Analytical Systems for the Determination of Metals in Food and Water Supplies

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INTRODUCTION

The analysis of food products or water supplies for toxic or potentially toxic elements, e.g., heavy metals, is the central problem addressed in this report. An evaluation of this problem in terms of the capabilities and limitations of applicable analytical techniques is the primary thrust of this report. Thus, recommendations regarding the selection of the best analytical methods and techniques for the determination of toxic elements are presented below. Before doing so, it is appropriate to delineate the criteria used in the development of the recommendations and to discuss philosophical rationales on which these judgmental guidelines are based.

The Analytical Process

Any chemical analysis can be divided into three sequential steps: 1) collection of the sample(s); 2) chemical and/or physical preparation of the sample(s) for analysis: and 3) measurement of the concentrations of the target constituents in the sample(s). Although these steps are interdependent and should not be considered otherwise, the present discussion will focus on sample preparation and measurement. Sampling should be discussed in the broader context of the overall problem of monitoring. The selection of an appropriate analytical technique must be based on the type of information desired and the purposes of collecting that information. In the present context, this may be delineated in fairly general terms.

The Monitoring Question

With rare exceptions, the central question associated with a monitoring program is:

- Are there one or more chemical entities present in the target material (e.g., food or water) in sufficient quantities to cause deleterious effects on the consumer population?

This question may be considered qualitative in that it actually requires only a yes or no answer. Given the knowledge that each of the chemicals being monitored must be present at or above some threshold[1] concentration before they individually or collectively produce observable effects on the recipient population, the answer is no if all are below their respective threshold effect levels and yes if one or more is above. As a result, it is quite common to use analysis approaches capable of determining or detecting the chemicals only at levels down to, but not below, their respective threshold levels. Although this practice can often be justified on an economic basis, it is subject to challenge for scientific reasons.

The astute recipient of the answer to the central question will immediately raise other questions regardless of whether the answer is positive or negative. If informed that all constituents of concern are below their individual or collective threshold levels, two questions are obvious:

- What degree of confidence can be assigned to the results?

[1]It must be noted that there is considerable disagreement whether or not a threshold concentration actually exists for any chemical that can deleteriously affect a recipient. Many argue, with at least some justification, that a chemical entity that affects a recipient deleteriously at any exposure concentration will cause the same effect(s) to a lesser degree even when the exposure is considerably less. While this view may prove to be totally valid, it is also certain that the effects produced must progress to certain stages before they become recognizable or measurable. In brief, the occurrence of a deleterious effect cannot be claimed unless it can be observed. The seriousness of the effect cannot be truly defined unless it can be measured. From this viewpoint, it is justifiable to define the threshold concentration as that required to produce an observable (measurable) effect on the recipient(s). This is the connotation of the term when used in this report.

- Are the concentrations of any chemicals increasing significantly over time for a particular monitor and/or a particular collection site?

When advised that one or more constituents are present above threshold levels, these same two questions are raised and a third becomes pertinent, i.e.,

- What is the cause or source of the observed contamination?

These questions are clearly quantitative in nature. Decisions regarding possible impacts on consumer populations, or the prevention thereof, should not be based on less than quantitative and defensible information. Adherence to this philosophy is complicated by the facts that reliable designations of threshold effect levels are often lacking and that two or more contaminants may act synergistically or antagonistically.

There is one general criterion that may be defined on the basis of the above discussion. All analyses should rely on analytical methods that are capable of determining all target contaminants at concentration levels below the threshold effect levels. While methods capable of making these measurements at concentrations 100 to 1,000 times below these levels would clearly be desirable, the use of methods providing measurement capabilities 10 to 100 times below the levels may prove more practical on an economic basis.

This discussion provides a basis for delineating a general protocol for the operation of the analytical laboratory responsible for answering the above questions. A brief discussion of this is presented here to provide a general basis for succeeding topics.

OPERATIONAL PROTOCOL FOR A MONITORING LABORATORY

A general flow diagram of the laboratory operation is presented in figure H-1. The analysis sequence given is depicted in the context of the monitoring questions, the checks required to validate the results, and the regulatory actions likely to prevail. Examination of this protocol indicates the probable need for inclusion of: 1) a quality-assurance program as a means of validating results; 2) complementary analytical methods to ensure that all required analyses can be completed and to provide comparative information relevant to validation of results; and 3) a data storage-retrieval system consistent with the requirements of the monitoring questions, the quality assurance program, and regulatory actions. The general utility of this protocol diagram and the actions it portrays will be expanded upon below.

CRITERIA FOR SELECTION OF ANALYSIS METHODS

The selection of an appropriate analysis approach must be based on the information requirements. These may be formulated on the basis of the answers to two questions:

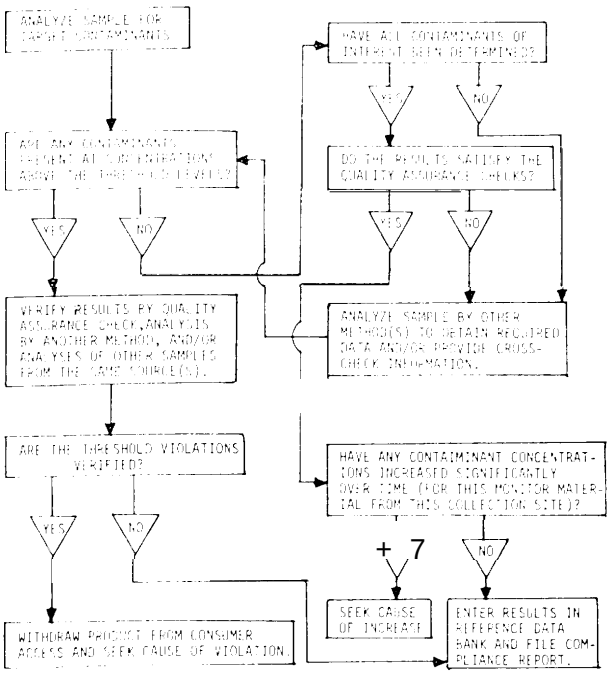
. What are the chemical entities that must be determined?

- What are the concentration levels (threshold levels) of primary concern for these target entities?

Although answering the first question should not be particularly difficult in most instances, the issue for toxic elements has often been obscured by the use of expedient analysis approaches which may or may not provide the information required. Historically, most analysis methods used for the determination of elements simply measure the total amount of an element present without differentiating between the various chemical states of the element that may be present. Such measurements may, in fact, be relatively nonspecific indicators of potential or actual deleterious effects on biological systems. In numerous instances, the identification and measurement of the active or functioning forms of the elements is actually needed. The following examples testify to the general importance of this statement.

1. Although-arsenic is toxic, the plus three oxidation state (As(III)) is clearly more toxic than the plus five state (As(V)); the compound arsine (AsH_3) is perhaps the most toxic chemical form of arsenic.
2. Although chromium is classified as a nutritionally essential element, Cr(III) is toxic while Cr(VI) is relatively innocuous.
3. A measurement of the level of vitamin B_{12} in animal tissue is ordinarily more useful than a

Figure H-1.—Flow Diagram of the General Operational Protocol of a Monitoring Laboratory



determination of total Co even though Co is the primary metal contained in B_{12}.

4. Alkyl (organic) mercury compounds apparently possess greater propensities for bioaccumulation and the associated health effects than the more common inorganic forms of mercury.

Clearly, chemical form or state is important! The selection of elemental analysis techniques capable of specifically measuring those chemical forms most important from a biological effects viewpoint should be a primary objective of any environmental monitoring program. The status of the present technology is inadequate for this purpose in many instances. As a result, the functioning forms of several elements are not well-known for various types of biological effects. Hence, the designation of the important (threshold) concentration levels for these cases has typically been rather crude. The capabilities and limitations of current analytical measurement systems are discussed in relation to the chemical form problem in the analytical instrumentation section. Research needs are also defined in that section

Although the measurement of the various chemical forms of each element is often important, it should be noted that the easier and more common measurement of the total concentration of each element (in all of its chemical forms) can be rationalized. For example, if the analysis indicates that the total concentration is below the threshold effect level for any one or combination of the particular chemical forms for an element, the need for measurement of the concentrations of the chemical forms is negated. The total concentration measurement consequently serves as a screening device indicative of the possible need for chemical form measurements.

Once the elements and the concentration ranges of interest for each have been defined, the criteria which must be invoked in the selection of an appropriate analysis method include: sensitivity, selectivity, reliability, scope, sample preparation requirements, and time-cost considerations. It would be folly to select an approach incapable of providing measurements a t or below the required concentration (threshold effect) levels. Thus, selection of a sufficiently sensitive method is of paramount importance. Although it is common to discuss sensitivity in the context of the term detection limit, it is also usually impossible to obtain a sufficiently accurate concentration measurement when said concentration is barely detectable. Thus, sensitivity should be considered in association with the term determination limit, i.e., the lowest concentration at which a sufficiently reliable concentration measurement can be carried out.

