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THE CHEMICAL SPECIATION AND TRANSFORMATIONS OF ARSENIC IN HUMANS AND IN THE ENVIRONMENT

Thesis submitted in accordance with the
requirements of the University of Glasgow
for the degree of Doctor of Philosophy
by Linda Rosemary Johnson

Department of Forensic Medicine and Science

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TO BARRY

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SUMMARY

The human metabolism and biotransformation of arsenic were studied by the analytical speciation of urinary arsenic excreted by volunteers in a series of laboratory-controlled experiments, the general population and several groups subject to enhanced environmental or occupational exposure to inorganic arsenic. In addition, the post-depositional remobilisation of arsenic in the aquatic environment was investigated by the establishment of the chemical forms of arsenic in the sediment porewaters of Loch Lomond and the Dubh Lochan and in the sediments of nearby Scottish sealochs.

Total arsenic concentrations were measured by hydride generation-atomic absorption spectrometry (HGAAS) following acid digestion of human urine and biological material and dry ashing of sediment samples. Urine samples were rapidly directly screened for the approximate sum of the hydride-forming species arsenate (As(V)), arsenite (As(III)), monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA), which were subsequently separated and accurately determined by ion-exchange chromatography/HGAAS, a procedure uninfluenced by the presence of the common dietary organoarsenicals from seafood.

In two experiments, the ingestion of several hundred micrograms of arsenic as the stable organoarsenical in seafood and as inorganic As(V) in mineral water resulted in the rapid gastrointestinal absorption and major urinary excretion of arsenic in under a week. A two-component model showed that urinary arsenic excretion was faster in the former, as reflected in first and second component half lives of 6.9 - 11hr and 3 days compared with 17 - 24hr and 7.1 - 8.6 days for the latter. However, while the seafood organoarsenical was excreted unchanged in urine, inorganic As(V) was rapidly reduced to As(III) and further biotransformed into the less toxic methylated metabolites MMAA and DMAA. After only 12hrs, DMAA became the predominant species to such an extent that over 7 days As(V) constituted only 9 - 10% of the total eliminated arsenic, As(III) 12 - 15%, MMAA 9 - 18% and DMAA 57 - 69%. In a subsequent experiment involving regular oral intake of As(V), an equilibrium was established in which 40 - 60% of the daily dose of 60 - 70ug As(V) was excreted in urine. As(V), As(III), MMAA and DMAA were eliminated in a steady speciation pattern with mean proportions of 5.8/15.2/14.3/64.7% respectively. Some possible longer-term tissue retention of As(III) was indicated.

For the general population, the concentration of the sum of the hydride-forming species As(V),

As(III), MMAA and DMAA rarely exceeds 10ug/g creatinine, of which DMAA is the dominant species (> 80%). With calculated intakes derived from urinary arsenic data comparable to M.A.F.F. dietary estimates and well below recommended limits, there is a negligible risk to the health of the general population from typical exposure to inorganic arsenic. In the mineralised south west of England, where it has been suggested that the highly enriched soil arsenic concentrations may at least be a cofactor in the increased incidence of skin cancer, urinary arsenic levels were only slightly elevated. The potential for increased uptake by the local population was, however, reflected in the more frequent occurrence of As(III) and MMAA in urine and, of especial significance, in two comparatively highly elevated sum of species concentrations of 48.7 and 20.8ugAs/g creatinine recorded for 2 pre-school children. Elsewhere, for workers occupationally exposed to inorganic arsenic, mean urinary arsenic excretion varied from < 10ug/g creatinine for those in the electronics industry to 67.4ug/g creatinine for timber treatment workers applying arsenical wood preservatives, to 79.4ug/g creatinine for a group of glassworkers using arsenic trioxide and 244.8ug/g creatinine for chemical workers involved in the manufacture of arsenical compounds. The maximum urinary arsenic (As(V)+As(III)+MMAA+DMAA)

concentration recorded was 956.4ug/g creatinine. For the most exposed groups, the average species proportions ranged from 1 - 6% As(V), 11 - 17% As(III), 15 - 18% MMAA, 60 - 70% DMAA. The highly elevated urinary arsenic concentrations for the chemical workers and glassworkers correspond to possible atmospheric arsenic concentrations in the workplace and intakes in excess of, or close to, recommended and statutory limits and those associated with increased incidence of lung cancer and other inorganic arsenic-related diseases reported elsewhere. Overall, the importance of distinguishing between occupational and typical dietary exposure to the different forms of arsenic by appropriate application of analytical speciation techniques is endorsed by the results of this study.

