled fluctuations in the system. These are mastered rather easily by the operator, as sample collection can be

carried out under exactly defined laboratory conditions, from the recording of environmental parameters and time to body posture and from the preparation of the subject to the selection of the most suited apparatus and technique. At each stage, effective measures are compulsory to keep contamination or loss of analytes at levels of no practical consequence.

During sampling, in particular, the greatest care should be devoted to the presence of dust, dirt, cosmetics, and industrial products on the surface of the body, of disinfectants, talc, and dust on the gloves of the operator, of metallic corrosion products and residues of previous actions on clinical tools, and of dust and disinfectants on working surfaces.[28] The use of devices capable of releasing the analytes sought for in the test is a highly risky source of contamination. A good example of this is found when blood is drawn with stainless steel needles, as the release of, for example, Cr, Mn, or Ni from this material when in contact with the fluid is on the magnitude order of ng ml^{-1} or a fraction thereof (i.e., equivalent to if not larger than the endogenous content in such elements of blood itself). To minimize this sort of contribution, plasticware (made preferentially of Teflon) should be employed throughout, or metal tools should be siliconized to make their surface hydrophobic. In both cases it is advisable to discard the first aliquot of liquid. As soon as the specimen becomes available due account should be taken of all preanalytical steps, i.e., any chemical and physical action necessary to stabilize it without spoiling the original information content, including adequate transport and storage precautions and what else may turn out to be crucial for the successful outcome of the undertaking.[29]

Furthermore, one should not use preservatives of any kind (formalin, heparin, etc.), for their content in some elements may be extremely high, as summarized in Table 2.[28] It is much more expedient to freeze the specimens down to −15° or −20°C, even though the plastic material of which most containers are made cannot be considered entirely impervious to water, so that a gradual loss of vapors through the walls cannot be excluded.

Freeze-drying is also advantageous provided that one takes into account the possible separation of the various components. This is less than a drawback when the whole sample is consumed for the analysis after being reconstituted with water. Also not to be overlooked is the mutual interference between container and fluid, as adsorption of analytes onto the inner surface of the container or release of elements from it may adversely influence the endogenous content of the specimen. From this standpoint, quartz, Teflon, or polythene are preferred to glass, although in all instances thorough cleaning of the container must be carried out, resorting to strong acids, as required by the particular combination material-determinand at hand, and finally rinsing the vessel with double distilled water as long as necessary. Although what comes from this point on is clearly of the utmost importance, as analytical determinations must be accomplished in a totally reliable way, no discussion of these otherwise fundamental issues follows because it is felt that extremely abundant and credible literature exists that can be easily retrieved on how accuracy of instrumental measurements can be pursued.

TABLE 2
Impurities in Preservatives

Element	Heparin (mg / kg)	Formalin (mg / kg)
Ba	2.5 - 12	—
Ca	300 - 2900	—
Cr	—	0.21
Cu	0.65	—
Fe	—	3.5
Hg	—	0.01
Mg	—	1.6
Mn	3.6	0.01
Sb	—	0.14
Se	—	0.03
Sr	5 - 92	—
Zn	28	6.2

From Reference 28, with permission.

2. Biological Tissues

The same general criteria hold for these kinds of samples as for those discussed in the preceding subsection, obviously wherever they are applicable to materials in the solid state. What is peculiar to tissue specimens is that differences may occur from site to site of the system considered, thus seriously jeopardizing the representativeness of a part for the whole. This is the case for pulmonary tissues, where variations in morphology result in changes of element levels, or of hair, where the growth rate causes elements to be accumulated at different extents along the shaft. From this point of view fluids possess a much higher degree of homogeneity which poses lesser problems in the sampling step. Thus, this phase must be carried out only after a strategy has been thoughtfully developed, taking into account the specific nature of the tissues to be sampled.

Additional difficulties arise in the actual process of specimen collection because the friction between the biological material and the tools employed to excise it can cause the release of significant amounts of elements that may considerably enhance the endogenous levels of those to be analyzed.

It is acknowledged, among the many possible examples, that the use of metal needles to obtain liver biopsies can alter completely the determination of Co, Cr, and Ni in this organ. Dissection with conventional surgical devices (knives, scalpels, scissors, etc.) may result in gross errors, which can be minimized or totally avoided only by resorting to devices made of alternative materials instead of the most frequently employed, stainless steel. Particularly suited to this aim are high purity tantalum, titanium, platinum-iridium alloys, quartz, or synthetic resins (e.g., polystyrene), which should be judiciously chosen with specific reference to the task at hand, i.e., when there is no analytical interest in the elements of which those instruments consist.[30-32]

Another expedient approach is based on coating metal tools with an extremely thin layer of a highly adhering substance such as titanium nitride, which acts as a barrier to the transport of analyte atoms from the tool surface to the sample, while preserving the mechanical properties of the instrument.[33] Subsampling virtually coincides in terms of precautions with that already outlined. As for storage, digestion, and analysis procedures, the previous considerations for biological fluids are equally valid in the case of solid samples. Nonetheless, it is worth mentioning what should be the rule in this field and regretfully is not: the full incorporation of the principles of quality control and assurance in all preanalytical and analytical steps. Only when adequate attention is paid to the need to achieve accuracy of measurements can credibility be attached to the obtained RVs. This implies that appropriate use of certified reference materials is made.

III. OVERALL SCENARIO

A. Of Elements and Man

Mineral imbalances can be ascribed both to geochemical effects and individual parameters such as the subject's health status, dietary habits, and lifestyle in general.[34] While this latter group of factors can be relatively well accounted for in the assessment of RVs by establishing specific sets of criteria to define normality for a given geographical reality, geochemical influences are more akin to a sort of undifferentiated background impacting on all subjects. To make things more difficult, these two classes of factors are not independent of one another, as the average composition of soil in a certain area clearly affects the nutritional state of individuals through the soil–plant–animal–man food chain.

As a rough indication, older, more acidic geological formations experience greater depletion of trace elements than young alkaline soils which have undergone less leaching and weathering processes. These effects combine in different ways with soil characteristics resulting for each geographical area in a unique set of conditions that trigger the uptake of elements by vegetables and ultimately by humans.[35-37]

This being the background, the following sections survey the major investigations carried out so far by different authors to ascertain RVs for trace elements in biological matrices. It also should be mentioned that a critical comparison of RVs available in various countries was attempted by Iyengar.[38] Furthermore, due attention is drawn to the fact that no claim of exhaustiveness is made, but that only representative investigations are quoted to illustrate specific cases. In order to facilitate its retrieval information is given according to the alphabetical order of elements by their chemical symbol.

B. Reference Values in Human Biological Fluids

1. Blood and Its Components

A number of investigations have been carried out over the years to assess, under a multiplicity of situations, RVs for elements in whole blood, plasma, serum, and erythrocytes. This is certainly not surprising, as blood is one of the biological fluids most frequently used for diagnostic purposes. Recently, a study was undertaken to ascertain the RVs of 12 elements (Ag, As, Bi, Cd, Co, Cr, Cu, Hg, Pb, Se, Ti, and Zn) in whole blood and of 15 elements (Ag, Al, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, Tl, V, and Zn) in serum or plasma of several hundreds of unexposed, healthy subjects living in the provinces of Brescia, Pavia, and Varese (Lombardy, northern Italy).[39] Use was made of flame atomic absorption spectrometry (AAS), electrothermal atomic absorption spectrometry (ETA-AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and neutron activation analysis (NAA). Measurements were made independently of each other by three laboratories. A thorough critical comparison with the data published by other authors was also carried out, which revealed striking differences for As, Cd, Co, Cr, and Ni in whole blood, mainly due to the different origin of the populations examined.[40] These data are included in Table 3.

As a rule Ag is said to be present at about 4 μg/l in serum.[41]

Much debate was raised in recent years over the "true" value of Al in serum.[42] Seriously prone to contamination phenomena, this kind of analysis seems now well under control, with concentrations in the healthy subject well below 10 μg/l. The case of Al is indeed indicative of how sample contamination could affect the achievement of correct values. At this point in time, concentrations of 2 to 7.5 μg/l in whole blood and 1 to 5 μg/l in plasma or serum are considered credible.[43] For this as well as for other elements, Morisi's group[44-48] investigated the problems associated with the quality assurance of trace determinations in blood. Several pluriennial interlaboratory quality assurance programs were set up to assess the performance of Italian analytical units in quantifying Cd and Pb in whole blood and Al, Cu, and Zn in serum. These interlaboratory exercises led to substantial improvements in the reliability of data and set the stage for obtaining RVs mirroring well the national situation and regional differences. Part of the results obtained by this team are given later in this sub-section.

Several studies of As revealed that the element content in blood and serum may considerably vary on a regional basis, the highest values occurring in people from the Far East.[49,50]

So far, only a few studies have dealt with B. One of these produced reliable values of 2.6 ± 2 μg/kg in erythrocytes, 31 ± 5.6 μg/kg in whole blood, and 22 ± 5 μg/kg in serum.[51]

Reeves[52] reports that plasma may contain from 80 to 400 μg/l Ba.

Woittiez[53] determined Br in serum at the level of 4.5 ± 1 μg/l. Values of 5 to 10 μg/kg in red blood cells and 1 μg/l were also detected.[53-55]

It is well known that hormonal control of hypo- and hypercalcemia keeps Ca at the normal level of 100 mg/l.[41] As a part of a project aimed at identifying risk factors of coronary heart disease and mortality, RVs for Ca, Cu, Mg, and Zn in serum were calcu-

TABLE 3
Overall Reference Concentration Ranges for Selected Elements in Blood and Its Components as Reported in the Literature

Element	Conc	Element	Conc
Ag	0.06 – 0.3 μg / l (p, s)	Mn	12 – 26 μg / kg (e)
	0.1 – 0.6 μg / l (wb)		0.1 – 2.9 μg / l (p, s)
Al	0.5 – 8 μg / l (p, s)		6.7 – 10.4 μg / l (wb)
	2 – 8 μg / l (wb)	Mo	0.58 – 0.91 μg / l (s)
B	0.6 – 4.6 μg / kg (e)		5 – 157 μg / l (wb)
	17 – 27 μg / l (s)	Na	2500 – 3560 ml / l (s)
	25 – 36 μg / l (wb)		
Ba	80 – 400 μg / l (p)	Nb	0.53 – 0.74 mg / l (s)
Be	0.04 – 0.3 μg / l (p, s)	Ni	0.05 – 1.3 μg / l (p, s)
Bi	0.1 – 0.8 μg / l (wb)		1.1 – 4 μg / l (wb)
Br	3.5 – 5.5 μg / l (s)		
Ca	91 – 106 mg / l (s)	Pb	0.08 – 0.48 μg / l (p)
			40 – 290 μg / l (wb)
Cd	0.04 – 0.4 μg / l (p, s)	Rb	150 – 560 μg / l (s)
	0.1 – 2 μg / l (wb)	Sb	≈ 0.8 μg / l (s)
	16 – 46 μg / kg (e)		0.3 – 3 μg / l (wb)
Co	0.08 – 0.45 μg / l (p, s)	Sc	< 0.03 μg / l (wb)
	≈ 20 μg / l (wb)		
Cr	0.04 – 0.43 μg / l (p, s)	Se	75 – 240 μg / kg (e)
	0.08 – 0.5 μg / l (wb)		30 – 105 μg / l (s)
	0.7 – 0.8 mg / kg (e)		80 – 140 μg / l (wb)
Cu	0.6 – 1.4 mg / l (p, s)		
F	0.06 – 0.26 mg / l (wb)	Sn	≈ 2 μg / l (p)
			120 – 140 μg / l (wb)
Fe	1.1 – 1.3 mg / l (p)	Sr	28 – 44 μg / l (p, s)
Hg	9 – 34 μg / kg (e)	Ti	20 – 70 μg / l (wb)
	0.1 – 3 μg / l (p, s)	Tl	0.05 – 0.3 μg / l (s)
	1.2 – 10 μg / l (wb)		0.15 – 0.6 μg / l (wb)
I	≈ 60 μg / l (s)		
K	≈ 8000 mg / kg (e)	V	0.1 – 0.9 μg / e (s)
	140 – 215 mg / l (s)		
Li	0.29 – 0.31 μg / l (p)	Zn	10 – 16 mg / kg (e)
	0.52 – 0.64 μg / l (wb)		0.6 – 1.2 mg / l (s)
			4 – 8 mg / l (wb)
		Zr	≈ 6.2 mg / kg (e)
			0.1 – 0.7 mg / l (s)
			0.01 – 0.02 mg / l (wb)
Mg	17 – 22 mg / l (s)		

e = erythrocytes, p = plasma, s = serum, wb = whole blood.

lated in a reference population of about 1500 elderly subjects aged 55 to 75 years.[56] A subgroup of 655 individuals was also selected after exclusion of people with clinical and biochemical signs of cardiovascular and liver diseases, metabolic disorders, cancer, and chronic renal and chronic respiratory failures. No differences being detected, 5th to 95th percentile intervals of 91.1 to 106 (Ca), 0.76 to 1.40 (Cu), 17.1 to 22.5 (Mg), and 0.67 to 1.13 (Zn) mg/l were achieved.

As regards Cd, this metal accumulates preferentially in erythrocytes, so that whole blood is the medium of election for diagnostic purposes. In fact, while in serum 0.1 μg/l is thought to be quite representative; whole blood may contain up to 0.3 to 1.2 μg/l for nonsmokers and ca. 1.7 μg/l for

smokers.[49,57,58] About 200 blood donors were selected in an attempt to define RVs for Cd and Pb in whole blood.[59] Rigorous criteria were adopted on the basis of which individuals were chosen for inclusion in the study, all being inhabitants of Brescia and surrounding areas of northern Italy. Clinical and biochemical parameters were systematically recorded for each subject. Average values of 0.52 ± 0.39 μg/l for Cd and 136 ± 89 μg/l for Pb were obtained. Sex and smoking and drinking habits largely affected result distribution.

Although poorly documented, normal concentrations of Co in red blood cells are reported to range from 16 to 46 μg/kg, whereas figures in whole blood and serum are close to 20 and 0.1 to 0.45 μg/l, respectively.[49] Strongly diverging values on the other hand were indicated by some authors, who reported ranges of Co in erythrocytes, whole blood, and serum as 10.5 to 206, 0.5 to 238, and 0.2 to 660 μg/l, respectively.[60]

Intervals of 0.038 to 0.35 μg/l of Cr in plasma and serum were given as values not affected by the gross errors that normally characterize other measurements as a consequence of contamination problems.[42] As for the other ubiquitous elements whose expected values in biological materials are at the trace and ultratrace levels, Cr poses a challenging task to the analyst. This makes the assessment of RVs for this metal problematic to a certain extent. Concentrations in serum as low as 0.2 μg/l were given by Greenberg and Zeisler.[61]

According to firmly established knowledge, Cu levels in healthy individuals are deemed to be around 1.0 mg/l in whole blood and 1.1 mg/l in serum, with a range of ca. 0.7 to 0.8 mg/kg in red blood cells. Female subjects reportedly show a tendency toward higher values in serum, further augmented during pregnancy or when assuming oral contraceptives.[62]

As for F, 60 to 260 μg/l have been detected in whole blood.[38]

Normal plasma levels of Fe are 1290 and 1100 μg/l for men and women, respectively, implying that transferrin is about one third saturated.[63]

Like other elements, Hg is almost totally bound to red blood cells, with values in the range 9 to 34 μg/kg, a median concentration of 9.5 μg/l in whole blood, and about 1 μg/l in serum.[64] Populations with diets rich in fish show figures shifted toward the upper limit.

