

Chapter 2

ENVIRONMENTAL HEALTH AND HUMAN EXPOSURE ASSESSMENT

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ABBREVIATIONS AND ACRONYMS

AAS	atomic absorption spectrometry
AES	atomic emission spectrometry
AFS	atomic fluorescence spectrometry
AOAC	Association of Official Analytical Chemists
APDC	ammonium pyrrolidine dithiocarbamate
As(III)	arsenous acid, arsenite
As(V)	arsenic acid, arsenate
AsB	arsenobetaine
ASV	anodic stripping voltammetry
AWWA	American Water Works Association
CD	criterion of detection
cm	centimetres
COMP	composite proportional sample procedure
CT	cold trapping
D Chart	mean of recovery chart
DD	duplicate diet survey
DHI	Water & Environment (Denmark)
DMA	dimethylarsine, dimethylarsinic acid
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organisation of the United Nations
g	gram
GFAAS	graphite furnace atomic absorption spectrometry
HCl	hydrochloric acid
HGAAS	hydride generation atomic absorption spectrometry
HPLC	high-pressure liquid chromatography
IAEA	International Atomic Energy Agency (Austria)
IARC	International Agency for Research on Cancer
IC	ion chromatography
ICP	inductively coupled plasma
ICP-AES	inductively coupled plasma atomic emission spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
IRMM	Institute for Reference Materials and Measurements, European Commission, Joint Research Centre

ISO	International Standardisation Organisation
ISO/CD	International Standardisation Organisation – committee draft
K	Kelvin
kg	kilogram
l	litre
LGC	Laboratory of the Government Chemist (United Kingdom)
LOD	limit of detection
LSE	liquid solid extraction
m	meter
M	molar
MB	market basket survey
mg	milligram
MIBK	methyl isobutyl ketone
ml	millilitre
mm	millimetre
mS	micro Siemens
MMA	monomethylarsonic acid
MS	mass spectrometry
NFCS	National Food Consumption Survey
NIST	National Institute of Standards and Technology (USA)
nm	nanometre
NMKL	Nordic Committee of Senior Officials for Food Issues
NRC	National Research Council (Canada)
NRCCRM	National Research Centre for Certified Reference Materials (China)
NWRI	National Water Research Institute (Canada)
pH	negative logarithm of hydrogen ion concentration
pKa	first dissociation constant
ppb	part per billion
PQL	practical quantification level
PTWI	provisional tolerable weekly intake
QC	quality control
R-chart	range chart
r-chart	relative range chart
RTD	random daytime sample
SD	standard deviation
SDDC	silver diethyldithiocarbamate
SM	Standard Methods for the Examination of Water and Wastewater
TDS	total diet study

USEPA	United States Environmental Protection Agency
USDA	United States Department of Agriculture
UV	ultraviolet
VKI	Institute for the Water Environment (Denmark)
WHO	World Health Organization
X-chart	control chart of mean
°C	degree Celsius
µg	micro gram
µl	micro litre

SUMMARY

Arsenic is naturally found in surface and groundwater, but the concentration levels differ widely depending, largely, on the underlying geology. In many parts of the world the concentration is well below the present WHO provisional guideline value for drinking water of $10 \mu\text{g l}^{-1}$. However, concentrations exceeding the guideline value by 10 to 200 fold have been reported in some parts of the world. Different forms of arsenic exist with different human toxicity. The inorganic forms of arsenic, which are the dominant forms in surface and groundwater, are the most toxic forms, while the organic forms, common in fish products, are much less toxic. Humans primarily take up arsenic from drinking water and food products. It is, therefore, important to measure the arsenic levels in water resources (i.e. groundwater and surface water), in order to identify potential problems before they give rise to adverse health effects within the population. Furthermore, there is a need to measure and control the arsenic in crops and other food products that are known to concentrate arsenic from water.

When ingested, arsenic is transported (via the blood) to different organs in the body and is excreted in the urine. It is possible to detect arsenic exposure through examination of biological samples of hair, nails, urine and blood. When analysing urine, in particular, high total arsenic concentrations may be a result of a high intake of organically bound arsenic from fish products, which are relatively non-toxic, this highlights the need for robust methods to distinguish between inorganic and organic forms of arsenic and, ideally, to identify the particular organic and inorganic forms present.

The following laboratory based analytical methods are well-described for routine analyses: hydride generation atomic absorption spectrometry (HGAAS), graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES or ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), atomic fluorescence spectrometry with hydride system (HGAFS), anodic stripping voltammetry (ASV), silver diethyldithiocarbamate spectrometric method (SDDC). The last-mentioned method (SDDC) has been widely used due to its simplicity regarding instrumentation (spectrometry). Depending upon the analytical principle employed, digestion, filtration, preservation and/or pre-reduction is carried out prior to the analyses. By use of chromatographic principles or a combination of different techniques it is possible also to determine the content of different arsenic species, although this is generally not performed on a routine basis.

Simple analytical techniques have been developed for analyses in the field and have been widely used for screening for many years. The mercuric-bromide-stain method is the principal method employed by field-testing kits. The analytical performance of field test kits is, unsurprisingly, lower than that of any of the above mentioned laboratory based methods and care must be taken when using data from field test kits. On the other hand, field test kits are useful for screening of groundwater in a highly contaminated geographical area.

Filtration or digestion of water samples may be prescribed. Where dissolved arsenic is to be determined, filtration through a $0.45 \mu\text{m}$ membrane filter as soon as possible after sampling is recommended. Where total arsenic concentrations are required and

the samples contain particles, pre-treatment may include sample digestion or digestion of the material caught on the membrane filter. Digestion procedures for determination of arsenic in water are described in analytical standards and will depend on the analytical procedure available for detection of arsenic.

Food and human biological samples for determination of arsenic need digestion prior to detection. Numerous digestion techniques are described in literature, although different digestion techniques may lead to different results and care must be taken when comparing results where different digestion techniques have been used. Apart from the digestion, the analytical techniques for the determination of arsenic are, in principle, the same for solid samples as for water.

Independent of the analytical techniques applied, whether laboratory based or field-kits, the quality of the procedures (including sampling) should be documented. Many countries have established an accreditation scheme, thereby ensuring that laboratories meet defined requirements, e.g. ISO/ICE 17025:1999. Quality requirements need be defined and related to the use of the data in each particular case. To be able to observe a statistically significant change in concentration over time it is necessary to define the minimum acceptable analytical quality. On the other hand, laboratories must document the analytical quality obtained for the actual analyses in order for them to be able to evaluate the analytical data.

Quality control (QC) is an important tool for monitoring, controlling and documenting analytical quality. By using internal QC laboratories are able to control the detection limit, accuracy and precision obtained. One important pre-requisite is the availability of control samples or reference materials for controlling the accuracy and traceability of the results. Reference materials for arsenic are available for some matrices and should at least be used during method validation. External QC (e.g. inter-laboratory comparisons and proficiency tests) is carried out on an international and national basis for some matrices. Participation in such exercises, on a regular basis, is an important means to determine equivalency of data and to determine the general analytical quality that may be expected for a certain group of laboratories.

The principal focus for future research centres on the need for more routine analysis of the different forms of arsenic, especially the inorganic forms. This requires concerted effort to develop robust and rugged international standard methods for the measurement of these forms in a range of matrices.

2.1 ASSESSMENT

The toxic nature of arsenic is well known and has long been recognised. The principle forms of human exposure are through ingestion, inhalation and skin absorption, however, concentrations in the environment vary widely, depending upon local geology and pollution sources. It is, therefore, important to be able to determine the levels of arsenic to which individuals and populations are exposed. Maximum levels have been prescribed for drinking water in many countries and a large part of the arsenic analysis conducted will be routine monitoring to ensure compliance with regulations. In some areas of the world arsenic in drinking water has been found to be a major (and unanticipated) problem, here examination of arsenic levels in a proposed drinking water source is a necessary pre-requisite to the development of that source. Clearly, as outlined above, water is not the only possible source of arsenic and it can be important to examine As levels in foodstuffs, especially seafood and rice which have proved to be rich sources of the trace metal. Analysis of arsenic in human samples, such as urine, blood, hair or nails can provide information on individual exposure. Although concentrating on drinking water issues, this section examines each of these assessment needs in turn.

2.1.1 Drinking water resource assessment – source screening and selection

The purpose of assessment of water resources is to identify potential problems before they give rise to serious adverse health effects. In developing any new drinking water source, it is important to ensure that any risks to health from various contaminants can be removed or minimised. At the beginning of the process, it is of significant benefit to consider the sources of possible contamination and to establish appropriate monitoring, taking into account the size of the population supplied and the local resources available. The aim is to provide a water supply that does not merely exchange one risk for another and that provides an acceptable quality of water in the most cost-effective way possible.

Arsenic is found widely in surface and groundwaters in many parts of the world. It is primarily of natural origin and, therefore may be a significant constituent even in areas in which industrial or other pollution is extremely limited. Prior to exploitation of a potential water source, it is prudent to determine potential sources of both microbiological and chemical contamination. As part of this process it is sensible to determine whether arsenic bearing rocks are present in the vicinity (see section 1.3.2) or, in the case of a surface water source, upstream of the anticipated water intake. Action taken at this planning stage is an important means of minimising the risk of requiring difficult and expensive remedial treatment at a later stage. Consultation with geologists and geochemists is recommended to ensure expert input with regard to inorganic contaminants such as arsenic. In the case of surface waters it is also relatively easy to obtain samples for a broad screen of water quality in advance of starting to plan water abstraction and treatment. In this case it might be helpful to examine both water and sediment samples since some arsenic species, both inorganic and organic, can adsorb to sediment which may act as a natural concentration step.

The primary problems with arsenic in drinking water have occurred in groundwaters (with levels of 3000 $\mu\text{g l}^{-1}$ and greater recorded – see section 3.1.1.4), particularly in deep wells that were drilled to avoid the problems of contamination from microorganisms in shallower and less protected sources. Since samples can be obtained less readily from such deep sources it is particularly important to carry out

enquiries as to possible problems with rocks bearing arsenic. In the absence of helpful geological information, or when a decision has been taken to use a groundwater source, it would be prudent to carry out test drilling in order to obtain appropriate samples of water for analysis, prior to full-scale development.

In finally selecting a source it is essential to take a holistic view and balance the various risks against the requirements of the population to be served. Microbiological contaminants pose an acute threat to health, while chemical contaminants such as arsenic pose what is essentially a chronic threat. While it is often easier to deal with microbiological contaminants the practicality of various solutions needs to be considered. In the case of arsenic, removal to obtain low concentrations is difficult and requires some kind of technological solution (see chapter 6).

In screening potential water supplies the WHO Guidelines for Drinking-Water Quality (WHO, 1996) provide an excellent basis for assessing the implications for health of the concentrations found. The provisional guideline value for arsenic is $10 \mu\text{g l}^{-1}$. This is based on both the potential risks of arsenic for health and the practical quantification limit. It is not, however, intended to be a hard and fast standard and must be used thoughtfully. In the case of a source that does exceed the guideline value by a small amount, the risk of possible long-term health effects may well be outweighed by the more immediate risks of microbiological contaminants.

An ideal protocol and important considerations in undertaking a source screening survey are outlined in Boxes 2.1.1 and 2.1.2; even if not all of the conditions can be fulfilled the authority concerned should do what it can.

Box 2.1.1 Conditions affecting the survey

In conducting a survey, as shown in Box 2.1.2, it is necessary to gather as much information as possible in three categories, shown below:

(1) Extent of the survey area

In order to determine the extent of the survey area, it is necessary to gather as much information as possible relevant to a survey of arsenic in ground water including the physical size of the survey area, population, environmental conditions, the state of damage occurred, number of sampling points, number of samples, condition of water use, amount of water available and seasonal variation in rainfall. A good idea of the overall scale of the survey can be obtained from analysis of this information.

(2) Supporting system for conducting the survey

Specific information relevant to developing the survey system includes information on the infrastructure such as electricity, gas and water supplies; transportation system for conducting the survey and transporting samples; training and training systems; concerns and understanding of central and local governments about conducting and continuing the survey; support system available for analysis; urgency, available resources and time limit of the survey. The direction of the strategy for conducting the survey must be determined from this information.

(3) Basic conditions of monitoring and measurement

Information on the basic conditions for conducting the survey includes information on the development level of analytical technology in the country and the region, availability of analytical equipment, possibility of securing personnel for the survey and analytical work, educational level of survey and analytical personnel, number of samples that can be processed and analysed, and water quality of the ground water. The monitoring system and analytical method must be determined based on this information.

However, some analysis is essential as early as possible as that is the early warning that a problem exists although one cannot be precise as to what concentration will give rise to effects in the exposed population.

Box 2.1.2 Conditions affecting the survey

Scale of the survey area

Overall information gathering

Assessing the survey scale

- Survey area
- Population
- Rate of success in assessing pollution conditions
- Rate of success in assessing the amount of damage
- Number of geographical survey points
- Attitude toward survey and success in continuing it
- Conditions of water use
- Seasonal fluctuations, including fluctuation of the amount of rainfall
- Map information
- Whether or not a geological survey was conducted

Surveying organization

General conditions relating to implementing the survey

- Assessing the policy of surveying organization
- Commercial availability and conditions of electricity, gas and water supplies
- Surveys, and transport of samples
- State of development of public transportation system
- Attitude of government toward conducting the survey
- Number of analyses per day
- Timing of end of survey
- Education and training facilities
- Urgency

Monitoring environmental information

Assessing the policy regarding analytical method

- Availability and condition of equipment
- Number of analytical technicians
- Success in acquiring analysis technicians
- Education of analysis technicians
- Setting of lower limit of detection
- Support system
- Number of people conducting the survey
- Number of samples

2.1.2 Water resource quality trend monitoring

It is quite possible for the concentration of contaminants to change with time, even in what might appear, at first, to be a stable groundwater. Pumping may pull in contaminants from more distant parts of the aquifer and changes in pH or oxygenation can cause changes in the solubility of inorganic contaminants. It is, therefore, prudent to establish a surveillance regime that is appropriate to the circumstances of a particular source. In the absence of arsenic, or concentrations well below the guideline value, in the source water it is probably appropriate to limit surveillance monitoring to a duplicate sample taken once every year. In the event that arsenic is present at concentrations close to the guideline value it would, ideally, be appropriate to begin by sampling more frequently, for example once per month or two months. Should the concentrations prove stable over the first year of pumping, then sampling could be reduced to once per year. However, if there is an increase in the pumping rate it may be prudent to take a check sample a few months after the commencement of increased

pumping. Should the concentration of arsenic begin to increase then the frequency of sampling should be increased. There are no hard and fast rules in choosing sampling frequency for such surveillance. However, in the event of the sampling being carried out to additionally ensure compliance with national drinking water standards, there may be a sampling regime specified appropriate authorities.

Clearly it is not possible to monitor all wells but it does require common sense to determine groups of wells that will be similar in their geology so that representative wells are sampled. Therefore, some understanding of the geology and hydrology is required. It also means that some knowledge is required of how similar the wells are in depth etc.

To be able to observe a statistically significant change in concentration over time it is essential to have reliable analytical quality (see section 2.7). The purpose of monitoring the water resource quality trend is to be able to identify fairly obvious increases from very low concentrations to higher concentrations (rather than minor variations), however, the quality of the analytical data is the limiting factor for observing such changes.

When designing a drinking water surveillance programme for public health protection it is also important to specify the type of sample to be taken (see section 2.5) and the way in which it should be treated in order to ensure that when it reaches the analytical laboratory it will not have changed (see section 2.5).

2.1.3 Human exposure assessment and public health surveillance

In areas with high concentrations of arsenic in the environment, there may be additional sources of exposure to arsenic other than the normal consumption of drinking water. For example, local customs of food preparation can give rise to an increased consumption of arsenic through cooking processes. In the case of inorganic contaminants such as arsenic, simmering and boiling to reduce the water in a local speciality dish (e.g. soups or stews) may lead to significant elevations in the arsenic concentration in foods.

Assessment of As levels in food can either be made on an individual *ad hoc* basis or, more usually, through the use of diet studies. Diet studies provide valuable population exposure data and consist of two main types (see Table 2.5.2):

- Duplicate diet studies
- Market basket studies

Some examples from the total diet study conducted in the USA are given in sections 2.5.2 and 2.8.1.