The scope of an analytical approach is defined on several bases. The ability to determine reliably large numbers of individual chemical constituents each of which may be present in a variety of sample types within a broad concentration range is the primary connotation, Thus, universal applicability (utility) is an alternate terminology implying the ideal case. It can also be argued that an analytical method which requires simple sample preparation operations offers greater scope than one requiring more complex preparative steps: the ideal case involves direct analysis of the samples without prior chemical treatment.

Analytical selectivity is usually interpreted synonymously with specificity. The analysis approach used must provide an unequivocal means of identifying each chemical constituent of interest irrespective of the compositional characteristics of the sample material being analyzed. In addition, the measurement of the concentration of a n y constituent of interest should provide accurate results independent of the variations in the concentrations of other constituents present in the sample materials. Analysis methods that do not satisfy these conditions lack selectivity (or

specificity): such methods are subject to what are often termed interference or matrix effects, In essence, reliability (accuracy) is the criterion which defines a sufficiently selective analysis; confidence in the results and the decision(s) based on them is explicit.

Minimization of the time-cost commitments must be an objective considered in the selection of an analysis approach. It is unfortunate that this consideration often leads to one of two extreme stances. Management often opts for the adoption of analytical technology of limited utility on the basis of lower initial capital costs and/or the fact that such technologies frequently can be applied by analysts with lower levels of expertise. Such selections are often economic errors when considered on a longer term basis, At the other extreme, management may invest in expensive facilities and expertise and then require that they be used to carry out analyses that can be accomplished more economically via another approach. This, too, can prove to be a false economy.

Summary

This background discussion has been presented as a preface to the evaluations presented below; the intent has been to provide a common basis for comments, evaluations, and suggestions which follow.

PREMISES FOR SELECTION OF LABORATORY FACILITIES

In considering the operation of a laboratory dedicated to monitoring food and water supplies for toxic elements, several premises may be established. These are essential in defining the necessary facilities and require discussion. The following list is not necessarily all inclusive nor is it set down in order of importance.

1. The majority of analysis requests will require the determination of several elements per sample.
2. To be most effective, the laboratory must be able to comply with these requests in a reasonable (short) time.
3. The analyses carried out should be accomplished at reasonable costs.
4. The analytical results must be sufficiently accurate to avoid challenge of the integrity of any decisions based on them.

The combination of these premises clearly implies that the ideal laboratory facility would be one capable of accurately analyzing for all constituents requested at concentration levels down to and below their respective threshold effect levels in a short period of time. The use of a system capable of simultaneously measuring all constituents of interest in each sample is definitely implied. The time and cost premises further imply the desirability of utilizing analysis techniques which do not require extensive sample preparation operations: the ability to directly analyze samples in an “as received” form may be considered ideal. Sample preparation operations are also primary sources of contamination or loss of the analytical constituents. The direct analysis, minimal preparations, capability is desirable from the accuracy standpoint as well. Finally, the accuracy requirement indicates the need for a highly specific analysis approach which is not subject to significant interference problems and the maintenance of a quality assurance program. No single analytical technique will necessarily satisfy all of these requirements for the elements and/or sample types of interest; a combination of techniques will be required. Properly selected, the techniques used may be complementary in terms of providing the range of elemental analyses required and in terms of providing redundant analyses for some elements. The latter will be useful for accuracy validation purposes (see discussion below).

In effect, these premises lead to the defensible conclusions that: 1) the laboratory facilities should be primarily comprised of multicomponent analysis systems; 2) more than one such system will likely be required: 3) the selection of systems that will provide some redundant analytical information is desirable; and 4) the inclusion of secondary analytical systems to be used in supportive capacities may be essential. The following evaluation is predicated on these bases.

EVALUATION OF ANALYTICAL TECHNIQUES FOR THE DETERMINATION OF TOXIC ELEMENTS

The qualitative identification and quantitative determination of toxic or potentially toxic elements in food products and water can be based on several different analytical techniques for which commercially available instrumentation exists. The primary techniques to be considered include:

1. neutron activation analysis (NAA);
2. molecular absorption and fluorescence spectrophotometry:
3. solids (spark source) mass spectrometry (SMS);
4. atomic absorption (AAS) and atomic emission spectrophotometry (AES);
5. electrochemical techniques
   —anodic stripping voltammetry (ASV),
   —differential pulse polarography (DPP);
6. plasma emission spectrometry (PES);
7. X-ray techniques
   —X-ray emission spectrometry (XES),
   —proton-induced X-ray emission spectrometry (PIXE).

Each of these are considered below.

Neutron Activation Analysis

This technique is perhaps the most sensitive of those available when all elements are considered. Absolute determination limits, expressed as nanograms (1 x 10^{-9} gm) that must be present in the sample for quantitative determination, are summarized in table H-1. Such determination limits should be converted to actual concentrations to lend them perspective for the present evaluation. For the analysis of food products or water, a 10-gm or 10-ml sample generally represents a reasonable upper limit on the sample size that can be activated for analysis. Thus, taking 0.5 ng as the determination limit for arsenic as an example (table H-1) this element can be determined at approximately 0.5 ng/10 gm = 0,05 ng/gm (rig/ml for water) or 0.05 parts per billion by NAA. Evaluation of the other elements of interest on this proximate basis indicates that most could readily be determined at required concentration levels via this technique. While this is an encouraging conclusion, there are other factors which detract from it.

Table H-1 .—Determination Limits for Neutron Activation Analyses[a]

Element	Absolute value, ng[b]	Concentration value, ppb[c]
Ag	0.05	0.005
As	0.5	0.05
Be	25	2.5
Bi	250	25
Cd	25	2,5
Co	2.5	0.25
Cr.	500	50
Cu	0.05	0.05
Fe	25,000	2,500
Hg	5	0.5
Mn	0.03	0.003
Mo	50	5
Ni	250	2.5
Pb	5,000	500
Sb	2.5	0.25
Se	2,500	250
Sn	250	25
Te.	25	2.5
Tl	—	—
V	0.5	0.05
Zn	50	5

[a]Data taken from R. K Skogerboe and G H Morrison, Trace Analysis E ssen tial Aspects, in *Treatise on Analytical Chemistry* (1 M Kolthoff and P J El ving, eds). New York Wiley and Sons, 1971, pp 5842-5843
[b]Nanograms of element that must be present in sample activated to Permit a quantitative determination; detection limits are approximately a factor of 5 lower Interference free measurement conditions are assumed
[c]Assuming a 10 gm S_{amp} le is act ivated, thus concen trat ion values absolute values – 10 These values should be Increased by a factor of 100 to 1 000 to compensate for the loss of neutron flux f a neutron accelerator were used in stead of a nuclear reactor

To achieve the sensitivity required, the use of a nuclear reactor providing a high flux of thermal neutrons is required. The acquisition cost of the reactor is several million dollars; the operational costs are also comparatively high. Considerable reduction of these can be achieved by replacement of the reactor with a neutron accelerator. The neutron fluxes available with such accelerators are, however, about a factor of 100 to 1,000 less than those typical of a reactor. The analytical sensitivity available is reduced in proportion [see footnote to table H-1).

If chemical separations are carried out after activation of the samples, proponents of NAA argue that analytical interferences can be virtually eliminated. This argument is often specious simply because the use of chemical separations is

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often a primary source of errors. Since such separations, even when free of errors, are time consuming and add to the expense; activation analysts usually prefer what is called the purely instrumental approach.

Two types of interferences are common to this approach. Direct interferences occur when two or more sample constituents emit radiation (gamma rays or beta particles) of nearly the same energy. If the emitting species undergo radioactive decay at significantly different rates, correction for these interferences can be based on measurement of the radiation at different times. Otherwise, the interfering species must be chemically separated prior to the measurement step. Indirect interferences due to contributions to the sample spectrum from Bremsstrahlung and Compton interactions are also common, Correction for these must be obtained by subtraction techniques. The net effect is, however, a reduction in the analytical sensitivity and/or an increase in the uncertainties associated with the measurements, While these instrumental approaches are widely used in NAA to avoid the need for chemical separations, such avoidance still restricts the potential scope of the analyses. They also tend to lengthen the time required to obtain analysis results. On these bases, NAA does not appear to be the best choice for a laboratory facility: capital costs, operational costs, and operator training requirements are primary weighting factors influencing this negative judgment.