Approximately 60 μg/l of I is considered normal.[53]

Thanks to the homeostatic control mechanism, serum shows a rather narrow range for K, 140 to 215 mg/l, while red blood cells present 20-fold larger values.[65]

Figures of 0.58 ± 0.06 and 0.3 ± 0.01 μg/l in whole blood were ascertained for Li.[51]

The level of Mg in serum is thought to be kept constant at $10{,}854 \pm 792$ mg/l by the two mechanisms of renal excretion and homeostatic regulation of gastrointestinal absorption.[66]

Careful minimization of external contamination led to the assessment of 0.36 to 1.04 μg/l Mn in serum, with figures 25 times higher in erythrocytes.[41] The average concentrations of this element in erythrocytes and serum are probably ca. 16 μg/kg and 0.6 μg/l, respectively, although such figures may be disputed.[38]

With reference to Mo, values ranging from 5 to 157 μg/l are considered the norm in whole blood.[41] The inveral of this element in serum was found to be 0.58 to 0.91 μg/l.[67,68]

For Na, the interval in serum is 200 to 3560 mg/l, with no significant sex differences.[65]

Evidence of 0.53 to 0.74 mg/l of Nb in serum was provided by Carson et al.[41]

Hopfer and co-workers[69] ascertained values between < 0.05 and 1.3 mg/l for Ni in sera of healthy individuals. In general, 2 to 4 μg/l Ni in whole blood is deemed the most frequent concentration of the metal in healthy individuals.[41]

According to Tsuchiya,[70] the Pb concentration in erythrocytes is about 16 times higher than in plasma. Differences in the hematocrit and in the average mass of food ingested justify the higher amount of Pb found in whole blood of males (136 μg/l) than in that of females (100 μg/l).[49] As a

result of the previously mentioned studies carried out by Morisi and co-workers,[71] median values of Pb in whole blood were found to be 153 μg/l in adult males and 100 μg/l in adult females, while for children below 14 years figures of 94 μg/l (males) and 86 μg/l (females) were ascertained. A total of 8635 subjects were taken into account and the correlation among Pb levels, alcohol consumption, and smoking habit was clearly supported by experimental data. Along with the same tendency observed in other countries, a 25% reduction of Pb concentration in whole blood was apparent when comparing present values to those of the 1979 to 1985 period. The Pb levels in whole blood were also measured in a group of 1800 nonoccupationally exposed men, aged 55 to 75 years, all inhabitants of the Rome area, who participated between 1989 and 1990 in an epidemiological survey for coronary heart disease.[72] A range of 74 to 180 μg/l was obtained (10th to 90th percentiles). The gradual decrease in Pb levels was ascribed only in part to a diminution of this metal in gasoline, while variations in wine consumption also had a major impact.

A number of investigations agree on RVs for Rb between 150 and 550 μg/l.[53]

Whole blood may contain up to 3 μg/l Sb, while 0.8 μg/l of the element in serum is considered normal.[73]

In Italy the Sc content of whole blood was found to be < 0.03 μg/l.[74]

The wide variability observed in whole blood and serum for Se clearly parallels geographic abundance. The poorest areas in the world are located in China, Egypt, Finland, and New Zealand.[62] On the other hand, age is known to strongly influence the normal levels of this element, which has been reported to pass from 30 μg/l in the sera of newborns to 100 μg/l for the elderly.[75-77] In red blood cells, where Se is mainly bound to glutathione peroxidase, concentrations range from 75 to 240 μg/kg. Morisi and Patriarca[78] found that mean values of Se in sera span the range from 87 to 93 μg/l for adults and from 78 to 83 μg/l for subjects below 15 years residing in different areas of Italy. Sex and geographical origin did not appear to play a significant role, while a trend toward lower concentrations was observed in adult males, which was followed by an enhancement in the elderly over 65. Whole blood concentrations of Se are reportedly 157 to 265 μg/l for U.S.A. residents.[41]

As concerns Sn, 120 to 140 μg/l whole blood were the figures detected in individuals in North and Central America, while the levels decreased to 2 μg/l in plasma.[41]

From 16 to 43 μg/l is the rule for Sr in plasma.[41]

Woittiez ascertained in RVs for Sr serum and plasma in the range 28 to 44 μg/l, while Ag, Au, Sb, and Sc were all < 1 μg/l.[53]

Ti levels in blood usually range from 20 to 70 μg/l.[79]

In whole blood and serum V was ascertained to be < 0.1 μg/l.[80] According to other authors, 95% of the blood V is in plasma, the whole blood concentration being < 1 μg/l.[63]

The mean serum concentration of Zn in both men and women was found to be about 1 mg/l, whole blood having a fivefold higher level.[41] Further studies confirmed that the concentration of Zn in serum is lower than in whole blood, as red blood cells are rich in this metal. Thus, while values in erythrocytes are in the interval 10 to 16 mg/kg and in whole blood around 6.5 mg/l, serum levels are close to 0.9 mg/l and are subjected to strong variations due to stress, medication, fasting, or pregnancy.[49]

Finally, whole blood shows Zr values of 0.01 to 0.02 mg/l, while serum encompasses 0.11 to 0.68 mg/l and erythrocytes contain 6.2 ng/l.[81]

All in all, data available would point to sets of RVs for selected elements as shown in Table 3. This table necessarily displays heterogeneous experimental information with the sole purpose of highlighting possible general trends and common patterns.

2. Urine

The importance of reliable data on RVs for elements in this biological fluid is obvious in considering two major aspects: the feasibility of monitoring undue exposure to poten-

tially toxic agents and the possibility of checking the proper performance of essential biochemical processes. In spite of this, there is a relative paucity of credible information of this type in the relevant literature of the last 2 decades.

A systematic and detailed study was undertaken to assess RVs for 46 elements (Ag, Al, As, Au, B, Ba, Be, Bi, Cd, Ce, Co, Cr, Cs, Cu, Eu, Ga, Gd, Hf, Hg, In, Ir, La, Lu, Mn, Nd, Ni, Pb, Pd, Pt, Rb, Sb, Sc, Se, Si, Sm, Ta, Te, Th, Ti, Tl, U, V, W, Yb, Zn, and Zr in the urine of unexposed Italian subjects living in the Lombardy region.[39] This investigation took into account several hundreds of individuals and largely exploited the wide analytical capabilities of ETA-AAS, ICP-AES, and NAA. The corresponding reference ranges arrived at for each element are incorporated in those given in Table 4. These authors also carried out a thorough comparison of the data obtained for 22 elements of those mentioned above with values reported by recent literature in this field.[40] While in the majority the agreement was satisfactory, the most striking differences were observed for Cu, Mn, and Ni, apparently due to diversities in the analytical approach as well as in the characteristics of the population tested.

Also, worth mentioning is a thorough survey accomplished by Snyder et al.[82] with the aim of estimating the total body burden and balance of elements in the so-called 70-kg reference human. Through a careful scan-

TABLE 4
Overall Reference Concentration Ranges for Selected Elements in Urine as Reported in the Literature

Element	Conc	Element	Conc
Ag	0.04 – 6 μg/l	Lu	1 – 220 ng/l
Al	2.3 – 110 μg/l	Mg	≈ 90 mg/l
As	2.3 – 100 μg/l	Mn	0.12 – 20 μg/l
Au	1 – 600 ng/l	Mo	≈ 100 μg/l
B	0.5 – 3.3 mg/l	Na	≈ 2200 mg/l
Ba	0.03 – 57 μg/l	Nb	≈ 240 μg/l
Be	0.04 – 0.76 μg/l	Ni	0.06 – 8 μg/l
Bi	0.8 – 1.6 μg/l	Pb	12 – 30 μg/l
Br	≈ 5 mg/l	Pd	< 0.15 μg/l
Ca	≈ 120 mg/l	Pt	< 1 μg/l
Cd	0.4 – 70 μg/l	Rb	1 – 4.1 mg/l
Ce	0.1 – 12 μg/l	Sb	0.19 – 1.8 μg/l
Co	0.2 – 135 μg/l	Sc	0.3 – 130 ng/l
Cr	0.04 – 50 μg/l	Se	2 – 160 μg/l
Cs	0.1 – 20 μg/l	Si	2.9 – 12 mg/l
Cu	42 – 50 μg/l	Sm	1 – 210 ng/l
Eu	3 – 360 ng/l	Sn	≈ 14 μg/l
F	0.3 – 1.0 mg/l	Sr	≈ 0.22 mg/l
Fe	≈ 0.17 mg/l	Ta	0.01 – 0.6 μg/l
Ga	< 0.5 μg/l	Te	≈ 360 μg/l
Gd	< 1 μg/l	Th	0.01 – 0.28 μg/l
Ge	≈ 0.95 mg/l	Ti	1.3 – 10 μg/l
Hf	0.01 – 1.3. μg/l	Tl	0.02 – 8.9 μg/l
Hg	0.1 – 24 μg/l	U	10 – 350 ng/l
In	< 0.15 μg/l	V	0.2 – 10 μg/l
Ir	0.5 – 54 ng/l	W	0.05 – 0.85 μg/l
K	≈ 1.9 mg/l	Yb	5 – 86 ng/l
La	0.015 – 36 mg/l	Zn	0.27 – 0.85 mg/l
Li	≈ 0.5 μg/l	Zr	2 – 100 μg/l

ning of relevant published information, these authors could assess the mean daily intake and excretion amounts through different pathways. From these figures and assuming an average volume of urine of 1.5 l, the expected concentrations for a number of elements in healthy subjects are reported hereafter. To allow for immediate identification, such values are marked with the superscript *c* (for calculated).

As regards Ag, values around 6^c μg/l are given as normal levels.[82]

Information on the normal 24-h excretion of Al is widely contradictory, with figures of between 13 and 110 μg/l being reported.[83]

Occupationally unexposed adults were found to excrete As at a rate of 100 μg/l or less.[63]

According to Stockinger's[63] findings, the normal levels of Au in urine are not higher than 20 μg/l.

Figures of 0.7^c mg/l for B, 0.03^c μg/l for Ba, 0.7^c μg/l for Be, and 1.0 μg/l for Bi were indicated by Snyder and associates.[82]

As regards Br, there is general agreement on the average urinary excretion per day, with values of about 5 mg/l.[54,84,85]

As deduced by Snyder and co-workers,[82] an average excretion of 120^c mg/l for Ca, of 70^c μg/l for Cd, of 135^c μg/l for Co, and 50^c μg/l for Cr can be considered acceptable (82). For Cs, the Snyder team provided a daily loss with urine in normal adults of ca. 6^c μg/l.[82] This value is proximal to the lower limit of the range of 8 to 20 μg/l ascertained by other experimentalists.[85,86] Approximately 35 μg/l Cu are excreted daily with urine.[82]

Only a few studies dealt with the normal values of F in urine; one provided evidence that this element ranges from 0.3 to 1.0 mg/l.[87]

For the normal adult, concentrations of 0.17^c mg/l of Fe, 0.95^c mg/l of Ge, up to 24^c μg/l of Hg, 1.9^c mg/l of K, 0.5^c μg/l of Li, and 90^c mg/l of Mg are deemed acceptable.[82]

As concerns Mn, two sets of values are given, centering respectively around 20^c μg/l and < 3 μg/l.[82,88] Furthermore, 100^c μg/l are the average excretion for Mo.[82] The action of aldosterone on renal tubular functions maintains Na homeostasis, with 2200^c mg/l concentrations.[82] As for Nb, 240^c μg/l are deemed to be excreted.[82]

Hopfer et al.[69] reported that Ni in the urine of healthy subjects can span the interval 0.3 to 4.6 μg/l. Synder et al.[82] believed urine normally contained 8^c μg/l of Ni.[82] Other authors report figures of 2 to 4 μg/l.[89] As for Pb, 30^c μg/l is the average concentration.[82]

Figures of 1 to 3 mg/l are given as the rule for Rb.[86,90] Analogously, the level of Rb was reported to be ca. 1.2^c mg/l.[82] Figures of 2.0 mg/l, in good agreement with the former, were also measured.[91]

The urine of normal individuals may contain from 0.35^c to 1.8^c μg/l Sb.[73] In general urine contains $< 7^c$ ng/l Sc.[74,91]

A wide variability is encountered for Se, with values from 7 to 160 μg/l.[92] This variation is confirmed by numerous authors, who found concentrations of ca. 34^c μg/l and of between 7 and 79 μg/l.[82,88]

Snyder and co-workers[82] found losses of Sn with urine, especially in the form of inorganic compounds, to be ca. 14 μg/l, but with regard to Sr and Th, the respective concentrations are around 0.22 mg/l and 0.07 μg/l. In the case of Te 360 μg/l is indicated as the average excretion.[82]

It was reported in 1983 that 10 μg/l of Ti are excreted daily.[79]

A more controversial case is that of Tl. This element is reportedly in the range of 0.02 to 1.0 μg/l.[82] Smith and Carson,[93] in turn, found values of 0.4 to 1.8 μg/l. On the other hand, the upper normal limit for Tl was judged by Brockhaus et al.[94] to be 0.8 μg/l.

As concerns *U*, the urine content was ascertained to range from 30 to 350 ng/l.[82,88] In contrast to these values, the average excretion of *U* was reported not to exceed about 10 μg/l in adults, while children may well show roughly one third of this value.[95]

Finally, average concentrations of 10^c μg/l for *V*, of 350^c μg/l for Zn, and 100^c μg/l for Zr were ascertained.[82]

This information is tentatively summarized in Table 4, and the same considerations hold as for Table 3.

3. Milk

The fundamental food of newborns, this biological fluid continues to be thoroughly investigated from both the standpoint of optimum content of and ratios among inorganic nutrients and levels of nonessential elements that through the mother can be transferred to the baby. Minor and trace elements in human milk are thus a matter of concern among scientists. Recommendations have been issued and are constantly being updated on desirable concentration ranges of essential mineral nutrients and indications of acceptable nontoxic levels of inorganic polluting agents.

A collaborative study, undertaken under the aegis of WHO/IAEA (International Atomic Energy Agency), was conducted to ascertain reliable RVs for 24 elements (As, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, Hg, I, K, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Sn, V, and Zn) in breast milk.[96] The centers collaborating in the project were located in Guatemala, Hungary, Nigeria, the Philippines, Sweden, and Zaire. Three subgroups of mothers were studied in each of the aforementioned countries: (1) urban well-to-do or economically advantaged mothers; (2) urban poor or economically disadvantaged mothers; and (3) rural mothers following a traditional lifestyle in families dependent mostly on subsistence agriculture and local marketing. All the specimens were collected by specially trained personnel on the basis of a strict and detailed protocol. Reliability of results was ensured by the use of: standardized procedures for the sample collection, a single reference analytical laboratory for each element, and appropriate analytical reference materials. In consideration of their quality, the data obtained in this multinational study form the basis for a sound comparison of RVs of elements in breast milk.

So far, only a few studies have reliably dealt with Al. This reflects the fact that this element is very sensitive to external contamination and, consequently, many erroneous results have been published in the literature for human milk and other clinical specimens. In two studies on Italian subjects Al median values were found to be around 150 μg/l.[97,98]

As regards As, there is very little information available. Owing to difficulties in determining this element reliably in biological materials, any values reported need to be interpreted with extreme caution.[99] Data varied from 3.20 to 36 μg/l.[92] Results obtained in the WHO/IAEA study (see above) are all on the low extreme of this range (median value < 2 μg/l), with the exception of the Philippines, for which the median value was around 19 μg/l. Dang et al.[100] found 0.41 μg/l As in mature milk from well-nourished Indian subjects. Kosta and associates[101] found very similar values in milk samples from Yugoslavian mothers—0.44 μg/l (range 0.09 to 0.73 μg/l). The As values found by Cortes-Toro[102] in northern Chile in a region with high natural concentrations of As in drinking water were somewhat higher, 3.04 μg/l (range 1.6 to 4.8 μg/l). On the other hand, in the Santiago region of Chile, the values were l < 0.1 μg/l. These last three investigations were conducted under the auspices of the IAEA and were characterized by rigorous analytical quality control procedures.

