Journal of Research of the National Bureau of Standards
Volume 91, Number 2, March-April 1986

Presampling Factors

G. V. Iyengar

Nuclear Research Center, D-517 Juelich, Federal Republic of Germany

Accepted: October 24 1985

Choosing the right kind of samples from human subjects for trace element studies poses many difficult problems. First of all, due to practical considerations, specimens with clinical relevance are restricted to a few such as whole blood, hair, nail, urine, and faeces. Although autopsies provide access to collect various organs, their usefulness is restricted to monitoring type of activities and not for clinical diagnosis. Besides these basic differences one is also confronted with procuring "valid" samples for analysis. Validity refers to both analytical and biological aspects and the material collected should satisfy both the demands to make the specimen meaningful. In practice this is not a simple task because a number of presampling factors need to be taken into account. Significant situations among these are the biological variations, post mortem changes, intrinsic errors resulting from internal contaminations, etc. The impact of these factors alters the status of the sample and calls for adequate discription of the specimen. In the absence of a well defined sample protocol accurate characterization of the material will not be possible and renders the analytical effort worthless. Solutions to these problems should be sought at interdisciplinary level and effective team work is mandatory to make any meaningful progress in our endeavours to answer public health questions.

Key words: biological systems; biological variations; biomedical; human tissues and body fluids; internal contamination; intrinsic errors; precision and accuracy; presampling factors; post mortem changes; reference values; sampling; trace element analysis; valid samples.

1. Introduction

There is mounting concern among trace element researchers dealing with biological systems that the elemental composition data, especially for trace elements at lower concentration levels, are inaccurate [1-8][1]. Major efforts are therefore necessary to generate reproducible and reliable results to build up a reference data base for human tissues and body fluids. Unless this is achieved, true variations in elemental concentrations arising from physiological changes, pathological influences, and occupational and environmental exposures remain submerged in the wide ranges of the largely uncontrolled analytical data. This being the case, very little progress can be achieved in applying the knowledge gained from trace element studies to practical problems in human health.

Several literature surveys on the elemental composition of biological systems have exposed the fact that reported data vary over a very wide range [9-10]. This feature is very effectively illustrated when analytical results for the same test material are pooled from various laboratories [11]. Generally speaking, the sources responsible for this kind of a situation in the reported information can be broadly classified into two groups as illustrated in figure 1: presampling factors and analytical and data handling errors. Identification of anal-

About the Author: G. V. Iyengar at present is with the National Bureau of Standards, Gaithersburg, MD 20899, U.S.A. He supervises a project concerned with the daily dietary intakes of minor and trace elements by human subjects, a joint undertaking of the Bureau, the International Atomic Energy Agency, the U.S. Food and Drug Administration, and the U.S. Department of Agriculture.

[1] Figures in brackets indicate literature references.