Seafood is a particularly rich source of arsenic in the diet. However, the form of arsenic is almost entirely in the form of arsenobetaine, an organic arsenic species, which is considered to be considerably less toxic than the inorganic forms.

Where exposure of an individual or population to arsenic is suspected examination of biomarkers can provide useful information and could be included in a public health surveillance programme. The most commonly used biomarkers are hair, nails, blood

and urine; with hair and nails being an indicator of past arsenic exposure, while blood and urine are markers of recent exposure. The use of these indicators, however, is not without problems, with hair and nails being particularly prone to external contamination. Measurement of total arsenic in urine does not give any indication of the relative intake of toxic inorganic arsenic and the less toxic organic forms. A better indication of the intake of inorganic arsenic can be achieved by measurement of inorganic arsenic and its two primary metabolites, methylarsonic acid and dimethylarsinic acid. However, even with these measurements, care must be taken in the interpretation since there are other sources of dimethylarsinic acid.

Care is required in establishing a public health surveillance programme in order to ensure that this is beneficial and cost effective. It is important that any programme has clear objectives, for example, is the programme intended to identify populations at greatest risk or is the programme intended to monitor the effectiveness of exposure reduction interventions such as changing water sources or the introduction of treatment? Much will depend on the availability of existing public health infrastructure, the populations covered and the extent of recording of symptoms, since it is preferable to build on the existing public health infrastructure. The public health surveillance programme could comprise food surveillance and compliance monitoring, where compliance monitoring is covering both drinking water as well as water for irrigation. Monitoring irrigation water might seem to be expensive but spot checks could be sufficient.

2.2 ANALYTICAL METHODS

Having established the need for measurements of arsenic levels in water, food and biological specimens in the previous section, this section gives an overview of the main analytical methods for the laboratory determination of total arsenic. Sample handling and pre-treatment are covered, on a matrix basis (i.e. water, food, biological samples), in sections 2.5. Although there is a need for the determination of levels of the different As species (because of their differing toxicity) the methods for this are still under development (see section 2.8) and it is not yet done on a routine basis, with most analyses limited to the determination of total As. Other non-routine methods, e.g. combination methods, are described in the literature and may provide excellent results (Eaton *et al.*, 1998).

Numerous methods are described in the literature, for the analysis of total arsenic both in water and in different digested matrices. Many of these essentially employ the same principles, but apply different reagents or concentrations. Preference, here, is given to widely used international or national standard methods, which are prescribed for use in many laboratories and have been validated prior to issue.

The possibilities for arsenic determination include:

- atomic absorption spectrometry (AAS)
 - using either a hydride system (HGAAS) or
 - graphite furnace (GFAAS) for atomisation,
- inductively coupled plasma
 - with either atomic emission spectrometry (ICP-AES)
 - or with mass spectrometry (ICP-MS),
- atomic fluorescence spectrometry (AFS),

- anodic stripping voltammetry (ASV) or
- spectrophotometry.

Both atomic absorption spectrometry and atomic fluorescence spectrometry, a relatively new and sensitive technique, are single element specific techniques with known and controllable interferences. The inductively coupled plasma techniques offer the possibility of examining a number of contaminants as they are multi-element techniques, again with known and controllable interferences. Anodic stripping voltammetry is a useful technique for samples containing only free dissolved arsenic, while the spectrophotometric method, which is also a single element technique, has the advantage of being relatively inexpensive in terms of equipment.

To achieve sufficiently low detection limits using any of the techniques all the reagents must have low arsenic contents. Water for preparation of all reagents should be at least of grade 2 (≤ 0.1 mS/m) or preferably of grade 1 according to ISO 3696 (≤ 0.01 mS/m) or better. The quality of the reagents needs to be carefully selected and evaluated to contribute as little as possible to the blank (see also sections 2.5 and 2.7). The labware used must be carefully cleaned by soaking in appropriate acid baths. The detection limits for arsenic range between less than $0.1 - 50 \mu\text{g l}^{-1}$ depending upon the technique employed. Lower detection levels can, in some cases, be obtained by the use of more laboratory intensive pre-concentration steps or the coupling of techniques; however, such methods are not used on a routine basis.

All the techniques (which are outlined below and in Table 2.2.1) require trained staff, generally analysts experienced in dealing with trace elements, with facilities to deal with potentially hazardous chemicals (such as sodium tetrahydroborate and chloroform). In addition to any specialised equipment specified for the analysis all the techniques require the use of standard laboratory equipment.

2.2.1 Hydride generation atomic absorption spectrometry (HGAAS)

The HGAAS technique is based on the atomic absorption measurement of arsenic generated by thermal decomposition of arsenic(III) hydride. As(III) is reduced to gaseous arsenic(III) hydride by reaction with sodium tetrahydroborate in a hydrochloric acid medium (ISO 11969:1996; SM 3114:1999). Trivalent and pentavalent arsenic have different sensitivities using this technique so any pentavalent arsenic must be reduced to trivalent arsenic prior to the determination. This pre-reduction is carried out using a solution of hydrochloric acid, potassium iodide and ascorbic acid.

If it is suspected that organic arsenic is present, acid digestion should be carried out prior to pre-reduction and analysis. However, if the organic arsenic content is insignificant, it is recommended that the digestion step be omitted, as digestion may compromise precision and, as a consequence, the detection limit. The method for digestion of samples stated in ISO 11969:1996 is considered tedious and outdated (see section 2.5).

Method performance

Practical experience suggests that if the blank values are minimal, it is possible to detect as little as $0.05 \mu\text{g l}^{-1}$ for samples in the absence of digestion. However, where samples have been digested prior to analysis detection limits are expected to be higher

due to the additional error introduced by addition of more reagents. Coefficients of variation representing repeatability and reproducibility are found to be 4-7 % and 12-19 %, respectively, for samples of ground water at concentrations between 1.4 and 8.0 $\mu\text{g l}^{-1}$ (ISO 11969:1996).

Interference and matrix effects

Most organic materials interfere and have to be removed by digestion (see section 2.6) prior to the arsenic analysis. Copper at a concentration $>2 \text{ mg l}^{-1}$, antimony at a concentration $>0.2 \text{ mg l}^{-1}$, selenium at a concentration $>0.05 \text{ mg l}^{-1}$, and nitrate at a concentration $>100 \text{ mg l}^{-1}$ interfere at an arsenic concentration of $1.0 \mu\text{g l}^{-1}$. The noble metals (e.g. platinum and palladium) may suppress the response of arsenic(III) hydride. The matrix effect can be corrected for by using standard addition calibration.

Sample size

Normally a minimum of 50 ml sample is needed for analysis, but the volume can be scaled down when using a continuous hydride system.

Equipment and consumable requirements

An atomic absorption spectrometer equipped with a hydride system, which is capable of operation in both a continuous system and a manual batch system is required. The continuous system can be automated by connection to an auto-sampler. The manual batch system has a higher consumption of reagents than the continuous system. In addition, an arsenic cathode lamp is required. The cost of a HGAAS system is between 20,000 and 100,000 US \$. Argon gas is required for the hydride system, and pump tubes (for the continuous hydride system). The major expense for operation in addition to the argon gas is the ultra pure hydrochloric acid, sodium borohydride and sodium hydroxide.

Throughput

The preparation of the samples, especially the pre-reduction procedure, is the time limiting step of the analysis. When using an auto-sampler and a continuous hydride system, the analytical throughput is in the range 30-60 samples a day. For the whole procedure, however, (including registration, sample handling, pre-reduction and reporting) 15-30 samples a day is more realistic.

2.2.2 Graphite furnace atomic absorption spectrometry (GFAAS)

A discrete sample volume is injected into a graphite tube, which is heated stepwise to dry and remove the main part of the matrix and finally to atomise arsenic, which is then measured in the spectral light from the cathode lamp used (ISO/CD 15586:2000; ISO 13812-1&2; SM 3113:1999; USEPA 7060A:1994; USEPA 1639:1996).

Care must be taken regarding optimisation of the method. For optimum efficiency it is recommended that the following be used (Julsham *et al.* 1996):

- a matrix modifier,
- pyrolytically coated graphite tubes with L'Vov platforms and
- peak area integration as measuring mode.

The role of the matrix modifier is to permit a sufficiently high pyrolysis temperature to remove other components without loss of arsenic; a mixture of Pd/MgNO₃ is

recommended. Sensitivity may be improved by the use of a pyrolytically coated graphite furnace tubes with the L'Vov platform. Improvement of the detection limits can be achieved by using multiple injections.

Addition of chemical modifiers to the sample causes background absorption to be increased and the measurement is performed in the low UV range, both of which call for an efficient background correction system on the instrument. Generally, great care must be taken in graphite furnace measurement to ensure that any possible interference is corrected. Likewise, it is necessary to check for matrix effects by use of the standard addition technique. The results obtained will indicate whether a calibration curve, a standard addition technique or an addition calibration is needed to correctly calibrate the measurements.

Method performance

Detection limits between 1 and 5 $\mu\text{g l}^{-1}$ are generally quoted. This detection limit can be improved under clean room conditions. Coefficients of variation representing repeatability and reproducibility are found to be 6-40 % and 12-43 %, respectively (SM 3113:1999).

Interference and matrix effects

Background correction may be used to control molecular absorption resulting from volatilisation of the matrix, especially from high concentrations of acids or dissolved solids. Temperature ramping can be used to decrease background interference. The use of a matrix modifier may control interference from other components, allowing a sufficiently high pyrolysis temperature to remove the interfering substances without loss of arsenic. Standard addition calibration can be used to compensate for matrix interference arising from, for example, chloride. Serial dilution is useful for verification of the absence of matrix or chemical interference. Contamination and cross contamination of samples can be a major source of error and special attention is drawn to the magnitude of the blank.

Sample size

A minimum sample volume of 1-2 ml is needed for the analysis. Care should be taken to achieve a representative sample and to avoid contamination.

Equipment and consumable requirements

An atomic absorption spectrometer equipped with simultaneous background corrector, graphite furnace is required. In addition, an auto-sampler is highly recommended for optimisation of the reproducibility. The price for a GFAAS system is approximately 40-100,000 US \$. The main expenses for the operation, apart from argon gas, are consumables such as graphite tubes and graphite contact cones. Chemical consumption is low.

Throughput

Sample preparation is minimal, the time consuming step of the analysis being the start up of the equipment and the change of graphite tubes. When using an auto-sampler the daily through-put for the equipment is in the range 50-100 samples a day, but 20-40 samples a day is a more realistic estimate with all the laboratory procedures included.

2.2.3 Inductively coupled plasma atomic emission spectrometry (ICP-AES or ICP-OES)

The liquid sample is transported as an aerosol to an argon plasma at 6000-8000 K, where arsenic is atomised and where a radio frequency inductively coupled plasma produces element-specific emission spectra. The spectra are dispersed by a grating spectrometer, and the intensity of the emission lines is determined (ISO/CD 11885:1996; SM 3120:1999; USEPA 6010B:1996). The technique is useful for multi-element determination and is applicable to all matrices, although solid samples and liquid samples containing precipitates must be digested prior to analysis. The terms ICP-AES and ICP-OES are synonymous.

Method performance

In general, detection limits of 35 - 50 $\mu\text{g l}^{-1}$ are quoted for arsenic by the instrument manufacturers. Reproducibility, expressed as relative standard deviations, is found in proficiency tests to be 8-23 per cent (EPA 6010B:1996).

Interference and matrix effects

Background emission and stray light can be controlled by background compensation. Spectral overlaps from other elements, ions or molecular band spectra may be avoided by using an alternative wavelength or can be compensated for by predetermined equations. Physical interferences may be caused by changes in viscosity and surface tension as well as by a high concentration of acid (< 10 volume %) or high concentrations of dissolved solids (< 1500 mg l^{-1}). These phenomena are controlled by the use of an internal standard or by dilution. Chemical interferences include molecular compound formation, ionisation effects and solute vaporisation effects. However, these are not significant and can be minimised by optimising the operating conditions, by buffering the sample, by matrix matching and by using standard addition calibration. Memory interferences originating from the previous sample can be minimised by flushing the system between each sample measurement.

Sample size

A minimum sample volume of 10-20 ml is needed for the analysis; however, it is essential that the sample is representative.

Equipment and consumable requirements

An ICP-AES equipped with a radio-frequency generator and simultaneous background corrector is required, with the cost being approximately 60-100,000 US \$. The main expense for the operation is argon gas, with the consumption of other chemicals being low.

Throughput

Sample preparation is minimal, the time consuming step of the analysis being the start up of the equipment and verification of the method procedure including checking the interference in the sample. When using an auto-sampler the daily through-put for the equipment is in the range 50-100 samples a day, but 20-40 samples a day is a more realistic estimate with all the laboratory procedures included.

2.2.4 Inductively coupled plasma mass spectrometry (ICP-MS)

The liquid sample is introduced into an argon-based, high-temperature radio-frequency plasma. Ions generated are extracted from the plasma and separated on the

basis of their mass-to-charge ratio by a mass spectrometer. The mass spectrometer can be a quadrupole or magnetic sector type (SM 3125:1999; USEPA 200.8:1994; USEPA 6020:1994; USEPA 1638:1996). The technique is useful for multi-element determination and, as with ICP-AES, solid samples and samples containing precipitates must be digested prior to analysis.

Method performance

In general, detection limits of 0.02 and 1 $\mu\text{g l}^{-1}$ are quoted. However, when the procedure includes sample digestion detection limits would be expected to be higher. Precision, expressed as relative standard deviation, has been reported to be 7-48 % (EPA 6020:1994). However, some of the results in the proficiency test were below the limit of detection resulting in the relatively high variation. The relative standard deviation from single laboratory tests has been reported as 0.5-16 % (EPA 200.8:1994; SM 3125:1999).

Interference and matrix effects

ICP-MS is a very sensitive analytical technique for multi-element analysis, but is susceptible to isobaric interferences, either from isotopes of different elements or from polyatomic ions in the form of molecular or doubly charged ions. For example, for arsenic analysis hydrochloric acid and perchloric acid should not (in general) be used for sample preparation because of the formation of argon chloride (from argon in the plasma), which can lead to measurement problems. ArCl has a mass of 75, the same as arsenic, which could (without correction) lead to errors. Correction for isobaric interferences is possible using either high resolution ICP-MS or correction equations after measurement of the possible interferences. Therefore, whenever possible, nitric acid should be used in sample preparation. However, it should be noted that chloride is present in most natural water samples at variable concentrations.

Physical interferences can be caused by changes in viscosity and surface tension, as well as changes in matrix composition or high concentrations of dissolved solids (0.2-0.5 % is acceptable). These phenomena are controlled by using an internal standard or by dilution. Memory interference originating from the previous sample can be minimised by flushing the system between each sample measurement. Ionisation interference is caused by moderate (0.1-1 %) amounts of a matrix ion changing the signal. This effect normally reduces the signal and can be controlled by the use of an internal standardisation technique.

Sample size

A minimum sample volume of 10-20 ml is needed for the analysis, however, it is essential that the sample is representative, and extreme care must be taken to avoid contamination of the sample.

Equipment and consumable requirements

An ICP-MS with peristaltic pump, mass-flow controller on the nebuliser gas supply and a water-cooled spray chamber is required. The ICP-MS should either have a data system that allows corrections for isobaric interferences or have a system capable of providing a resolution of 10,000. An auto-sampler is a useful addition. The price for an ICP-MS system is approximately 150,000-400,000 US \$. Apart from argon gas, the main operational expense is the use of consumables (such as cones). However, general running costs are relatively high, and when ICP-MS instruments are used for

the determination of a single element in samples, the procedure is not cost effective in comparison to other techniques.

Throughput

Sample preparation is minimal, the time consuming step of the analysis being the start up of the equipment. When using an auto-sampler the daily throughput for the equipment is in the range 20-100 samples a day depending upon the number of elements to be determined.