The values for Ba found in two studies conducted on Italian mothers are in the interval 13 to 26 μg/l.[97,98]

An element moderately documented in the scientific literature is Cd, mainly because reliable results became available only in the last several years. The published data vary widely, from 0.10 to 19.0 μg/l.[92] A study of Yugoslavian subjects detected figures of about 0.30 μg/l in mature milk.[101]

An interval of 300 to 400 mg/l was ascertained for Cl.[92]

Co is also poorly documented in the scientific literature, with values between 1 and 8.6 μg/l.[92] Dang et al.[103] found values of around 0.5 μg/l in mature milk from 14 Indian mothers. Cortes-Toro[102] reported similar data in the Santiago region of Chile, namely 0.37 ± 0.22 μg/l, with a range of 0.03 to 0.77 μg/l. Somewhat lower values of about 0.1 μg/l in colostrum and transitional milk specimens were observed in Yugoslavian subjects.[101]

The reliable determination of Cr at the low levels present in biological matrices is difficult.[99,104] Consequently, there is scarce published information on its concentration in breast milk. The fact that the values obtained through the WHO/IAEA study (median values range from 0.78 to 4.35 μg/l) are all lower than the data cited by Iyengar and Woittiez[92] is not in itself very probative. The Cr content of breast milk in Italy (median value < 2 μg/l) was found to be inferior to that usually reported in the literature of concern.[97] Further evidence in support of the assumption that normal levels of Cr in human milk are lower than previously reported is provided by Kumpulainen et al.[105] and Casey and Hambidge. The former group measured a value of 0.49 ± 0.07 μg/l for a pooled sample of milk from Finnish subjects. The latter authors reported a mean concentration of 0.30 ± 0.17 μg/l for breast milk from mothers of the United States at various stages of lactation. These new sets of values are even lower than the values reported in the WHO/IAEA study, and raise some suspicions about the validity of that study; e.g., was the study affected by systematic analytical errors or even by contamination? The analytical quality assurance schemes adopted should exclude the occurrence of such errors. Analytical precision was so poor, however, that the questions cannot at this stage be completely resolved. If any conclusion is to be drawn, it should favor the fact that actual Cr concentrations in human milk are probably equal to or less than the values obtained in the WHO/IAEA study.

Moving on to Cu, numerous data are available as the determination of this element in breast milk does not pose difficulties. The general findings of the WHO/IAEA study are in agreement with values retrieved in the literature, as ascertained by Iyengar and Woittiez.[92] Dang et al.[100] found 340 ± 80 μg/l Cu in mature milk from well-nourished Indian subjects. Kosta and co-workers[101] found 253 ± 52 μg/l in mature milk from Yugoslavian subjects. Another study gave 249 ± 54 μg/l, with a range of 139 to 333 μg/l, from the region of Santiago, Chile, and 397 ± 105 μg/l, with a range of 210 to 629 μg/l, in northern Chile, which is characterized by Cu mining activities.[102] In Italy the Cu content of breast milk was found to be equal to 387 μg/l.[97,98] These data are consistent with results reported from other studies in which variations between different geographical areas were observed. Values as high as 500 μg/l have been reported for India.[107]

The data reported in the WHO/IAEA study for *F* pointed out great variations between different countries, with median concentrations ranging from 6.8 to 117.7 μg/l. Most values, however, are < 25 μg/l, thus being comparable to those reported by Esala et al.[108] for milk specimens from Finnish mothers (5.0 and 8.9 μg/l, median values, for subjects living, respectively in low and high *F* areas), and by Backer et al.[109] in The Netherlands.[108,109] Exceptionally high values were recorded in the Philippines.[96] The wide difference in *F* concentrations observed for human milk in all these studies can be traced back to the *F* content of foods grown in zones with diverse geochemical characteristics.

As regards Fe, the WHO/IAEA survey reported values between 346 and 720 μg/l, with relatively small differences between the various areas investigated. Such values fall within the range of 200 to 1300 μg/l retrievable in the literature.[92] Some differences between rural and urban groups were observed, although no definite trend could be detected. In Zaire rural mothers and children with signs of malnutrition showed a higher concentration of the element (851 μg/l) than the urban group (388 μg/l). In general, Fe concentration in breast milk appear to be unrelated to the element status or to the socioeconomic status of the mother.[98,110,111]

For Hg, the findings of the WHO/IAEA study are in good agreement with the data cited by Iyengar and Woittiez.[92] At the lower end of the range, values of 1.0 ± 0.9 μg/l for mature milk from Yugoslavian subjects were reported.[101] The country with the highest median value was Sweden, where fish consumption is relatively frequent. The in-

creased Hg content of human milk may find explanation in this fact.

In most countries the concentration of *I* is remarkably similar, with values of 50 μg/l (range 40 to 80 μg/l).[112-114] The only exception is Zaire with values close to 15 μg/l. In Zaire, the study was conducted in the Kivu province, which is notoriously an endemic goiter and low *I* intake area. Other authors[101] found 88 ± 19 μg/l in mature milk from Yugoslavian subjects. Much higher levels, with a mode of 150 μg/l and a range of 80 to 7000 μg/l, were reported in human milk specimens from Japanese subjects who consumed dietary algae.[115]

For Mg, values ranging from 29 to 38 mg/l are considered normal in human milk. For this metal there appears to be little difference between different groups and countries.

For Mn there is a modest amount of related documentation, although data are reasonably self-consistent. Concentrations of 4 μg/l in mid-lactation (2 to 6 months) and 6 to 8 μg/l in late lactation were reported.[116,117] A later study found a value of 23.0 ± 8.3 μg/l in mature milk from Indian subjects.[100] Somewhat lower values were detected for mature milk samples collected in Yugoslavia, namely 3.3 ± 2.1 μg/l.[101] Also, in two studies conducted in Italy the median values, 6 and 4 μg/l, were found to be lower than those normally reported.[97,98]

As for Mo, this element is in general very poorly documented, with occasional exceptions. A value of 6.4 ± 3.8 μg/l was found in mature milk from Indian subjects.[100] Concentrations of the metal in food vary considerably depending on the environment in which the food was grown.

The results reported for Na in the WHO/IAEA study are lower than those usually given in the literature.[92] The WHO/IAEA study documented median values oscillating around 100 μg/l, whereas Iyengar and Woittiez give a figure of around 160 μg/l. The findings of the WHO/IAEA study are, however, more credible due to the quality assurance criteria adopted.

In the case of Ni little information can be retrieved from the literature and no meaningful comparison can be made. A range of median values of 4.9 to 16.1 μg/l can be assumed.

For *P* and *K* the normal median values range from 135 to 155 μg/l and from 410 to 550 μg/l, respectively.

The variation observed in Pb concentrations of human milk in different populations can be ascribed to wide differences of intake related to environmental conditions, although it cannot be excluded that at least a part of the variation may be caused by analytical errors and contamination with laboratory airborne dust, which is rich in this element. The values for Hungary, the Philippines, and Sweden (median concentrations ranging from 15 to 22 μg/l) appear to be higher than those checked in Guatemala, Nigeria, and Zaire (median concentrations ranging from 3 to 6 μg/l).

In the case of Se values of around 20 μg/l were found in mature milk from Indian mothers.[103] Cortes-Toro[102] found 20.0 ± 3.9 μg/l, with a range of 14.6 to 26.6 μg/l in milk samples collected around Santiago, Chile. The WHO/IAEA study reports values in the range of 13 to 24 μg/l. Somewhat lower values (8.5 ± 1.9 μg/l, with a range of 6.6 to 11.9 μg/l) were reported in milk from Yugoslav mothers.[101] Levels of Se in foodstuffs are known to vary widely according to the geochemical environment in which plants are grown.

The concentration of Sn in breast milk is approximately 1 μg/l, as reported in the WHO/IAEA study. Much lower values, < 0.3 μg/l, were reported in four samples of mature milk collected from Yugoslavian mothers.[101]

For Sr, a recent study[118] suggested a value of 60 μg/l in transitory milk.

The few data available for *V* are often controversial, mostly due to contamination problems. Kosta et al.[101] reported values of 0.15 μg/l in mature milk collected from Yugoslavian mothers. Median values in the WHO/IAEA study are reported to range from 0.1 to 0.3 μg/l.

As for Zn, values of 2.4 ± 1.2 mg/l were measured in mature milk from Indian mothers.[106] Cortes-Toro[102] found significantly

higher values, 3.6 ± 1.0 mg/l, with a range of 2.0 to 5.4 mg/l, in milk samples collected in the area around Santiago, Chile. Higher values (median concentration equal to 3.2 μg/l) also were found after an investigation of Italian subjects.[98] Somewhat lower values of 1.44 ± 0.11 mg/l were reported in milk from Yugoslavian mothers.[101]

Table 5 collects the data described in detail in this section.

C. Reference Values in Human Biological Tissues

1. Hair

The diagnostic potential of human hair has still to be fully exploited. The former lack of reliable RVs for minor and trace elements has in the vast majority of cases substantially hindered the development of systematic approaches in this field. Hair analysis is undergoing a sort of renaissance, as witnessed by the preparation and commercialization of multielemental reference materials suited for this kind of determination.[119,120]

Among recent studies of major importance, the one by Iyengar and Woittiez[92] is worth mentioning. These authors investigated the problems raised by the assessment of RVs for 14 elements in human hair as determined by fluorimetry, AAS, NAA, and ICP-AES.

Kamakura[121] also employed ICP-AES in the analysis of 21 elements in samples of human hair taken from 1899 Japanese subjects (mean age 39.4 ± 16.1).

Nishiyama et al.[122] determined Cr, Fe, Rb, Se, and Zn in the hair of autopsy samples of 48 Japanese individuals, ages 22 to 66 (45 males and 3 females), all of whom were in good health before their accidental deaths.

Takey et al.[123] compared the data of 21 elements in people aged from 3 to 80 years and stemming from five different countries (Canada, India, Japan, Poland, and the U.S.).

A number of elements were also taken into account for the assessment of RVs in an urban area of Rome, namely Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Pb, Se, Ti, V, and Zn.[56,124,125] This study was carried out by ICP-AES on a population of young subjects (3 to 15 years), and partitioning depending upon either age or sex was accomplished.

As regards Ag, an average value of 0.25 μg/g was found by examining 246 adults living in the U.S., while a range of 0.165 to

TABLE 5
Overall Reference Concentration Ranges for Selected Elements in Human Milk as Reported in the Literature

Element	Conc	Element	Conc
Al	39 – 250 μg/l	Mg	29 – 38 mg/l
As	0.2 – 36 μg/l	Mn	3 – 40 μg/l
Ca	220 – 300 mg/l	Mo	0.3 – 19.5 μg/l
Cd	0.7 – 4.6 μg/l	Na	90 – 130 mg/l
Cl	320 – 410 mg/l	Ni	1.5 – 39 μg/l
Co	0.15 – 3 μg/l	P	135 – 1 mg/l
Cr	0.4 – 5.1 μg/l	Pb	2 – 30 μg/l
Cu	180 – 751 μg/l	Sb	1 – 4 μg/l
F	7 – 17 μg/l	Se	10 – 62 μg/l
Fe	202 – 1710 μg/l	Sn	1 μg/l
Hg	0.2 – 13 μg/l	V	0.1 – 0.3 μg/l
I	41 – 168 μg/l	Zn	0.7 – 4.0 mg/l
K	410 – 550 mg/l		

0.205 μg/g was found for a population from metropolitan New York. With reference to this metal, values of 0.7 μg/g for Italian female subjects are reported by Clemente et al.[126] For a population living in Tokyo an RV of 0.2 μg/g was ascertained.[127]

In another study on trace elements in human hair of some local populations in Japan the concentration of Al was found to range from 3 to 10 μg/g.[128,129]

Much experimental information is available for As, concentrations being in the interval 0.1 to 1 μg/g.[130-133] Smith employed NAA to measure As concentrations on a large number of samples. He found that the element content of human hair ranged from 0.03 to 0.74 μg/g, with a mean of 0.81 μg/g and a median of 0.51 μg/g.[134] This study pointed to a statistically significant difference between male and female subjects, the mean value for the former being 0.62 μg/g, while for the latter a value of 0.37 μg/g was obtained. This element was also determined in 259 Hungarians consuming water with on As content of < 25.0 μg/l.[135] The average As concentration was < 0.5 μg/g, with the exception of males over 60 years.

The B concentration in metropolitan New York youngsters (0 to 15 years) was ascertained to be 0.881 μg/g.[136] For older individuals the element content is slightly higher, 0.981 μg/g.

In the same study Ba was also measured, thus leading to RVs of 0.76 μg/g for the 0 to 15 years-old group and of 1.41 μg/g for the older group.

A certain variability was observed for Br, with values of 35.4 μg/g for a population in Toronto, 12.3 to 13.2 μg/g for inhabitants of England and Wales, and 16.4 μg/g as the mean for 333 normal individuals from Japan, and lower figures (2 to 10 μg/g) for some local populations and a mean value of 2.30 μg/g for a student population in India.[127,129,130,137,138]

A number of authors measured the concentrations of Ca in human hair. The reported mean concentrations cover the range 250 to 4693 μg/g.[139] Eatough et al.[139] ascertained that the average Ca concentrations in hair for females are approximately five times higher than the average value for males.[139] Bacsó[140] tested a group of individuals aged 20 to 35 years and found that males had average Ca concentration of 714 ± 933 μg/g (scalp hair) and 554 ± 529 μg/g (pubic hair). In the case of female subjects the figures were 1139 ± 851 μg/g (scalp hair) and 623 ± 532 μg/g (pubic hair).

The interest in Cd determination in hair has increased remarkably in the environmental health literature over the years. Petering et al.[141] suggested that this metal in a general, not occupationally exposed population is in the range 0.5 to 2.5 μg/g in both sexes.

Only a few reports on Cl in hair were published. Arunachalam et al.[130] reported a mean adult concentration of 3190 μg/g, as averaged from 221 Indian subjects, with a range of 120 to 14,000 μg/g.

The concentration values of Co in human hair are all within the range of 0.07 to 1.7 μg/g.[129,130] Other Co values in hair reported are 0.37 (U.S.),[142] 0.41 to 0.48 (Canada),[137] 0.35 (South Africa),[143] 0.084 (Japan),[127] and 0.28 μg/g (Iraq).[131] Moreover, Cole and Carson[60] reported 0.5 μg/g as an RV for Co in hair.

Levels of Cr in hair were ascertained by many investigators, as this determination is deemed more indicative of the body burden of the element than serum Cr in fasting patients. Studies in Denver, Colorado (U.S.) and in Chandigarh (India), as reported by Hambidge et al.[189] support the assumption that Cr levels decline with age. These authors reported a mean Cr value of 0.9 μg/g for normal young adults living in Denver.[145,146] Ashurbekov[147] determined Cr concentrations for males and females in the 20 to 25 year age bracket and recommended an RV of 1.03 ± 0.18 μg/g. Creason et al. reported that Cr is significantly correlated with Ba, Mn and Ni in human hair.[136]

Cs is very poorly documented for almost all human tissues and body fluids. In human hair Iyengar et al.[84,148] reported a range of 0.1 to 1.0 μg/g. Perkons et al.[132] found a value of 0.36 to 0.38 μg/g for Amazonian Indians, while Clemente[74] reported an inter-

val of 0.05 to 0.1 μg/g for a population in Italy.