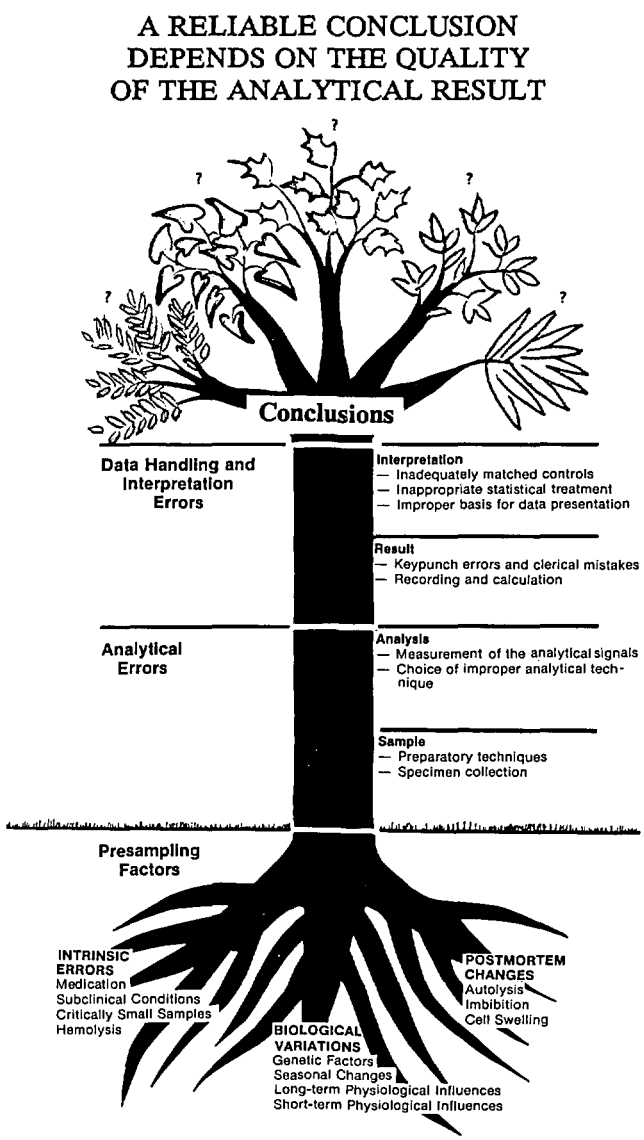

Figure 1–The elemental analysis of biological systems.

ytical errors mainly arising from choosing an inappropriate methodology or paucities due to sampling and sample handling have received considerable attention by the analytical community especially in the past few years [12]. However, an understanding of the impact of presampling factors on the entire analytical sequence is sadly lacking and awaits immediate recognition since the "validity of the sample" itself becomes questionable if the influence of presampling factors is neglected.

Procurement of normal human tissues for elemental analysis is complicated by some inherent difficulties. Only a few samples, such as blood, excretory products, and occasional biopsy samples are obtainable from living subjects; most other samples must be sought at autopsy. Irrespective of the mode of collection, the samples are subject to the influence of presampling factors. The purpose of this report is to identify the special difficulties associated with biomedical systems destined for elemental composition studies. The main emphasis will be on the quality of the sample by elaborating the role of presampling factors, which have great effect on the overall accuracy of the analytical result but are yet to be fully appreciated.

2. Definition of Presampling Factors

Essentially, presampling factors may be defined as events associated with biological specimens in situ and before the arrival of the samples at the laboratory for analysis. A host of circumstances such as biological variations, post mortem changes, intrinsic and inadvertent errors arising from internal contaminations for specific elements, and situations such as preferential accumulation of elements in selected organs or even within an organ, etc., fall in this group. As illustrated in figure 1, a number of components are involved in the process of acquiring a biological specimen to study its elemental composition and emphasize the complexity involved in contrast to the situation dealing with static inorganic systems. Thus, there exist numerous pitfalls and shortcomings associated with presampling factors leading to erroneous result and conclusions in acquiring and intepreting elemental analysis data.

3. Biological Variations

These may be divided into genetic factors, long and short term physiological influences, and seasonal changes.

Genetic predisposition of human subjects belonging to different racial groups is an example in this context. However, because of the overriding influences of environmental and other factors, changes of elemental concentrations in body components directly attributable to genetic predisposition are somewhat difficult to identify. This has been illustrated in a recent survey involving Zn in serum covering black and white populations. The results showed that blacks generally tended to have lower serum Zn values than did whites but there were inconsistencies [13]. On the other hand, in animals, changes arising from differences in breed are well recognized. Commonly encountered examples include variations in the composition of I in cow milk [14] and Cu in the blood of sheep [15].

Long term physiological influences include age, sex, habit geographical and environmental factors, diet, pregnancy and lactation. The definition of long and short term influences is somewhat arbitrary since factors such as diet and smoking habits overlap. For example, habitual diet may be regarded as a long term effect,

while a single meal may be looked upon as a short term effect.

The age of the examined individuals is an important factor in elemental analysis studies since shifts in concentrations occur as a function of age. Zn in serum is a good example in this context. It has been shown that as a function of age, lowest values in serum are found in babies under one year, while the concentration reaches a peak around 20 to 40 years and starts to fall at the age of 50 and above [16]. Similar tendencies have been observed in the second National Health and Nutrition Examination Survey (NHANES II) in the U.S.A., involving 14,770 subjects and completed recently [13].

An example of differences due to sex is that of Fe in liver [17]. It has been shown that men have higher concentrations of this element than women. The recorded concentrations on dry weight basis are 913 and 700 micro g/g, respectively. It has also been shown that serum Zn levels in males is significantly higher than in females in several age groups with the difference being greatest in the 20-44 year olds [13].