2.2.5 Atomic fluorescence spectrometry with hydride system (HGAFS)

Acidified sample solutions are treated with sodium tetrahydroborate to generate the gaseous hydride arsine. The hydride and excess hydrogen are swept out of the generation vessel, using a stream of argon, into a chemically generated hydrogen diffusion flame. The hydrides are atomised and the resulting atoms are detected by atomic fluorescence spectrometry (CEN/TC/230/WG1/TG 12 N 3, 1999). Prior to the measurement a sub-sample is treated with hydrochloric acid, potassium iodide and ascorbic acid. This reduction process ensures quantitative reduction of arsenic(V) to arsenic(III).

Method performance

A detection limit of $0.01 \mu\text{g l}^{-1}$ is obtainable using high purity chemicals. Figures for precision from proficiency tests are not available but the relative standard deviation is typically less than 5 % for concentrations greater than 20 times the method detection limit.

Interference and matrix effects

The hydride generation AFS technique is prone to interference by transition metals. Recovery tests can be used to reveal any interference. It is essential to convert all arsenic(V) to arsenic(III) prior to the measurement since arsenic(V) gives a significantly lower response than arsenic(III). Organoarsenic compounds require vigorous digestion since these compounds do not react with sodium tetrahydroborate.

Sample size

Generally a minimum sample volume of 40-50 ml is required, but the volume can be scaled down when using a continuous hydride system and an autosampler.

Equipment and consumable requirements

An atomic fluorescence spectrometer equipped with a hydride generation system is required, the price for such a HGAFS system is approximately 20-25,000 US \$. Other than argon gas for the hydride system, consumables such as pump tubes (for the continuous hydride system) and an arsenic lamp are needed. Apart from argon gas, the main operational expense is the ultra pure hydrochloric acid, sodium borohydride and sodium hydroxide.

Throughput

The time consuming step of the analysis is the pre-treatment of the samples. When using an auto-sampler the daily throughput for the equipment is in the range 30-60 samples, but 15-30 samples per day is a more realistic estimate when all the laboratory procedures are included.

2.2.6 Anodic stripping voltammetry (ASV)

Free dissolved arsenic is collected on a conditioned gold metal film deposited on a glassy carbon electrode. Arsenic is quantified by anodic stripping at a potential of +145 mV with respect to the saturated calomel electrode. The technique is very sensitive but only free dissolved arsenic is determined, and interferences and high sensitivity can cause severe limitations (USEPA 7063:1996).

Method performance

A detection limit of $0.1 \mu\text{g l}^{-1}$ is quoted. Precision data in the form of standard deviation from proficiency testing is not available but the relative standard deviation is typically 2-15 %.

Interference and matrix effects

Major interferences include intermetallic compound formation, overlapping stripping peaks, adsorption of organics, and complexation. Dissolved antimony, bismuth and copper (concentrations greater than 1 mg Cu l^{-1}) are known as positive interferences. Turbid samples must be filtered ($0.45 \mu\text{m}$) prior to analysis. The technique cannot be used if the total metal concentration is over 10 mg l^{-1} .

Sample size

A minimum sample of 25-50 ml is needed for the analysis.

Equipment and consumable requirements

Anodic stripping voltammetry equipment including potentiostat, glassy carbon electrode and saturated calomel electrode, stirrer and sampler stand are required, which will cost approximately 10-20,000 US \$. The main operational expense is the cost of the chemicals.

Throughput

Sample preparation is minimal, the time consuming step of the analysis being the start up of the equipment and in particular deposition and conditioning of the glassy carbon electrode with gold film. When using an auto-sampler the daily throughput for the equipment is in the range 25-50 samples, but 15-30 samples a day is a more realistic estimate with all laboratory procedures included.

2.2.7 Silver diethyldithiocarbamate spectrometric method (SDDC)

The silver diethyldithiocarbamate spectrometric method (SDDC) has been widely used due to the simplicity of the instrumentation. The method described in ISO 6595:1982 oxidises organic arsenic by heating with potassium permanganate and potassium peroxodisulphate. Pentavalent arsenic is reduced afterwards by potassium iodide and tin(II) chloride. Reduction of trivalent arsenic to arsenic trihydride (arsine) is carried out by nascent hydrogen in an acidic medium. Arsine is absorbed in a chloroform or pyridine solution of diethyldithiocarbamate and 1-ephidrine. A spectrophotometric measurement of the red-violet complex determines the amount of arsine evolved from the solution.

Another method, based on the same principle, is described in SM 3500-As A: 1999. Arsenic(V) is reduced to arsenic(III) by using hydrochloric acid, with arsenic(III) converted to arsine gas by means of sodium tetrahydroborate. The generated arsine gas is stripped off using oxygen free nitrogen and the arsine is collected in an

absorber solution of silver diethyldithiocarbamate and morpholine dissolved in chloroform. The stripped arsine gas is passing through glass-wool impregnated with lead acetate to remove interfering hydrogen sulphide before being absorbed in SDDC solution. The absorbance of the developed red coloured compound is measured by spectrophotometry at 520 nm and is equivalent to the amount of total inorganic arsenic in the sample. Without the hydrochloric acid treatment in the above procedure, the method can be used to measure arsenic(III).

Method performance

Detection limits between 1 and 10 $\mu\text{g l}^{-1}$ are quoted. Data from proficiency tests are not available, but the relative standard deviation is typically less than 10 % for concentrations greater than ten times the detection limit.

Interference and matrix effects

Hydrogen sulfide interferes, but can be removed relatively simply with lead acetate. Chromium, cobalt, copper, mercury, molybdenum, nickel, platinum, silver and selenium interfere at high concentrations. Antimony also forms a coloured complex with an absorption maximum at 510 nm and so interferes with the arsenic determination. In this method methylarsenic compounds are not determined as part of the total arsenic. The method is limited to water samples.

Sample size

A minimum of 100 ml is needed for the analysis.

Equipment and consumable requirements

A spectrophotometer or filter photometer capable of measurement at 520 nm and equipped with 1 cm glass cells will cost approximately 2-10,000 US \$. Apart from the oxygen-free nitrogen, the main methodological expense is the large consumption of chemicals.

Throughput

The time consuming step of the analysis is the chemical reactions prior to the measurement. The daily throughput for the equipment is in the range 20-30 samples, but 10-20 samples a day is a more realistic estimate with all procedures included.

2.2.8 Summary

The key points of each of the above techniques is summarised below in Table 2.2.1.

Table 2.2.1 Summary of Analytical Methods

Technique	LoD ($\mu\text{g l}^{-1}$)	Sample size (ml)	Major equipment cost (US\$)	Analytical throughput	Comments	Selected references
HGAAS	0.05	50	20 – 100,000	30 – 60	Single element	ISO 11969 SM 3114
GFAAS	1 – 5	1 – 2	40 – 100,000	50 - 100	Single element	ISO/CD 15586 SM 3113
ICP-AES	35 – 50	10 – 20	60 – 100,000	50 – 100	Multi-element	ISO/CD 11885 SM 3120
ICP-MS	0.02 – 1	10 – 20	150 – 400,000	20 – 100	Multi-element	SM 3125 USEPA 1638
HGAFS	0.01	40 – 50	20 – 25,000	30 – 60	Single element	CEN/TC/230/WG1/TG 12 N 3
ASV	0.1	25 – 50	10 – 20,000	25 – 50	Only free dissolved arsenic	USEPA 7063
SDDC	1 - 10	100	2 – 10,000	20 - 30	Limited to water samples	SM 3500 ISO 6595

2.3 FIELD MEASUREMENT OF ARSENIC IN WATER

Although field methods play an important role in environmental analytical chemistry few papers appear in the literature dealing with such testing methods. Unfortunately, field-testing methods for arsenic in drinking water have been restricted to very simple and crude testing protocols.

The most important characteristic for field measurement is that analytical results can be obtained on the site where the sample is taken. This can be done using relatively simple field-testing kits or very sophisticated instrumental methods installed in a van. This section examines only the relatively simple field-testing methods.

Field-testing methods are normally the method of choice in a number of cases such as:

- emergencies,
 - when analytical results are required in a hurry.
- inadequately developed country infrastructure;
 - where the number of laboratories with competent personnel and equipment is small;
 - where the sampling sites are too far away and where poor logistics do not guarantee that the sample results can be made available in a timely fashion to responsible persons who have to take or recommend a course of action.

A further reason to choose a field method could be the extent of the monitoring programme in a highly contaminated geographical area, where the lower quantification limit specifications of field equipment would be sufficient for a major part of the samples. For example, the present demand to monitor a huge number of possibly contaminated tube wells in a country like Bangladesh is a strong argument for the use of reliable field methods.

2.3.1 Screening and confirmatory methods

Field-testing methods used for screening purposes are methods that are used to detect the presence of arsenic at, or above, the level of interest. This level is normally the maximum allowable concentration (country standard) for drinking water. Screening methods could be used to identify highly contaminated sources and develop priority programmes to lower the health risk of the affected population. Screening methods normally have a high sample throughput capacity.

There are also more sophisticated field-testing kits on the market, which are capable of determining the actual arsenic concentration with a specified level of accuracy and precision.

2.3.2 Quality control

Irrespective of which field equipment is chosen, accuracy should always be checked by comparison to laboratory equipment (with specified accuracy) prior to the routine use of the equipment. Detection limits, in a normal sense, are difficult to establish using field-testing kits where the human eye is used for quantification. Such a limit may be estimated by performing analyses of a number of natural samples, covering the concentration range of interest, using both the field-testing equipment and validated laboratory equipment. The quantification limit may be estimated from the number of false positive/false negative results at each concentration level.

For field methods the same criteria (accuracy and precision) are required as for laboratory methods (see section 2.7). This relates to the fact that the authorities responsible for sample collection and analyses have to recommend the maximum tolerable bias and standard deviation of analytical results throughout the concentration range. Accuracy, precision and estimates of quantification level for the equipment could be established during the training of working groups in laboratories after the appropriate statistical evaluation has been completed. During the field operation quality control data must ensure that the control limits are met. Depending upon national capabilities and available resources, a certain number of samples should be analysed by laboratory methods and the results compared to those from the field methods. This not only allows the confirmation of the results found in the field but should also detect any errors at an early stage.

In addition to analytical and field competence, it is important to follow a strict protocol which allows the collection and encourages the recording of additional data, which is vital for the correct interpretation of results.

2.3.3 Methods suitable for field analyses

Unfortunately, there are few analytical methods for arsenic that are suitable for field analysis. In fact, there is only one useful field method, which appears in different configurations with the same basic chemistry. It should also be noted that over the past 100 years nothing new has been published on classical analysis of arsenic. However, on account of the large demand for field-testing and a growing market potential for field-testing kits, many new kits are currently being developed and marketed. As such, no specific trademarks will be mentioned and only the fundamental principles and specific differences known at the time of writing will be discussed. For specific details of the new kits the reader is referred to the internet (e.g. www.arsenator.com; www.merck.de; www.hach.com).

Generally, all of the known photometric methods could be adapted for use in the field. Most of the companies that sell photometers also offer battery driven units. If a higher detection limit and less precision can be tolerated by the authorities concerned, the measurement could be simplified to a colorimetric method using a comparator and the human eye for quantification. In the case of the silver diethyldithiocarbamate (SDDC) method (Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998) the use of a toxic volatile organic solvent creates an additional problem. On the whole, the photometric methods have not been successfully adapted for use in the field.

The Gutzeit method

The only widely used field-testing method is the so-called Gutzeit Method. This was proposed in 1879 as a qualitative proof for the presence of arsenic in aqueous solutions. Subsequently, this method was improved for a quantification of lower amounts of arsenic in water and other beverages (Gutzeit, 1891). This quantification was achieved, when the formation of a yellow compound was observed when arsine gas passes a crystal of silver nitrate. The first practical quantitative analysis was developed when the crystal was replaced by a paper strip, which had been impregnated with mercuric-chloride (Sanger and Black, 1907). Shortly afterwards the method was slightly improved by changing from mercuric-chloride to mercuric-bromide. This method has dominated arsenic field-testing for the last 100 years.

Methods based on the mercuric-bromide stain

Field methods, based on the mercuric-bromide-stain, consist of three different major parts, which are carried out stepwise. The first part of the procedure is to remove serious interference caused by hydrogen sulfide. As most of the water samples from arsenic rich areas originate from reducing environments they are likely to contain various amounts of sulfur in its lowest oxidation state (hydrogen sulfide). This odoriferous compound reacts with mercuric-bromide to form a greyish-black precipitate, which makes the determination of arsenic ambiguous. Field kits that do not have a way to negate this interference are useless. Two methods to remove hydrogen sulfide are currently in use; either the sulfide is oxidized to sulfate and the excess oxidizing reagent removed prior to the hydride generation step or, as is done in the larger number of available kits, the hydrogen sulfide is filtered out by passing the gas stream through a filter impregnated with lead acetate during the hydride generation step. The lead salts, which react with hydrogen sulfide, form insoluble lead sulfide, while the arsine gas passes the filter unchanged. Other interferences at this step of the analyses are normally not expected for drinking water analysis. When the kit is used for waste-water analysis then the larger concentrations of heavy metals (such as copper and nickel) may interfere the hydride generating process and/or other hydride generating elements, e.g. antimony and selenium.

The second step, the generation of the arsine gas, is the most important one. Again, different field kits offer a variety of possible ways to achieve this. The classical way is the use of zinc metal and hydrochloric acid, which produce the 'nascent' hydrogen, which is the actual reducing agent. The zinc metal has to be of a definite grain size (20-60 mesh) to ensure that the reaction progresses at a certain rate: not too fast – in order to ensure the maximum yield at the reagent paper, and not too slow – in order to

allow the determination to be completed in a practicable time. If a kit is used for the first time or the chemicals are refilled, the operator should perform blank tests to determine that the zinc metal is arsenic free, because as in nature, zinc and arsenic occur together. If kits contain zinc of poor quality they are likely to show an unacceptable blank value. At room temperature arsenate is reduced to arsenite, which is then reduced to arsine gas. The reduction of arsenate to arsenite is accomplished by adding a small amount of potassium iodine and stannous chloride and, depending on temperature, needs at least 15 minutes to be completed. This stage may not be as complete or as fast as advertised in the kit directions (Dedina and Tsalev, 1995). Hydrochloric acid could be replaced by sulfamic acid, which is solid and avoids a major disadvantage of having to handle an aggressive liquid in the field. Another drawback when using zinc is the time needed for one determination. The arsine production needs at least 30 minutes and, together with the pre-reduction step, the time needed for one analysis adds up to almost one hour.

A completely different approach for the production of the volatile arsine gas is to take advantage of a more strongly reducing compound, namely sodium tetrahydroborate (NaBH_4), which is normally used for hydride generation atomic absorption spectroscopy (HGAAS) in the laboratory. Mixed with an inert medium for dilution it could readily be prepared in the form of tablets, one for each determination. The other essential chemical is amidosulfonic acid. This chemical is also available in tablet form. The advantage of this combination is the fact that no liquid reagent is used, and the whole analysis can be completed in less than ten minutes. The comparison of hundreds of field sample analyses with laboratory data have demonstrated satisfactory results for this system (Kosmus and Kinniburgh, 2001).

In the field, purge gas is not available to transport the released arsine gas to the reagent paper. Therefore an excess amount of the reduction reagent is required to produce a sufficient amount of hydrogen gas to strip the arsine gas out of the solution and to transfer it to the mercuric-bromide paper. Attention is necessary if excessive amounts of an oxidizing compound, such as nitrate, is present in the sample. Additional reduction reagent may solve this problem.

Generally, two systems are in common use for the reaction of the arsine gas with the reagent paper impregnated with mercuric-bromide. The original version from 1907, still being sold, inserts a small reagent strip into a tube and the arsine gas passes over the surface of this paper. Because the contact between the two reactants is not very intense, this method lacks sensitivity and has low reproducibility. Many tests, both in laboratories (Greschonig and Irgolic, 1997) and in the field (Milton, 1999), have shown that this method is not sufficiently reliable to test for low arsenic concentrations. If the flow of the arsine gas passes through, rather than over, the reagent paper then lower detection limits can be reached.