The content of Cu in hair samples reflects geographical differences, but is normally between 10 and 20 μg/g.[49]

In the case of Fe, determinations are quite easy as concentrations are normally in the range 10 to 100 μg/g. Figures reported by Ashurbekov[147] are 30.21 ± 1.5 μg/g (males, 20 to 25 years) and 36.2 ± 2.1 μg/g (females, same age). Reportedly RVs (μg/g) for Fe in some countries are 29.9 (U.S.),[144] 41.0 (Canada),[137] 128 to 293 (Italy),[126] 30 to 50 (Zambia),[149] 60 (India),[130] 35 (Japan),[127] and 122 (Iraq).[131] According to Iyengar,[49] most values for Fe in hair range from 20 to 60 μg/g. For African countries the range results between 150 and 900 μg/g, the consequence of geochemical factors.

The element Ga is poorly documented. Perkons and Jervis[150] reported a range of 1 to 250 μg/g as calculated for 770 individuals in Canada.

Birke et al.[151] determined normal levels of total Hg and methylmercury in persons having no, low, or moderate intake of fishes. Total Hg levels in hair were in the range of 0.76 to 3.0 μg/g (mean, 1.6 μg/g). The levels of Hg in hair are about 300 times higher than those in whole blood.

The content of I in hair is 0.03 to 0.14 μg/g, according to Yurachek et al.[152] Other authors reported a mean value of 0.85 μg/g for male subjects and 0.6 μg/g for female subjects living in the U.S.[143]

For K it is well known that values decrease with age. In fact, Aal et al.[153] reported much higher values with significant decrease with age. For males this value was *ca.* 265 μg/g in the early years, which then decreased to *ca.* 92 μg/g at an age of 51 to 70 years. For females the corresponding values were 458 and 91 μg/g.

Creason et al.[136] determined Li in children (0 to 15 years old). A range of 0.009 to 0.3 μg/g was found, with a mean value of 0.044 μg/g. For older subjects the mean was found to be 0.056 μg/g.

An interval of 24.3 to 380 μg/g for Mg was ascertained.[154,155] Slightly different results were reported by Ashurbekov,[147] who reported a value of 236 μg/g for males 20 to 25 years old. The value for females of the same age was 2.3 times higher, 538 μg/g.

For Mn Ashurbekov listed μg/g values of 1.57 ± 0.01 (males, 20 to 25 years), 2.5 ± 0.26 (males, 70 years), 1.94 ± 0.23 (females, 20 to 25 years), 1.49 ± 0.16 (females, 60 years), and 4.85 ± 0.31 (pregnant females, 20 to 25 years). For metropolitan New York children (0 to 5 years of age) the RV is 0.56 μg/g.[136]

Imahori et al.[129] studied Mo in the hair of a Tokyo population. A range from 0.058 to 1.84 μg/g was found for males, with a mean value of 0.38 μg/g, while for female subjects the range of content spanned from 0.026 to 2.14 μg/g, with a mean value of 0.49 μg/g.

For Na, the lowest value reported so far is 23 μg/g, whereas concentrations as high as 2019 μg/g were ascertained for females aged 13 to 20 years.[153,156]

The Ni content in the hair of female subjects is significantly higher than that of male individuals. Chattopadhyay and Jervis[137] reported 0.1 μg/g as a mean for a group of 76 subjects, while Katz et al.[157] established 1.01 μg/g as the RV for male subjects and 4.05 μg/g as the RV for female subjects. More recent investigations[158] assumed the Ni content of hair to be 1.25 ± 0.46 μg/g in healthy control subjects.

Aal et al.[153,156] investigated the P content of hair in Egyptian individuals. These authors studied subjects aged 3 months to 70 years. The concentrations of P were found to be 127.1 μg/g for males and 130.3 μg/g for females.

In healthy persons the concentration of Pb in scalp hair is said to be from 2 to 5 times greater than in bones, about 10 to 50 times greater than in blood, and from 100 to 500 times greater than that excreted in urine.[159,160] Kopito et al.[161] showed that the Pb range in hair is 2 to 95 μg/g (mean, 24 μg/g) in healthy children.

The concentration of Pd in human hair as determined in 150 subjects living in California (U.S.) (1 to 35 years) was found to be < 0.02 μg/g.[162]

In the case of Pt there is only an upper limit of 0.05 μg/g reported by Johnson et al.[162]

Clemente et al.[126] reported Rb at the 1.7 μg/g level for Italian females. To the contrary, Arunachalam et al.[130] reported a mean value of 1.25 μg/g for 121 subjects living in India. Similar values were found by Sky-Peck and Joseph.[163]

Generally, the Sb concentration in hair is < 1 μg/g. Imahori et al.[129] reported that the range for inhabitants of Tokyo is 0.049 to 0.058 μg/g.

Values of Se in human hair were reported by several authors, all in the range 0.3 to 6.4 μg/g for subjects living in Tennessee (U.S.).[164] The lowest reported mean value is 0.3 μg/g for Italian individuals.[74] Other studies reported values of 0.67 μg/g for 248 U.S. adult subjects, 0.303 μg/g for metropolitan New York inhabitants older than 16 years, and 0.32 μg/g for younger children in New York.[142,136] Other values of interest are 1.8 to 1.98 μg/g (Canada),[137] 0.36 μg/g (former East Germany),[165] 1.4 μg/g (Iraq),[131] 1.28 μg/g (India),[130] and 1.18 μg/g (Japan).[127] Clemente et al.[166] reported the range of concentration in Italian subjects to be 0.002 to 0.13 μg/g.

The median value of Sr for Finnish populations, according to Forsseén and Erämetsä,[167] is 160 μg/g, with a range from 20 to 860 μg/g. Othman and Spyron[154] reported a mean value of 8 μg/g for 40 Kenyan males and 14 μg/g for 31 Kenyan females.

Creason et al.[136] determined Sn in the hair of children aged 0 to 15 years from New York City, and found a range of values from 0.034 to 8.30 μg/g, with a mean of 0.561 μg/g. For adults the mean reported was 0.785 μg/g.

Turkstra et al.[144] reported a Ti concentration in the hair of U.S. subjects of 3.5 μg/g (144). On the other hand, for Japanese hair Takeuchi et al.[127] indicated an interval from 5 to 12 μg/g.

A range of 90 to 160 μg/g for V was ascertained by Bederka et al.[128] and by Heydorn.[168] Creason et al.[136] reported values of 0.2 μg/g for males and 0.37 for females in subjects aged 1 to 15 years, while for adults there was an interval from 0.072 to 2.2 μg/g.

For Zn an RV of 175 μg/g was reported by Iyengar.[49]

The overall ranges of RVs for the various elements are summarized in Table 6.

2. Organs

The role and fate of trace elements greatly differ depending on the specific organ considered. From this standpoint, kidneys, liver, and lungs should be considered of particular importance as they are, respectively, the site of excretion, of metabolic transformation, and of entrance of airborne substances. No wonder that these three organs were, more frequently than others, the subject of studies aimed at establishing RVs.

a. Kidneys

In an investigation undertaken to define element concentrations and to attempt correlations among element contents in different human organs (kidneys, liver, and lungs) Coni et al.[169] proposed RVs for Al, Ba, Cd, Cr, Cu, Li, Mg, Mn, Ni, Pb, Sr, and Zn as evaluated from a total of 36 autoptic specimens of urban subjects. In order to guarantee reliable data, strict criteria for sampling, pretreatment, and analytical conditions were set. Only in a few cases did the variability exceed 50% of median, with the widest excursion showed by pulmonary tissues.

The group headed by Zhuang and Cheng,[170,171] in the framework of a study aimed at clarifying the relationship between the concentration of various elements in hair and organs, determined levels of As, Cd, Hg and Se in the kidney cortex of 24 male persons (35 to 60 years of age) who died accidentally. A significant positive correlation was found between the As concentration in the kidney cortex (0.004 to 0.065 μg/g, wet weight) and that in the hair (correlation coefficient, $r = 0.751$), with an element ratio of hair to kidney equal to 4.10.

TABLE 6
Overall Concentration Ranges for Selected Elements in Hair as Reported in the Literature

Element	Conc	Element	Conc
Ag	0.16 – 0.7 μg/g	Mg	1.49 – 567 μg/g
Al	0.1 – 36 μg/g	Mn	0.04 – 24 μg/g
As	0.03 – 25 μg/g	Mo	0.03 – 2.16 μg/g
B	0.88 – 0.98 μg/g	Na	0.02 – 2.02 mg/g
Ba	0.76 – 1.41 μg/g	Ni	0.002 – 4.05 μg/g
Br	2 – 35 μg/g	P	88.9 – 773 μg/g
Ca	0.17 – 4.69 mg/g	Pb	0.004 – 95 μg/g
Cd	0.04 – 5.3 μg/g	Pd	< 0.02 μg/g
Cl	0.12 – 14 mg/g	Pt	⩽ 0.05 μg/g
Co	0.07 – 1.7 μg/g	Rb	0.06 – 5.34 μg/g
Cr	0.08 – 2.50 μg/g	S	773 μg/g
Cs	0.05 – 1 μg/g	Sb	0.05 – 0.06 μg/g
Cu	6.0 – 293 μg/g	Se	0.002 – 6.6 μg/g
Fe	10 – 900 μg/g	Sn	0.036 – 8.30 μg/g
Ga	1 – 250 μg/g	Sr	1.7 – 860 μg/g
Hg	0.3 – 12.2 μg/g	Ti	0.13 – 12 μg/g
I	0.03 – 4.2 μg/g	V	0.04 – 160 μg/g
K	4 – 700 μg/g	Zn	53.7 – 327 μg/g
Li	9 – 460 ng/g		

Concentration levels of Cd deemed normal were 4.43 to 65.6 (mean, 24.5) μg/g (wet weight).[170] A large portion of Cd absorbed by the organism is concentrated in renal tissues and in the liver, in association with metallothioneins. An expected increase in Cd concentration of kidney tissues also could be observed in smokers. In this context a measure of the critical concentration of Cd in the kidney cortex was proposed.[172] This measure is the so-called population critical concentration (PCC), which has a clearly specified rate. For example, the PCC-10 is the concentration of Cd (180 to 220 μg/g, wet weight) that produces a given response in 10% of the population.

As regards Co, an extensive literature compilation updated to 1980 pointed to a representative value of 0.1 μg/g (dry weight).[81]

Zhuang et al.[170] reported the Hg content in kidney tissues, as determined in 24 Chinese subjects, to range from 4.43 to 65.6 (mean, 24.5) μg/g (wet weight).

According to Oster et al.[173], on a per-weight unit basis, Se is more concentrated in kidneys than in other organs, even if the renal content corresponds to only 4% of total Se body burden. In fact, about 50% of this element is judged to be stored in the skeletal muscle mass. This evidence was obtained from an investigation on the content of the element in various tissues of German victims of traffic accidents. The 14 subjects under study were aged 19 to 61 years, with a mean of 39 years, and showed Se kidney levels of 771 ± 169 ng/g (wet weight). This is comparable to the Se content in a New Zealand population, a low-Se country, and significantly lower than that observed in organs of American, Canadian, and Japanese people. A similar value (0.75 μg/g, wet weight) was found by Cheng et al.[171]

These sets of data are displayed in Table 7.

b. Liver

In a reevaluation of literature data of element values in clinical specimens Iyengar and Woittiez[92] identified reliable baseline data for liver concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, and Zn.

TABLE 7
Overall Reference Concentration Ranges for Selected Elements in Kidneys as Reported in the Literature

Element	Conc	Element	Conc
Al	0.55 – 1.31 μg/g	Li	0.01 – 0.05 μg/g
As	4.00 – 65.0 ng/g	Mg	68.5 – 98.1 μg/g
Ba	0.04 – 0.19 μg/g	Mn	0.28 – 0.60 μg/g
Cd	3.24 – 65.6 μg/g	Ni	0.01 – 0.16 μg/g
Co	≈ 0.1 μg/g	Pb	0.11 – 0.41 μg/g
Cr	0.04 – 0.09 μg/g	Se	≈ 0.8 μg/g
Cu	1.17 – 2.15 μg/g	Sr	0.04 – 0.12 μg/g
Hg	4.45 – 65.6 μg/g	Zn	15.0 – 31.8 μg/g

The main objective of the authors was to focus as much as possible on the quality of the results rather than to include a large quantity of data. For other elements, such as Al, Br, F, I, Ni, and V, limited credible information was available. For B, Li, Si, Sn, and U reliable information as of 1988 was very scarce.

A comprehensive approach was attempted by Zeisler and co-workers[174] to determine seven elements in human liver. The approach was to apply a combination of modified instrumental NAA with or without radiochemical separations. The specimens were investigated within the framework of the program of the National Environmental Specimen Bank. Thirty human livers were analyzed and data critically compared as to Ag, As, Cr, Mo, Sb, Sn, and Se levels. Liver reference values for 12 elements (Al, Ba, Cd, Cr, Cu, Li, Mg, Mn, Ni, Pb, Sr, and Zn) were also ascertained in autoptic samples of 36 urban Italian subjects.[169]

As regards Al, substantial progress has been made in mastering contamination problems during the analysis of biological matrices. In spite of this, analytical conditions should be further optimized before a definitive assessment can be attempted. According to Zeisler and associates,[175] Al concentrations in liver range from 0.3 to 2.0 μg/g. Other authors found ranges of 1.06 to 1.82, 1.08 to 2.16 and 1.00 to 2.45 μg/g (10th to 90th percentiles, wet weight) for female and male nonsmokers and male smokers, respectively.[169]

Baseline levels of 0.027 to 0.22 μg/g (wet weight) of As were found in the kidney cortex of 24 male persons (35 to 60 years old), who died accidentally.[170]

Versieck[54] determined normal levels of Br in the liver by means of NAA, with values of 1 to 2 μg/g.

As regards Cd, concentrations of 0.790 μg/g (interval 0.280 to 1.16 μg/g, wet weight) and 2.62 μg/g (interval 0.51 to 6.13 μg/g) were proposed as RVs by two different teams.[169,170]

Information on normal Co liver content shows wide variability and may be referred, at least partially, to different intakes with food. This organ, in fact, stores vitamin B_{12}, the biologically active Co compound. However, from an extensive literature compilation updated to 1980, a representative value of 1.0 μg/g (dry weight) was given.[81]

Analytical problems still considerably affect the determination of Cr, and thus no reliable RV can be indicated. Concentrations between 5 and 10 μg/kg were suggested by Iyengar.[49] On the other hand, a mean of 98 μg/kg was found more recently for inhabitants of an heavily industrialized area.[169]

Cs is a metal conveniently determined by means of radiochemical NAA. Using this technique Lievens and co-workers[176] ascertained a range of 10 to 20 μg/kg for this element in the liver of normal subjects.

For Hg, a baseline value of 0.11 μg/g (interval 0.027 to 0.22 μg/g) in the liver from Chinese subjects could be identified.[170]

Very few data are reported for the normal levels of I in liver as well as in other body compartments.[84,177] In any case the con-

centration of this element in fluids and tissues is strictly correlated with the dietary status. Therefore, information on food intake becomes mandatory when evaluating experimental data on I.