The influence of geographical and environmental factors has been established in several cases. Elevated blood Pb levels in several urban areas of the world is an ideal example with gasoline considered as one of the main sources where unleaded fuel is not mandatory [18]. Other known examples in this context include a dramatic accumulation of various trace elements, in particular Sc, found in lung tissue from people living in Duisburg in Germany due to the environmental pollution from the steel furnaces operating in this area [19]. Populations living near Cu smelting areas have had greatly elevated As concentrations in urine than those living far away [20]. In a recent investigation dealing with human milk distinct geographical differences were shown for As, Mn, Se and Zn, among others, between Guatemala, Hungary, Nigeria, Phillipines, Sweden, and Zaire [18,21].

Several examples have been cited to record the influence of diet on the elemental composition profile of tissues and body fluids [22]. Some elements show profound effects as exemplified by I. When elemental and foods rich in I are consumed, the concentration of this element in blood and other tissues is elevated to very high levels and remains so for several weeks [23,24]. Recently it has been reported that consumption of dietary algae by nursing women in Japan elevated the concentration of I in milk ranging from 80 to 7000 micro g per L with the highest frequency at 150 micro g per L [25].

Smoking tobacco is a good example of the impact of habit on trace element picture. It induces variations in Cd level as seen from a number of studies; its level in blood [18,26], kidney [27] and placenta [28] is elevated.

Pregnancy induces several physiological and biochemical changes of which an increase of plasma volume by as much as 50% and a decrease of plasma protein up to 30%, are of great consequence from a trace element point of view. These changes partly account for the shifts observed in electrolytes and Zn, all of which are consistently low during pregnancy. The reverse tendency of elevated Cu in serum in pregnant women is well documented [29].

Concerning lactation, it is well established that both humans and animals show a large decline in the concentration levels of several elements with the progress of lactation and therefore, it is essential to define precisely the stage of lactation if comparison of results between different milk samples is desired. These aspects have been exclusively discussed by Iyengar in a separate report [30].

Short term physiological influences can be regarded as those which affect the system over short periods. Thus, circadian rhythm, recent food, fasting, posture and stress during or just before sampling can be grouped under this.

Circadian rhythm or the diurnal variation has been examined for electrolytes and for certain trace elements such as Zn, Fe, and F. In healthy adults on normal diet, the excretion rate for Ca and Mg is usually greater during the day than during the night. P and Na reach maximum in the evening, while K follows the opposite trend. A rapid rise also occurs in the excretion of Ca and Mg after a meal [31].

Concerning trace elements, although there is some disagreement with respect to the exact time of the day at which peak concentrations are reached, there is considerable information for a few elements. For example, there is clear evidence that Zn concentration of serum reaches maximum concentration in the mornings [13,32] and that Fe in plasma reaches high levels during the night and early morning [33]. A short term Fe rhythm in children showing variations up to 40% in two consecutive estimations within 30 minutes has also been identified [34]. F does not undergo any cyclic variation. The effect of circadian rhythm has also been observed for Cd in urine. On five of the seven days Cd was measured in seven subjects it was found that the concentration was highest between 10–11 hours [35].

The influence of recent and specific foods is also an important factor. Amino acids present in the foods may chelate some elements in the blood and remove them from the stream. This appears to be the case with Zn in serum which drops to less than 70% of its initial facting level within three to six hours following a large meal [36]. A recent comprehensive survey also reported the same tendency but to a lesser degree in a large group of subjects [13].

Recent dietary intakes can act as internal contaminants for certain elements. Ingestion of a specific food, namely fillet of plaice, elevated As levels in blood serum by more than an order of magnitude. Even after 12 hours its level did not return to fasting value [37]. This is important in the context of accepting overnight fasting values as normal levels. Another good example of the short and intermediate term influences from food is seen for the case of Hg in hair. In four groups of subjects consuming fish once a month, every week and every day, the concentration of Hg in hair was found to be 1.4, 1.9 and 11.6 micro g/g, respectively, thus distinctly reflecting the effect of the frequency of fish consumption [38].

Fasting before sampling and posture and stress during sampling also contribute to significant shifts in concentration levels of fluids such as serum. There is evidence to show that fasting values for Zn in serum are elevated [39]. A set of examples is shown in table 1 to illustrate the influence of "status at sampling" on the concentration of selected elements in serum and blood. Basically, this table reveals that samples collected under a given condition may not necessarily be suitable for carrying out multielement determinations and great care is needed in interpreting the variations observed for different elements in such samples. Furthermore, it is known that even changes from an upright to a recumbent postion affect the concentrations of plasma proteins inducing alterations in the concentrations of protein bound elements such as Zn and Se [40]. Stress, imparted through venous acclusion applied prior to venepuncture has been shown to increase plasma protein up to 30%

Table 1. Influence of "status at sampling" on the concentration of selected elements in human subjects: some examples.