Molecular Absorption and Fluorescence Spectrophotometry

These techniques have been used extensively for elemental analysis. They generally rely on carrying out a reaction of the element of interest with a reagent (or series of reagents) to form a product which has properties required for the absorption of light. Identification of the element incorporated into the light-absorbing product is based on the wavelength of light absorbed while measurement of the concentration relies on the extent of absorption; hence the term molecular absorption, Some light-absorbing species regain a more stable energy configuration by release of the light energy absorbed as light (fluorescence or phosphorescence). Measurement of the wavelength at which this occurs and the intensity of the light emitted is used to identify the species responsible and the amount present,

The use of these techniques requires that specific or semispecific chemical reactions be used for the formation of the absorptive and/or fluorescent products, While such reactions are generally available for the elements of interest, the analysis of any one sample for several constituents would necessarily have to rely on carrying out several individual reactions. Even then, there are only limited instances for which a reaction will occur for only one constituent (a specific reaction). Most reactions involving a particular reagent set tend to occur for each of several elements having similar properties and, as a result, their absorption of fluorescence spectra tend to be quite similar. Such spectra are subject to some degree of wavelength coincidence such that spectral interferences are not uncommon; the measurements are less specific than desirable, While other features of these techniques could be discussed, their chemical reaction requirements coupled with the specificity problem are deleterious from the multicomponent analysis standpoint. The techniques should not be classified as essential for the present elemental analysis purposes.

Solids Mass Spectrometry

This analytical technique offers sensitivity competitive with that characteristic of NAA (see table H-1) for a wide range of elements. To utilize it, nonconducting samples must be rendered electrically conducting. The constituents of an aqueous sample would ordinarily be analyzed after the water was evaporated away; the residue would be mixed with a conductor for analysis. Solid materials such as foods are also typically nonconductive; by mixing them with a conductor such as graphite or silver powder they become conductive. Food products must also be oxidized (by wet or dry oxidation techniques) to destroy the organic constituents which cause serious spectral interferences in the analysis step.[2] These sample preparation steps are quite extensive and can obviously be the sources of serious errors unless carried out with caution.

In practice, the analyses must be based on two sample mixtures to obtain a complete analysis subject to less interference problems. Elemental subset A may be determined based, for example, on mixing the sample residue or ash with high-purity graphite. However, the carbon polymers of which graphite is composed are observed in the mass spectrum and preclude the possibility of analyzing for those elements that would normally

[2] See the following" reference for a more complete discussion of the problems and capabilities: C. A. Evans and G. H. Morrison, Anal. Chem., 40, 869 (1969).

be measured at these mass positions. The analysis for elemental subset B would subsequently be based on the use of high-purity silver, gold, copper, or aluminum powder as the conducting matrix. Again, the use of any of these choices to achieve sample conductivity results in spectral interferences which necessitate analyses based on two or more subsets of elements. The conductive materials used must be high purity, i.e., 6-9s or 99.9999-percent pure. This requirement limits the possibilities and affects their costs, Finally, it should be noted that solid mass spectrometric analyses require unusually long times even though it is possible to obtain analyses for -40 to 60 elements on each sample. Although the sample preparations required are time consuming, the analysis itself is also rather slow. Given samples ready for analysis, a well-organized SMS laboratory would be hard-pressed to analyze more than 5 to 10 samples per man-day. These factors, coupled with high acquisition costs (~$250,000) and high operational and instrument maintenance costs, place solids mass spectrometry in a negative posit ion relative to other possibilities,

Atomic Absorption and Emission Spectrophotometry

Recent instrument sales figures indicate that only gas chromatography is more widely used than atomic absorption spectrophotometry. When a flame is used as the energy source required to produce the gaseous atomic populations from the sample dissolved in aqueous solution, atomic absorption offers favorable analysis capabilities for a reasonably impressive array of elements (see table H-2]. Atomic emission from the same atom populations in the flame is totally complementary and supplementary to atomic absorption. Factors which affect absorption also tend to affect emission; the ultimate sensitivity achieved with either measurement approach is limited by the ability to produce the atomic populations. As a result, it can be shown that atomic absorption is generally most favorable (on the basis of sensitivity) for the determination of those elements requiring more energy to produce atomic emission, i.e., an excitation potential above approximately 4.5 electron volts (eV). Elements with lower excitation poten-

table H-2.—Determination Limits for Flame Atomic Absorption and Emission and Furnace Atomic Absorption'

Elements	Flame methods[b] Atomic absorption	Flame methods[b] Atomic emission	Flame AA analysis of tissue digests[c]	Furnace atomic absorption	Furnace AA analysis of tissue digests[c]
Ag	002	0.04	0.2	000004	0.0004
As	05	5	5	0.02	0 2
B e	001	0.5	0.1	00002	0002
Bi	02	2	2	0.001	0.01
C d	0005	2	0.05	0.00002	0.0002
co	003	0.1	0.3	0.001	0.01
Cr	002	002	0.2	0.001	0.01
Cu	001	005	0.1	0.001	0.01
Fe	0.02	0.1	0.2	0.0006	0.006
Hg	10	2.0	10	0.02	0.2
Mn	001	0.02	0.1	0.0001	0.001
Mo	02	05	2	0,008	0.08
Ni	002	0.1	0.2	0.002	0.02
Pb	0.05	05	0.5	0.001	0.01
Sb	05	2	5	0.006	0.06
Se.	05	10	5	0.02	0 2
Sn	01	2	1	0.01	0.01
Te	05	200	5	005	0 5
Tl	01	0.1	1	0.0006	0.06
V .	01	0.05	1	002	0 2
Zn	001	0.2	01	0.00002	0.0002

[a]Data from J D Winefordner et al *Appl Spectrosc Revie* ws 712J *147* (1973) A ll va lues Given in μ9 m l for aqueous solu tions
[b]Defined for best flame conditions for each element Values in μg ml (ppm)
[c]Based on dissolvin q 10 q m o f biological tissue (wet weight) per 100 m l of ac ld va lues in μg/g m (ppm) wet weig h t
[d]Defined for optimun furnace conditions , n eac h case and use of a 25 μl sample inj ection Values In μg m l (ppm)

tials are best determined by atomic emission when flames are the energy media. These generalizations require the use of an instrument that is well-designed for both absorption and emission measurements (two of the three major U.S. atomic absorption manufacturers supply such instruments).

In spite of the combined capabilities offered by flame emission and absorption, they are frequently inadequate for the types of analyses in question unless preconcentration and/or separation procedures (e.g., solvent extraction) are used. These inadequacies may be due to a lack of sufficient sensitivity and/or to the occurrence of interference effects. These, coupled with the difficulties attendant to separation/preconcentration procedures, have led to the development of what is often called nonflame atomic absorption. The first significant development in this area involved the reduction of mercury ion in aqueous solution to atomic mercury (Hg) such that it could be carried directly to the gas phase into the optical path of an AA instrument for measurement. The measurement actually involves separation of the Hg from the sample under conditions less subject to interferences. If carried out under appropriate conditions so that the Hg arrives at the measurement cell rapidly, an effective preconcentration is also realized. In effect, the measurement sensitivity is determined by the concentration of atoms delivered to the measurement cell per unit time. The nonflame methods are all designed to take advantage of this thereby enhancing the ability to analyze at lower concentrations; methodological developments for this purpose have taken two general tacts, i.e., chemical generation and furnace vaporization.

Beyond the Hg method described above, the hydride generation method for the determination of arsenic, selenium, germanium, lead, tellurium, tin, antimony, and bismuth has received wide attention. The hydrides of these elements are rapidly formed by reaction in acid media with sodium borohydride. The metal hydrides, being gases at ambient temperatures, are readily transported via carrier gas to a flame or a heated (- 800° C) quartz cell for atomization and measurement. The success of this general approach has led to the commercial availability of hydride generator accessories for atomic absorption. It has also been adopted in commercial autoanalyzer systems. Detection limits of less than 1 ng metal/ml (1 ppb) solution are readily obtained.

Furnaces, fabricated from graphite or tungsten, that can be temperature ramped by resistance heating have been developed to high levels of refinement. Liquid samples are delivered to a furnace, which is resident in the optical path of an AA unit, and a programed heating cycle is initiated, In this cycle, the liquid is first evaporated at ~120° C; the salt residue remaining is "ashed" to convert it to a "common" chemical form at -3000 to 500°C; and the ash is rapidly vaporized and atomized at -2,0000 C for the absorption measurement. The use of such systems provides improvements in the analytical sensitivity for most elements (see table H-2). The extent of improvement is limited by the amount of sample that can be placed in the furnace (~25 to 50 µ1) but generally amounts to a factor of 10 to 100 when compared with flame AA capabilities.

These capabilities combined with the general simplicity of operation and lower instrument costs have been largely responsible for the widespread acceptance of atomic absorption. During the principal time of AA development, the primary competing techniques were flame, arc, and spark-emission spectrometry. The last two were rather quickly abandoned by the analytical spectrometry community because they were "notably subject to interference problems associated with the vaporization-atomization system. It is rather ironic that the same community, in less than 10 years, reverted to the use of furnace AAS systems which are subject to the same interference problems for the same reasons. It is also ironic that an extensive fraction of the furnace AA research reported in the past 5 to 10 years has dealt with the study of interference effects and means for their elimination. A large percentage of these studies are reaching the same conclusions and developing the same compensatory methods that resulted from arc and spark spectrometry interference effect studies before the advent of atomic absorption. Nevertheless, atomic absorption analysis is well-established and here to stay. The furnace methods, in particular, clearly offer the required sensitivities for a wide range of analysis problems.