Similarly, the concentration of Se in fluids and tissues is influenced by even short-term variations in intake.[92] Oster and coworkers[173] reported a mean value of 291 ± 78 ng/g (wet weight) based on an investigation of 18 German victims of traffic accidents, aged 19 to 61 years (mean, 39 years). Analogous results were obtained by Cheng et al.[171] in Chinese subjects with values of 0.43 μg/g (interval 0.23 to 0.65 μg/g) (wet weight).

From data on 27 subjects living in New York a mean value of 0.2 ± 0.3 μg/kg of U was measured by Fissenne and Welford[178] with alpha spectrometry.

Finally, V is probably present at very low concentrations in clinical samples, although contamination problems hinder reliable determination. From studies carried out by means of NAA techniques, which are particularly suited to obtain accurate results, reasonable estimates of 5 to 10 μg/kg can be assessed.[176,179]

Table 8 collects the information avalaible for RVs in liver.

c. Lungs

A critical review of published data for 67 elements was carried out by Vanoeteren et al.[180] in 1986 to assess baseline concentrations in lung tissues. The authors found serious inconsistencies, only part of them explainable by the effect of inhomogeneous distribution of elements throughout the organ or differences in the population. Thus, only for a group of elements, including Ag, Al, As, Br, Ca, Cd, Cl, Co, Cr, Cs, Cu, Fe, K, Mg, Mo, Na, P, Pb, Rb, Se, and Zn, could indicative baseline data be reported. On the other hand, information on B, I, In, Li, Nb, S, Te, and Y was found to be insufficient for inclusion in the list of indicative values, while for all other elements simply speculative values were suggested.

Matsumoto et al.[181] ascertained the levels of Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, P, Si, Ti, and Zn in autoptic samples from 48 subjects (main age, 47.4 years). High correlations also were found between pairs of elements in each of the groups K, Mg, P, and Zn; Al, Si, and Ti; Cr, Fe, and Mn; and Cd and Zn.[182]

A series of investigations was undertaken to assess trace element concentrations in

TABLE 8
Overall Reference Concentration Ranges for Selected Elements in Liver as Reported in the Literature

Element	Conc	Element	Conc
Ag	⩽ 1.00 – 34.0 ng/g	Mg	91.6 – 134.0 μg/g
Al	1.00 – 2.45 μg/g	Mn	0.50 – 2.10 μg/g
As	2.00 – 53.0 ng/g	Mo	0.332 – 1.41 μg/g
Ba	0.05 – 0.16 μg/g	Ni	9.00 – 380 ng/g
Br	1.00 – 2.00 μg/g	Pb	0.250 – 2.30 μg/g
Cd	0.28 – 6.13 μg/g	Sb	2.00 – 24.0 ng/g
Co	6.0 – 151 ng/g	Se	0.19 – 0.70 μg/g
Cr	8.00 – 160 ng/g	Sn	0.05 – 2.42 μg/g
Cs	10.0 – 20.0 ng/g	Sr	0.01 – 0.04 μg/g
Cu	2.48 – 9.90 μg/g	Zn	3.20 – 93.3 μg/g
Fe	46.0 – 307 μg/g	U	≈ 0.20 ng/g
Hg	0.033 – 0.49 μg/g	V	5.00 – 10.0 ng/g
Li	0.01 – 0.06 μg/g		

lungs and other human organs of inhabitants of Italian urban sites, for example, Rome and Terni, the latter being a medium-sized industrial city in central Italy. The elements considered were Al, B, Ba, Cd, Co, Cr, Cu, Li, Mg, Mn, Ni, Pb, Si, Sr, V, and Zn. All subjects (13 for Rome and 36 for the Terni area) were persons deceased from causes not related to lung pathologies and with no occupational exposure history.[33,169,182-184] The authors took into consideration all possible precautions to generate reliable analytical results; in fact, analysis-related factors such as sampling, storage, sample handling and pretreatment, as well as detailed information on tested subjects were systematically reported. The data obtained pointed out that there was a rather limited range of concentrations for each element in the whole pulmonary mass that in general did not exceed one order of magnitude. Moreover, with only a few exceptions, there were no significant differences between subjects from the two areas. Classification of the subjects on a smoking habit basis showed that elements such as Cr, Ni, and Sr were significantly higher in smokers. On the contrary, it was surprising that the Cd content was not significantly higher in smokers, as would be expected.[169]

Al is a clear example of enrichment in lung tissues due to airborne particles. This fact can partly justify the rather large spread of Al concentration in the pulmonary tissue as compared to other organs. Analytical problems cannot be excluded in the work of Sweet et al.,[185] who reported values as low as 0.012 μg/g, with a variation of more than four orders of magnitude for 100 U.S. inhabitants. In two other investigations,[169,185] thanks to the special care devoted to minimizing contamination and analytical errors, an Al mean concentration in lung tissue of 8.39 ± 2.5 μg/g and a 10th to a 90th percentile range of 1.61 to 11.5 μg/g could be estimated.

In the case of As, values are influenced by regional and food intake differences. Higher levels in liver and in other human tissues and fluids were found in some Far East countries, which may reflect excess intake as well as analytical problems.[49,180] A mean value of 0.02 μg/g (wet weight, interval 0.003 to 0.04 μg/g) was reported from an evaluation carried out on 24 Chinese subjects.[170]

For Ba, an RV for unexposed persons of 140 μg/kg (wet weight) with a 10th to a 90th percentile range equal to 0.060 to 320 μg/kg was suggested.[169]

As regards Br, literature data, although scarce, appear to be consistent, with ranges of 3.74 to 6.52 and 2.65 to 10.6 μg/g.[187,188] An analogous behavior is shown by Ca because this metal displays a relatively small variation in lung tissues. The data vary from 210 ± 76 μg/g, as reported by Bartsch et al.,[189] to 214.5 ± 142 μg/g, as determined by Teraoka[190] on 12 Japanese subjects, and to 88 ± 63.5 μg/g, as proposed by Tipton and associates[191] for a population of a Near East region.

Because of its toxicity, many literature data are available on Cd levels in lungs. From papers published over the last 15 years, baseline values were found to range from 0.016 μg/g for infants to 0.506 μg/g for persons aged 60 to 69 years from New Zealand.[192] Other authors[185] found a range of 0.016 to 1.3 μg/g, as estimated from 100 U.S. subjects. On the other hand, an interval of 0.005 to 0.274 μg/g was evaluated by Vuori et al.[193] for inhabitants of Finland. The lowest RV reported is 0.00378 μg/g and is the mean of 23 determinations of infant tissues.[194] In a study aimed at correlating element concentration in various organs from Chinese victims of traffic accidents, Zhuang and co-workers[170] found values from 0.03 to 1.28 (mean, 0.36) μg/g (wet weight), with an individual difference of more than one order of magnitude. These authors found a significant increase in Cd content in lungs depending on smoking habits. This pattern was not confirmed by Coni et al.,[169] according to whom an RV of 0.18 μg/kg (wet weight) was more acceptable.

An RV for Co of 0.2 μg/g (dry weight) was estimated from an extensive literature compilation.[81] A large interval of between 0.0006 and 2.64 μg/g was observed by other authors.[180] The lowest values, from 0.0006 to 0.84 μg/g, were obtained by Brown and Tay-

lor,[195] who analyzed more than 100 specimens from normal subjects by spark source mass spectrometry. A similar variation of more than three orders of magnitude is shown by the results of Sweet et al.,[185] from 0.0001 to 0.84 μg/g, while Vanoeteren et al.[188] determined a Co concentration varying between 0.0021 and 0.058 μg/g.

The influence of environment on Cr lung concentration is clearly illustrated by literature data. In fact, the large spread of Cr in lungs can be explained through different exposure levels. The Cr content was determined in lung samples taken through autopsy of 50 subjects, randomly selected from rural and urban areas and not known to have been professionally exposed.[196] As a rule, the Cr levels were well correlated with age. On the average, for subjects younger than 40 years the concentration of Cr was approximately 2 μg/g, while for older people Cr ranged from 5 to 15 μg/g (dry weight). Lower concentration data are usually reported by other authors. Kollmeier and co-workers[197] ascertained a value of 0.7 ± 0.5 μg/g (dry weight). A mean of 0.506 μg/g was also proposed.[169] Levels of 1.5 ± 2.0 μg/g (dry weight) in lung tissue from 30 subjects were also reported.[198] Reference values of 0.117 to 0.57 μg/g for Belgian subjects, and of 0.231 to 0.39 μg/g for Japanese individuals, were given.[188-190,199] A range of 0.004 to 5.2 μg/g, also was reported.[185]

As a rule, literature data on Cs refer to occupational or heavily industrialized conditions. The results of Vanoeteren et al.[188] indicate the range of 0.00315 to 0.0123 μg/g as the baseline for the normal population.

Cu appears to be present in normal lung tissue at a level of some μg/g, varying from 1.50 μg/g for Belgians to 4.78 ± 3.89 μg/g for Indian subjects.[189,200,201] A range for U.S. inhabitants of 0.166 to 12.4 μg/g was ascertained by Sweet et al.[185] A total of 36 subjects from an industrialized area in central Italy was investigated and an interval of 0.710 to 1.65 μg/g (wet weight) was reported.[169]

Despite being the most abundant heavy metal in the human organism, Fe is subject to some procedural errors when its levels in lungs are quantified. These errors are due mainly to the fact that the residual postmortem blood present in tissues may considerably alter the actual element concentration. For this reason, the literature data display a variation of more than two orders of magnitude. Vanoeteren et al.[188] ascertained a mean value of 173 ± 183 μg/g; however, this value was affected by high variations of 40 to 1170 μg/g. As a rule, RVs between 100 and 300 μg/g were obtained.[189,190,199,200]

Hg also shows a large variability in pulmonary tissues. The metal RV in lungs was proposed to range from 0.003 to 0.04 μg/g (mean value, 0.02 μg/g, wet weight) from a study on 24 Chinese subjects.[170]

The considerations made above also hold for K. Literature data go from 1000 to 2000 μg/g, a range that can be accepted as the baseline.[189,199,200]

With the exception of the 1972 paper by Hamilton and co-workers,[90] and reporting for Li a mean of 0.06 ± 0.03 μg/g, for years no further RVs were assessed for this element. More recently, other authors[169] obtained from the analysis of 36 subjects a mean value of 0.073 μg/g (wet weight), with a 10th to a 90th percentile interval of between 0.030 and 0.090 μg/g.

As regards Mg, an RV in the interval between 20 and 200 μg/g appears reasonable.[185,199,201] Another group found an overall mean of 62.2 μg/g, with a 10th to a 90th percentile range of 43.9 to 79.9 μg/g.[169]

Literature data for Mo display a considerable variation—about three orders of magnitude. Furthermore, some of the reported RVs are close to detection limits, thus pointing to inadequate analytical methodologies.[185,187,191] The lowest ranges obtained are < 0.02 to 0.05[202] and 0.008 to 0.74 μg/g.[185] The highest RV was assessed by Vanoeteren et al.,[188] 0.110 to 1.10 μg/g.

A general consensus appears to mark Na with an RV of between 1 and 3.5 mg/g.[188,199,201]

On the other hand, there is no agreement among published data on Ni content in lungs, as this analysis is strongly affected by detection problems as well as by considerable contamination due to ambient air. Average RVs of 0.08 ± 0.05, 0.12 ± 0.05, and 0.16 ±

0.094 μg/g were reported.[190,200,202] A range of 0.0086 to 0.0722 μg/g was ascertained by Kollmeier et al.[197] Caroli and his team[169] reported a mean value of 0.22 μg/g, with a 10th to a 90th percentile range from 0.055 to 1.79 μg/g.

For Pb, an RV of 0.279 μg/g (with a 10th to a 90th percentile range of 0.142 to 0.380 μg/g) was given for subjects of an industrialized area of Italy.[169] Data of other authors confirm the susceptibility of the Pb content in lungs to the pollution of living or working environments. Such RVs vary from 10 ng/g for young people or inhabitants of nonpolluted areas, to 0.5 μg/g for heavily or long-term exposed subjects.[194,202-205]

The available information on Rb shows concentration values varying from 0.5 to 6.0 μg/g, with a mean around 2.0 μg/g (wet weight).[187,188,199,200]

In a comparison of enzyme activities and Se content in normal and neoplastic human adult lung tissue, normal Se levels were determined in 18 control subjects aged from 41 to 75 years (mean, 57 years).[206] Baseline Se concentration was 148 ± 71 ng/g (wet weight, with a range of between 7 and 275 ng/g). Oster et al.[173] determined Se content in the lungs of German victims of traffic accidents. The 14 subjects studied were aged 19 to 61 years, with a mean of 39 years, and showed an Se level of 132 ± 33 ng/g (wet weight). This value is comparable to the Se content in the population of New Zealand, while significantly lower than that observed in lungs of American, Canadian, and Japanese people. Regarding the Chinese, Cheng et al.[171] reported an Se RV in 0.19 μg/g lungs (0.1 to 0.33 μg/g, wet weight) from a study on 24 subjects.

Few data are available on the Sr levels in lungs. Reference values reported are 0.2 and 0.09 μg/g.[169,189,190]

As regards Ti, striking differences were noted in lung tissue depending on geographic residence.[63] Snyder and co-workers[82] reported an RV of 220 μg/g for U.S. inhabitants.

Finally, an abundant literature exists on RVs for Zn in lungs, the mean value lying in the interval between 1 and 30 μg/g, as suggested by Vanoeteren et al.[180] The most reliable RV appears to be ca. 10 μg/g.[169,188-190,192]

The information detailed above for element RVs in lungs is summarized in Table 9.

IV. CONCLUSIONS

The diversity of RVs retrievable from the literature on this subject clearly reflects the variety of specific local situations. In this sense diversity is entirely acceptable, being inherent in the very concept of baseline figures for elements in biological systems. On

TABLE 9
Overall Reference Concentration Ranges for Selected Elements in Lung Tissue as Reported in the Literature

Element	Conc	Element	Conc
Al	2.21 - 15.3 μg/g	K	1.00 - 2.00 mg/g
As	0.03 - 0.04 μg/g	Li	3.00 - 9.00 ng/g
B	≈ 265 μg/g	Mg	20.0 - 200 μg/g
Ba	0.06 - 0.32 μg/g	Mn	0.02 - 1.58 μg/g
Br	2.65 - 10.6 μg/g	Mo	≤ 2.00 - 1,100 ng/g
Ca	124.3 - 214.5 μg/g	Na	1,000 - 3,500 μg/g
Cd	4.00 - 1,300 ng/g	Ni	0.055 - 1.79 μg/g
Co	0.10 - 2,640 ng/g	Pb	1.00 - 500 ng/g
Cr	4.00 - 15,000 ng/g	Rb	0.50 - 6.00 μg/g
Cs	3.00 - 12.0 ng/g	Sr	0.040 - 0.160 μg/g
Cu	0.16 - 12.4 μg/g	Ti	0.31 - 220 μg/g
Fe	100 - 300 μg/g	Zn	1.00 - 30.0 μg/g
Hg	3.00 - 40.0 ng/g		

the other hand, it cannot be ignored that discrepancies may well be caused by poor, if not definitely erroneous, and in any case certainly not harmonized approaches to the assessment of RVs. The availability today of sophisticated and extremely powerful analytical techniques further complicates this investigational area in that users are not always fully aware of the pitfalls and limitations posed by them, while at the same time they are dazzled by the ease and rapidity with which an enormous mass of experimental data can be generated.

With this in mind, further, substantial progress in the achievement of sound and comparable RVs is to be envisaged mainly through the adoption of internationally agreed upon procedural schemes and a common will to fill existing gaps in present knowledge. Preventive medicine can only benefit from the progressive accomplishment of these goals.