Sample	Element	Condition	Influence	Remarks
Serum	Zn	Fasting	Elevated	Even overnight fasting
		After normal food	Lowered	During first few hours
		After high Zn food,	Elevated	e.g., Oysters, liver, etc.
		Low Zn intake	Lowered	Rather sudden effect
		Stress	Elevated	e.g., Standing posture
		Non-stress	Lowered	e.g., Recumbent position
		Pregnancy	Lowered	Progressive decline
	F	Fasting	Normal	Overnight fasting
		After normal food	Variable	If sampled immediately
		Tea consumption	Elevated	If sampled immediately
		Low F intake	Lowered	Over a few days
	I	High intake, e.g., sea foods, iodinated salts etc.	Elevated	Uptake is rapid but decline is slow since biological half life is 2.5 weeks
		Low I intake	Lowered	Over a few days
	As	Fish intake	Elevated	Well absorbed and slowly excreted
Blood	Cd	Tobaccao smoking	Elevated	Remains chronically high in smokers
	Pb	Alcohol consumption	Elevated	Especially wine drinkers

with concomitant increase in Zn value [41]. Such a situation might call for controlling the pressure used on the arm and using standard conditions in serial samples.

Seasonal changes can be grouped under two categories: physiologic and climatic. The combined influence of these two factors has been previously illustrated through various examples [22]. In humans, changes brought about by summer may be envisaged through recreational activities, differences in diets due to the availability of fresh fruits and vegetables, and changes in the excretory patterns becausee of excessive sweating.

Seasonal changes due purely to climatic conditions are very well illustrated in animals that are confined to stalls in winter and graze out in the fields in summer. This leads to dietary and other changes that lead to altered elemental composition of tissues and fluids. Depending upon the soil composition of the fields, concentrations of Cu, F, I, Mn, Mo and Se in milk are affected [18]. For example, I content in cow's milk fluctuates throughout the year, reaching minimum levels in summer. These variations are demonstrated in figure 2 by plotting data from [14].

In a number of species such as birds, reptiles, amphibia, and fish, different physiological states such as moulting, wintering, laying, or spawning bring about specific changes in the metabolism of certain elements, e.g., Ca. There are also great internal shifts in tissue composition of fat and protein in organs such as liver, resulting in extreme variations of elemental concentrations. Therefore, any intersample comparison has little relevance. For example, physiologicallyinduced seasonal variations in the concentrations of Zn, Cu, Fe, Cd, and Hg in starling liver has been identified [42]. Therefore, it follows that if the object of an investigation is to make an assessment of the extent to which an animal might be polluted, then it is necessary to take the samples at more than one time of the year.

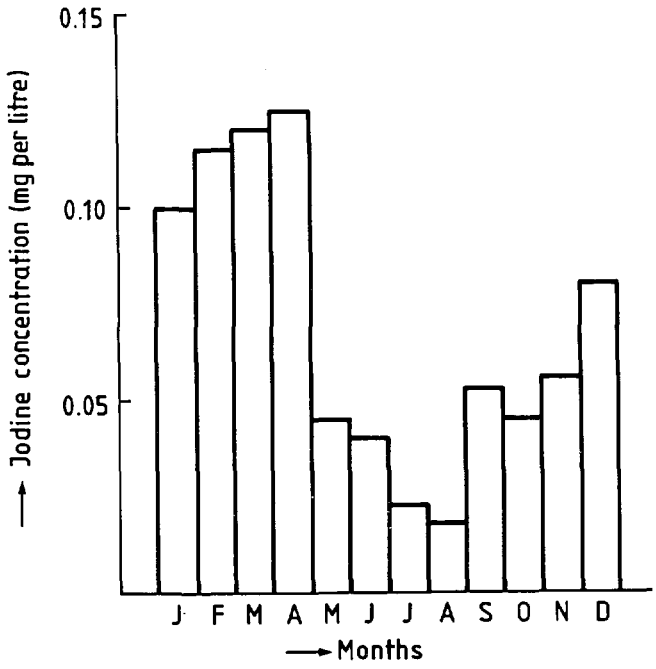

Figure 2–Variation in the iodine content of cow's milk.