A primary historic limitation has been that atomic absorption has been basically a single element analysis technique; analyses for several elements in a sample are carried out sequentially in time. The emergence of plasma emission spectrometric systems which allow the simultaneous analysis of several elements (to be discussed below) has forced the atomic absorption community to the development of multicomponent analysis systems. Only one commercial instrument offering this capability for more than two elements is available at this date, This unit is, in fact, a se-

quential analysis system since the elements of interest are not measured simultaneously. Its use saves analysis time but not as much as it would in the simultaneous mode. At least one other manufacturer will introduce a truly simultaneous multielement AA analysis system in the next 1 to 2 years. Prototypes of such a unit have been developed and tested at Colorado State University.[3] The tests have shown that sets of 5 to 10 elements can be determined simultaneously without sacrifice of analytical sensitivity. The availability of such instrumentation will advance the state of the art for atomic absorption.

The inclusion of atomic absorption in an elemental analysis laboratory may be considered worthwhile as a secondary facility at least. If said instrument offers the simultaneous measurement capability, the inclusion may be justified on a better economic basis.

Electrochemical Techniques

These techniques are perhaps the most classical of those considered herein, And yet, electrochemistry has been reborn in the past two decades largely through the development of what may be classified as pulse or differential techniques. The electrochemical techniques of primary interest in the present context rely on the measurement of the Faradaic current (FC) produced or used during oxidation or reduction reactions involving the element to be determined; the potentials at which FC changes occur depend on the elements (ions) involved in the reactions. Such reactions are almost universally accompanied by non-Faradaic processes involving other sample constituents which also result in the production or utilization of current. In classical direct current polarography or voltammetry, the ability to measure a low Faradaic current (low analyte concentration) is limited by the magnitude of the non-Faradaic current changes occurring simultaneously. The revitalization of electrochemistry has been largely based on the fact that when the potential required to induce a redox reaction is removed, the non-Faradaic current decays more rapidly than the Faradaic current. Thus, by "pulsing" the potential up to the reaction level required, shifting it back to below the reaction level, and waiting an appropriate time period for the non-Faradaic current to decay; the Faradaic to non-Faradaic current ratio can be significantly improved. A variety of these differential pulse approaches have been developed to achieve analytical measurements at concentrations 10 to 1,000 times lower than previously possible. The techniques which appear to offer the most significant capabilities are differential pulse polarography (DPP) and differential pulse anodic stripping voltammetry (DPASV). At least one commercial instrument offering both of these measurement capabilities is available.

The electrochemical literature indicates that -16 to **20** elements can be determined by DPP, DPASV, or cathodic stripping voltammetry (CSV). This is true, but all such elements cannot be determined under a single set of experimental conditions. Some must be determined using a gold working electrode (for example) while others require the use of a mercury electrode. Some determinations require the use of a specific supporting electrolyte solution while others do not. Finally, the redox potentials of some entities are sufficiently similar that they cannot be determined in the presence of each other without the use of correction techniques. In some cases, prior separations are required.

The electrochemical analysis literature indicates that Cd, Cu, Pb, Zn, Tl, and Fe can usually be determined simultaneously in a single supporting electrolyte solution using mercury as the working electrode material, These elements can be determined at levels of 1 ng/ml or less in the electrolyte solution by DPP or DPASV. As a result, these techniques are quite widely used for the analysis of the above elements. Although Cu and Zn interfere with each other via formation of the Cu:Zn intermetallic in the mercury electrode and the reduction of Fe(III) to Fe(II) interferes with the Cu determination, these can be corrected via use of expedient instrumental procedures. Other elements frequently determined are As(III) by DPASV or DPP and Se(H) by CSV. Again, analyses at or below the part per billion concentration level are common.

The electrochemical methods detect only the electroactive species, e.g., the ions, This statement must be qualified in terms of the time-scale of the measurement step. To illustrate, consider an electroactive metal ion (M^{+n}) which may be present in the sample solution primarily as a complexed or molecular species designated, for example, by MX_n. To measure the total M^{+n} plus MX_n concentration, the following reaction must occur either prior to or during the measurement period:

$$MX_n \rightarrow M^{+n} + nX^-$$

If the duration of the measurement is short, the above reaction must go to a reproducible state of

[3] See for example: F. S. Chuang, D. F. S. Natusch, and K. R. O'Keefe, Anal, Chem. 50, 525 [1978).

completion within that time period; in such cases the complexed/molecular entity may be classed as labile. Thus, there is a growing investigative effort in the electrochemical community involving the use of this conceptual approach as a means for the identification of the chemical forms of metals in natural systems. The general thrust of these efforts involves either the use of chemical or electrochemical means for systematically shifting the above type of reactions toward the formation of an electroactive species for identification purposes. A typical electrochemically induced shift experiment, for example, might involve ASV. By electrodepositing from the sample solution for successively longer periods and measuring the amount of M^{+n} reduced during each period, the time required to drive the above type of reaction to completion might be deduced. This may be indicative of the "lability" of the complexed/molecular form, i.e., its thermodynamic or kinetic stability, Extensive research must be completed to establish the potential utility of such approaches. However, these possibilities combined with the high sensitivities that can be achieved with modern electrochemical techniques suggest that such facilities should prove to be valuable laboratory facilities. (See further discussion below.)

Plasma Emission Spectrometry

It has been suggested that atomic emission spectrometry offers what may approximate the ideal approach to multielement analyses. Indeed, the use of the radio frequency inductively coupled plasma (ICP) as the atomic excitation source in combination with a direct-reading emission spectrometer permits the simultaneous determination of numerous elements at low concentration levels.[4] Similarly, the use of a direct current plasma (DCP) excitation source for direct-reading emission spectrometry shows comparable promise. The evolution of such systems over the past decade has brought emission spectrometric analysis back to the forefront of analytical capabilities. Such plasmas are principally used to excite analytical constituents delivered to them via solution nebulization (aerosol production) systems. The conversion of solid samples to aqueous solutions for analysis (e.g., by wet oxidation) results in elimination of many of the major compositional differences between samples such that interference effects due to matrix differences may be eliminated or reduced. As a result, a single set of operational conditions may be used for the simultaneous determination of 20 to **60** elements,

Quantitative determination limits for the ICP- and DCP-Multielement Atomic Emission Analysis Systems (MAES) are listed in table H-3. Examination of these data indicates that these systems are adequate for the simultaneous determination of a major fraction of the elements of interest at levels commensurate with the anticipated threshold effect concentrations. This is one reason why the ICP-MAES and DCP-MAES manufacturers have enjoyed significant annual sales improvements over the past 5 years.

Some elements of high concern, because of their toxicities or propensities for bioaccumulation, cannot be determined at low enough concentrations by direct solution analysis, e.g., Hg, As, and Se. However, the hydride generation approaches used to solve this problem when AAS is the analysis method, are equally applicable to MAES. In fact, all hydride-forming elements can

Table H-3.—Multielement Atomic Emission Determination Limits for Two Common Plasma Excitation Systems[a]

	ICP-MAES		DCP-MAES	
Element	In solution, μg/ml	For tissue digest in μg/gm (ppm)[b]	In solution, μg/ml	Tissue digest g/gin (ppm)[b]
Ag	0.01	0.1	—	—
As	0.3	3	0.2	2
B	0.02	0.2	0.02	0.2
Be	0.002	0.02	0.05	0.5
Bi	0.2	2	—	—
Cd	0.01	0.1	0.05	0.5
Co	0.01	0.1	0.08	0.8
Cr	0.02	0.2	0.02	0.2
Cu	0.005	0.05	0.01	0.1
Fe	0,01	01	0.01	0.1
Hg	0.1	1	0.01	0.1
Mn	001	01	0.05	0.5
Mo	0.03	0.3	0.05	0.5
Ni	0.05	0.5	0.05	0.5
Pb	0.1	1	0.05	0.5
Sb	1	10	—	—
Se	0.2	2	0.2	2.0
Sn	1	10	1	10
Te	0.4	4	—	—
Tl	1	10	—	—
U	0,2	2	0.5	5
V	0,02	0.2	0.02	0.2
Zn	0.01	0.1	0.02	0,2

[a] Data for the ICP from R K Winge et al Spectrochim Acta, *32B* 327 (1977) Data for the DCP from R K Skogerboe H E Taylor and G W Johnson, Spectrochim Acta, in press

[b] For 10 gm of biological tissue (wet wt) dissolved in 100 ml of solution

4 A good introductory review has been presented by V.A. Fassel and R. N. Kniseley, Anal. Chem, 46. 11 10A (1974). See also, Science, 202, 183(1978).

be determined at required concentrations on a simultaneous basis. (Tissue detection limits of 0.01 µg/gm (ppm wet weight) can be achieved.)