ACKNOWLEDGMENT

The patience and expertise of Mrs. Anna Maria Chierici Castelli in typing the various drafts of this monograph are gratefully acknowledged.

REFERENCES

1. *OECD and the Environment*, Organization for Economic Cooperation and Development, Paris, 1986.
2. **Huismans, J. W.,** The international register of potentially toxic chemicals, *Ecotoxicol. Environ. Safety*, 4, 393, 1980.
3. **EEC Directive 79 / 831**, *Off. J. Eur. Comm.*, 22, 1979.
4. **Prasad, A. S.,** Role of zinc in humans, in *Ultratrace Metal Analysis in Biological Sciences and Environment*, Adv. Chem. Ser., No. 172, Risby, T. H., Ed., American Chemical Society, Washington, D.C., 1979, 197.
5. **Kurland, L. T., Faro, S. N., and Seidler, H.,** Minamata disease, *World Neurol.*, 1, 370, 1970.
6. **Mediterranean Health Related Environmental Quality Criteria. Report on a Joint Group** WHO/FAO/UNEP, Meeting in Bled (Yugoslavia), 12–16 September 1988.
7. **Solberg, H. E.,** The theory of reference values. Part 1. The concept of reference values, *Clin. Chim. Acta*, 165, 111, 1987.
8. **PetitClerc, C., and Wilding, P.,** The theory of reference values. Part 2. Selection of individuals for the production of reference values, *Clin. Chim. Acta*, 139, 205F, 1984.
9. **Solberg, H. E.,** The theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits, *Clin. Chim. Acta*, 139, 97F, 1984.
10. **Dybkaer, R.,** The theory of reference values. Part 6. Presentation of observed values related to reference values, *Clin. Chim. Acta*, 127, 441F, 1983.
11. **Berg, B., Nilsson, J. E., Solberg, H. E., and Tryding, N.,** Practical experience in selection and preparation of reference individuals: empirical testing of the provisional Scandinavian recommendations, in *Reference Values in Laboratory Medicine. The Current State of the Art*, Gräsbeck, R., and Alström, T., Eds., John Wiley & Sons, Chichester, 1981, 55.
12. **Reed, A. H., Cannon, D. C. Winkelman, J. W., Bhasin, Y. P., Henry, R. J., and Pileggi, V. J.,** Estimation of normal ranges from a controlled sample survey. I. Sex and age-related influence on the SMA 12/60 screening group of tests, *Clin. Chem.*, 18, 57, 1972.
13. **Wilding, P., Rollason, J. G., and Robinson, D.,** Patterns of changes for various biochemical constituents detected in well population screening, *Clin. Chim. Acta*, 41, 375, 1972.
14. **Alström, T., Gräsbeck, R., Hjelm, M., and Skandsen, S.,** Recommendations concerning the collection of reference values in clinical chemistry and activity. Report by the Committee on Reference Values of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, *Scand. J. Clin. Lab. Invest.*, 35(Suppl.), 144, 1975.
15. **Winkel, P., and Statland, B. E.,** Reference values, in *Clinical Diagnosis and Management by Laboratory Methods*, Henry, J. B., Ed., W. B. Saunders, Philadelphia, 1979, 29.
16. **Solberg, H. E.** Statistical treatment of collected reference values and determination of reference limits, in *Reference Values in Laboratory Medicine*, Gräsbeck, R., and Alström, T., Eds., John Wiley & Sons, Chichester, 1981, 193.
17. **Solberg, H. E.,** The theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits, *Clin. Chim. Acta*, 137, 97F, 1984.
18. **Boyd, J. C., and Lacher, D. A.,** A multistage gaussian transformation algorithm for clinical laboratory data, *Clin. Chem.*, 28 1735, 1982.
19. **Box, G. E. P., and Cox, D. R.,** An analysis of transformations, *J. R. Stat. Soc. Ser. B*, 26, 211, 1964.
20. **Elveback, L. R., Guiller, C. L., and Keating, F. R.** Health, normality and the ghost of Gauss, *JAMA*, 211, 69, 1970.

21. **Stephens, M. A.,** EDF statistics for goodness of fit and some comparison, *J. Am. Stat. Assoc.*, 69, 730, 1974.
22. **Elveback, L. R., and Taylor, W. F.,** Statistical methods of estimating percentiles, *Ann. N. Y. Acad. Sci.*, 161, 538, 1969.
23. **Gabrielli, E. R.,** Enhancing the meaning of clinical laboratory data, *Crit. Rev. Clin. Lab. Sci.*, 1, 65, 1970.
24. **Starmer, C. F., and Lee, K. L.,** A mathematical approach to medical decisions: application of Bayes' rule to a mixture of continuous and discrete clinical variables, *Comput. Biomed. Res.*, 9, 531, 1976.
25. **Caroli, S.,** The accuracy syndrome in trace element analysis of biological samples, in *Proceedings of the 5th Italo-Hungarian Symposium on Spectrochemistry: Quality Control and Assurance in Life Sciences, Pisa 9–13 September 1991*, Caroli, S., and Fuoco, R., Eds., *Microchem. J.*, 45, 257, 1992.
26. **Statland, B. E., and Winkel, P.,** Effects of pre-analytical factors on laboratory test results, *Crit. Rev. Clin. Lab. Sci.*, 8, 105, 1977.
27. **Young, D. S., and Bermes, E. W.,** Specimen collection and processing and sources of biological variation, in *Textbook of Clinical Chemistry*, Tietz, N. W., Ed., W. B. Saunders Philadelphia, 1986, 478.
28. **Katz, S.A.,** Collection and preparation of biological tissues and fluids for trace elements analysis, *Int. Biotechnol. Lab*, 3, 10, 1985.
29. **Masironi, R., and Parr, R. M.,** in *Proceedings of the International Workshop on Biological Specimens Collection*, Luxembourg, April 1977.
30. **Vanoeteren, C., Cornelis, R., Versieck, J., Hoste, J., and De Roose, J.,** Trace element patterns in human lung tissues, *J. Radioanal. Chem.*, 70, 219, 1982.
31. **Koch, H. J., Jr., Smith, E. R., Shimp, N. F., and Connor, J.,** Analysis of trace elements in human tissues, *Cancer*, 91, 499, 1956.
32. **Forssén, A.,**Inorganic elements in the human body, *Ann. Med. Exp. Biol. Fenn.*, 50, 99, 1972.
33. **Caroli, S., Coni, E., Alimonti, A., Beccaloni, E., Sabbioni, E., and Pietra, R.,** Determination of trace elements in human lungs by ICP-AES and NAA, *Analusis*, 16(9–10 Suppl.), 75, 1988.
34. **Iyengar, G. V., and Gopal-Ayengar, A. R.,** Human health and trace elements including effects on high-altitude populations, *Ambio*, 17, 31, 1988.
35. **Adriano, D. C.,** *Trace Elements in the Terrestrial Environment*, Springer-Verlag, New York, 1986.
36. **Lag, J.,** A comparison of Se deficiency in Scandinavia and China, *Ambio*, 13, 286, 1984.
37. **Thornton, I.,** Ed., *Applied Environmental Geochemistry*, Academic Press, London, 1983.
38. **Iyengar, G. V.,** Reference values for elemental concentrations in some human samples of clinical interest: a preliminary evaluation, *Sci. Total Environ.*, 38, 125, 1984.
39. **Minoia, C., Sabbioni, E., Apostoli, P., Pietra, R., Pozzoli, L., Gallorini, M., Nicolaou, G., Alessio, L., and Capodaglio, E.,** Trace element reference values in tissues from inhabitants of the European Community. I. A study of 46 elements in urine, blood and serum of Italian subjects, *Sci. Total Environ.*, 95, 89, 1990.
40. **Sabbioni, E., Apostoli, P., and Minoia, C.,** Assessment of reference values for 22 trace elements in biological fluids by GFAAS with Zeeman correction, in *Applications of Zeeman Graphite Furnace Atomic Absorption Spectrometry in the Chemical Laboratory and in Toxicology*, Minoia, C., and Caroli, S., Eds., Pergamon Press, Oxford, 1992, 351.
41. **Carson, B. L., Ellis, H. V., III, and McCann, J. L.,** Eds., *Toxicology and Biological Monitoring of Metals in Humans, Including Feasibility and Need*, Lewis Publishers, Chelsea, MI, 1986.
42. **Versieck, J.,** Trace element analysis. A plea for accuracy, *Trace Elem. Med.*, 1, 2, 1984.
43. **Cedergren, A., and Ferewck, W.,** Determination of aluminum in biological materials by graphite-furnace AAS, *Pure Appl. Chem.*, 59, 221, 1987.
44. **Morisi, G., Patriarca, M., and Taggi, F.,** Evaluation of the performance of Italian laboratories in the determination of cadmium levels in blood, in *Bioelements: Health Aspects*, Caroli, S., Iyengar, G. V., and Muntau, H., Eds., *Ann. Ist. Super. Sanità*, 25, 449, 1989.
45. **Morisi, G., Patriarca, M., and Taggi, F.,** Comparable laboratory performances in the analysis of lead in control samples and in fresh human blood, in *Bioelements: Health Aspects*, Caroli, S., Iyengar, G. V., and Muntau, H., Eds., *Ann. Ist. Super. Sanità*, 25, 417, 1989.
46. **Morisi, G., Patriarca, M., and Taggi, F.,** The interlaboratorial quality assurance program for blood lead determination. An evaluation of methods and results, in *Bioelements: Health Aspects*, Caroli, S., Iyengar, G. V., and Muntau, H., Eds., *Ann. Ist. Super. Sanità*, 25, 405, 1989.
47. **Menditto, A., Patriarca, M., and Morisi, G.,** Organization of interlaboratory quality assurance programs for the analysis of trace elements in blood: evaluation of procedures and analysis of data, in *Proceedings of the 5th Italo-Hungarian Symposium on Spectrochemistry: Quality Control and Assurance in Life Sciences, Pisa 9–13 September 1991*, Caroli, S., and Fuoco, R., Eds., *Microchem. J.*, 46, 137, 1992.
48. **Patriarca, M., Menditto, A., and Morisi, G.,** The performance of Italian laboratories in the determination of trace elements in blood, in *Proceedings of the 5th Italo-Hungarian Symposium on Spectrochemistry: Quality Control and Assurance in Life Sciences, Pisa 9–13 September 1991*, Caroli, S., and Fuoco, R., Eds., *Microchem. J.*, 46, 147, 1992.
49. **Iyengar, G. V.,** Concentrations of 15 trace elements in some selected adult human tissues and body fluids of chemical interest from several countries: results from a pilot study for the estab-

lishment of reference values, Report of the Nuclear Research Center, Jülich, 1985.
50. **Iyengar, G. V.**, Regional reference range of elemental concentration levels in some adult samples of clinical interest: a preliminary evaluation, in *Chemical Toxicology and Clinical Chemistry of Metals*, Brown, S. S., and Savory, J., Eds., Academic Press, New York, 1989, 153.
51. **Clarke, W. B., Koekebakker, M., and Barr, R. D.**, Analysis of ultratrace lithium and boron by neutron activation analysis and mass spectrometric measurement of helium-3 and helium-4, *Appl. Radiat. Isot.*, 38, 735, 1987.
52. **Reeves, A. L.**, Barium, in *Toxicology of Metals*, Vol. 2, National Technical Information Service, Springfield, VA, 1977, 71.
53. **Woittiez, J. R. W.**, *Elemental Analysis of Human Serum and Serum Protein Fractions by Thermal Neutron Activation*, Dissertation, University of Amsterdam, Amsterdam, 1983.
54. **Versieck, J.**, Accuracy in trace element analysis, *Crit. Rev. Clin. Lab. Sci.*, 22, 97, 1984.
55. **Krivan, V., Geiger, H., and Franz, H. E.**, Spurenelemente in Plasma und Erythrozyten von niereninsuffizienten Patienten und Haemodialysepatienten, *Verh. Dtsch. Ges. Inn. Med.*, 89, 909, 1983.
56. **Caroli, S., Alimonti, A., Delle Femmine, P., Petrucci, F., Senofonte, O., Violante, N., Menditto, A., Morisi, G., Menotti, A., and Falconieri, P.**, Role of inductively coupled plasma atomic emission spectrometry in the assessment of reference values for trace elements in biological fluids, *J. Anal. At. Spectrom.*, 7, 859, 1992.
57. **Friberg, L., and Vahter, M.**, Assessment of exposure to lead and cadmium through biological monitoring: results of a UNEP/WHO global study, *Environ. Res.*, 30, 95, 1980.
58. **Iyengar, G. V.**, Radiochemical separations for inorganic trace elements in some biological reference materials, foods, tissues, and body fluids, *J. Radioanal. Nucl. Chem.*, 110, 503, 1987.
59. **Alessio, L., Apostoli, P., and Ferioli, A.**, Identification of reference values for metals in general population groups, *Toxicol. Environ. Chem.*, 27, 39, 1990.
60. **Cole, C. J., and Carson, B. L.**, Cobalt in the food chain, in *Trace Metals in the Environment*, Vol. 6, *Cobalt*, Smith, I. C., and Carson, B. L., Eds., Ann Arbor Science Publishers, Ann Arbor, MI, 1980, 925.
61. **Greenberg, R., and Zeisler, R.**, A radiochemical procedure for ultratrace determination of chromium in biological materials, *J. Radioanal. Nucl. Chem.*, 124, 5, 1988.
62. **Underwood, E. J.**, *Trace Elements in Human and Animal Nutrition, 4th ed.*, Academic Press, New York, 1977.
63. **Stockinger, H. E.**, The metals, in *Patty's Industrial Hygiene and Toxicology*, 3rd ed., Vol. 2A, Clayton, G. D., and Clayton, F. E., Eds., John Wiley & Sons, 1981, 1493.
64. **Iyengar, G. V., and Kollmer, W. E.**, Some aspects of sample procurement from human subjects for biomedical trace element research, *Trace Elem. Med.*, 3, 25, 1986.
65. **Henry, R. J.**, *Clinical Chemistry: Principles and Technics*, Harper and Row, New York, 1964.
66. **Crounse, R. G., Pories, W. J., Bray, J. T., and Mauger, R. L.**, Geochemistry and man: health and disease. 1. Essential elements, in *Applied Environmental Geochemistry*, Thorntain, I., Ed., Academic Press, London, 1983, 309.
67. **Friberg, L., Nordberg, G. F., and Vouk, V. B.**, Eds., *Handbook on the Toxicology of Metals*, North-Holland, Amsterdam, 1979.
68. **Versieck, J., Hoste, J., Barbier, F., Vanballenberghe, L., De Rudder, J., and Cornelis, R.**, Determination of molybdenum in human serum by neutron activation analysis, *Clin. Chim. Acta*, 87, 135, 1978.
69. **Hopfer, S. M., Sunderman, F. W., Jr., and Fay, W. P.**, Nickel concentrations in body fluids of healthy adults and hemodialysis patients in Sudbury, Ontario, Canada, in *Trace Elements in Health and Disease, Extended Abstracts of the Nordic Symposium*, World Health Organization, Copenhagen, 1987, 74.
70. **Tsuchiya, K.**, Lead, in *Toxicology of Metals*, Vol. 2, National Technical Information Service, Springfield, VA, 977, 243.
71. **Morisi, G., Patriarca, M., Carrieri, M. P., Fondi, G., and Taggi, F.**, Lead exposure: assessment of the risk for the general Italian population, *Ann. Ist. Super. Sanità*, 25, 423, 1989.
72. **Morisi, G., Menditto, A., Spagnolo, A., Patriarca, M., and Menotti, A.**, Association of selected social environmental and constitutional factors to blood lead levels in men aged 55–75 years, *Sci. Total Environ.*, 126, 209, 1992.
73. **Elinder, C. G., and Friberg, L.**, Antimony, in *Toxicology of Metals*, Vol. 2, National Technical Information Service, Springfield, VA, 1977, 15.
74. **Clemente, G. F.**, Trace element pathways from environment to man, *J. Radioanal. Chem.*, 32, 25, 1976.
75. **Lambeck, I.**, The selenium state of healthy children, *Eur. J. Pediatr.*, 125, 81, 1977.
76. **Plantin, L. O., and Mauerling, S.**, The concentration of trace elements in blood from healthy newborns, in *Proceedings of Trace Element Analytical Chemistry in Medicine and Biology*, Vol. 1, Brätter. P., and Schramel, P., Eds., Walter de Gruyter, Berlin, 1980, 243.
77. **Verlinden, M.**, *De Bepaling van Selenium door Atomaire Absorptis Spectrometrie*, Dissertation, Universitaire Binstelling, Antwerp, 1981.
78. **Morisi, G., and Patriarca, M.**, Variations in the serum levels of Se according to age and sex in a