4. Post Mortem Changes

Literature reveals that elemental concentrations based on autopsy samples generally show great variations in contrast to blood or serum which are obtained from living subjects (fig. 3). It should be recognized that autopsy sampling from humans involves a certain time lapse between death and sample collection and that, immediately after the death of an organism several post mortem changes set in with varying rapidity depending upon environmental temperature, humidity, body temperature at the time of death, insulating effect provided by the fat layers in the body, time elapsed before the body was put under cooling, and the storage time. Of the many changes that occur, cell swelling, tissue dehydration, imbibition, putrefaction, and autolysis are of particular significance to the analyst since they influence the "tissue status" as long as the organs remain inside the body. By implication, post mortem changes (which are inevitable to a certain extent in human situations) could have important bearings on the elemental composition profiles of individual organs.

Rapidly metabolizing organs such as liver, spleen, kidney and heart are severely affected by cell swelling, imbibition, and autolysis. The former two events produce changes in organ volume due to fluid influx and expulsion while the latter accounts for the actual tissue degeneration. Recently an animal model study illustrated the effects of post mortem changes on the elemental concentrations in liver [43].

According to the above mentioned study retention of rat liver inside intact carcasses for prolonged periods of time at ambient temperature induced significant changes in its weight due to post mortem tissue degeneration. Livers from animals that were frozen at -15 °C also showed significant decrease in weight when they were thawed on the third day. The effect of these changes on the elemental concentrations of various elements depended on the association of the elements with extracellular fluid and intracellular components. For example, concentration of $K+$ was affected more by the lysis of the cell and sustained losses up to 30% in relation to the control values, while the total content was reduced by more than 40% as a result of both lysis and tissue liquefaction. For $Na+$, differences ranging from $+10$ to

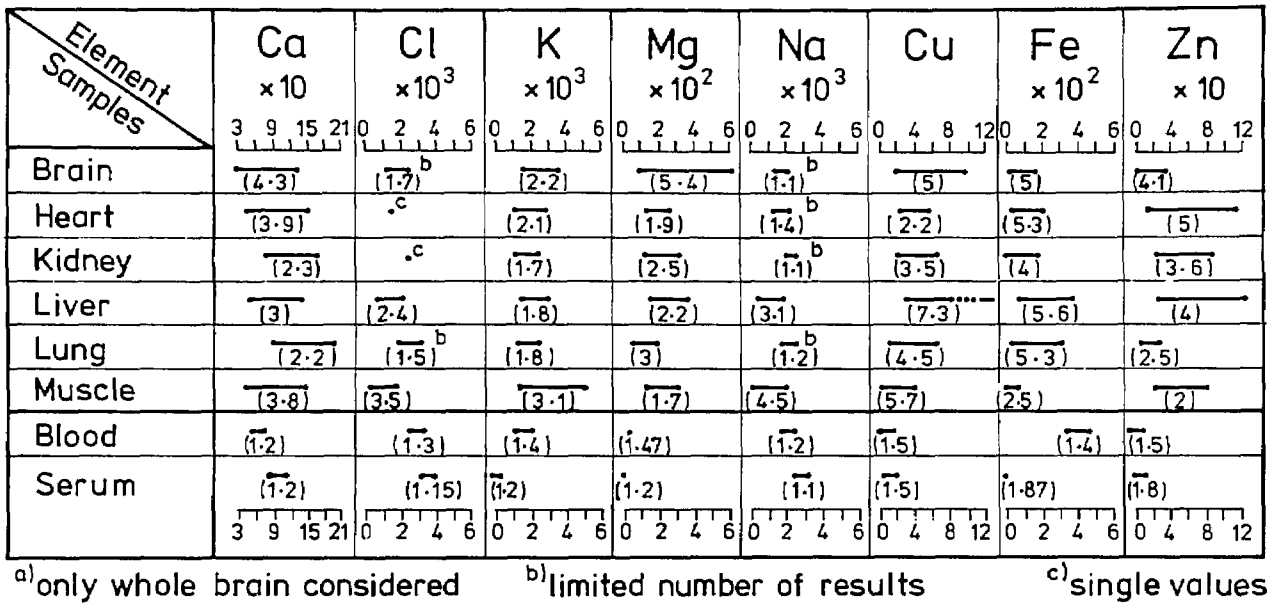

Figure 3–Variations in the elemental concentrations (mg/kg fresh tissue and mg/liter fluid) of normal adult human tissues reported in the literature (Iyengar, Kollmer, Bowen 1978). Figures in parentheses represent the ratio maximum/minimum.