The third methodological step is the quantification of the arsenic concentration. The arsine gas produces a yellowish spot on the reagent paper. Quantification of the arsenic concentration is by visual comparison with a colour chart. This ability differs from person to person. Unfortunately, the faint yellow colour is not very discernible for the average human eye. Visual acuity tests can eliminate individuals who are not sufficiently sensitive to this wavelength region of visible light. For persons passing

the visual acuity test it should be possible to distinguish concentrations in steps of ten ppb in the lower concentration range.

The yellow compound formed when the arsine gas reacts with the reagent paper is light sensitive. Therefore, the analysis has to be performed away from direct sunlight and the final visual comparison with the colour chart made without undue delay. The subjective nature of such analysis could be overcome by utilizing a pocket-size battery driven instrument, which can measure the colour intensity on the reagent paper in an objective way. Blue light, which is complementary to yellow, is less reflected if the spot becomes more intensively coloured. With the help of modern electronics such small instruments could provide instant results in the correct concentration units.

2.3.4 Further requirements

It should be noted that personnel using field kits should be fully aware of the fact that they are performing sophisticated trace element analysis. During training they need to become familiar with the general requirements to be able to conduct such analysis, including exactness, clean environment and critical performance.

In order to select appropriate field-testing methods that meet local conditions, logistical and technical support systems are extremely important in developing countries. In this connection, it would be desirable to provide more detailed technical information on the validation of field test data as well as a protocol for the selection of appropriate field testing methods and training materials.

2.4 SELECTING APPROPRIATE ANALYTICAL METHODS FOR ARSENIC

In nature arsenic can exist in a number of states. Inorganic arsenic can occur as both As(III) and As(V) while several organically bound species are also known to occur, many of these being methylated compounds. In natural waters much of the arsenic is in the inorganic form but in foodstuffs, blood, urine etc. appreciable quantities of arsenic occur in organic compounds. Most methods for arsenic determination are designed to give results for total arsenic, however, as different arsenic species have been found to have varying effects on human health, more recent methods have attempted to determine the various species of arsenic in the media of interest. It is essential when determining total arsenic that pre-treatment liberates all of the arsenic from organic compounds.

Many methods have been proposed for the determination of arsenic (section 2.2) with gravimetric and colorimetric methods being utilised historically. While colorimetric methods, particularly the SDDC method, still find favour, the most common method of arsenic determination is atomic absorption spectrometry, using either hydride generation (HGAAS) or a graphite furnace (GFAAS) for atomisation. More recently, both inductively coupled plasma atomic emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS) have been applied to arsenic determinations. Hydride generation has been coupled with both of these techniques to determine total arsenic, to give greater sensitivity and to negate interference effects. Hydride generation has also been coupled with atomic fluorescence for the determination of total arsenic. Finally, the technique of anodic stripping voltammetry has also been applied to arsenic determination.

As stated above it is becoming increasingly important to obtain information on the various species of arsenic present in samples. Hydride generation coupled to atomic absorption, ICP-AES or ICP-MS has been used to determine As(III) and As(V) species. Best results for arsenic speciation, however, have been obtained from coupling hydride generation with high performance liquid chromatography (HPLC) to separate the various species, followed by determination with atomic absorption or ICP (AES or MS).

As outlined in section 2.2 many of the methods listed are suitable for the determination of total arsenic in the various media, however, it has to be stated at the outset that to obtain good quality data for arsenic analyses, by whatever method, requires experienced and skilled analysts.

As arsenic is generally present as a trace constituent in the media of interest, the methodology applied must be capable of accurately determining arsenic at low concentrations. For example in most natural waters arsenic is present at a concentration of less than $5 \mu\text{g l}^{-1}$, and as far as drinking water is concerned it is necessary to be able to accurately determine values of less than $1 \mu\text{g l}^{-1}$. In recent times the use of sophisticated instrumentation such as ICP-MS has offered very sensitive methodology, however, the limited availability and high cost of this instrumentation (see Table 2.2.1), limits its usefulness. In a review of the analytical chemistry of arsenic in drinking water Eaton *et al.* (1998) point out that atomic absorption instrumentation is that most freely available in analytical laboratories. Therefore, it is likely that most laboratories would use atomic absorption for arsenic determination with atomisation performed by either graphite furnace or hydride generation.

The SDDC colorimetric method, following hydride generation of arsine was used for a rapid survey of drinking waters in Bangladesh (British Geological Survey/Mott MacDonald, 1999). This technique was compared with results using hydride generation ICP-AES determinations. In general the colorimetric technique was found to be fairly reliable and as such for waters containing in excess of about $10 \mu\text{g l}^{-1}$ represents a cheap and fairly rapid method.

Field methods for the determination of arsenic in water samples, as dealt with in section 2.3, are limited and are generally only of use for relatively arsenic-rich waters, being unreliable for samples containing less than $40 \mu\text{g l}^{-1}$ (Greschonig *et al.*, 1997). However, such methods are of some use in providing immediate data on potentially high arsenic drinking waters in areas such as Bangladesh and Bengal where information is required fairly rapidly. Such surveys can be followed up with more sensitive and accurate laboratory methods in follow up surveys if necessary.

As previously stated it is essential for future studies to be able to obtain data on arsenic speciation in the various media. This would seem to be particularly important in studies of groundwater, as it has been shown that where these are of a reducing character much of the arsenic is present as As(III). Such groundwaters are used for drinking supplies in Bangladesh and Bengal, and other areas. As(III) has been found to be considerably more toxic to humans than As(V). As mentioned earlier, several methods have been suggested for determining various species (Chen *et al.*, 1994; Chatterjee *et al.*, 1995; National Academy of Sciences, 1999). However, it perhaps

pertinent to point out that care needs to be exercised when samples are collected. While organically bound As and As(V) are unlikely to change during storage, such samples as groundwater from reducing environments will undergo rapid change when exposed to oxidising conditions with the As(III) being oxidised to As(V). It has been suggested that freezing samples, while limiting oxidation will not completely prevent this. Yalcin and Lee (1998) overcame problems of storage by separating As(III) and As(V) on site by passing the water sample through disposable ion exchange cartridges.

To summarise no one method can be considered universal for arsenic determination in the sample media outlined. The method used is, at least in part, dictated by the analytical equipment available, staff experience and the relative costs. In addition, the choice of method is going to be influenced by the problem to be solved, as in the application of field methods for rapid monitoring of drinking water in high arsenic areas.

2.5 SAMPLE TYPES AND SAMPLING

Even the most accurate and precise analytical technique is useless without suitable samples, taken in an appropriate manner with thought given, prior to sampling, to the question that requires answering. This section examines water, food and human biological samples and highlights issues for consideration when designing a sampling strategy. Consideration of sample type is also important when planning the analysis as the form of arsenic present will vary according to the matrix under examination.

2.5.1 Water samples

2.5.1.1 Arsenic species in water

The most important arsenic compounds in terms of drinking water supplies (surface water and ground water) are the inorganic species As(III) and As(V). These species are usually associated with low concentrations of dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA). In well-oxygenated water and sediments nearly all arsenic is present in the thermodynamically more stable pentavalent state (arsenate). In chlorinated drinking water supplies, arsenic salts are in the pentavalent state as a result of oxidation by free chlorine.

Arsenic dissolved in water can undergo either reduction or oxidation, depending upon conditions. Arsenic tends to be sorbed by iron oxyhydroxides, aluminium hydroxides, manganese compounds, organic materials and clays. There is potential for arsenic release, when there is a fluctuation in oxidation-reduction potential and/or pH. As(III) is more mobile than As(V) because it is less strongly sorbed on most mineral surfaces than the negatively charged As(V) oxi-anions.

In lake water methylarsenicals can contribute a significant part of the total arsenic content as can the 'hidden arsenic species', which are species that are not determined using HGAAS, HGAFS and SDDC, unless the samples are digested (Eaton *et al.*, 1998).

2.6.1.2 Sampling

The collection of water samples may seem a relatively simple task. However, more is required to obtain representative water samples and to preserve their integrity until samples are analysed than simply dipping a container into water. A representative

sample can easily be obtained from rivers, which are relatively homogeneous, whereas many bodies of water have significant spatial and temporal variations and the collection of a representative sample becomes much more complex. Ground water, too, can present problems as suggested in section 2.1.

The quality of data collected depends first and foremost on the quality of the sample, i.e. how well it represents the quality of the body of water from which it was collected.

Regulated drinking water supplies

Ideally, drinking water samples should be representative of the amount of arsenic ingested by consumers during a period of time. In some situations it is likely that the average weekly intake of arsenic via drinking water may differ due to variations in water composition and behaviour of the consumer. For example, an investigation carried out by the Danish EPA showed that certain brass fittings used in household installations could leach up to $10 \mu\text{g l}^{-1}$ arsenic into water left in the tap for a 12 hour period (*REF*), thus construction of installations should be taken into account when planning the protocol for tap water samples. The composite proportional sample procedure (COMP) is the only method that takes into account all variations within a test period and is, therefore, the only method capable of determining the weekly intake in a representative way (provided that the sampling device is used correctly). However, even this procedure only reflects the average intake by consumers using the tested tap during the period of sampling and it is impractical for regular sampling at a large scale. An alternative is provided by the random daytime sample (RTD, defined as unannounced sampling taken during office hours by a professional sampler), which has been shown to give representative results for lead and allows the detection of significant problems regarding installations (Discussion paper for the Drinking Water Committee meeting, 18 June 1999 - preparation for Directive 98/83/EC). Random daytime samples have poor reproducibility, but they are relatively inexpensive, practical and acceptable to consumers. A more reproducible sampling strategy is provided by 30-minute stagnation samples (after flushing the tap is left with water for 30 minutes before sampling). These samples are representative and enable the detection of installation problems, but they are not very practical and are inconvenient for consumers. The fully flushed sample is not representative and does not allow detection of problems regarding installations.

Generally, it is recommended that random daytime sampling or 30-minute stagnation samples should be taken, as these will provide reasonably representative results and highlight any possible installation problems (which clearly will not be seen if measuring water leaving the water treatment works). When the sampling is being done to determine compliance with drinking water regulations it is likely that a sampling level and frequency will also be laid down, but at least 20 randomly selected properties should be sampled in order to detect a 10 % non-compliance level (at 95 % confidence).

Sampling plan

A sampling plan should be used so that any changes seen between sampling rounds can be attributed to changes in environmental conditions and not to changes in sampling procedure. The sampling plan should contain details on the objective of the sampling, identification of water body (e.g. well, tap), number, size and types of

samples to be collected, flushing or stagnation time (if applicable), control samples, filtration, storage conditions and storage time (see ISO 5667). Each sample taken should have details noted of any sources of interference, problems during sampling or other information that may influence the quality or representativeness of the sample. Box 2.5.1 illustrates the ideal level of detail. The sampling sheet (or a copy of it) must follow the sample(s) to the laboratory and the information must be stored and documented in accordance to the laboratory quality management system.

**Box 2.5.1 Inspiration for preparation of a sampling sheet for groundwater.
(Based on GEMS/VKI 1997)**

- Objective of sampling including sampling programme
- Identification and description of the sampling spot
- Sampling by date, time and person performing the sampling
- Field measured parameters including their instrument calibration
- Description of filtration; filtration type, filter information (manufacturer, pore size, material), cleaning procedure, time for filtration after sampling
- Sampling equipment and procedures; equipment identification, pre-pumped volume and time prior to sampling
- Sample specifics such as container material, collecting volume, preservation in field or in lab, quality control
- Storage conditions and time until delivery to laboratory
- Transportation; mode and shipping date
- Remarks regarding quality control and in general
- Laboratory

2.5.1.3 Sample containers

Samples should be collected in pre-cleaned polyethylene containers. Glass containers are not recommended as some glass materials contain arsenic. Containers can be cleaned by soaking in a solution of 10 % nitric acid and rinsed with lab water. Blank samples should be prepared in cleaned sample containers in order to ensure that there is no significant contamination of the containers.

2.5.1.4 Filtration of water samples

Environmental water samples may contain significant amounts of suspended matter. The arsenic content of the solid phase may be appreciable and the decision on whether to filter depends upon the use of the results. Differing levels of suspended solids between samples may compromise result comparability.

Where interest is confined to dissolved arsenic, filtration through a 0.45 µm membrane filter, as soon as possible after sampling and prior to acid preservation, is recommended. Filtration of water samples prior to arsenic speciation analysis may be relevant in most cases depending upon the different species or form of arsenic. The concentration of total arsenic in the solid phase (i.e. suspended solid) may be determined in the material caught on the membrane filter after digestion of the filter. The filtration procedure must be described in the sampling or analytical report. Determination of suspended solids must be carried out on separate samples. Filtration of anoxic groundwater must take place immediately after sampling (preferentially online) to avoid oxidation/precipitation of oxidised iron and co-precipitation of arsenic species.

Clearly, care must be taken to avoid contamination and loss during the filtration step. Ideally, filtration of the samples should be carried out parallel to filtration of blank samples, and it is vital that filtration units, including membrane filters, are clean prior to use.

2.5.1.5 Sample preservation

In order to prevent adsorption losses, samples for the analysis of total arsenic are preserved by acidification to $\text{pH} < 2$. The acid used will depend upon the subsequent analytical procedure. For ICP-MS, GFAAS and ASV measurement nitric acid is recommended whereas hydrochloric acid is the choice for sample preservation prior to analysis using the HGAAS, HGAFS, and SDDC methods. ISO 11885:1997 (ICP technique) prescribes the addition of 5 ml concentrated nitric acid to each litre of water sample and ISO 11969:1996 (HGAAS technique) prescribes the addition of 20 ml of 50 % hydrochloric acid to each litre of water sample.

As with all reagents used, the acid should be checked for contamination with arsenic; this can be done by preserving a blank sample. Preservation should preferably be carried out in the laboratory in a clean atmosphere in order to avoid contamination. It is recommended that preservation should take place a minimum of 24 hours prior to measurement (see also the individual standards and ISO 5667-3:1994).

Storage of samples for arsenic speciation studies should be as short as possible due to the oxidation/reduction of As(III)/As(V). Results from preservation studies on drinking water at low concentration levels (few $\mu\text{g l}^{-1}$) using 0.005 M HCl, ascorbic acid and no preservation, indicate that there is no universal preservation that is effective for all matrices (AWWA, 1998). Even when an effective preservative is identified, it is recommended that samples are stored in the dark at 4°C and speciation work is conducted as soon as practically possible.

2.5.1.6 Digestion of water samples

As mentioned earlier, water sample digestion may compromise the detection limit, accuracy and precision of the analyses. In cases where groundwater or tapwater samples are filtered or samples have been obtained without significant levels of suspended solids, analyses should be carried out directly on preserved samples. If, however, suspended solids are present and data representing the total arsenic concentrations in the water body are required, digestion of a representative sub-sample should be carried out prior to analysis.

Acid digestion is the basic method, which has been widely employed for wet digestion of samples prior to trace element analysis. Different acids, different amounts and mixtures and different heating systems have been described in the literature (as outlines in Table 2.5.1).

Table 2.5.1 Outline of digestion principles as given in standards (ISO 15587:1999, Standard Methods for Examination of Water and Wastewater and U.S. Environmental Protection Agency).

Method	Principle
ISO/DIS 15587-1 (Aqua regia), 1999	25 ml water sample + 6 ml HCl, concentrated + 2 ml HNO ₃ Digestion in open system using electrical heating or microwave or in a closed system using microwave or autoclave
ISO/DIS 15587-2 (Nitric acid), 1999	25 ml water sample + 6.25 ml HNO ₃ , concentrated Digestion in open system using electrical heating or microwave or in a closed system using microwave or autoclave
SM 3030 E (Nitric acid), 1999	10.0 ml water sample + 0.50 ml HNO ₃ , concentrated Digestion in open system using electrical heating
SM 3030 F (Aqua regia), 1999	A measured sample volume + 2.0 ml HNO ₃ , (1+1) + 10 ml HCl, (1+1) Digestion in a open system using electrical heating or steam bath
SM 3030 K (Nitric acid), 1999 USEPA 3015 (Nitric acid), 1994	45 ml water sample + 5.0 ml HNO ₃ , concentrated Digestion in a closed system using microwave
USEPA 3005A (Aqua regia), 1992	100 ml water sample + 2.0 ml HNO ₃ , concentrated + 5.0 ml HCl, concentrated Digestion in open system using electrical heating or steam bath

The acid for digestion has to be chosen according to the analytical technique to be employed, thus nitric acid is recommended for samples to undergo analysis by ICP or GFAAS, while aqua regia digestion is the choice for samples prior to HGAAS or AFS as hydrochloric acid (part of aqua regia) is used in the last step of the determination as well as in the reduction of As(V) to As(III) prior to the measurement.