sample of Italian population, *Acta Chim. Hung.*, 128, 581, 1991.
79. Titanium, *IRPTC Bull.*, 6, 12, 1983.
80. **Simonoff, M., Conri, C., and Simonoff, G.**, Vanadium in depressive states, *Acta Pharmacol. Toxicol.*, 59, 463, 1986.
81. **Smith, I. C., and Carson, B. L.**, *Trace Metals in the Environment*, Vol. 3, *Zirconium*, Ann Arbor Science Publishers, Ann Arbor, MI, 1978.
82. **Snyder, W. S., Cook, M. J., Nasset, E. S., Karhausen, L. R., Howell, G. P., and Tipton, I. H.**, International Commission on Radiological Protection, *Report of the Task Group on Reference Man.*, 1975, 23.
83. **Jones, K. C., and Bennet, B. G.**, Exposure of man to environmental aluminium: an exposure commitment assessment, *Sci. Total Environ.*, 52, 65, 1986.
84. **Iyengar, G. V., Kollmer, W. E., and Bowen, H. J. M.**, *Elemental Composition of Human Tissues and Body Fluids*, Verlag Chemie, Weinheim, 1978.
85. **Cornelis, R., Specke, A., and Hoste, J.**, Neutron activation analysis of bulk and trace elements in urine, *Anal. Chim. Acta*, 78, 317, 1975.
86. **Wester, P. O.**, Trace element balances in relation to variations in calcium intake, *Atherosclerosis*, 20, 207, 1974.
87. **Jeszke, M., Letewka, B., and Kubiack-Limu, K.**, in *Proceedings of the 5th Trace Elements Symposium*, Karl Marx University, Leipzig, and Friedrich Schiller University, Jena 1986, 757.
88. **Lauwerys, R. R.**, Biological monitoring of exposure to inorganic and organometallic substances, in *Industrial Chemical Exposure: Guidelines for Biological Monitoring*, Davis, C. A., Ed., Biomedical Publications, Santa Ana, California, 1983, 9.
89. **United States Environmental Protection Agency**, Ambient Water Quality Criteria for Nickel, Pub. No. 81-117 715, National Technical Information Service, Springfield, VA, 1981
90. **Hamilton, E. I., Minsky, M. J., and Cleary, J.**, The concentration and distribution of some stable elements in healthy human tissues from the United Kingdom, *Sci. Total Environ.*, 1, 341, 1972–1973.
91. **Clemente, G. F., Rossi, L. C., and Santaroni, G. P.**, Studies on the distribution and related health effects of the trace elements in Italy, in *Trace Substances in Environmental Health*, XII, Hemphill, D. D., Ed., *Proceedings of University of Missouri's 12th Annual Conference on Trace Substances in Environmental Health*, June 6 to 8, University of Missouri, Columbia, 1978, 23.
92. **Iyengar, V. G., and Woittiez, J.**, Trace elements in human clinical specimens: evaluation of literature data to identify reference values, *Clin. Chem.*, 34, 474, 1988.
93. **Smith, I. C., and Carson, B. L.**, *Trace Metals in the Environment*, Vol. 1, *Thallium*, Ann Arbor Science Publishers, Ann Arbor, MI, 1977.
94. **Brockhaus, A., Dolgner, R., Ewers, U., Freier, I., Jermann, E., and Wiegand, H.**, Repeated surveillance of exposure to thallium in a population living around a cement plant emitting thallium containing dust, *Proceedings of the 3rd International Conference on Heavy Metals in the Environment*, 1981, 482.
95. **Kelly, W. R., Fasset, J. D., and Hotes, S. A.**, Determination of picogram quantities of uranium in human urine by thermal ionization mass spectrometry, *Health Phys.*, 52, 331, 1987.
96. **Minor and Trace Elements in Breast Milk**, Report of a Joint WHO/IAEA Collaborative Study, World Health Organization/International Atomic Energy Agency, Geneva/Vienna, 1989.
97. **Coni, E., Stacchini, A., Caroli, S., and Falconieri, P.**, Analytical approach to obtaining reference values for minor and trace elements in human milk, *J. Anal. At. Spectrom.*, 5, 581, 1990.
98. **Coni, E., Falconieri, P., Ferrante, E., Semeraro, P., Beccaloni, E., Stacchini, A., and Caroli, S.**, Reference values for essential and toxic elements in human milk, *Ann. Ist. Super. Sanità*, 26, 119, 1990.
99. **Parr, R. M.**, Quality assurance of trace element analysis, health effects and interactions of essential and toxic elements, *Nutr. Res.*, I (Spec. Suppl.) 5, 1985.
100. **Dang, H. S., Jaiswal, D. D., Wadkwani, C. N., and Somasundaram, S.**, Breast feeding: Mo, As, Mn, Zn, and Cu concentrations in milk of economically poor Indian tribal and urban women, *Sci. Total Environ.*, 44, 177, 1985.
101. **Kosta, L, Byrne, A. R., and Dermelj, M.**, Trace elements in some human milk samples by radiochemical neutron activation analysis, *Sci. Total Environ.*, 29, 261, 1983.
102. **Cortes-Toro, E.**, A Study of Trace Elements in Mother's Milk and Food for Suckling Infants, Final report to the IAEA on research contract No. 2738, IAEA, Vienna, 1984.
103. **Dang, H. S, Desai, H. B., Kayasth, S. R., Jaiswal, D. D., Wadhwani, C. N., and Somasundaram, S.**, Daily requirements of Fe, Co and Se during infancy, *J. Radioanal. Nucl. Chem.*, 84/1, 177, 1984.
104. **Parr, R. M.**, Problems of chromium analysis in biological materials: an international perspective with special reference to results for analytical quality control samples, *J. Radioanal. Chem.*, 39, 421, 1977.
105. **Kumpulainen, J., Lehto, J., and Koivistoinen, P.**, Determination of chromium in human milk, serum and urine by electrothermal atomic absorption spectrophotometry without preliminary ashing, *Sci. Total Environ.*, 31, 71, 1983.
106. **Casey, C. E. and Hambidge, K. M.**, Chromium in human milk from American mothers, *Br. J. Nutr.*, 52, 73, 1984.
107. **Belavady, B.**, Lipid and trace element composition of human milk, *Acta Paediatr. Scand.*, 67, 566, 1978.

108. **Esala, S., Vuori, E., and Helle, A.**, Effect of maternal fluorine intake on breast milk fluorine content, *Br. J. Nutr.*, 48, 201, 1982.
109. **Backer, O., Jongeling-Eindhoven, J. M. P. A., Flissebaalse, T. D., and Gedalia, I.**. Total and free ionic fluoride in human and cow's milk as determined by gas liquid chromatography and the fluoride electrode, *Caries Res.*, 8, 181, 1974.
110. **Kermarkar, M. G. and Ramakrishnan, C. V.**, Studies on lactation. Relation between the dietary intake of lactating women and the chemical composition of milk with regard to principle and certain inorganic constituents, *Acta Paediatr.*, 49, 599, 1960.
111. **Murray, M. J., Murray, A. B., Murray, N. J., and Murray, M. B.,**, The effect of iron status of Nigerian mothers on that of their infants at birth and 6 months, and on the concentration of Fe in breast milk, *Br. J. Nutr.*, 39, 627, 1978.
112. Composition of Mature Human Milk, Report on Health and Social Security, No, 12, Her Majesty's Stationery Office, London, 1977.
113. **Gushurst, C.A., Mueller, J. A., Green, J. A., and Sedor, F.**, Breast milk iodide: reassessment in 1980s, *Pediatrics*, 73, 354, 1984.
114. **Altman, P.L. and Dittmer, D. S.**, Eds., *Biology Data Book*, Federal American Society of Experimental Biology, Bethesda, MD, 1974.
115. **Muramatsu, Y., and Parr, R. M.**, Stable iodine contents in human milk related to dietary algae consumption, *Hoken Butsuri*, 18, 113, 1983.
116. **Vuori, E.**, Copper, Iron, Manganese and Zinc in Breast Milk, *Ph.D. thesis*, University of Helsinki, Helsinki, Finland, 1979.
117. **Vaughan, L. A., Weber, C. W., and Kemberkling, S. R.**, Longitudinal changes in the mineral content of human milk, *Am. J. Clin. Nutr.*, 32, 2301, 1979.
118. **Li, Z. J., Yoshinaga, J., Suzuki, T., Abe, M., and Morita, M.**, Mineral and trace element content of human transitory milk identified with inductively coupled plasma atomic emission spectrometry, *J. Nutr. Sci. Vitaminol.*, 36, 65, 1990.
119. **Okamoto, K., Morita, M., Quan, H., Uehiro, T, and Fuwa, K.**, Preparation and certification of human hair powder reference material, *Clin. Chem.*, 31, 1592, 1985.
120. **BCR CRM 397**, The Certification of the Contents (Mass Fraction) of Cd, Hg, Pb, Se and Zn in Human Hair, *Rep. EUR 13433 EN*, Luxembourg, European Communities Publications, 1991, 45.
121. **Kamakura, M.**, A study of the characteristics of trace elements in the hair of Japanese. Reference values and the trace element patterns for determining normal levels, *Jpn. J. Hyg.*, 38, 823, 1983.
122. **Nishiyama, K., Miki, T., Sakurai, S., Sumino, K., Suzuki, Y., Tanaka, S., Terai, M., Tomura, K., Tsunoda, H., Yoshikawa, H., Yasumoto, M., and Yukawa, M.**, Distribution of trace elements in normal human organs and tissues determined by neutron activation analysis, *2nd Research Coordinating Meeting*, International Atomic Energy Agency, Munich, 1985.
123. **Takey, Y., Matsuda, S., Imai, S., Ohmori, Y., Masuda, T., Vinson, J. A., Mehra, M. C., Puri, B. K., and Kaniewski, A.**, Trace elements in human hair: an international comparison, *Bull. Environ. Contam. Toxicol.*, 36, 793, 1986.
124. **Senofonte, O., Violante, N., Fornarelli, L., Beccaloni, E., Powar, A., and Caroli, S.**, Reference values for elements of toxicological, clinical and environmental interest in hair of urban subjects, *Ann. Ist. Super. Sanità, Bioelements: Health Aspects*, 25, 385, 1989.
125. **Caroli, S., Senofonte, O., Violante, N., Fornarelli, L., and Powar, A.**, Assessment of reference values for elements in hair of urban normal subjects, *Microchem. J.*, 46, 174, 1992.
126. **Clemente, G. F., Cigna Rossi, L., and Santaroni, G. P.**, Trace element intake and excretion in the Italian population, *J. Radioanal. Chem.*, 37, 549, 1977.
127. **Takeuchi, T., Hayashi, T., Takada, J., Koyama, M., Kozura, H., Tsuji, H., Kusaka, Y., Ohmori, S., Shinogi, M. et al.**, Survey of trace elements in hair of normal Japanese, *Proceedings of the Symposium on Nuclear Activation Techniques in the Life Sciences, IAEA*, Vienna, 1978, 545.
128. **Bederka, Jr., J. P., Lueken, T. M., Brudno, S., and Waters, R. S.**, Elemental balance in the human, in *Proceedings of the Conference on Trace Substances in Environmental Health*, Vol. 19, Hemphill, D. D., Ed., University of Missouri, Columbia, 1985, 304.
129. **Imahori, A., Fukushima, I., Shiobara, S., Tomura, K., Suzuki-Yasumoto, M., Yukawa, M., and Terai, M.**, A study on trace elements in hair of some local Japanese populations. I. Study on trace element concentration levels in human hair of inhabitants in Tokyo metropolitan area, *IAEA-SM-217 / 16, Proceedings of the Symposium on Nuclear Activation Techniques in the Life Sciences, IAEA*, Vienna, 1978, 563.
130. **Arunachalam, J., Gangadharan, S., and Yagnasubramanian, S.**, Elemental data on human hair sampled from Indian student population and their interpretation for studies in environmental exposure, *IAEA-SM-227 / 24, Proceedings of the Symposium on Nuclear Activation Techniques in the Life Sciences, IAEA*, Vienna, 1978, 499.
131. **Al-Shahristani, H., Shihab, K. M., and Jalil, M.**, Distribution and significance of trace element pollutants in hair of Iraqi population, *IAEA-SM-227 / 7, Proceedings of the Symposium on Nuclear Activation Techniques in the Life Sciences, IAEA*, Vienna, 1978, 515.
132. **Perkons, A. K., Velandia, J. A., and Dienes, M.**, Forensic aspects of trace element variation in the hair of isolated Amazonas Indian tribes, *J. Forensic Sci.*, 22, 95, 1977.
133. **Jervis, R. E., and Tiefenbach, B.**, As accumulation in gold smelter workers and nearby residents,