Although the measurement of atomic emission offers a high degree of qualitative specificity, there are two common types of interferences which affect the quantitative specificities of such systems. Both plasma types are highly efficient excitation media. As a result, extremely intense radiation from common elements such as Ca and Mg is delivered to the spectrometer-measurement system, The result is the observation of stray light interference effects for some other elements. The magnitudes of these effects depend on: the design characteristics of the spectrometer used, the concentrations of the elements from which the stray light originates, the concentrations of the elements subject to the interferences, and the types of approaches used to alleviate the effects. An expedient means of correcting for these effects may be based on determination of the concentrations of the causative elements. To illustrate, let C_m represent the measured concentration of a particular element (analyte) subject to interference due to a concomitant constituent present at concentration C_C. The true (corrected) concentration (C_T) of the analyte may be determined from:

$$C_T = C_m - aC_C$$

where a is a correction coefficient determined by simple experimental procedures.

The other type of interference effect involves interelemental processes in which the presence of one constituent changes the extent of excitation of another in the plasma, While such interelement effects are less common, the above correction procedure can also be used for compensation. Commercial ICP- and DCP-MAES systems are routinely equipped with minicomputer or microprocessor systems for control, data acquisition, and data correction purposes. The use of the types of corrective procedures described above is, thus, easily automated.

In effect, the general capabilities of plasma-MAES are such that it should be considered a primary facility.

X-Ray Emission Techniques

The bombardment of samples with X-rays to produce X-ray fluorescence (XRF) has remained in wide usage. In fact, the development of the lithium drifted germanium, Ge(Li), or silicon, Si(Li), detectors has advanced the status of this approach by the reduction of spectral interference problems and a general increase in the sensitivity available. Such energy-dispersive detection systems have rather rapidly replaced the conventional wavelength-dispersion units. The production of X-ray fluorescence (emission) by X-ray bombardment and measurement with an energy dispersive detector offers reasonable analysis capabilities for several elements of interest (see table H-4).

In 1970, the potential of heavy, charged particles for X-ray excitation was recognized, and improved capabilities have been demonstrated. The capability improvements of accelerator (particle) beams are due to: 1) the high particle fluxes available, z) the relatively low background radiation associated with the excitation process: 3) the fact that the excitation cross-sections of many elements for particles are higher than for photons or electrons, and 4) a single charged particle can induce emission of several X-ray photons as it pene-

Table H-4.—Determination Limits for X-Ray Fluorescence Techniques

	X-ray flourescence[a]		Proton induced X-ray emission[b]	
Element	ng/cm²[c]	0.1cc dry tissue, µg/gm[d]	ng/cm²[d]	0.1cc dry tissue, µg/gm[d]
Ag	—[e]	—[e]	50	0.3
Al	800	4	—[e]	—[e]
As	8	0.04	5	0.03
Ba	120	0.6	—	—
Bi	—	—	20	0.1
Cd	20	0.1	50	0.3
Co	—	—	3	0.02
Cr	—	—	3	0.02
Cu	—	—	4	0.02
Fe	25	0.1	3	0.02
Hg	—	—	20	0.1
Mn	—	—	3	0.02
Mo	—	—	30	0.2
Ni	—	—	4	0.02
Pb	25	0.1	20	0.1
Sb	—	—	150	0.8
Se	10	0.05	5	0.03
Sn	25	0.1	150	0.8
Sr	10	0.05	—	—
Te	—	—	150	0.8
Tl	—	—	20	0.1
V	50	0.3	3	0.02
Zn	15	0.08	4	0.02

[a]Data from J. M. Jaklevic and R. L. Walter in "X-ray Flourescence Analysis of Environmental Samples" (T. G. Dzubay, ed.) Ann Arbor Publishers, Inc., 1977. p. 68. Excitation with Cr or Rh X-ray tubes and measurement with a Si(Li) energy dispersive detector.

[b]Data from C. J. Umbarger et al., "Advances in X-Ray Analysis," vol. 16 (L. S. Birks et al., eds.), Plenum Press, N.Y., 1973, pp. 102-110. 3 Mev protons used.

[c]Nanograms element per square cm of sample surface, could be converted to solution analysis capabilities by evaporating X ml of liquid on a 1 cm² area for analysis.

[d]1 cm² × 0.1 cm thick dry tissue; wet weight of 0.2 gm assumed to estimate determination limit in µg/gm (ppm) wet weight.

[e]Failure to cite a value due to lack of data, does not necessarily indicate analysis is impossible.

trates the sample. As a result, proton induced X-ray emission analysis (PIXE) has rapidly emerged as a sensitive analysis approach which may be subject to fewer interference problems than X-ray induced emission. Thus, the X-ray source is replaced by a van de Graaff accelerator to produce proton beams in the **2.5 to 3.0** Mev energy range.

A potentially significant capability associated with PIXE analysis is that of direct analysis of biological tissue sections or blood. Recent publications[5,6] have shown that Cl, K, Fe, Cu, Zn, Pb, Se, Br, Sr, and S could be directly measured in a 30 µm thick section of human kidney; that the same elements plus Mn, Ni, Hg, Rb, and Zr could be determined in a thick section of carp muscle; and that several elements can be directly measured in tissue sections of liver, kidney, lung, and bone. The analysis of liquid- or wet-digested materials may be based on evaporation of the liquid phase to leave a residue deposit on an appropriate analytical substrate, Ions in solution may also be preconcentrated for analysis using filters impregnated with ion exchange resins. ' Determination capabilities for XRF and PIXE are listed in table H-4,

The general capabilities of these techniques are such that they can be used in a laboratory of the type considered. It should be emphasized, however, that quantitation of the measurements is subject to difficulties particularly for direct measurements. Proponents of the techniques argue that these problems can be readily overcome; others (cynics??) argue that this will require extensive development efforts. The truth appears to be intermediate between these extremes. The ultimate decision to include the X-ray capabilities in the laboratory facility should probably be based on the essentiality (desirability) of being able to analyze solid samples (e.g., tissue thin sections) directly for several elements of potential interest. This capability may be considered by some to be advantageous simply from the semiquantitative screening standpoint.

[5] J. L. Campbell et al. "Advances in X-ray Analysis, " vol. 17 (C. L. Grant et al., eds.), Plenum Press, New York, 1974, pp. 457-466.

[6] P. S. Ong et al., [bid., vol. 16 (L. S. Birks et al., eds.), 1973, pp. 124-133.

S. L. Law and W. J, Campbell, "Advances in X-ray Analysis, " vol. 17, Plenum Press, New York, 1974, pp. 457-466.

PREPARATION OF **SOLID MATERIALS** (BIOLOGICAL TISSUE) FOR ANALYSIS

Many techniques require that solid samples be converted to solution form for analysis. The literature on methodology for sample decomposition is immense, The procedures cited find both wet-(acid digestion) and dry-(ashing) oxidation methods extensively used. Dry-ashing methods are usually implicated when problems with recovery or losses of analytical constituents are reported. In comparing wet- and dry-ashing methods, the paucity of data specific to real-life samples makes it inappropriate to state categorically that one method is superior to another. Some generalizations can, however, be made on the basis of procedural differences. Wet oxidation has the advantage of requiring a minimum of apparatus and is less prone to volatilization and retention losses than dry-ashing. Wet-ashing may suffer in that relatively large amounts of reagents having significant levels of contamination may be required and contact with glassware may account for a higher risk of contamination than dry-ashing. Dry-ashing requires few, if any, reagents and handling of larger samples presents less problems. The risk of volatilization, convection, and retention losses is higher, however, unless ashing conditions are carefully controlled, These risks have led to the fairly widespread adoption of low temperature ashing (LTA) based on reaction of the sample of the oxygen free radical generated via a radio frequency field. While it is true that materials can be converted to oxides under these conditions, it is also apparent that several elements may still be lost by volatilization at typical operational temperatures of 100° **to 150° C.** These are largely elements with a tendency to form volatile chlorides, oxychlorides, or hydrides, Thus, the extent of their loss may be particularly influenced by the halogen (chloride) content of each sample, The use of programed-temperature ashing furnaces has been shown to be effective in preventing or reducing volatilization losses. Raising the temperature at a rate permitting slow charring and oxidation of the sample is to be highly recommended.

Nitric acid is a universally used wet-oxidant, The azeotrope boils at 120° C, a factor which assists in its removal after oxidation but also limits

its effectiveness in completing the oxidation process. The most effective medium for wet oxidation is a mixture of nitric and perchloric acid; the azeotrope boils at 180° C to force oxidation. Extraordinary care to avoid explosions and fume hoods is required. The precaution of keeping sufficient nitric acid present until all easily oxidized material is gone (cessation of brown fumes) is particularly important in wet digestion of tissues having lipid (fat) contents. Because the acids used are never absolutely free of metal contamination, the objective of wet oxidation should always be to complete the oxidation with the smallest possible amount of acid. This minimizes the blank problem. The use of reflux digestion apparatus equipped with condensers is focused on this objective as well as that of minimizing losses. Recent systems which rely on microwave ovens for heating the acid-sample mixture also show considerable promise.[8,9] The microwave system heats the solution rapidly and prevents bumping and frothing. Recovery studies run on a range of elements suggest that loss problems are minimal. Bovine liver is notorious for being difficult to wet digest. The report[10] that 2 gm (wet weight) or 1 gm (dry weight) of beef liver can be digested in 10 ml of nitric/perchloric acid in 2 to 3 minutes indicates an attractive capability that will surely guarantee extensive use of the microwave system.