The international standard (ISO/CD 15587, part 1 and 2, 1999) as well as a number of national standards (USEPA and SM) has been developed in order to harmonise digestion for different elements and different water types in an effort in increase comparability between results.

2.5.2 Food samples

For the majority of the population, diet is one of the main sources of arsenic exposure, however the concentrations, even within the same foodstuffs, vary widely (see Table 2.5.2).

In any risk assessment of dietary arsenic, it should first be determined whether arsenic exposure from foods makes a significant contribution to the total exposure (i.e. from air, water, food, and smoking). If dietary exposure is significant then the key foods

that contribute the majority of the total arsenic food exposure must be identified and analysed for total arsenic concentrations (see Table 3.3.3). In the USA, the US Total Diet Study (TDS) provides information on average dietary exposure to a range of contaminants in the American population (see Box 2.5.1). It is unlikely that individuals will be habitually exposed to arsenic at levels consistently greater than the population mean over a long period of time (except for some religious or cultural related dietary habits, i.e. vegetarians). When national diets are low in total arsenic levels, a simple conservative exposure estimation based on total arsenic exposure will suffice as an assessment of the safety of the food supply.

Box 2.5.1 Dietary exposure estimates (total diet study in the USA) and arsenic in foods in various countries

Population, or per-capita, average exposures are estimated by the mean concentrations of total arsenic analysed in the 264 core foods that make up the TDS and the quantities of these foods consumed for various age-sex groups. Quantities of these foods consumed are determined based upon the food consumption data from the 1987-1988 US Department of Agriculture's (USDA) National Food Consumption Survey (NFCS) for each of the 14 age/sex groups. The mean analytical residue concentrations are used for estimation of dietary exposures because over a lifetime the mean concentration reflects residue levels the population would be exposed to.

Consumer exposures are estimated by the analytical results of the mean concentrations of total or inorganic arsenic found in rice and shrimp and the quantities of rice and shrimp consumed by individuals who eat average, or higher amounts of these foods (i.e. mean, 90th, and 95th percentile consumers). Food consumption data are based upon USDA Three-day 1989/90, 1990/91, and 1991/92 NFCS for selected age-sex groups.

The results from the most recent total diet study indicated that among the 264 foods analysed, seafood had the highest concentration of total arsenic, followed by rice and/or rice cereals, mushroom, and poultry (Tao and Bolger, 1999). Like results from previous TDS market baskets, seafood and rice/rice cereals contributed the majority of the estimated dietary exposure to total arsenic in US diets. Of the estimated dietary total arsenic exposure, seafood contributes 42 per cent for infants, 76-90 per cent for children (2-10-year olds), 79-85 per cent for 14-16-year olds, and 89-96 per cent for adults (\geq 25-30-year olds); rice/rice cereals contributes 31 per cent for infants, 4-8 per cent for children, 8 per cent for 14-16-year olds, and 1-4 per cent for adults (\geq 25-30-year olds). Similar findings were also reported in other countries.

Table 2.5.2 summarises the estimated dietary exposure to total arsenic in many countries. Similar to the findings observed in US diets, seafood or fish is the major contributor to dietary total arsenic in other countries. It contributed from 52 per cent of the dietary total arsenic in The Netherlands to 96 per cent in Spain.

Since it is difficult to analytically differentiate the chemical forms of arsenic in food, most currently available exposure estimates are for total arsenic, which includes both inorganic and organic forms. However, speciation of arsenic in food may be important when general exposure is high, or in specific sub-populations with elevated exposure (such as high seafood consumers or those using rice as their staple food).

Table 2.5.2 Estimated average dietary arsenic intakes by adults and contributions from seafood in different countries

Country	Period	Method ^a , population	Total As intake µg/day	Total As intake from seafood ^b % total As intake	Reference
Belgium		DD	12 (median)		Buchet <i>et al.</i> , 1983
Brazil	1992 Sept-Nov	DD (3-d), 19 children (12 boys, 7 girls); 23 elderly (7 males, 16 females)	12.4 6.9	No info on seafood intake	Maihara <i>et al.</i> , 1998
Canada	1981	DD (5 cities), adults	16.7 (2.6-101)		Dabeka <i>et al.</i> , 1987
Canada	1985-1988	MB, 7 collections from 6 cities, 112 food composites all ages, males and females	38.1		Dabeka <i>et al.</i> , 1993
Canada	1985-1988	MB, 20-39-yr males	59.2	64	Dabeka <i>et al.</i> , 1993
Croatia	1988-1993	MB, per capita	11.7	75.8 (19.4 g/d)	Sapunar-Postruznik <i>et al.</i> , 1996
Denmark	1983-1987		118	72 (26 g/d)	NFAD, 1990
Japan	1991	DD (3 d), 39 females (33-67 yr)	260		Tsuda <i>et al.</i> , 1995
Japan	1992	DD (3 d), 40 females (28-66 yr)	210		Tsuda <i>et al.</i> , 1995
Japan	1991	MB, 14 grps, 170 foods	160		Tsuda <i>et al.</i> , 1995
Japan	1992	MB, 14 grps, 170 foods	280		Tsuda <i>et al.</i> , 1995
Netherlands	1976-1978	MB, 16-18-yr males	15	86.7 (10 g/d)	de Vos <i>et al.</i> , 1984
Netherlands	1984-1986	MB, 18-yr males LD=0.005 ppm	38	52 (9 g/d)	van Dokkum <i>et al.</i> , 1989
Spain (Basque Country)	1990-1991	MB, 16 grps, 91 food. water not included	Mean=286 Max=291	96 (89 g/d)	Urieta <i>et al.</i> , 1996
Sweden	1988	DD, 15 females (27-46 yr)	60		Jorhem <i>et al.</i> , 1998
United Kingdom	1982	MB, all ages	67-90	71	MAFF, 1998
United Kingdom	1983		70		MAFF, 1998
United Kingdom	1991	MB, 20 grps, 115 food, all ages	56-67	62	MAFF, 1998
United Kingdom	1994	MB, 20 grps, 115 food, adults	120		MAFF, 1997
United Kingdom	1994	MB, 20 grps, 115 food, all ages	63	89	MAFF, 1997; Ysart <i>et al.</i> , 1999
United Kingdom	1997	MB, 20 grps, 115 food, all ages	65	94	MAFF, 1999
United States	1984-1986	MB, 25-30-yr males	58.1	87	Gunderson, 1995a
United States	1986-1991	MB, 25-30-yr males	38.6	88	Gunderson, 1995b
United States	1991-1996	MB, 25-30-yr males	56.6	92	Tao and Bolger, 1999

^a DD = duplicate diet Study; MB = market basket survey; ^b Seafood intake in parenthesis

2.5.2.1 Sample collection

To provide the most useful information, samples should be representative of the food consumed in a population. Thus, preparation and cooking should be carried out in a manner similar to those carried out by the consumer. In the USA, both rice and shrimp samples were portions of samples collected for the US total diet study. Details of collection and preparations have been described previously (Pennington and Gunderson 1987; Pennington *et al.* 1996). Briefly, rice and shrimps are two of the 264 core food items (i.e. foods most commonly consumed), identified in the total diet study, reflecting the average US household diet (Pennington, 1992). Samples for the US total diet study were collected four times a year, from retail stores in one of each of the four geographic locations by FDA inspectors. For each collection or 'basket', identical food items were purchased from three designated cities. For analysis, a composite was made for each food item after preparation or cooking (in a manner similar to those that would be used at home) if appropriate. For arsenic speciation analysis, a total of eight basket samples collected over a two-year period from 1997 to 1998 were used (see section 2.8).

2.5.2.2 Sample pre-treatment

Homogenisation and sub-sampling

Care must be taken to ensure that the laboratory sub-sample is representative, which generally means that homogenisation is necessary. It is not possible to give a general rule that applies to the homogenisation procedure for all food sample types. Freeze-drying may be a useful precursor to homogenisation as the freeze-dried material is often easier to homogenise. Various commercial homogenisers or mills are available and convenient to use. The draft European standard prEN 13804:1999 provides general considerations about the special requirements for sample preparation, materials and reagents for heavy metal analyses.

Digestion

Numerous digestion techniques are described in literature for biological materials (*REFS*). The digestion techniques differ in mode of energy transmission, amounts and combination of digestion media used. The energy transmission technique can be chosen according to the available equipment, provided that the energy level is sufficient and care is taken to avoid loss or contamination during the digestion process. Options include open and closed microwave digestion techniques, closed bomb systems and Kjeldahl digestion equipment. As mentioned in section 2.6.1.6 the choice of digestion media will be guided by the subsequent analysis technique. The Nordic countries have developed a method for arsenic determination in seafood products using nitric acid and hydrogen peroxide as digestion media with microwave digestion prior to GFAAS measurement (Julsham *et al.*, 1996). The method is expected to achieve the status as Nordic NMKL standard method. The draft European standard prEN 13805:1999 specifies a method for digestion under pressure and the method has been collaboratively tested in combination with GFAAS, HGAAS, ICP-MS, ICP-AES and voltammetry.

The use of nitric acid for digestion of marine food samples in a conventional microwave oven has been shown to be inadequate for HGAAS analysis (Øygard *et al.*, 1999) as only 10 % of the arsenic content was recovered from a certified seafood reference material. This digestion method, however, is satisfactory for GFAAS measurement (Øygard *et al.*, 1999). When using a mixture of nitric and sulphuric

acid for digestion temperatures need to be higher, however, using either high-pressure vessels in a microwave oven or Kjeldahl digestion equipment gives satisfactory results for certified seafood reference material.

2.5.3 Human samples for assessing body burden

Human samples for which determination of arsenic is most relevant include urine, blood, hair and nail samples. Each of these biomarkers provide information on the absorbed dose of arsenic, although how recent that exposure varies according to the biomarker examined.

Guidance regarding patient preparation, specimen collection, transportation and processing for the measurement of trace elements (including arsenic) in a variety of biological matrices can be found in an American guideline (NCCLS C38-A, 1997). Normally, where human samples are concerned, the sample size is limited (especially for blood and organ tissue samples) thus 'micro' techniques are most appropriate for the analytical steps. This section examines issues involved with the collection and handling of human samples (see also National Research Council, 1999).

2.5.3.1 Collection and handling of urine samples

The background concentration of arsenic in urine is relatively low ($<10 - 50 \mu\text{g l}^{-1}$ arsenic) and the same precautions as mentioned for water samples are relevant for samples of urine (2.5.1.3) to avoid contamination during the sampling process.

Procedure for preservation of urine samples depends on the analytical procedure chosen. If speciation studies are to be carried out it is recommended that the sample be divided into two containers, one of which can be preserved with acid for analysis of total arsenic.

It has been shown that the stability of As(III), As(V), MMA, DMA and AsB at a concentration level of each $50 \mu\text{g/L}$ is maintained for up to two months when kept refrigerated or frozen (*REF*). Stability for longer periods has been shown to be dependent upon the urine matrix. Acidification with nitric acid alters the speciation of inorganic arsenic and may cause de-methylation. Arsenic stability studies of speciation of water samples at lower concentrations have shown alterations in speciation for storage periods shorter than two months, depending upon the water matrix. It is recommended that urine samples for speciation measurement be stored for as short a period as possible. Refrigeration or frozen conditions are recommended.

2.5.3.2 Collection and handling of blood samples

Blood is a hazardous material and precautions must be taken when dealing with patients or blood products to ensure that both the patient and the analyst are protected against infection. All supplies must be one-use only and must be disposed of in biohazard containers for incineration or sterilization. If venous samples are required only trained phlebotomists or medical personnel should collect the samples.

As the concentration of arsenic in blood samples is low, care must be taken in order to avoid contamination and to minimise loss of trace amounts from the samples. To avoid contamination rigorous cleaning of the puncture site is required. As with water and urine samples, polyethylene sample containers should be used and pre-cleaned as outlined in 2.5.1.3.

Few studies on arsenic speciation have been reported, and no stability studies on the storage of blood samples for speciation analyses have been reported.

2.5.3.3 Collection and handling of hair and nail samples

Hair and nail sampling have the advantage that they are non-invasive, although of all the biomarkers they are probably the most likely to suffer from contamination problems. With background concentrations of arsenic in hair and nails of 0.02-10 mgkg⁻¹, a sample size of about 1 gram of nail or hair is needed.

As the concentration of arsenic in the hair from the same person may vary, hair should be cut from several places on the head, to decrease the chances of contamination areas such as the nape of the neck and close to the scalp are recommended. Unsurprisingly, arsenic concentration in hair may vary with the distance from the hair root, therefore the length of the hair to be analysed should be specified in the sampling programme. Toenail samples are recommended over fingernails to decrease contamination problems and increase sample size.

Stability of arsenic in hair and nail is expected to be high. No special method of storage is recommended.

2.5.3.4 Evaluation of human biological materials

The different biomarkers reflect different timescales of exposure to arsenic, with hair and nails reflecting past or long-term exposure, while blood and urine levels reflect recent exposure. Measurements of total arsenic in urine need to be treated with caution as a large proportion of this could be relatively non-toxic organic forms. Hair and nails suffer from contamination problems and it is difficult to ascribe what is likely to be due to external exposure.

Concentrations of arsenic in urine, blood, fingernails and hair from persons with no known exposure to arsenic are low in relation to the expected detection limits. Detection limits for water, which is a relatively simple matrix compared to human samples, is expected to be about 0.5 - 1 µg l⁻¹ for the routine analytical methods normally applied. The practical quantification limit (PQL) has been estimated to 1 - 4 µg l⁻¹ for water. As hair and fingernails and in some cases also blood and urine are digested prior to determination, and as interferences from other constituents in the samples are expected, PQLs for these sample types may be five times (or more) higher than for water samples. This implies that results at the background concentration for urine, blood, hair and fingernails and in elevated levels for blood may be influenced by increased errors.

2.6 DATA PRESENTATION AND INTERPRETATION

Data presentation together with interpretation represents the final stage of any environmental monitoring exercise. Where a large number of samples have been collected and analysed it will generally be necessary to use a statistical approach for data handling. However, prior to any such approach it is necessary to design the study with this in mind. Thus it is necessary to obtain sufficient independent data points and these need to be representative of the population or populations being sampled.

2.6.1 Presentation

Prior to the advent of computers data sets were stored in written form and such a method is still applicable to small data sets. However, for large data sets information needs to be stored in computers. When storing analytical data details must include the type of sample medium and the units of measurement, together with as much background information as possible. This information should include items such as dates and times of sampling, method of collection, type of sample preservation and pre-treatment performed, date of analysis, details of methodology together with a measure of data quality, including values for precision and accuracy and limits of detection and so on. For water samples it is also necessary to include exact data on geographical location of the samples, type of water sampled and in the case of groundwaters the depth from which they were collected.

Data can be presented in a variety of forms ranging from simple tables through to graphical displays. Results for groups of data from similar media are frequently summarised by quoting maximum and minimum values together with the arithmetic mean and the standard deviation. However, in the case of data that are not normally distributed, which is the case for most data from environmental sources, it is better practice to quote geometric mean or median values. However, any statistical treatment of data needs careful thought, prior to commencing the sampling programme and, as stated above, requires careful pre-planning. In some cases, for example the distribution of arsenic in surface or groundwaters, data can be displayed in a series of maps thus enabling the distribution of high arsenic waters, which could pose potential problems if they are used as potable supplies, to be easily identified.

2.6.2 Interpretation

Arsenic has long been known to be toxic to humans and has been classified as a human carcinogen (IARC, 1980), with chronic ingestion associated with skin cancer while inhalation is associated with lung cancer. Serious health effects resulting from relatively low doses of arsenic, derived from its natural occurrence, have been highlighted during the latter part of the twentieth century. The greatest health concern with regard to regular exposure to arsenic derives from drinking waters containing elevated concentrations.