−20% in concentrations and +20 to −40% in total content were observed which could be explained by the movement of fluid in and out of the organ. Among trace elements, variations observed for Fe ranged from −20 to +40% while both Cu and Zn were found to fluctuate between −20 and +20%. The loss observed in the total content in the liver for the five trace elements studied (Cu, Fe, Mn, Rb and Zn) was found to be about 20 to 40% [43,44]. Similar differences were observed also for Co, Cs and Se [45].

This study indicates the need for standardizing the sampling time in order to obtain reliable results for elemental analysis in human autopsies. This step would reduce fluctuations due to volume changes. Once the organ is removed from the body, careful freezing, or freeze drying permits prolonged storage.

5. Internal Contamination, Intrinsic and Inadvertent Errors

Internal contamination of tissues and body fluids from elements may arise due to a number of reasons governing many aspects of presampling factors and lead to intrinsic errors.

Intrinsic errors reflect the factors inherently present in the sample that may falsify the results. These errors, as the definition itself suggests, are difficult to detect and the analyst has little or no control over them. Good examples in this context are medication, haemolysis, prevalence of subclinical conditions and certain inescapable medical restrictions.

Certain types of medication, e.g., chelation therapy, are recognized for their role in upsetting the balance of trace elements in various body pools. Also, there are certain baffling situations such as prior exposure to I containing drugs or x-ray contrast media which generally elevate the tissue I levels with varying retention times in body compartments. Widespread use of I containing drugs (some as prophylaxis) and x-ray contrast media signal a formidable source of internal contamination for this element. Careful evaluation of case history is necessary to minimize such errors. Antinausea drugs and sleeping pills are additional examples.

Haemolysis is another source of intrinsic errors. Normal plasma contains much less hemoglobin in relation to serum which may contain 10 to 20 mg/mL, an equivalent of 350 to 700 ng of Fe per mL. Since the naked eye cannot distinguish hemolysis in serum below a certain degree, errors of this kind virtually go unnoticed at sampling stage. Depending upon the methodology used (measuring total Fe or exclusively transferrin Fe) the analytical values differ. These and other situations of intrinsic errors are discussed elsewhere [22].

Besides intrinsic errors sometimes errors may also be introduced inadvertently.These may happen while dealing with critically small samples (e.g., needle biopsies) due to changes in humidity in the sample environment thereby presenting formidable difficulties in assessing the correct weight of the sample material. The need for retaining viability of cells (e.g., platelets) is yet another example. Several such examples are discussed [22].

6. Differences Between Different Segments of an Organ

Significant differences in trace element concentrations between specific segments of an organ such as kidney, brain, and bone are well known. For example, sectioning of kidney into pure cortex and medulla is necessary since metal levels (e.g., Cd and Zn) differ significantly between tissue sections [46]. Similarly, great regional variations have been reported for Cu, Mn, and Zn between epidermis and dermis, an important factor while dealing with skin samples. The differences cover a wide range and may be of the order of 2 and 7 micro g/g for Cu, 0.1 and 1 micro g for Mn and 24 and 132 micro g/g for Zn, all on dry weight basis for dermis and epidermis, respectively [47]. Careful sectioning of hair to account for the distance from the scalp is another example of site dependency of elemental composition.

7. Conclusions

It is obvious from the foregoing discussions that presampling factors contribute their share to the overall variability of an analytical finding in biological systems.

The specimen to be sampled should be chosen with regard to the aim of the investigation and biological implications. Most often, the significance of the data can be strengthened if the sample characterization is thorough and if samples can be obtained at the same time from different body fluids and tissues.

There is a need for standardizing sampling and storage conditions for autopsy samples destined for elemental composition studies. It is important to incorporate the various aspects discussed in this report so that the true biological variations of the elemental composition of tissues may be easier to reveal.

Finally, it is imperative that analysts be associated with trace element investigations in biological systems at the planning stage and not just from the moment a sample arrives at the laboratory.

8. References

[1] Iyengar, G. V., Human health and trace element research: problems and prospects. Sci. Total Environ. **19**, 105–109 (1981).