For any of the analysis techniques which require sample dissolution, the approximate upper limit of "dissolved salts" that can be tolerated is 2 percent (wt/vol). Thus, for a wet biological tissue which yields 10 percent ash, this upper limit would be equivalent to dissolving 20 *gm* of the tissue (wet wt) per 100 ml of analytical solution. Working with half that weight per 100 ml would have practical advantages.

[8] A. Abu-Samra, J. S. Morris, and S. R. Koirtyohann, Anal. Chem., 47, 1475 (1975).

[9] U. S. Patent No. 4,080.168 (Mar. 21, 1978).

[10] "Report to the U.S. FDA (Contract No. 223-75-2268] Dec. 2, 1976.

ASSURANCE OF ANALYTICAL ACCURACY

Quality assurance in an analysis laboratory refers specifically to the question: Are the analytical results valid (accurate or reliable]? Obtaining the answer is complicated by the fact that all analyses are subject to random (indeterminate) errors and may also be subject to systematic (determinate) errors. Errors are cumulative; those which characterize the accuracy of any analysis result may include the composite contributions of the random and systematic errors inherent in the analysis method(s) used and those characteristic of each analyst, Laboratory, or set of equipment involved in the analysis. To maintain quality assurance, an operating laboratory must base its program on some combination of the possible strategic approaches. These include:

1. Recycling of submitted samples to obtain cross check analyses,
2. Recycling of certified or secondary standard reference materials to estimate analytical accuracy,
3. Spike-recovery studies to estimate accuracy,
4. Participation in collaborative test programs, and
5. Comparison of results obtained by independent analysis methods.

Each of these provides specific types of relevant information but all should be included in the program. Moreover, any of the above tests should be carried out incognito. Otherwise, the analysts may be tempted to devote inordinate attention to the check analyses.

All of these methods add to the expense of lab operation. It is necessary, however, for the lab personnel to be acutely aware of the ways in which they or their analytical methods can fail and (ideally) know when they have failed. Otherwise, their products—the analysis results—will be subject to challenge. Experience with the legal process suggests that the agreement between results obtained by two or more independent analytical methods is a primary indicator of success (accurate analyses). Thus, the planned inclusion of analysis redundancy (5 above in particular) in the laboratory operation is to be recommended; a typical quality assurance program would involve check analyses amounting to 5 to 10 percent of the total load.

[1] I An expanded discussion of quality assurance programs and the necessity thereof is given in the paper by R. K. Skogerboe and S. R. Koirtyohann, "Accuracy Assurance in the Analysis of Environmental Samples," NBS Special Bulletin 422 (1976).

LABORATORY INSTRUMENTATION AND FACILITIES REQUIREMENTS

Consideration of the above discussion indicates that the majority of the analytical requirements can be satisfied by plasma emission spectrometry or by atomic absorption spectrometry with heavy reliance on furnace atomization systems. The inclusion of more than one measurement technique in the laboratory can also be justified on various bases. The advantages, limitations, and tradeoffs involved will be considered below. Regardless of which instrumentation facilities are chosen as primary; the space requirements, ancillary facilities requirements, and personnel requirements are comparable. Thus, discussion of these prior to consideration of the analytical instrumentation facilities is appropriate,

Personnel, Space, and Ancillary Facility Requirements

The general design of the laboratory should include four physically separated types of space: 1) office space for personnel, 2) a sample receiving and storage room, 3) a sample preparation laboratory, and 4) the analysis laboratory. Working desk space should be included in the last three types of space in addition to the facilities discussed below. All lab facilities will require temperature control to plus or minus 5° F and humidity control (less than 50 percent).

It is recommended that computer capabilities should be a primary ancillary facility included in the laboratory operation. This is based on several rationales including:

1. several types of instrumentation likely to be present are most effectively used under a computer control-data acquisition mode of operation:
2. personnel requirements, time commitments, and human errors can be reduced in a computer-oriented operation; and
3. a computer system may be essential as a data management, quality assurance evaluation, warning assessment, and report preparation tool in a laboratory operation of the size likely for the present program.

Although the computer configuration selected will be dependent on the laboratory purpose(s), the analytical facilities installed, the size of the operation, and other subsidiary factors, it is likely a computer acquired with one of the instruments described below could be adapted for use in an intralaboratory, interactive (time-shared) mode and tied to a larger computer system (external to the laboratory) which would perform those data management functions that need not be carried out on a real-time (fast response) basis. Systems which use internal, instrumentation-coupled, computers for control and data acquisition purposes and transmit the data to central management computers are presently in operation at the USGS Water Resources Laboratory in Denver and the EPA Laboratories in Cincinnati. Although these operations may not be the best model examples, the conceptual approach which they embody is recommended for the present operation. Further comments relating to this are inserted in appropriate sections which follow.

Sample Receiving and Storage Room—The receiving operation will necessarily include facilities for logging in samples and the associated analytical work requests. A computer terminal should provide the most effective capability. The storage facilities should include: shelving or cabinets for those samples that can be stored under ambient conditions, a cold room (4°C) for storage of water samples, and a freezer for storage of certain biological samples. The size of the room and storage facilities required will depend on the anticipated sample load and variations in the sample submission rate. Since such data have not been supplied, size estimates and costs are not included in this report,

Sample Preparation Laboratory—The design of this laboratory will be sample type and sample load dependent. If the sample types to be analyzed include those which must be ground in the dry form such that atmospheric contamination can occur, the grinding facilities should be physically isolated from the other sample preparation operations, In addition, the sample preparation lab should basically be a clean room[12] operation or it should be equipped with laminar flow, filter hoods in which certain sample preparation operations can be carried out. Both wet- and dry-oxida-

[12] Clean room or clean hood environments are classified on the basis of controlling the concentrations of particles (dust) in air which can contaminate the samples by fallout. A class-100 clean room, for example, must have no more than 100 particles in the 0.5- to 5- micrometer diameter size range per cubic foot of air. A class-1,000 room permits 1,000 particulates per cubic foot in the above range. To meet these specifications, air filtration is required. See the 1963 Federal Standard No. 209:1,

tion facilities should be included in the laboratory plan to permit handling of various sample types in the most expeditious manner. A list of sample preparation facilities likely to be required is given with their approximate costs in table H-5. Access to a computer terminal to permit convenient entry of sample preparation data is also recommended.

Analysis Laboratory—The instruments selected will influence the facilities and size requirements of this laboratory. It is assumed that the prepared samples will be delivered to this lab in closed containers and will be handled in ways that will minimize the probability of contamination via atmospheric contact or fallout. Thus, although rigorous clean room operation has not been recommended for this lab, it will be necessary that all analytical operations which can result in simple vaporization be carried out under ventilation conditions where the discharge is external to the lab.

Personnel Requirements and Performance—Stipulation of exact personnel requirements will again be dependent on the sample load and the types of samples received. The sample logging operation can be handled by an individual with no chemical training but with secretarial or keypunch skills: this individual might also perform some of the simpler sample preparation operations, e.g., weighing and grinding. The preparation laboratory should be staffed by individuals with B.S. degree training in chemistry with 1 to 3 years of wet chemical experience.[13] One such individual can typically prepare 15 to 30 biological tissue samples per day by wet- or dry-oxidation techniques depending on the complexity of the preparative steps involved. The analysis lab should also be staffed by chemists with at least B.S. degree training plus 2 to 4 years of experience.[14] The experience should preferably be in the general area of instrumental trace analysis dealing particularly with the types of instrumentation to be used. A working familiarity with basic electronics and minicomputers would be desirable. The availability of major repair capabilities through instrument maintenance contracts should be insured.

For the lab personnel, it may be assumed that the average analysis production hours per man-day will range between 5 and 6. The remaining time will be utilized for preventive maintenance of equipment, recordkeeping, cleanup, etc. Although there are nominally 260 working days per annum, this reduces to about 200 days when vacations, holidays, sick leave, and refresher training time are taken into account. The number of personnel required and the sample throughput capabilities of the lab must be defined on this basis.