As drinking water is a major pathway into the human body analysis of such waters for arsenic has become essential. The WHO guideline for arsenic in drinking water is $10 \mu\text{g l}^{-1}$ and this is exceeded in many areas of the world. The U.S. EPA has suggested that this is too high and has suggested that the concentration of arsenic in drinking water should be no more than 5 or even $2 \mu\text{g l}^{-1}$. Thus results from water samples need to be compared with these values.

As urine is the major pathway for excretion of arsenic from the human body this medium is analysed to estimate exposure. While it has been demonstrated (Calderon *et al.*, 1999) that there is a good correlation between arsenic in urine and that in the corresponding drinking water this has been a matter of some debate (Brown and Chen, 1995; Goessier *et al.*, 1997). Other workers have suggested that human hair and nails can give estimates of arsenic body load (Chowdhury *et al.*, 1997). It is therefore sometimes necessary to compare analytical data for several media and further to compare these with data from unexposed populations.

It has been shown that arsenic exists in several forms, in the media of interest. Most arsenic in drinking waters is inorganic, this occurring as As(III) and As(V) with As(III) being more toxic than As(V). However, in other media such as urine much of the arsenic occurs in organically bound methylated forms. Once again, therefore, where speciation data are available it is important to interpret results in terms of the forms of arsenic occurring in the various media.

2.7 QUALITY MANAGEMENT

Decisions on activities intended to improve environmental conditions often rely on measurement results. To ensure that legislation and regulations regarding arsenic levels are adhered to and that enforced mitigation measures are indeed producing improvements, monitoring must be able to produce reliable data about the state of the environment. Growing concern with poor laboratory data comparability and money wasted on monitoring projects where the resulting data have been seen as unreliable has led, in a number of countries, to the establishment of laboratory structures designed to ensure that data produced are reliable, accurate and adequate for the intended purpose.

Minimum laboratory requirements are outlined in ISO/IEC 17025:1999 "General Requirements for the Competence of Testing and Calibration Laboratories". This standard includes the requirements of a quality system but does not include details relating to the required or appropriate quality to be attained. The standard ISO/DIS 15189:1998 "Quality management in the medical laboratory" provides advice especially for laboratories analysing human samples. An overview of the requirements regarding the quality management system related to laboratory activities as required in ISO/IEC 17025:1999 is shown in Table 2.7.1. As can be seen from the table the requirements may be divided into management requirements and technical requirements. Both are equal parts of a system with the purpose to create confidence in the data produced by the laboratory. This section examines some of the technical requirements for an adequate quality management system.

Table 2.7.1 Requirements regarding quality management system

	Description
Management requirements	<ol style="list-style-type: none"> 1. Organisation and management 2. Quality system 3. Document control 4. Review of request, tender or contract 5. Subcontracting of tests and calibrations 6. Purchasing services and supplies 7. Service to the client 8. Handling of complaints 9. Control of non-conforming testing and/or calibration work 10. Corrective action 11. Preventive action 12. Records 13. Internal audits 14. Management review
Technical requirements	<ol style="list-style-type: none"> 1. General 2. Personnel (e.g. competence) 3. Accommodation and environmental conditions 4. Test and calibration methods including method validation

-
5. Equipment
 6. Measurement traceability
 7. Sampling
 8. Handling and transport of test and calibration items
 9. Assuring the quality of test and calibration results
 10. Reporting results
-

It is important that the quality management system includes all activities that may influence the quality of the data, including sampling. However, sample collection (and related data recording and record keeping) may not be within the scope of the laboratory's responsibilities. Some field records, therefore, may not be available to or maintained by the laboratory. Either the laboratory or the organisation responsible for the sample collection should keep records describing how the sample containers were prepared (i.e. cleaning methods), how preservatives were prepared and used, and how the samples were collected. In all cases, the sample tracking record and shipping form (if available) should accompany samples to the laboratory, and the laboratory should, at a minimum, maintain this documentation. Documentation should be available to verify that a given sampling method was used for sample collection and that the sample collectors were properly trained.

2.7.1 Personnel

All persons involved in sampling and analyses of arsenic should be qualified through appropriate education, training, experience and/or demonstrated skills. It is important that field survey researchers and field workers are able to accurately assess conditions at and around the survey site. In this regard, they must be knowledgeable on how to collect the specific samples and how to describe the sampling conditions. Where field test kits are to be used they should be trained in all aspects of using and maintaining the kits. It is suggested that training for between two and four weeks (including training in the field) would be necessary for new personnel, who will be in charge of sampling and analysis with field test kits. Any training should ensure, and document, that the required level of technical skills and understanding are achieved.

Personnel who operate laboratory analytical equipment must be trained in quality management, including all aspects of handling the sample in the laboratory from sample receipt to the data reporting. It is desirable that the person responsible for the laboratory management system and data quality be qualified, to degree level, in analytical chemistry or similar. The laboratory management must ensure the competence, in terms of general and specific laboratory skills, of staff operating specific equipment. Specific training needs for conducting arsenic analysis will vary according to the techniques to be used, but for the more advanced techniques (such as AAS and ICP) it should be expected that training and supervision will amount to several months working fulltime on the instrument.

2.7.2 Accommodation and environmental conditions

It is necessary to ensure that the working environment does not invalidate the results through contamination or loss. Particular care should be taken during sampling and sample handling at the sampling site. Loss of arsenic during sampling, transport and storage is avoided by preservation, by choice of proper sampling material and by minimising the time between sampling and analyses. Sampling conditions, choice of sampling equipment including bottles, transport, and storage must be fully documented

2.7.3 Sampling and analytical methods, validation

Generally, it is highly recommended that the laboratory select appropriate methods, which have been published either in international, regional or national standards documents, or by reputable technical organisations. Furthermore, where appropriate, it is recommended that the laboratory apply the latest version of the method. In many cases the standard may be supplemented by additional details to ensure that the method is properly understood and applied correctly in the laboratory. A rewriting of the standard is not recommended, except translating if necessary. Standard methods for field measurements are rare and often the user is left to apply the method given in the manufacturers' manual.

Validation of a method is established by systematic laboratory studies. This process shows whether the performance characteristics of the method meet the specifications required for the intended use of the analytical results. The amount of validation necessary to be made by the laboratory prior to carrying out routine analytical work depends on the status of the method. Well-documented international standards need less validation than new methods that have been developed and implemented by the laboratories themselves. Table 2.7.2 lists the validation requirements in different situations.

Table 2.7.2 Schematic guideline for establishing the extent of method validation depending on type of method

	Method described in international or national standard	In house developed method or other non-validated method (e.g. from manufactories manual)
Selectivity		*
Limit of detection	*	*
Accuracy	*	*
Precision	*	*
Linearity	*	*
Sensitivity		*
Range		*
Ruggedness		*

As a minimum, the following performance criteria should always be analysed and documented by the laboratory prior to carrying out routine analytical work:

- limit of detection
- accuracy
- precision, and
- linearity

Additional for arsenic speciation:

- mass balance, if possible

Analyses should include natural samples representative of those sample types for which the method will be applied. It is important to emphasise that interferences are likely to occur for all analytical methods on arsenic, whether field-based techniques or laboratory-based techniques and whether simple or sophisticated techniques are applied. Therefore the validation is of equal importance for all methods applied (a practical approach to method validation is given in Lund, 1997).

Limit of detection

The limit of detection is calculated from the standard deviation of the results of at least six determinations of a natural sample with a concentration close to the expected limit of detection (LOD). A sample of a concentration less than five times the LOD will generally be adequate. The determination must be made under repeatable conditions.

Common types of detection limits as applied to methods are summarised below:

- Criterion of detection (CD) - this is the measured concentration at which there is a stated probability (i.e. 95, 99 or 99.7 per cent) that an analyte is present. The CD prevents false positives beyond the specified probability.
- Limit of detection (LOD) – this is the lowest analyte concentration required to be present in a sample to ensure an analytical response that will exceed the CD with stated probability (usually the same as above). The LOD = 2 x CD. The LOD prevents false negatives beyond the specified probability.

Arsenic measurement usually involves the measuring of a blank value and subtracting this from the measured value for the test sample to give the result for the test sample. The standard deviation for this difference is

$$s_{result} = s_{blank} \sqrt{1 + \frac{1}{n}} \quad \text{Eq. 1}$$

The CD is then the upper end of the confidence interval for a sample whose true concentration is zero:

$$CD = t_{1-\alpha}(df) \bullet s_{blank} \bullet \sqrt{1 + \frac{1}{n}} \quad \text{Eq. 2}$$

Where n is the number of blank measurements used for blank correction in routine analysis, s_{blank} is the standard deviation of repeated blank measurement. Normally, s_{blank} will not be measured in each batch of analyses but will be obtained either from a larger number of determinations made in a separate experiment, from method validation or from internal quality control. Thus, n is normally different from the number of measurements determining df, which is the number of degrees of freedom

in the estimation of s_{blank} . Values for $t_{1-\alpha}$ can be found in statistical tables or in Lund, 1997.

The confidence level can be chosen to suit the specific measurement situation. Generally a 95 per cent confidence level is chosen, i.e. α of 5 per cent. When estimating the limit of detection it is customary to choose the same level of confidence as for the criterion of detection. In this case the limit of detection is simply two times the criterion of detection.

A practical quantification level (PQL) has been defined by USEPA as the “lowest concentration of an analyte that can be reliably measured within specified limits of precision and accuracy during routine laboratory operating conditions” (*REF*). PQLs are set at a point where at least 75 % of laboratories can perform the analysis within an acceptable level of precision and accuracy. Acceptance limits set at ± 20 % of the measured value may be used for accuracy, but are dependent upon conditions. The PQL may be determined through inter-laboratory studies. It can be used in the assessment of the ability of routine laboratories to analyse at a specific limit according to set quality criteria.

The practical quantification level for the determination of total arsenic in drinking water as determined in Lund, 1997 and VKI 1997 is relatively high ($1-4 \mu\text{g l}^{-1}$) compared to background levels of arsenic. Even higher PQLs are expected for more complex media such as urine and blood. Results from laboratories quoting lower detection limits than the PQLs may be reasonable, but it should always be remembered that the detection limit calculation does not include the systematic error component.

Accuracy

For the determination of accuracy a number of methods exist which are applicable in different situations. The methods in general used are:

- Recovery test. A known amount of the analyte is added to a natural sample giving a spiked sample. Both the original natural sample and the spiked sample are analysed using the whole procedure several times. The difference between the results of the two samples gives the recovery. In this way interferences affecting the sensitivity are revealed.
- Comparison with results of another validated and generally accepted method. This procedure is valuable when changing from one method to another or when making modifications of an existing standardised method.
- Inter-laboratory studies. If relevant inter-laboratory studies are available this is a valuable method, but unfortunately this is not the case in most countries.
- Analysis of certified reference materials. Such materials are available and the procedure is highly recommended (see Table 2.7.4 – later).

For method validation it may be an advantage to use a combination of the above techniques.

Precision

The precision is expressed in terms of standard deviation and consists of repeatability, reproducibility and a number of intermediate measures of precision such as standard deviation between batches.

Every laboratory must establish its own repeatability and the between batch standard deviation. Variability between laboratories should be examined when preparing a method for standardisation but is generally not necessary for methods used in a single laboratory. The following deals only with repeatability and between batch standard deviation.

Representative samples are chosen and each sample is measured n times in each of m batches. The choice of the number of replicates (n) and the number of batches (m) needs careful consideration in order to ensure that the dominant source of uncertainty will be evaluated. Too few analyses will not provide a worthwhile estimate of standard deviation. The uncertainty of an estimate of standard deviation depends on the number of associated degrees of freedom. Tests that are likely to provide an estimate of standard deviation with much less than six degrees of freedom may prove uninformative.

The experimental design recommended for general use is to make n equal to two and m equal to between six and ten. Such a design provides estimates of repeatability and between batch standard deviation with an approximately equal number of degrees of freedom. For most arsenic methods the between batch standard deviation may be assumed to be dominant. The product $n * m$ should not be less than ten and should preferably be 20 or even higher.

Samples used for precision tests must be stable over the period of time in which the experiments are to be made. Stability of total arsenic can generally be assumed for natural samples (although some sample types need preservation). The samples must also be sufficiently homogeneous to ensure that inhomogeneity does not significantly contribute to the estimated standard deviation.

For trace element analyses (including those of arsenic) the variability from analyses of natural samples differs considerably from that of synthetic samples and it is therefore insufficient to base measures of precision on synthetic samples and standard solutions.

The precision generally depends on the concentration in the sample and this dependence must be documented. A basis for determination of precision measures could be:

- Two natural samples, one in the middle and one at the lower end of the range of the method.
- One or two other samples, synthetic or preferably natural, with concentrations distributed within the range of the method and the same samples spiked with arsenic.

Linearity

For at least one of the batches, calibration is then performed by means of duplicate (or more) measurements of at least six calibration solutions. The purpose of the linearity

investigation is in most cases to document the linear range of a method, this being the range within which a simple calibration can be used in routine analysis. Matrix interferences may influence the calibration, thus it needs to be carried out by using standard addition or addition calibration. This is the case when a simple calibration solution produces a calibration curve where the slope is different from a slope produced when plotting data from analyses of solutions containing the matrix of interest.

Sensitivity

The sensitivity is calculated on the basis of the data generated in the determination of linearity, using the slope of the line produced by linear regression, or in some cases as the concentration giving a certain response. This concentration is also calculated from the slope. Matrix interference may influence the slope, which in turn will influence the sensitivity. This aspect should be taken into account.

Range

For the range estimation, the accuracy and precision (especially at the upper and lower ends of the range) are needed. The validated range does not necessarily have to be the total theoretical range of the method. It is sufficient to validate the range within which the method is normally used.

Selectivity

The interferences in analytical procedures may be caused by:

- components adding a positive or negative response to that of the analyte,
- components affecting the sensitivity resulting in different responses from the analyte in a standard solution and in a natural sample.

Such interferences affecting sensitivity (for example matrix effects in AAS) are relatively easy to detect by spiking a natural sample with a known concentration of the analyte. For interferences adding a positive or negative response it is necessary to identify the possible interfering substances and subsequently investigate their effect.

If matrix reference materials are available they should naturally be included in a check for interference. Comparison with a reference procedure is also an obvious check. However, reference materials and reference procedures are scarce and, therefore, in most cases, the selectivity must be investigated by experimental check of possible interferences.

The extent of selectivity investigations is completely dependent upon the method in question, but, as a minimum investigations should always include spiking experiments at the upper and lower end of the range of the method and considerations on possible additive interferences. The evaluation of spiking experiments for natural samples implies knowledge of recovery from synthetic standard samples.

Ruggedness

Ruggedness is not an essential performance characteristic for every laboratory to measure. When applying a standardised method it is reasonable to assume that the ruggedness of the method has been determined in connection with the standardisation procedure. The effect could, for example, be an unusually large variation between

different analysts in the laboratory or a very large between laboratory variations in inter-laboratory studies.

When planning a ruggedness test, every parameter that could be critical to the outcome of the analysis is identified. The analysis is then performed the necessary number of times, inducing a deliberately small variation in each of the critical parameters. The number of parameters to be investigated is generally large and a statistical approach to the experimental plan is recommended. Ruggedness testing is described in books of statistics (e.g. Youden and Steiner, 1975).

Determination of the ruggedness and the selectivity of a method may be a tedious process involving a range of analyses to be carried out on samples of different composition. Regarding new techniques, all possible and relevant interferences in the sample type of question must be investigated to document the selectivity of the method.

Various events, which take place from time to time in every laboratory, will trigger a method validation. A number of such events and guidelines for the extent of method validation in each case are given in Table 2.7.3.