[2] Talanta Special Issue 29, 963-1056 (1982).

[3] Iyengar, G. V., Presampling factors in the elemental composition of biological systems. Anal. Chem. **54**, 554A–558A (1982).

[4] Friberg, L., and M. Vahter, Assessment of exposure to Pb and Cd through biological monitoring: results of a UNEP/WHO global study. Environ. Res. **30**, 95–128 (1983).

[5] Stoeppler, M., Processing biological samples for metal analysis. Proc. Chemical Toxicology and Clinical Chemistry of Metals. Brown, S. S., and J. Savory, eds., Academic Press: New York, 31–44 (1983).

[6] Heydorn, K., Neutron activation analysis for clinical trace element research (Vol. 1, CRC Press, Boca Raton, 1984).

[7] Versieck, J., Trace element analysis: a plea for accuracy. Trace Ele. Med. **1**, 2–12 (1984).

[8] Cornelis, R., and B. Wallaeys, Chromium revisited. Proc. Trace Element Analytica Chemistry in Medicine and Biology (Braetter, P., and P. Schramel, eds.) de Gruyter Co: Berlin (1984).

[9] Iyengar, G. V.; W. E. Kollmer and H. J. M. Bowen, The elemental composition of human tissues and body fluids, Verlag Chemie: Weinheim (1978).

[10] Versieck, J., and R. Cornelis, Normal levels of trace elements in human blood plasma or serum. Anal. Chim. Acta. **116**, 217–54 (1980).

[11] Parr, R. M., The reliability of trace element analysis as revealed by analytical reference materials. Proc. Trace Element Analytical Chemistry in Medicine and Biology (Braetter, P., and P. Schramel, eds.) 631–55, de Gruyter Co: Berlin (1980).

[12] Trace element analysis of biological materials, IAEA Technical Report 197, IAEA: Vienna (1980).

[13] Assessment of Zn nutritional status of the U.S. population based on data collection in the second national health and nutrition examination survey, 1976–1980 (Pilch S. M., and P. R. Senti, eds.). Report prepared for FDA, Washington D.C., 1984) Contract No. FDA 223-83-2384.

[14] Broadhead, G. D.; I. B. Pearson and G. M. Wilson, Seasonal changes in Iodine metabolism I. Iodine content of cow's milk, Br. Med. J. **i**, 343-48 (1965).

[15] Woolliams, J. A.; N. F. Suttle, G. Wiener, and A. C. Field, Genetic and dietary factors in Cu accumulation by sheep, Proc. Trace Element Metabolism in Man and Animals (Mac Howell, J.; J. M. Gawthorne and C. L. White, eds.) 137–140. Australian Academy of Sciences: Canberra (1981).

[16] Kasperek K.; L. E. Feinendegen, I., Lombeck and H. Bremer, Zinc concentrations in serum of children. Eur. J. Pediat. **126**, 199–202 (1977).

[17] Van Eijk, H. G.; W. E. Wittink, G. Boss, and J. P. Goosens, Iron concentrations in liver. Clin. Chim Acta. **50**, 278–280 (1974).

[18] Iyengar, G. V., Concentrations of 15 trace elements in some selected adult human tissues and body fluids of clinical interst from several countries: results from a pilot study for the establishment of reference values. KFA Report 1974, Nuclear Research Center: Juelich, FRG (1985).

[19] Persigehl, M.; K. Kasperek, H. J. Klein and L. E. and Feinendegen, Influence of industrialization on trace element concentrations in human lungs. Beitr. Path. Bd. **157**, 260–268 (1976).

[20] Ghelberg, N. W., and E. Bodor, Arsenic levels in the environment and in human body in a copper metallurgy plant area. Proc. Management of Heavy Metal in the Environment, CEP Consultants: Edinburgh, 163–66 (1979).

[21] WHO/IAEA coordinated research programme on trace elements in human milk. Final Report, IAEA: Vienna (in preparation).

[22] Iyengar, G. V., Problems in trace element analysis of biological systems. Ref. [15], 697–701.

[23] Carr, E. A., and D. S. Riggs, Protein bound iodine in extrathyroidal tissues. Biochem. J. **54**, 217–25 (1953).

[24] Kiltgaard, H. M.; H. W. Dirks, W. R. Garlick, and S. B. Banker, Protein bound iodine in various tissues after injection of elemental iodine. Endocr. **50**, 170–73 (1952).