Table H-5.— List of Sample Preparation Facilities Required

Preparation facility	Approximate cost, $[a]
Analytical balance, ± 0.1 mg	$2,500
Lyophilization unit	8,000 automatic 3,000 manual
Sample grinding unit[b]	4,000
Wet oxidation unit (10 to 15 sample capability)	4,000
plus perchloric acid hood	8,000
Dry oxidation unit (programed temperature)	6,000
Laminar flow (class 100) clean hoods, 2 each[c]	8,000
Reagent purification units[d]	
Quartz and sub-boiling stills	4,500
Electrolysis apparatus	2,000
Miscellaneous teflon and glassware[e]	$5,000-10,000

[a]Estimates based on 1978 catalog prices, installation costs for hoods are not included because they are affected by other features of the building.
[b]Use in a separate room to reduce cross-contamination problems may be advisable.
[c]If laboratory is to be a new building, it may be economically advisable to construct a clean room rather than install clean hoods.
[d]Purification of reagents in-house is economically advisable when sufficient quantities are required.
[e]Teflon ware is often required to prevent contamination and adsorption-loss problems. Although the initial costs of glassware are less, the long-term costs may be more expensive than Teflon due to breakage.

[13]These should be considered minimal requirements with emphasis placed on experience at any degree level.
[14]Again emphasis should be placed on experience at any degree level.

ANALYTICAL INSTRUMENTATION

The previous discussion has presented the general analytical features, capabilities, and limitations of the various analytical techniques considered applicable to the present problem. A summary of the cost, space, and throughput features of these types of instrumentation is given in table H-6. Although the data presented in that summary have been based on personal experience and discussions with other practicing analysts, they must be considered as estimates only. This applies in particular to the cost per analysis because it will be strongly influenced by the applicable salary

Table H-6.—Summary of Cost-Productivity Estimates for Various Analytical Techniques

Instrument facility	Approximate acquisition cost, $[a]	Space requirements sq. ft.[b]	Estimated no. analyses per man-day[c]	Approximate cost per analysis, $[d]	Data system requirement[e]	Estimated down time, %[f]
ICP-MAES	100,000	200-400	1,500-2,000	0.1-0.3	Yes"	3-5%
DCP-MAES	60,000	200-400	1,200-1,500	0.1-0.3	Yes*	3-5%
Flame & furnace AAS						
Single element mode[g]	25,000	200-400	100-200 (flame)	0.7- 1.0	Desirable	3-5%
			50-100 furnace	1.0- 1.5	Desirable	3-5%
Multielement mode[h]	40,000	200-400	400-800 (flame)	0.3-0.5	Yes'	3-5%
			200-400 (furnace)	0.5-0.8		
Electrochemical[i]	25,000	200-400	50-100	1.0- 1.5	Yes*	3-50/0
N. A.A.[j]	40-50,000[k]	200-400	200-700	?	Yes*	?
X-ray fluorescence	60-100,000	200-400	200-700	7	Yes*	3-50/0
PIXE[j]	300,000[l]	300-500	400-800	7	Yes*	?

[a] For 30-element analysis capabilities including instrument installation
[b] In addition 10 sample preparation and office space but allowing for adjacent working space.
[c] Samples Previously prepared for analysis, data management system available, 5 hours on the Instrument per 8-hour day, one analysis defined as determination of one element per sample, approximately 10 percent of total analytical load involving quail ty assurance assumed, all analyses to Involve simultaneous multicomponent determinations of 10 to 15 elements on each sample
[d] Cost per element per sample, samples Previousl, prepared for analysis, approx 5-year instrument depredation assumed, see footnote [c] above
[e] Instrument control, data acquisition and data management system considered essential for all facilities; * indicates inclusion of a minicomputer or microprocessor of at least 8K memory In acquistion costs
[f] An upper limit estimate intended to include preventive maintenance
[g] Analyses based on determination of one element at a time Cost estimate includes hollow cathode lamps for 30 elements and for automatic sample injection but not for a computer/microprocessor system
[h] Analyses based on determination of 5 t. 7 elements at a time Cost estimate includes hollow cathode lamps for 30 elements, an automatic sample injector, and a data acquisition and control system
[i] Limited principally to analyses for As, Cd Cu, Fe, Pb, Se, Tl, and Zn; other metal analyses possible but not widely practiced
[j] Same type of readout facilities could be used for both techniques Radiation protection required particularly for NAA and PIXE systems
[k] Does not include reactor costs
[l] Reduce b, -2513000 if surplus van de Graaff accelerator is available

structure, to actual types and numbers of analyses to be performed, the efficiencies of the employees involved, and the final laboratory design configuration, The instrument acquisition estimates include initial installation and assume that the experience of the operating analyst will minimize startup time requirements. The estimated number of analyses per 8-hour day assume samples have been previously prepared for the analysis; that the instrument operation will rely on computer-controlled data acquisition and management; that the salary of the operation would approximate $20,000 to $24,000 per annum; and that the instrument would depreciate completely over a 5-year period. These estimates do not allow for laboratory refitting or remodeling costs or for overhead costs above the direct instrument operational costs. Such factors have been excluded because the costs involved are highly contingent on the extent of lab refitting required, the location of the lab, and the wide variations in overhead charges. It should also be noted that the overhead costs will be essentially the same regardless of the lab instrumentation facilities selected. The footnotes to table H-6 provide further qualifying information.

The decision to exclude estimates of the costs of preparing the samples for the analyses has been based on several factors including: 1) lack of information regarding sample types and numbers, 2) the influences of the preparation procedures actually selected on costs, and 3) the fact that the sample prep costs will be essentially the same for each of the analytical techniques considered.

Comparisons of the estimates presented indicate that the plasma emission techniques offer superior economic advantages which are complementary to the sensitivity advantages previously discussed. Although more analyses per day can be carried out with the plasma techniques, the present estimates have been based on the expectation that it will be necessary to analyze each sample via two different sample introduction methods to achieve the required sensitivity. Thus, several elements may be determined by direct nebulization of the aqueous sample solutions. A second set of elements, e.g., As, Bi, Hg, Sb, Se, and Sn, may have to be determined using the hydride generation method to achieve the required sensitivity (low-threshold effect levels). A similar situation is anticipated for atomic absorption spectrophotometry; direct flame measurements will be adequate for some elements while furnace measurements will be required for others. Analysis in the single-element mode for AAS is clearly more costly. The inclusion of the simultaneous multiele-

ment AAS estimates is based on present experience at Colorado State University. Conversion of single-element AAS units to the multielement capability can be anticipated in the near future. The long-term cost advantages are obvious.

The restricted capabilities of the electrochemical system tend to remove it from competition except as a supplementary or specialized capability. Primary arguments for the use of NAA or the X-ray techniques are based on the ability to directly analyze tissue (solid) samples without dissolution. Unless this capability is important, the cost differentials evident in table H-6 argue against their use.

In view of these data, the use of the ICP-MAES system as the primary instrument appears quite rational, The inclusion of an AAS system and an electrochemical (EC) system as support (e.g., cross-check) techniques should be recommended. The cost estimates given for the AAS and EU units in table H-6 were based on the expectation that each might be the primary lab facility. When their roles are reduced to a secondary (support) level, less sophisticated (high versatility and production) units may be acceptable. The cost of an adequate AAS facility could thus be reduced by -$8,000 to $10,000 and that for the electrochemical unit by -$5,000 to $10,000. It should also be noted that these lower cost units could be interfaced to the ICP-MAES computer system preferably using inexpensive ($300 to $500) microprocessors as buffers.

ESTIMATED ANNUAL SAMPLE THROUGHPUT LOAD

The above estimates of sample throughput capabilities may be used to approximate annual analytical productivities. These are based on a total of 260 working days per annum minus 60 days for vacations, holidays, downtime, quality assurance time, etc., leaving 200 effective 8-hour days. The projection below is based on the assumption that the primary load will involve the analysis of biological (organic) tissues; such analyses probably represent the most rigorous time/cost requirements,

Using the estimate of 15 to 30 samples per day as the load that can be handled by a sample preparation technician, the annual preparative capability ranges from 3,000 to 6,000 samples. Analysis of these samples for 30 elements (as an upper limit example) using the plasma emission capability involves performing 90,000 to 180,000 analyses. Applying the lowest analytical throughput (1,500 analyses/day; table H-6) suggests that 60 to 120 days would be required for these analyses once the samples were prepared. In essence. this emphasizes a fairly universal observation, i.e., preparation of samples for analysis is often the factor which limits laboratory productivity. Several inferences may be drawn from the above sample preparation and analysis estimates:

1. The analytical capability proposed is sufficient to keep 2 to 4 sample preparation personnel busy if biological tissues comprise the principal workload of the lab.
2 The sample preparation methods used need to be upgraded in terms of throughput per unit time.
3. The analytical facility could be used for additional types of analyses which do not require sophisticated (time consuming) sample preparation operations, e.g., water samples.

Comments on the last two possibilities are appropriate.