Table 2.7.3 Guidelines for extent of method validation for various events in the laboratory (Lund, 1997)

EVENT	ACTION
New method developed in the laboratory	Complete method validation as defined in Table 2.7.2
Implementation of a standardised method	Ruggedness and selectivity can be assumed to be established as part of the standardisation process. The effort can be concentrated on showing that the remaining performance characteristics give the same quality as that described in the standard
Change of method	The demands are the same as for implementation of a standardised method but the practical performance may be different
Slight modification of method	The method must, after modification, give the same or better performance on all matrices as the original method
New matrix	Accuracy and precision must be tested for example by means of reference materials, by participation in inter-laboratory studies, or by analysis based on alternative methods
New instrument/ transport of instrument	Limit of detection, precision, linearity and range must be tested by means of certified reference materials or laboratory reference materials
New analyst	Limit of detection and precision must be tested by means of certified reference materials or laboratory reference materials. Furthermore, a close surveillance must be kept on accuracy until the analyst has the proper understanding of the methods and problems of interference

2.7.4 Equipment

When received equipment, including that used for sampling and on-site measurement, should be checked to establish that it meets the prescribed quality requirements. Quality requirements are statements in respect of LOD, precision and so on. The quality requirements may be those given by the manufacturer or those demanded by the laboratory and accepted by the manufacturer. It is recommended that quality requirements form part of the procurement contract for any relevant equipment.

Instructions on the use and maintenance of the equipment as well as a logbook should be established for all instruments significant to the analyses (which in many cases will include analytical balances as well as volumetric equipment). Reference is made to ISO 17025:1999 for further details.

2.7.5 Measurement traceability

Experience from evaluations of inter-laboratory comparison data shows that data from laboratories on the analyses of trace elements, including arsenic, are subject to systematic errors, some of which can be traced back to calibration errors.

Arsenic analysis requires a calibration against an arsenic standard, so that the primary analytical response (e.g. absorbance) can be transformed into an arsenic concentration. It is emphasised that the uncertainty of the measurement can be worse than that of the calibration, it can never be better. The calibration routinely applied to an analytical system nearly always involves one or more assumptions about the behaviour of the system (e.g. on linearity). If these assumptions are not justified, the analytical results will be subject to errors (either systematic or random, or both). When statistical control has been verified, calibration uncertainties can be the major source of discrepancies between laboratories. Thus, biased measurement data can be the result when using unbiased methodology because of calibration bias. Laboratories should give maximum attention to this important operation and critically evaluate it in every measurement situation.

One important prerequisite of good calibration is of course a good quality arsenic calibration standard. Arsenic stock solution for total and inorganic trivalent arsenic determination can be prepared from arsenic(III) oxide of sufficient quality, previously dried over silica gel to constant weight. As(V) stock solution for inorganic pentavalent arsenic determination can be prepared from arsenic pentoxide trihydrate (do not dry!). Arsenic stock solutions, including some organic arsenic compounds, can be purchased from reputable commercial sources.

With most of the analytical techniques, the performance of arsenic analysis is matrix dependent. Herein lies an often-overlooked element of uncertainty. The standard may satisfy all requirements for traceability but may not correspond fully to the unknowns in the matrix and full correspondence may be very difficult (if not impossible) to achieve in a given situation. Accordingly, the analyst must use matrix modifiers, apply corrections for matrix effects or remove the component of interest from the matrix and/or carry out calibration by using standard addition or addition calibration. As a general rule for atomic absorption, standard addition or addition calibration should be applied, unless it can be shown unnecessary on natural samples.

The accuracy of low-level trace determinations is seriously affected by the degree of control of the analytical blank, i.e. contamination of the sample from all external sources.

The degree of control can also affect the ultimately attainable accuracy in high-accuracy analyses. Most of the sources of the blank are variable and it is this variability that determines the uncertainty of the blank correction and, often, the lower limit of trace concentration that can be determined with reliability. To improve both the accuracy and lower limit of trace determination, it is imperative to control the variability of the analytical blank. The only practical way to accomplish this is to reduce the size of the blank itself by controlling the sources of the blank and by always determining the blank value.

By applying a well-described analytical standard some of the above-mentioned precautions regarding calibration will be taken into consideration, but many interferences may be instrument specific and analytical instruments are equipped with different correction systems. Therefore, in many cases corrections are left for the experienced analyst to overcome. This is a major reason why analyses made by means of AAS, ICP-AES, ICP-MS or other advanced equipment should always be carried out by very experienced analysts.

Chemical traceability to SI units of measurements is achieved in two ways: First, by use of pure chemical standards and, secondly, by control of the measurement by means of typical matrices in which the amount of analyte present is well characterised. This latter type of standard is known as reference material and is often known as a matrix reference material. The use of reference materials is covered below and in Table 2.7.4. The consistent use of appropriate reference materials together with control charts can secure laboratories against unacceptable biases in the measurement of calibration.

Regular participation in a suitable programme of inter-laboratory comparison or proficiency testing is another way of controlling systematic errors and comparing results with other laboratories. For areas where monitoring programmes are set up that involve a number of laboratories and institutions regarding the analyses, inter-laboratory comparisons should regularly be conducted among all participating laboratories. The programmes should include both synthetic (where relevant) and natural matrices at appropriate concentration levels. For areas where test kits are used for the analyses of arsenic, these should also be included in the programme. Results from inter-laboratory comparisons on natural samples may be used to estimate the overall between laboratory variation of data. In Europe, a database for proficiency test schemes (www.eptis.bam.de) has been established and can be used by the laboratories to find an appropriate proficiency test.

2.7.6 Sampling and handling of samples

As outlined in section 2.5.1.2, the laboratory or relevant body responsible for sampling should have a sampling plan and sampling procedure for each specific sample type. Requirements regarding different sample types are outlined in section 2.5.

2.7.7 Assuring the quality of test results

Quality control is an evaluation programme included in routine laboratory activities designed to assess the accuracy and precision of measurement and check that the analytical system is in statistical control. Quality control is part of the QC-cycle of a test method and is, as such, applied during routine analyses after a method has been demonstrated to be appropriate for the given application.

2.7.7.1 Quality control of field sampling

The combined estimate of error in quantitative analytical chemistry includes the sampling error, which is most often forgotten. Field quality control requires the submission of blanks to test the purity of chemical preservatives and to check for contamination from sample containers, filters, filtering equipment or any other equipment used in sample collection or handling. Duplicate sampling assists in the estimation of the random errors associated with sampling. The timing and frequency of blanks and replicate samples must be established in the sampling plan. Field quality control samples measure the combined performance of the field sampling process and the laboratory methods. The results of the field quality control should be documented in the sample report.

2.8.7.2 Quality control of the analytical procedure

A routine analytical quality control system based on the experience gained during the validation step should be designed for the analytical measurement. Laboratory quality control samples measure the laboratory method performance.

Control and evaluation of blanks

The quality control programme must give special emphasis to blank control whenever a blank correction is significant. Environmental control can range from simple good housekeeping practices to the conduct of all operations under ultra-clean conditions. The latter is relevant when carrying out arsenic analyses at background levels in water, human biological material and low-level food products. The ultimate goal is to keep blanks reproducible with arsenic concentrations as low as possible and reproducible.

Reagent blanks are derived from all of the chemicals and the laboratory water that may contact a sample as well as the cleanliness of the labware. Those operations, such as dissolutions or extractions, where relatively large quantities of chemicals are involved can make large contributions to the reagent blank. This is often seen in the case of reagents used for determination of arsenic by HGAAS and SDDC. Good practice dictates that reagents used for a particular set of measurements should come from the same manufacturing lot. Records should be kept of all chemicals used whenever blanks are of consideration.

Appropriate control charts (see later) provide the means to evaluate the stability and variability of the blank. Blank control charts should be analysed in the same manner as any other control chart and systematic trends and outliers must be looked for. If good records are kept, it might be possible to correlate abnormalities with other experimental information to discover assignable causes and corrective measures necessary to obtain acceptable blanks.

Whenever the blank correction becomes significant, as is normally is the case for arsenic analysis, it is necessary to measure it with sufficient care. As a matter of course, the blank measurements need to be made with the same amount of effort as the sample itself as the concentration in the sample approaches the concentration in the blank. Analysts who make a limited number of blank measurements while devoting most of their efforts to sample measurement often overlook this fact.

Blank corrections become increasingly important in the case of measurements close to the limit of detection. The effect of a small variability in the blank is magnified in this case. Likewise, even small constant blanks can result in the differing of two quantities approaching each other in magnitude. The absolute value of the blank would appear less important than its accurate evaluation. However, it is a necessary correction and good measurement practice dictates that it should be kept within reasonable limits. An empirical rule, in the case of trace element analysis, is to limit the blank correction to no more than ten times the acceptable limit of error for the measurement (preferably considerably less) and, furthermore, never to exceed the concentration level expected in the sample. The logic behind the first condition is that up to a 10 % error in estimation of the blank would cause no serious difficulties. The second condition is to prevent minor errors in the two measured quantities from introducing large errors in the difference, which is the quantity of practical interest.

There is no way to correct for any blank other than the so-called reagent blank. Rather, one should look for assignable causes and eliminate or minimise them as far as possible. Ordinarily, such sources are variable and provide unpredictable effects. Good records are essential if they are to be identified.

Control and evaluation of precision and accuracy

Control charts are a visual tool for checking that the process is in statistical control, for surveillance of the measurement process and for diagnosing measurement problems. Furthermore, they serve as a documentation of bias (or preferably, lack of bias), precision and limit of detection. A practicable approach to quality control is given in Lund, 1997.

Selection of control charts for analyses of total arsenic

The R-chart (and the r-chart) gives the possibility for control of the part of random error due to within batch variations. The R-chart (absolute range chart) is used for control of field blanks, as described above. In some cases, where no pre-treatment is carried out, the field blank can be taken as the blank, which may control the whole process. Furthermore, if the control sample is a blank, a calculation of the limit of detection is possible.

It is recommended in every batch of analysis to analyse at least one sample in replicate, the results of which should be used to control the within batch variation. The r-chart (relative range chart) is used for this purpose.

Control samples, for which the content is known, should be analysed in any case and the analyses should preferably be carried out in duplicate or triplicate. Synthetic control samples (solutions) for total arsenic with known contents (certified reference materials) are commercially available from a range of suppliers. For some methods only small sample quantities are required, so the reference material can be used regularly. Alternatively, the laboratory can prepare synthetic control solutions from well-defined chemicals, however, it is important not to use the same arsenic compound as the one used for the preparation of the calibration solution. Verification of the laboratory-prepared control sample should preferentially be carried out against a certified reference material. However, the relevance of the control depends upon it being different to the one used for calibration purposes, or at least obtained from a different producer. If this is not the case, any error introduced, e.g. from an impure

standard substance, will be cancelled and this important source of error will not be controlled.

The results of an analysis of the control sample should be introduced into an X-chart (control chart of mean). Appropriate synthetic reference material controls the calibration substance. In the case of water samples it also controls accuracy and between batches precision, provided that no naturally occurring interferences in natural water are encountered. If interferences are observed, which is to be investigated during the validation of the method, it is recommended to use the method of recovery from a spiked natural sample as a control, in which case a D-chart (control chart for mean of recovery) can be established.

Synthetic solutions (whether having the status as certified reference material or laboratory made) are rarely sufficient to control the accuracy and precision for complex matrix samples, e.g. blood and solid samples. In the ideal case, certified reference material of the same matrix type as the natural samples should be used. Furthermore, certified reference materials are used to control the traceability (section 2.7.5). In Table 2.7.4 an overview is given of commercially available certified reference materials for the sample matrices included in this chapter. There is an international need for reference materials certified for arsenic species, especially for the inorganic toxic species.

The systematic error is checked by means of the \bar{X} -chart (and the D-chart) that moreover gives the possibility of checking for trends and other non-random distribution of the measurements. Furthermore, the \bar{X} -chart gives information of the part of random error assignable to between batch variations.

Table 2.7.4 Reference material for internal quality control of arsenic analysis

Matrix	Reference material	Certified value, As	Value not certified, As
<i>WATER:</i>			
Estuarine water	NRC SLEW-2	0.792 µg/L	
Estuarine water	LGC 6015 & LGC 6016	In preparation	
Fortified water	NWRI TM23, & TM24, TM26, TM27 & TM28, TMDA51 & TMDA52	9.9 & 2.7 µg/L 4.4 , 2.2 & 3.14 µg/L 14.8 & 26.9 µg/L	
Fresh water	NIST SRM 1643d	56,02 µg/L	
Groundwater	IRMM CRM 609	1.29 µg/kg	
Groundwater	IRMM CRM 610	10.8 µg/kg	
Hard drinking water	LGC 6010	52 µg/L	
Natural water	NIST SRM 1640	26.67 µg/kg	
Rain water	NWRI TMRAIN 95	1.07 µg/L	
Rain water, fortified	NWRI TMDA 54	20.5 µg/L	
River water	NRC SLRS-4	0.68 µg/L	
Seawater	IRMM CRM 403		19.5 nmol/kg

Matrix	Reference material	Certified value, As	Value not certified, As
Seawater, near shore	NRC CASS-3	1.09 µg/L	
Seawater, open ocean	NRC CASS-5	1.27 µg/L	
Water	VKI QC METAL LL1	29.7 µg/L	
Water	NRCCRM GBW08605	0.500 mg/kg	
Crops and animal derived food:			
Cod muscle	IRMM CRM 422	21.1 mg/kg	
Crab paste	LGC 7160	11 mg/kg	
Dogfish liver	NRC DOLT-2	16.6 mg/kg	
Dogfish muscle	NRC DORM-2	18.0 mg/kg	
Lobster hepatopancreas	NRC LUTS-1	2.83 mg/kg	
Lobster hepatopancreas, partially-defatted	NRC TORT-2	21.6 mg/kg	
Mussel	IRMM CRM 278R	6.07 mg/kg	
Mussel tissue	NIST SRM 2976	13.3 mg/kg	
Mussel	NRCCRM GBW08571	6.1 mg/kg	
Oyster tissue	NIST SRM 1566b	In preparation	
Prawn	NRCCRM GBW08572	1.42 mg/kg	
Bovine liver	IRMM CRM 185	24 µg/kg	
Bovine liver	NIST SRM 1577b		0.05 mg/kg
Bovine muscle	NIST RM 8414		0.009 mg/kg
Pig kidney	IRMM CRM 186	63 µg/kg	
Single cell protein	IRMM CRM 274	132 µg/kg	
Non-fat milk powder	NIST SRM 1549		1.9 µg/kg
Non-fat milk powder	NRCCRM GBW8509		0.013 mg/kg
Whey powder	IAEA-155		49 µg/kg
Whole milk powder	NIST RM 8435		0.001 mg/kg
Whole egg powder	NIST RM 8415		0.01 mg/kg
Apple leaves	NIST SRM 1515	0.038 mg/kg	
Brown bread	IRMM CRM 191		23 µg/kg
Cabbage	NRCCRM GBW08504	0.056 mg/kg	
Corn bran	NIST RM 8433	0.002 mg/kg	
Durum wheat flour	NIST RM 8436		0.03 mg/kg
Olive leaves	IRMM CRM 062		0.2 mg/kg
Peach leaves	NIST SRM 1547	0.060 mg/kg	
Peach leaves	NRCCRM GBW08501	0.34 mg/kg	
Pine needles	NIST SRM 1575	0.21 mg/kg	
Rice flour	NIST SRM 1568a	0.29 mg/kg	
Rice flour	NRCCRM GBW08502	0.051 mg/kg	

Matrix	Reference material	Certified value, As	Value not certified, As
Spinach leaves	NIST SRM 1570a	0.068 mg/kg	
Tea	NRCCRM SBW07605	0.28 mg/kg	
Tomato leaves	NIST SRM 1573a	0.112 mg/kg	
Wheat flour	NIST SRM 1567a		0.006 mg/kg
Wheat flour	NRCCRM GBW08503	0.22 mg/kg	
Wheat gluten	NIST RM 8418		0.02 mg/kg
Human biological materials:			
Bone ash	NIST SRM 1400		0.4 mg/kg
Bone meal	NIST SRM 1486		0.006 mg/kg
Human hair	IRMM CRM 397		0.31 mg/kg
Human hair powder	NRCCRM GBW07601	0.28 mg/kg	
Human hair	NRCCRM GBW09101	0.59 mg/kg	
Urine (two levels)	NIST SRM 2670	0.48 mg/L	0.06mg/L

Frequency

Matrix reference materials, however, are costly and as the bias of a method would not be expected to vary considerably from day to day the use of reference materials in every batch of analyses is generally not required. If matrix reference materials are available it is recommended that they are included at intervals specified in the measurement method and to use control charts for interpretation of the data.