[25] Maramatsu, Y.; M. Sumiya and Y. Ohmomo, Stable iodine contents in human milk related to dietary algae consumption. Hoken Busuri **18**, 113–117 (1983).

[26] Stoeppler, M., and K. Brandt, Rapid determination of Cd in whole blood by electrochemical atomization. Ref. [20], 183–186.

[27] Le Baron, J. G., PhD thesis, University of Waterloo, Canada (1977).

[28] Peerboom, J. W. C.; P. de voogt, B. V. Hattum, W. V. D. Velde, and J. H. W. C. Peerboom-Stegeman. The use of human placenta as a biological indicator for Cd exposure. Ref. [20], 8–10.

[29] Underwood, E. J., Trace Element Nutrition in Human and Animal Nutrition. Academic Press: New York (1977).

[30] Iyengar, G. V., Elemental composition of Human and Animal Milk: A Review. IAEA-TCDOC-269, IAEA: Vienna (1982).

[31] Heaton, F. W., and A. Hodgkinson, External factors affecting diurnal variation in electrolyte excretion with particular reference to Ca and Mg. Clin. Chim Acta. **8**, 246–54 (1963).

[32] Battacharya, R. D., Circadian rhythmic aspects of urinary Zn excretion in presumably healthy subjects. Pan Med. Torino **21**, 201–203 (1979).

[33] Stengle, J. M., and A. L. Schade, Circadian variation of Fe in plasma. Br. J. Haematol. **3**, 117–20 (1957).

[34] Werkmann, H. P.; J. M. F. Trijbels, and E. D. A. M. Schretten, Short term Fe rhythm in serum of children. Clin. Chim. Acta. **53**, 65–68 (1974).

[35] Subramanian, K. S., and J. E. Meranger, Diurnal variations in the concentration of Cd in urine. Clin. Chem. **39**, 1110 (1984).

[36] Kasperek, K.; H. Schicha, V. Siller, L. E., Feinendegen, and A. Hoeck, Trace element concentrations in human serum: diagnostic implications. Proc. IAEA Symp. Nuclear Activation Techniques in the Life Science. IAEA: Vienna, 517–526 (1972).

[37] Heydorn K.; E. Damsgaard, N. A. Larson and B. Nielsen, Sources of variability of trace element concentrations in human serum. Ref. [36], 129–42.

[38] Airey, D., Total Hg concentrations in human hair from 13 countries in relation to fish consumption and location. Sci. Total Environ. **31**, 157–180 (1983).

[39] Henry, R. W., and M. E. Elmes, Plasma Zn in acute starvation. Br. Med. J. **4**, 625–626 (1975).

[40] Behne, D., and H. Juergensen, Determination of trace elements in human blood serum and in the standard reference material bovine liver by instrumental neuton activation analysis. J. Radioanal. Chem. **42**, 447–453 (1978).

[41] Juswigg, T.; R. Batres, N. W. Solomons, O. Pineda, and D. B. Milne, The effect of temporary venous occlusion on trace mineral concentrations in plasma. Am. J. Clin. Nutr. **36**, 354–58 (1982).

[42] Osborn, D., Seasonal changes in the fat, protein and metal content of the liver of the starling Sturnus Vulgaris. Environ. Pollut. **19**, 145–55 (1979).

[43] Iyengar, G. V., Autopsy sampling and elemental analysis: errors arising from post mortem changes. J. Path. **134**, 173–80 (1981).

[44] Iyengar, G. V., Post mortem changes of the elemental composition of autopsy specimens: variations of K, Na, Mg, Ca, C., Fe, Zn, Cu, Mn and Rb in rat liver. Sci. total Environ. **15**, 217–36 (1980).

[45] Iyengar, G. V.; K. Kasperek and L. E. Feinendegen, Post mortem changes of the elemental concentration of autopsy specimens: variations of Co, Cs and Se in rat liver. J. Radioanal. Chem. **69**, 463–72 (1982).

[46] Livingstone, H. D., Distribution of Zn, Cd and Hg in human kidneys. Proc. Trace Substances in Environmental Health-V (Hemphill, D. D. ed.) University of Columbia, Mo, pp. 399–411 (1971).

[47] Molokhia, M. M., and B. Portnaoy, Neutron activation analysis of trace elements in skin. IV Regional variations in Copper, Manganese and Zinc. Br. J. Derm. **82**, 254–255 (1970).