IAEA-SM-227 / 67, Proceedings of the Symposium on Nuclear Activation Techniques in the Life Sciences, IAEA, Vienna, 1978, 627.
134. **Smith, H.**, The interpretation of the As content of human hair, *Forensic Sci. Soc. J.*, 4, 192, 1964.
135. **Bozsai, G., Deák, Z., Csanádi, M., Ring, J., Horváth, A., and Kárpáti, Z.**, Determination of arsenic content of hair samples to assess the role of drinking water in the arsenic load of population, *Egeszsegtudomany*, 33, 115, 1989.
136. **Creason, J. P., Hinners, T. A., Bumgarner, J. E., and Pinkerton, C.**, Trace elements in hair as related to exposure in metropolitan New York, *Clin. Chem.*, 21, 603, 1975.
137. **Chattopodhyay, A., and Jervis, R. E.**, Hair as an indicator of multielement exposure of population groups, *Trace Subst. Environ. Health*, 8, 31, 1974.
138. **Coleman, R. F., Cripps, F. H., Stimson, A., and Scott, H. D.**, The trace element content of human hair in England and Wales and the application to forensic science, *Proceedings of the 1st International Conference on Forensic Activation Analysis*, San Diego, 1967, 203.
139. **Eatough, D. J., Christensen, J. J., Izatt, R. M., and Hartley, C.**, Level of selected trace elements in human hair, *The First Human Hair Symposium*, Brown, A. C., Ed., Medcom Press, New York, 1974, 377.
140. **Bacsó, J.**, Significance of hair mineral analysis as a means for assessing internal body burdens of environmental mineral pollutant, *2nd Research Coordination Meeting*, International Atomic Energy Agency, Munich, 1985.
141. **Petering, H. G., Yeager, D. W., Witherup, S. O.**, Trace metal content of hair. II. Cadmium and lead of human hair in relation to age and sex, *Arch. Environ. Health*, 27, 327, 1973.
142. **Gordus, A. A.**, Factors affecting the trace metal content of human hair, *Proceedings of the 2nd International Forensic Activation Analysis Conference*, Glasgow, 1972, *J. Radioanal. Chem.*, 5, 229, 1973.
143. **Turkstra, J., Retief, D. H., and Cleaton-Jones, P. E.**, Activation analysis in biological materials, *S. Afr. Med. J.*, 49, 191, 1975.
144. **Hambidge, K. M., Walravens, P., Kumar, V., and Tuchinda, C.**, Cr, Zn, Mn, Cu, Ni, Fe and Cd concentration in the hair of residents of Chandigarh, India and Bangkok, Thailand, *Trace Subst. Environ. Health*, 8, 39, 1974.
145. **Hambidge, K. M., Franklin, M. L., and Jacobs, M. A.**, Hair Cr concentration effects of sample washing and external environment, *Am. J. Clin. Nutr.*, 25, 384, 1972.
146. **Hambidge, K. M., Franklin, M. L., and Jacobs, M. A.**, Changes in hair Cr concentration with increasing distances from hair roots, *Am. J. Clin. Nutr.*, 25, 380, 1972.
147. **Ashurbekov, T. R.**, Elemental composition of human hair in relation to sex, pregnancy, and presence of climateric, *Sud. Med. Ekspert.*, 17, 23, 1974.
148. **Kasperek, K., Iyengar, G. V., Feinendegen, L. E., Hashish, S., and Mahfouz, M.**, Multielement analysis of fingernails, scalp hair and water samples from Egypt: a preliminary study, *Sci. Total Environ.*, 22, 149, 1982.
149. **Baumslag, N., and Petering, H. G.**, Trace metal studies in Bushman hair, *Arch. Environ. Health*, 31, 254, 1976.
150. **Perkons, A. K., and Jervis, R. E.**, Hair individualization studies, *Proceedings of the International Conference on Modern Trends in Activation Analysis*, University of Texas, College Station, 1965, 295.
151. **Birke, G., Johnels, A. G., Plautin, L. O., Sjöstrand, B., Skerfoing, S., and Westermark, T.**, Studies on humans exposed to methyl mercury through fish consumption, *Arch. Environ. Health*, 25, 7, 1972.
152. **Yurachek, J. P., Clemena, G. G., and Harrison, W.**, Analysis of human hair by spark source mass spectrometry, *Anal. Chem.*, 41, 1666, 1969.
153. **Aal, H. A., Soliman, A. A., El Mahdym, H., and El Saeei, L.**, Study of some minerals in normal Egyptian scalp hair, *Acta Derm. Venereol.*, 56, 55, 1976.
154. **Othman, I., and Spyron, N. M.**, Elemental concentration in hair and nail from a selected population group in the Machakos district of Kenya, *IAEA-SM-227 / 33, Proceedings of the Symposium on Nuclear Activation Techniques in the Life Sciences*, IAEA, Vienna, 1978, 583.
155. **Hambidge, K. M., and Baum, D.**, Cr, Fe, Zn and Mg concentrations in newborn hair, *Clin. Res.*, 19, 220, 1971.
156. **Aal, H. A., Soliman, A. A., El Mahdym, H., and El Saeei, L.**, Study of some minerals in scalp hair and blood serums of psoriatics, *Acta Derm. Venereol.*, 56, 265, 1976.
157. **Katz, S. A., Bowen, H. J. M., Comaish, J. S., and Samitz, M. H.**, Tissue Ni levels and Ni dermatitis: Ni in hair, *Br. J. Dermatol.*, 92, 187, 1975.
158. **Hildebrand, H. F., Ostapczuk, P., Mercier, J. F. et al.**, Orthopaedic implants and corrosion products. *Biocompatibility of* Co-Cr-Ni *Alloys*, NATO-ASI Ser., Hildebrand, H. F., and Champy, M. Eds., Plenum Press, New York, 1988.
159. **Lockeretz, W.**, On the Pb content of human hair (1871–1971), *Science*, 180, 1080, 1973.
160. **Rosmanith, J., Einbrodt, H. J., and Gordon, T.**, Beziehungen zwischen Blei- und Zinknierschlägen und den Schwermetallgehalten (Pb, Zn, Cd) im Blut, Urin und in den Haaren bei Kindern, *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg.*, 161, 125, 1975.
161. **Kopito, L., Shwachman, H., and Williams, L. A.**, Measurement of Pb in blood, urine, and scalp hair by AAS, *Stand. Methods Clin. Chem.*, 7, 151, 1972.
162. **Johnson, D. E., Tillery, J. B., and Prevost, R. J.**, Levels of Pt, Pd and Pb in population of southern California, *Environ. Health Perspect.*, 12, 19, 1975.

163. **Sky-Peck, H. H., and Joseph, B. J.,** The use and misuse of human hair in trace metal analysis, in *Chemical Toxicology and Clinical Chemistry of Metals*, Brown, S. S., and Savory, J., Eds., Academic Press, New York, 1983, 159.
164. **Jervis, R. E.,** Activation analysis in forensic science: survey paper, WM-91/67, *Proceedings of the Symposium on Nuclear Activation Techniques in the Life Sciences*, IAEA, Vienna, 1967, 645.
165. **Wiesemer, W., Goerves, W., and Niese, S.,** Changes in trace element concentration in hair and other organs in relation to disease and metal burden, *IAEA-SM-227 / 73, Proceedings of the Symposium on Nuclear Activation Techniques in the Life Sciences*, IAEA, Vienna, 1978, 307.
166. **Clemente, G. F., Cigna Rossi, L., and Santaroni, G. P.,** Trace element composition of hair in the Italian population, *IEAE-SM-227 / 13, Proceedings of the Symposium on Nuclear Activation Techniques in the Life Sciences*, IAEA, Vienna, 1978, 527.
167. **Forsseén, A., and Erämetsä, O.,** Inorganic elements in human body: Ba, Br, Ca, Cd, Cu, K, Ni, Pb, Sn, Sr, Ti, Y, and Zn in hair, *Ann. Acad. Sci. Fenn. Ser. A5*, 1, 162, 1974.
168. **Heydorn, K.,** Aspects of Precision and Accuracy in Neutron Activation Analysis, RISO-R419, RISO National Laboratory, Roskilde, Denmark, 1980.
169. **Coni, E., Alimonti, A., Bolis, G. B., Cristallini, E., and Caroli, S.,** An experimental approach to the assessment of reference values for trace elements in human organs, *Trace Elem. Med.*, 11, 86, 1996.
170. **Zhuang, G. S., Wang, Y. S., Tan, M. G., Zhi, M., Pan, W. Q., and Cheng, Y. D.,** Preliminary study of the distribution of the toxic elements As, Cd and Hg in human hair and tissues by RNNA, in *Biological Trace Element Research,* Schrauzer, G. N., Ed., Humana Press, Clifton, NJ 1990, 729.
171. **Cheng, Y. D., Zhuang, G. S., Tan, M. G., Zhi, M., and Zhou, W.,** Study of correlation of Se content in human hair and internal organs by INAA, in *Biological Trace Element Research,* Schrauzer, G. N., Ed., Humana Press, Clifton, NJ, 1990, 737.
172. **Kjellstrom, T., Elinder, C. G., and Friberg, L.,** Conceptual problems in establishing the critical concentration of cadmium in human kidney cortex, *Environ. Res.*, 33, 284, 1984.
173. **Oster, O., Schmiedel, G., and Prellwitz, W.,** The organ distribution of selenium in German adults, *Biol. Trace Elem. Res.*, 15, 23, 1988.
174. **Zeisler, R., Greenberg, R. R., and Stone, S. F.,** Radiochemical and instrumental NAA procedures for the determination of low level trace elements in human livers, *J. Radiochem. Nucl. Chem.*, 124, 47, 1988.
175. **Zeisler, R., Harrison, S. H., and Wise, S. A.,** Trace elements in human liver using quality control in the complete analytical process, *Biol. Trace Elem. Res.*, 6, 31, 1984.
176. **Lievens, P., Versieck, J., and Cornelis, R.,** The distribution of trace elements in normal human liver determined by semi-automated radiochemical neutron activation analysis, *J. Radioanal. Chem.*, 37, 483, 1977.
177. **Frey, H.,** Iodine status in Scandinavia, *Acta Pharmacol. Toxicol.*, 59, 111, 1986.
178. **Fissenne, I. M., and Welford, G. D.,** Natural uranium concentrations in soft tissues and bone of New York residents, *Health Phys.*, 50, 739, 1986.
179. **Cornelis, R., Mees, L., and Hoste, J.,** Neutron activation analysis of vanadium in human liver and serum, in *Proceedings of the Symposium on Nuclear Activation Techniques in the Life Sciences*, IAEA, Vienna, 1979, 65.
180. **Vanoeteren, C., Cornelis, R., and Sabbioni, E.,** Critical evaluation of normal level of major and trace elements in human lung tissue, in *Environment and Quality Life*, Final Report V, 1986, 1.
181. **Matsumoto, M., Nakano, A., and Ohta, Y.,** Element analysis of human lung tissue, *Res. Rep. Nat. Inst. Environ. Stud.*, 7, 131, 1984.
182. **Alimonti, A., Coni, E., Caroli, S., Sabbioni, E., Nicolau, G. R., and Pietra, R.,** Critical comparison of performances of inductively coupled plasma atomic emission spectrometry and neutron activation analysis for determination of elements in human lungs, *J. Anal. At. Spectrom.*, 4, 577, 1989.
183. **Sabbioni, E., Nicolau, G. R., Pietra, R., Beccaloni, E., Coni, E., Alimonti, A., and Caroli, S.,** Inductively coupled plasma atomic emission spectrometry and neutron activation analysis for the determination of element reference values in human lung tissue, in *Nuclear Analytical Methods in Life Sciences*, Zeisler, R., Ed., Humana Press, Clifton, NJ, 1990, 757.
184. **Alimonti, A., Fornarelli, L., Coni, E., Bolis, G. B., Cristallini, E., and Caroli, S.,** Assessment of trace element concentrations in human lungs of urban subjects, *Microchem. J.*, 46, 191, 1992.
185. **Sweet, D. V., Crouse, W. E., and Crable, J. V.,** Chemical and statistical studies of contaminants in urban lungs, *Am. Ind. Hyg. Assoc. J.*, 39, 515, 1978.
186. **Vanoeteren, C.,** Spoorelementbepaling in Menselijke Long, *Ph.D. thesis*, State University of Ghent, Ghent, Belgium, 1984.
187. **Filby, R. H.,** Elemental Analysis of Human Lung Tissue and Other Selected Samples Utilizing NAA, Hew Pub. No. 75, NIOSH, Cincinnati, No. 75, 1975, 187.
188. **Vanoeteren, C., Cornelis, R., and Versieck, J.,** Evaluation of trace elements in human lung tissue. I. Concentration and distribution, *Sci. Total Environ.*, 54, 217, 1985.
189. **Bartsch, P., Collignon, A., Weber, G., Robaye, G., Delbrouck, J. M., Roelandts, I., and Yujie, J.,** Distribution of metals in human lung: analysis by particle induced X-ray emission. *Arch. Environ. Health,* 37, 111, 1982.

190. **Teraoka, H.,** Distribution of 24 elements in the internal organs of normal males and the metallic workers in Japan, *Arch. Environ. Health,* 36, 155, 1981.
191. **Tipton, I. H., Schroeder, H. A., Perry, H. H., and Cook, M. J.,** Trace elements in human tissue. Part III. Subjects from Africa, the Near and Far East and Europe, *Health Phys.,* 11, 403, 1965.
192. **Casey, C. E., Guthrie, B. E., and McKenzie, J. M.,** Copper, manganese, zinc and cadmium in human tissues: effects of age, in *Proceedings of New Zealand Workshop on Trace Elements,* University of Otago, Dunedin, New Zealand, 1981, 210.
193. **Vuori, E., Huunan-Seppala, A., Kilpio, J. O., and Salmela, S. S.,** Biologically active metals in human tissues. II. The effect of age on the concentration of cadmium in aorta, heart, kidney, liver, lung, pancreas and skeletal muscle, *Scand. J. Work Environ. Health,* 5, 16, 1979.
194. **Erickson, M. M., Poklis, A., Gantner, G. E., Dickinson, A. W., and Hillman, L. S.,** Tissue mineral levels in victims of sudden infant death syndrome. I. Toxic metals: lead and cadmium, *Pediatr. Res.,* 17, 779, 1983.
195. **Brown, R., and Taylor, H. E.,** Trace Element Analysis of Normal Lung Tissue and Hilar Lymph Nodes by Spark Source Mass Spectrometry, HEW Publ. No. 75, NIOSH, Cincinnati, 1975, 129.
196. **Wirnsberger, G., Wawschinek, O., Pruegger, F., and Hoefler, H.,** Chromium content of normal and pathological altered lung tissue, *Biol. Trace Elem. Res.,* 14, 43, 1987.
197. **Kollmeier, H., Wittig, C., Seermann, J., Wittig, P., and Rothe, R.,** Increased chromium and nickel content in lung tissue, *J. Cancer Res. Clin. Oncol.,* 110, 173, 1985.
198. **Raithel, H. J., Schaller, K. H., Reith, A., Svenes, K. B., and Valentin, H.,** Investigation on the quantitative determination of nickel and chromium in human lung tissue. Industrial, medical, toxicological and occupational medical expertise aspects, *Int. Arch. Occup. Environ. Health,* 6, 55, 1988.
199. **Yukawa, M., Suzuki-Yasumoto, M., Amano, K., and Terai, M.,** Distribution of trace elements in the human body determined by NAA, *Arch. Environ. Health,* 35, 36, 1980.
200. **Weber, G., Robaye, G., Bartsch, P., Collignon, A., Beguin, Y., Roelandts, I., and Delbrouk, J. M.,** Trace element analysis by PIXE in several biomedical fields, in *Proceedings of Third International Conference on Particle Induced X-Ray and Nuclear Instrumental Methods,* Martin, B., Ed., North-Holland, Amsterdam, 1984, 326.
201. **Soman, S. D., Joseph, K. T., Raut, S. J., Mulay, G. D., Parameshwaran, M., and Panday, V. K.,** Studies on major and trace element content in human tissues, *Health Phys.,* 19, 641, 1970.
202. **Sumino, K., Hayakawa, K., Shibata, T., and Kitamura, S.,** Heavy metals in normal Japanese tissues, *Arch. Environ. Health,* 30, 487, 1975.
203. **Nordberg, G. F., Wester, P. O., and Brune, D.,** Tissue levels of 25 elements in smelters workers, in *Proceedings of International Symposium on the Control of Air Pollution in the Working Environment,* Lyber Tryck, Stockholm, 1978, 261.
204. **Ophus, E. M., and Mylius, E. A.,** Lead levels in human lungs, *Bull. Environ. Contam. Toxicol.,* 18, 734, 1977.
205. **Mylius, E. A., and Ophus, E. M.,** Pulmonary distributions of lead in human subjects, *Bull. Environ. Contam. Toxicol.,* 17, 302, 1977.
206. **Di Ilio, C., Del Boccio, G., Casaccia, R., Aceto, A., Di Giacomo, F., and Federici, G.,** Selenium level and glutathione-dependent enzyme activities in normal and neoplastic human lung tissue, *Carcinogenesis,* 8(2), 281, 1987.