The frequency for measurement of control samples depends on a number of factors, the most important of which is the stability of the measurement process. Normally, one set of control samples will be included in every batch, unless the batch is large. As a guideline, one set of control samples will generally be adequate for batches of less than 20 samples. However, the importance of decisions based on the results should also be taken into account, as all data produced in the period from last control sample known to be in control to the first out of control will be of an unknown and therefore questionable quality. Finally, the cost of analysing control samples must be balanced against the cost of repeated measurement of samples in out-of-control situations.

The minimum level of control will normally involve an X-chart and an R-chart from a reference material or synthetic control sample. For the majority of environmental measurements a blank sample should be included in every batch, and the best use of the data is made by including it in X- and R-charts, especially if concentrations normally measured in 'real' samples are low.

When looking at the recovery of spiked natural samples, the spike should be large enough to ensure a reasonable precision when calculating the difference between the spiked and the unspiked sample and, on the other hand, small enough that the standard deviations of the two measurements do not differ significantly. A spike higher than five (preferably ten) times the within batch standard deviation and below 20 % of the concentration in the natural sample should be adequate.

2.8 ON-GOING RESEARCH AND OUTSTANDING QUESTIONS

The current chapter focuses on the needs for and aspects of the analyses of arsenic, especially total arsenic. This reflects the state of the art in a general sense, as robust speciation analyses are currently not fully developed or implemented for routine purposes. Nevertheless, as alluded to in a number of previous sections, there is a high demand for investigations and monitoring studies that call for the availability of robust, sensitive and reliable speciation methods for arsenic analyses. This is the principle need in terms of on-going research, and many of the outstanding questions will not be answered until speciation is more routinely adopted. An example of the utility of speciation is given in relation to food, before details of some speciation approaches are given.

2.8.1 Elevated total inorganic arsenic exposure in water and food

As has been noted the toxicity of arsenic varies with the chemical form and valence of the element, it is very important to identify major sources of arsenic exposure and factors that may influence its uptake and toxicity. Food samples may need to be tested for specific arsenic species as there can be striking differences between the same product derived from different sources. While the relative inorganic arsenic to total arsenic is uniformly present in seafood it is very low (<1 % in fish), however, in rice it is quite variable depending upon the cultivation environment (18 % in the USA, 75 % in Taiwan).

In areas where water contaminated with high levels of arsenic is used for irrigation of major food crops, such food products will significantly contribute to the total inorganic arsenic exposure and could be of health concern. Moreover, water used for cooking must be taken into account as it contributes significantly to the total inorganic arsenic exposure (see section 2.1).

Some comparisons of arsenic in rice

Total arsenic and total inorganic arsenic concentrations in boiled white rice in total diet study (TDS) samples (USA - see section 2.5.2) are presented in Table 2.8.1. The moisture content of the boiled rice samples was relatively consistent (65.4 - 75.5 %) with a coefficient of variation of 4.5 %. Correction of water content was not made and data are, thus, presented on a wet-weight basis. The total arsenic concentrations of these eight rice samples (mean of 68.5 ngg⁻¹) are quite consistent with those found in the previous TDS samples (average of 72.7 ngg⁻¹ of the preceding 17 basket samples). For inorganic arsenic, three to five independent replicate determinations were made in each of the eight rice samples. Total inorganic arsenic concentration ranged from 0.9 - 26 ngg⁻¹ with an average of 12.4 ngg⁻¹. The variations in the inorganic arsenic contents were twice as high as those of total arsenic concentrations. The percentage of total arsenic as inorganic arsenic in rice was found to vary from 1 to 27 % with a mean of 18.

Table 2.8.1 **Total arsenic and total inorganic arsenic concentrations in boiled white rice**

Market Basket	Moisture (%)	Total As ng/g wet	Inorganic As replicates	Inorganic As ng/g wet	Standard error of the mean	Inorganic As % total
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K971	73.29	63	3	10.1	1.00	16.03
K972	75.6	82	3	0.9	0.55	1.10
K973	65.4	98	3	26	3.7	26.53
K974	73.4	43	5	6.2	2.09	14.42
K981	69.65	64	5	12.6	0.43	19.69
K982	73.48	79	5	12	4.1	15.19
K983	71	55	5	14.9	0.31	27.09
K984	69.7	64	3	16.3	1.33	25.47
Average	71.44	68.5		12.4		18.19
Minimum	65.4	43		0.9		1.10
Maximum	75.6	98		26		27.09
Std. Dev.	3.20	17.18		7.39		8.65
Coef. Var.	4.48	25.08		59.74		47.57

The TDS rice samples were purchased domestically (i.e. within the USA) and because only 2 - 4 % of the rice supply is imported (Economic Research Service, 1999), it can be assumed that the majority of the samples are US rice cultivated in a relatively unpolluted agricultural environment. Few published data on inorganic arsenic in white rice are available, however, Schoof *et al.* (1999) made a comparison of total and inorganic arsenic concentrations in uncooked rice purchased from USA, Taiwan, and Canada. While total arsenic concentrations varied from 120 - 303 ngg⁻¹, the inorganic arsenic levels were relatively consistent, ranging from 74 - 110 ngg⁻¹. Interestingly, there was a striking difference in the proportions of inorganic arsenic to total arsenic in rice from different sources, 24 % in rice from US compared to 69-73 % from Taiwan. Based on only one sample, 42 % of the total arsenic in rice from Canada was inorganic (Yost *et al.*, 1998).

When comparing results of inorganic arsenic concentrations in rice from different sources, several variables need to be taken into account, such as analytical method (sensitivity and specificity) used, moisture content, source of rice (cultivar types, geographical origins, agronomy conditions and agricultural environment, etc.). For example, when the TDS white rice is adjusted for moisture content (9 %, Taiwanese raw rice, Schoof *et al.*, 1998), the inorganic arsenic is 39.5 ngg⁻¹. This value is still considerably lower than all the values reported by Schoof *et al.* (1999). Whether the differences in inorganic arsenic concentration and the fraction of inorganic arsenic in rice from different sources are due to the cultivar types, the agronomy and environmental conditions, or other factors awaits further study. In addition, more studies are needed to see if there is a constant ratio of inorganic to total arsenic in rice cultivated under the same specified conditions.

In rice plants, the highest concentration of arsenic is found in the root followed by the straw and then the grain and the extent of environmental pollution can significantly increase the arsenic concentration in the rice grain. A 20-fold higher concentration of arsenic was reported in rice grown in polluted soils than that grown in non-polluted soils (Chen, 1992). Should this elevated concentration be due to inorganic arsenic, the total contribution to inorganic arsenic exposure could be significant. In addition, the contribution of arsenic from water used in cooking should be taken into account in the exposure/risk assessment especially in areas where arsenic concentrations in drinking water is relatively high. The moisture content of cooked rice is approximately 75 %, roughly a three-to-one ratio of water-to-rice. For a population that uses rice as their staple food, an average consumption of 250 g rice per day cooked in water containing

50 ngml⁻¹ of arsenic would contribute approximately 9 µg of inorganic arsenic from the cooking water alone. Clearly, the contribution would be even higher for the above-average consumers. The average daily consumption of rice varies among countries with the amount of rice consumed in countries like Bangladesh, Indonesia, Korea and Thailand easily reaching as much as 500 to 600 g per person per day (Rivai *et al.*, 1990). This will increase the inorganic arsenic exposure from the cooking water alone by at least 2 fold up to about 21 µgday⁻¹. This level of exposure is in addition to the 100 µgday⁻¹, which would be derived from drinking water (assuming a consumption of 2 litres a day). The Joint Expert Committee on Food Additives have decided a provisional tolerable weekly intake (PTWI) of 0.015 mg kg⁻¹ body weight for inorganic arsenic; this is equivalent to 129 µgday⁻¹ for a 60-kg adult (WHO, 1989). It is therefore likely, that if also considering the exposure from the rice itself people would exceed recommended maximum levels of intake.

2.8.2 Arsenic speciation analysis

As illustrated in the previous section, there is a growing demand for quantitative information on speciation analysis of arsenic. However, there is no generally accepted definition of 'speciation'. In this context it is defined as the description of the amount of defined arsenic-containing molecular species present. The species may be defined functionally, operationally, or as specific chemical compounds or oxidation states. Some techniques determine inorganic species, some techniques determine some organic species and the inorganic species and others again differentiate between organic species.

2.8.2.1 Solvent extraction of biological material

For speciation of arsenic in biological material, extraction into a solvent is required prior to analysis in order to bring arsenic species in solution for subsequent analysis. The content of arsenic species in solution may be dependent upon the extraction media used. Acids, water, chloroform, methanol, benzene and toluene have been used as extraction media (*REFs*). For food analyses, HCl extraction followed by distillation or extraction with a mixture of methanol and water has been widely used (National Research Council, 1999). Some of the techniques described in the literature are as follows:

- Microwave digestion with nitric acid and hydrogen peroxide followed by extraction with water (Mattusch *et al.*, 2000)
- Solubilisation with hydrochloric acid and extraction with chloroform (Muñoz *et al.*, 1999)
- Distillation with hydrochloric acid and potassium iodide (Øygaard *et al.*, 1999)
- Extraction with methanol/water mixture (Le *et al.*, 1994)
- Extraction with chloroform-methanol-water (Larsen *et al.*, 1993).

Speciation analysis regarding biological material is still considered to be developmental. Compared to water matrices, speciation of arsenic in biological material includes at least one additional analytical extraction procedure and, consequently, the results may include additional errors that could be significant.

2.8.2.2 Analyses

In principle, the techniques for speciation analyses in water can also be used for speciation analyses of extracts from biological material. The commonly used hydride-

generation method is employed for the determination of arsenic species in many applications. Volatile arsines are produced from a range of inorganic and methylarsenicals in both oxidation stage 3 and 5. An important feature of the hydride-generation is its pH-dependence. All the As(III) compounds produce arsines at about pH 6. At about pH 1, all the arsenicals are reduced to arsines. By using the difference in reactivity, As(III) and As(V) can be determined in the sample (National Research Council, 1999).

Another feature of the reactivity of As(III) used for speciation is that As(III) forms a complex with sodium diethyldithiocarbamate or ammonium pyrrolidine dithiocarbamate (APDC). The complex may be extracted into an organic solvent (e.g. MIBK) or separated on, for example, C-18 disks (Eaton *et al.*, 1998).

Chromatography is commonly used for the initial separation of the arsenicals, e.g. HPLC or ion chromatography (Larsen *et al.*, 1993; Le *et al.*, 1994; Muñoz *et al.*, 2000). Quantification of the fractions separated can be carried out by means of hydride generation methods, ICP-MS or ICP-AES. A number of hyphenated methods are described in literature, but so far none of these methods has obtained status as routine analytical methods. One reason may be instrument cost, another the demand for very skilled operators.

Preference here is for analytical methods that may be amenable to instrumentation available in laboratories capable of performing total arsenic analyses using HGAAS or GFAAS (minor additional investments may be necessary), with the following two methods selected for presentation:

- Liquid solid extraction and GFAAS detection (LSE-GFAAS): Chelation of As(III) with ammonium pyrrolidine dithiocarbamate (APDC) coupled with liquid-solid extraction.
- Determination of As(III) using HGAAS after extraction with cold trap HGAAS (CT-HGAAS): Pre-concentration by use of cold trap prior to determination of arsenic species using HGAAS.

The American Water Works Association in collaboration with water utility laboratories has carried out method evaluation and validation for both methods. The methods are fully described in Eaton *et al.*, 1998.

Liquid-solid extraction and GFAAS detection

As(III) is complexed with APDC and is partitioned from the water sample on a C-8 disk. Arsenic compounds are then eluted from the disk with acidic methanol, evaporated to near dryness, diluted with 1 % HNO₃ and analysed by GFAAS using normal operating conditions for As analysis. The method provides procedures for the determination of arsenite, arsenate and total inorganic As in finished drinking water, groundwater or drinking water in any treatment stage at concentrations from 0.05 µg l⁻¹ to greater than 10 µg l⁻¹.

As(V) is determined in the water sample after extraction of As(III). The As(V) in the sample is reduced to As(III) by addition of sodium thiosulphate. The sample is filtered through a C-8 disk and the procedure outlined above is repeated. When total inorganic or As(V) is being determined approximately 60 % of all monomethyl arsenate present

is also reduced to As(III) and measured. Other organic arsenicals do not interfere. Because monomethyl arsenate does not normally occur at significant concentrations (compared to inorganic arsenic) in drinking water this interference is, in many cases, not considered important.

The lower limit of detection for this method is limited by the sample size that can be processed and by blank values. With a 1-litre sample concentrated to 5 ml, it is reported that the method can be used for accurate determination of arsenic concentrations as low as $0.05 \mu\text{g l}^{-1}$.

Cold trap and hydride generation AAS

A cold trap unit is used for pre-concentration for arsenic determination by hydride generation and is based on the reduction of the arsenic species by sodium borohydride to their corresponding arsines: As(III) and As(V) to arsine, MMA to methylarsine, and DMA to dimethylarsine. The reduction reaction is pH dependent and related to the pKa of the arsenic acids.

To fully speciate the four targeted arsenic compounds, the reduction needs to be carried out at two different pHs: At acidic pH, both As(III) and As(V), as well as methylated organic arsenic, are reduced by sodium borohydride and the gaseous arsines are purged from the solution by a carrier gas (helium). They are collected at a liquid nitrogen cold trap filled with chromatography packing material. When the reaction is complete, the liquid nitrogen is removed and the cold trap is slowly warmed up to allow sequential vaporisation of the various arsine according to their boiling points. The carrier gas then sweeps the arsines into a heated quartz cell, where detection is made by AAS. The inorganic arsine measured in this technique consists of As(III) and As(V). At near neutral pH, only As(III) is reduced to arsine to any significant degree by sodium borohydride. As(V) is determined by subtracting the inorganic arsenic at near-neutral condition from that at acid conditions. This method determines four arsenic species: arsenite As(III), arsenate As(V), monomethyl arsonite, and dimethyl arsenate. It is reported to be applicable to analyses of drinking water in the range of 0.05 to $0.8 \mu\text{g l}^{-1}$.

Biological materials

In the arsenic speciation of rice in the total diet study mentioned above, food samples were digested with concentrated hydrochloric acid, and then extracted with toluene in the presence of potassium iodide. After centrifugation, water was added to the toluene aliquot in a 1 to 1 ratio, mixed, and centrifuged again to transfer the extractable arsenic components into the water phase for liquid chromatography. The inorganic arsenic was retained on a Waters IC-Pak HR anion exchange column, 4.6 mm x 75 mm, maintained at 350°C using 10 % 10 mM NaOH and 90 % water as an eluting mobile phase at a flow rate of 1 ml min^{-1} . After eight minutes, inorganic arsenic was eluted with a mobile phase containing 10 % 10 mM sodium carbonate, 10 % 10 mM NaOH and 80 % water. Arsenic was then determined in the separated arsenate anionic species by ICP-MS (Yasui *et al.*, 1978). A total of six test samples were fortified with assayed inorganic arsenic compounds from which a recovery of 87 % was obtained.

Using HGAAS for speciation studies in foods requires at least acid treatment and extraction (Le *et al.*, 1994; Muñoz *et al.*, 1999; Øygaard *et al.*, 1999). Whereas using HPLC-ICP-MS or IC-ICP-MS for speciation studies these can be performed after an

extraction procedure (Larsen *et al.*, 1993; Le *et al.*, 1994) or after digestion followed by extraction (Mattusch *et al.*, 2000).

2.8.3 Conclusions

The hydride generation technique can be used to quantify a limited number of water-soluble arsenic species. However, recent developments permit the analysis of a much wider range of species by use of HPLC-ICP-MS. Inter-laboratory studies have been carried out for two relatively simple speciation techniques on drinking water. The results, however, were not very encouraging as the data were not considered to be very reliable, and at present it would seem that arsenic speciation studies should only be carried out at very experienced personnel. In terms of the further development of speciation techniques more reference materials are needed.

Although the more routine application of speciation techniques is likely to be increasingly important, the development of protocols stating the quality measures to be used for the validation of field equipment and test kits as well as quality control of field data is also an important on-going research need.

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