Primary factors which affect the sample preparation time/cost requirements include: the elements to be determined and the types of samples. The determination of toxic elements in natural and effluent waters is perhaps the simplest case. The water samples must be filtered and appropriate preservatives added; these operations must be carried out as soon after sample collection as possible and preferably in the field so essentially no lab prep operations are required, Such analyses could occupy the additional time available on the instrumentation. The preparation of animal tissues (particularly liver) is at the other extreme. These contain varying amounts of fatty materials (lipids) which are difficult to decompose by wet oxidation techniques thereby requiring that rigorous conditions be used. Although dry oxidation essentially circumvents this problem, the fact that several elements may be lost by volatilization forces the use of slower oxidative methods, e.g., temperature-programed furnaces, low-temperature ashing units, or sealed [high-pressure) bomb systems. The estimates given above have been predicated on the use of wet oxidation (reflux) techniques in common use; these appear to be less susceptible to problems than the dry techniques. It has also been assumed that complete destruc-

tion of lipids present is required. An examination of the literature, however, has not produced convincing evidence that this is essential. The point raised is simply: Can the most significant fraction (e.g., >90 percent) of the elements of interest be "extracted" from the tissue matrices via wet digestion without complete destruction of the fat, lipids, or cellulose present? If so, the sample preparation times can be prominently reduced. Finally, it should be noted that the wet ashing of biological materials in microwave ovens shows promise in alleviating the digestion time problem.[15] Spike-recovery studies carried out when food products were digested via this method are very encouraging. The ability to reduce sample digestion times by a factor of two or more appears to be a reasonable estimate. Factors of this nature must be considered in the final planning stage for the present program.

Adel Abu-Samra, J. S. Morris, and S. R. Koirtyohann, Anal. Chem., 47, 1475 (1975). See also reports on USPH contract No. 223-75-2268 and U.S. Patent 4,080,1 68.

ANALYTICAL ACCURACY COMPARISONS

As emphasized above, the validity (accuracy) of the measurements strongly influences the integrity of any decision based on the results, Any measurement is subject to errors which may be influenced by several factors including the measurement methods used and the analyst responsible. This is the primary reason for stressing the importance of a quality assurance program. The general degree of accuracy that can be achieved with analytical techniques discussed above can be anticipated to be essentially the same for all techniques. This first approximation expectation is based on the following. First, each of the techniques discussed essentially requires the same sample preparation procedures. Since these are likely to prove to be significant (primary?) sources of analytical errors, the errors which may accrue during the preparative steps will be essentially the same for all techniques. Second, the analysts involved can be responsible for the cause or prevention of errors depending on the expertise and caution they exercise. The analyst that tends to use poor technique or judgment when applying analysis method A will, in all probability, do the same for method B; the errors for which he is responsible will be comparable in both cases. Finally, the measurement accuracies of each technique discussed above are generally similar. As a result, there are no clear-cut, easily defensible reasons for suggesting preference for one approach over another on the basis of improved accuracy.

RESEARCH NEEDS ASSOCIATED WITH THE PRESENT PROGRAM

In the section discussing the criteria involved in the selection of an analytical method, the general importance of being able to identify and measure the chemical forms or oxidation states of several elements was emphasized. This emphasis was based on the fact that all segments of the ecological system are affected to varying extents by the elements (e.g., metals) present. Indeed, the statement that "the life processes of every living cell are conditioned by the types and amounts of metals present" would be accepted by a majority of scientists. Some metals are essential to the health of living systems and, yet, they may also be insidious pollutants because of their generally nonbiodegradable nature. Only a few metals are completely nontoxic at any concentration level; most cause deleterious effects at some exposure level. The ultimate definition of: 1) what constitutes a deleterious effect?; 2) what actually causes it?; and 3) what are the operative threshold-effect concentration levels? cannot be considered trivial problems. It is unfortunate that simply measuring the total amount of a particular element present in an ecological system may, in fact, be only a gross indicator of potential or actual deleterious effects, What is often needed is the identification of the active or functioning forms of the elements in question. Although numerous examples which support this statement can be cited, the fact remains that chemical form is often extremely important.

For this reason, the development and refinement of analytical methods and techniques for the identification of chemical form (chemical speciation), at the trace to ultratrace concentration levels so often of interest, has received considerable attention in the past decade, Although this is

clearly an important area of research, progress has been slow for several reasons. These include:

1. The analytical techniques most suitable for the determination of chemical form are also generally those which lack sufficient sensitivity for use at the low concentrations characteristic of many elements in biological systems.
2. Biological systems are inherently very complex mixtures of a wide variety of chemical constituents. It is a truism that the complexity of the identification problem is dramatically enhanced by the compositional complexity of the target system.
3. While it can be argued that chemical identification techniques are necessary for the delineation of what chemical forms are biologically or toxicologically important, it appears equally valid to argue that such knowledge on an *a priori* basis is highly beneficial as a n aid i n focusing the development t work.
4. The analytical chemical community is often only partially aware of what types of chemical speciation measurements are considered most important by the medical, toxicology, and ecology communities. Similarly, the latter are often only peripherally aware of the most promising emergent analytical technology.
5. The scientific communities involved in this chemical speciation question have often been forced by the pressing circumstances so frequently associated with deleterious effects to expend their efforts on the use of less than satisfactory approaches leaving less time for the required types of development.

Although other contributing factors could be cited, the case in point is simply that the chemical speciation capabilities so badly needed are available in only limited instances. The development of adequate speciation technology should be a central thrust of research efforts involving collaborative efforts between the toxicology, biological effects, and analytical chemical communities. Governmental and private funding agencies should strongly encourage these efforts, Because propogation of chemical speciation developments will ultimately be essential to the success of the overall monitoring program, the laboratory facilities available at the outset should take advantage of present capabilities. Moreover, the prime stance of the program should be that of providing feedback to the relevant scientific communities as one means of focusing speciation research efforts on those elements which may appear to be more important from the effects standpoint.

Technology presently exists for the differentiation between the organic (alkylated) and inorganic forms of the toxic elements: arsenic, lead, mercury, and selenium. These rely on the fact that the inorganic forms can be easily converted to gaseous forms (mercury vapor and the hydrides) by reaction with borohydride while the organic forms must first be photodecomposed by exposure to ultraviolet (UV) radiation, Thus, analysis of the gaseous reaction products before and after UV irradiation provides measurements of the relevant organic and inorganic concentrations. This chemical differentiation approach has been interfaced with atomic absorption and emission spectrometry as the measurement tools; inclusion of this capability in the laboratory is recommended.

Similarly, the ability to differentiate between the possible oxidation states for some metals is important, e.g., As(III) versus AS(V) or Cr(III) versus Cr(VI), Such differentiation for As, Cr, and some other metals can be accomplished by use of selective oxidation-reduction (redox) reactions. The redox reactions may be controlled by proper selection of chemical reagents or by judicious use of electrochemical (polarographic or voltammetric) principles. Electrochemical measurements can also be used to differentiate between electroactive and nonelectroactive (chemically bound) forms of some elements and between “labile” and ‘‘nonlabile”’ bound forms of some elements. In this context, labile and nonlabile refer to the thermodynamic and/or kinetic stabilities of the metal-ligand systems in question. Although this type of differentiation may be only semiquantitative or semiempirical in nature, such measurements have been shown in some instances to be pertinent in experimentally defining bioavailability, transfer mechanisms, transfer rates. etc. It is certain that research must still be done to further delineate the diagnostic potentials of such types of differential measurements, Present knowledge seems sufficient, however, to support the inclusion of polarographic and voltammetric instrumentation in the laboratory facilities for “lability’” and/or oxidation state measurements.

A limited number of other possibilities exist for chemical speciation measurements. These are largely very specialized in terms of applicability to certain types of samples and will not be discussed herein. It is certain that the need for chemical speciation exists and that its importance will become even more apparent as more definitive

monitoring programs evolve. It is also certain that no single analytical technique for speciation or any other purpose will be a universal panacea. The ultimate solutions to the majority of speciation problems will involve the application of some combination of chemical and physical principles, The research programs most cognizant of this are most likely to be successful.

GLOSSARY OF ABBREVIATIONS

AAS—atomic absorption spectrophotometry
AES—atomic emission spectrometry
ASV—anodic stripping voltammetry
cc—cubic centimeters
cm--square centimeters
DCP—direct current plasma
DPP—differential pulse polarography
FC—Faradaic or analytical current
Ge(Li)—designates a germanium crystal which has lithium drifted into it to give it uniquely defined properties
gin-gram
ICP—inductively coupled (radiofrequency) plasma
MAES—multielement atomic emission spectrometry
Mev--million electron volts
ml—milliliter
NAA—neutron activation analysis
Ng—nanograms—one billionth of a gram
PES—plasma emission spectrometry
PIXE—proton induced X-ray emission (spectrometry)
ppb—parts per billion (1×10^{-9} gms/gm on a weight basis or 1 x 10-j µg/ml on a weight per unit volume basis)
ppm—parts per million (1×10^{-6} gm/gm or 1 µg/ml as for ppb)
Si(Li)--a silicon crystal which has lithium drifted into it to provide unique physical properties
SMS—solids mass spectrometry
µg—one millionth of a gram
µl—one millionth of a liter
µm—one millionth of a meter
XES—X-ray emission spectrometry
XRF—X-ray fluorescence (emission spectrometry)