Total arsenic concentrations (maximum 283mg/kg) were substantially enhanced, in association with peaks in the iron and manganese profiles, in the near-surface sediments of a southern Loch Lomond core. Porewater arsenic concentrations peaked (76.1 - 103.4ug/l) in the reduction zone of the sediment column, consistent with the solubilisation of iron compounds at depth and release of arsenic, followed by upward migration, oxidation, and re-adsorption in the near-surface aerobic layers. Porewater arsenic speciation showed that As(III) was the dominant

species throughout, even in the surface oxic layers, where preferential adsorption of oxidised As(V) species onto oxides and hydroxides of iron in the sediments depletes the overall porewater arsenic concentrations and prevents release, under current conditions, to the overlying water column. Although trends in the sedimentary arsenic, iron and manganese profiles were less well defined for the much smaller Dubh Lochan, speciation of the porewaters from an oxygenated site showed As(III) to be the dominant species at depth, consistent with the diagenetic remobilisation hypothesis. During anoxic conditions at a deeper site, As(III) predominated throughout the porewaters until reoxygenation of the bottom waters and the appearance of As(V) in the near-surface sections. The similarity in the trends of near-surface sedimentary enrichment profiles for arsenic, iron and manganese in the sealoch, Loch Goil, with those of Loch Lomond, in which diagenetic remobilisation of arsenic was unambiguously confirmed by porewater speciation data, suggests that similar redistributional mechanisms for arsenic prevail in the adjacent coastal marine environment.

ABBREVIATIONS

As(V)	Inorganic pentavalent arsenic
As(III)	Inorganic trivalent arsenic
MMAA	Monomethylarsonic acid
DMAA	Dimethylarsinic acid
AAS	Atomic Absorption Spectrometry
HGAAS	Hydride Generation Atomic Absorption Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
nm	nanometres
ng	nanogram
ug	microgram
mg	milligram
kg	kilogram
ul	microlitres
ml	millilitres
l	litres
w/v	weight to volume
v/v	volume to volume
b.w.	body weight

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PREFACE

Arsenic occurs as a trace constituent in the atmosphere, hydrosphere, lithosphere and biosphere. Its distribution, behaviour and transport within and between each of these systems are influenced significantly by transformations between its different chemical forms.

The research described here can be divided into three main subject categories, all dependent on the development and application of appropriate analytical speciation techniques for arsenic:

1) examination of the human metabolism and excretion of various chemical forms of arsenic.
2) determination of the extent to which adults and children, under diverse environmental and working conditions, are exposed to this potentially toxic element.
3) investigation of processes controlling the mobility of arsenic in the sediments of aquatic environments.

Chapter 1 introduces these three subject areas and outlines the objectives of the work while the analytical methods employed are described in Chapter 2. Chapter 3 reports on the results of a number of laboratory-controlled experiments on the human

metabolism of different forms of arsenic. Chapter 4 considers the extent of arsenic intake and excretion by several populations exposed to different levels of inorganic arsenic. Finally, Chapter 5 is concerned with the cycling of arsenic in the freshwater bodies of Loch Lomond and neighbouring Dubh Lochan, and in the marine environment along the west coast of Scotland.

CHAPTER 1

INTRODUCTION

1.1 HISTORY

The poisonous nature of arsenic has been known for centuries. As long ago as 2000 B.C. arsenic trioxide obtained from copper smelting was used both as a drug and as a poison. It was known to the ancient Greeks and Romans and the word arsenic appears to have been derived from the Greek word 'arsenikon', meaning potent (Winship, 1984). Its use by Nero and the Borgias for homicidal purposes is well known, it being a popular poison because of its tasteless and odourless nature, ease of administration and the fact that the symptoms are readily confused with other illnesses (Emsley, 1985). The release of arsenical vapours from wallpaper dyed with Scheeles Green, containing copper arsenite, is thought by some (Jones, 1982) to have contributed to the ill health of Napoleon and perhaps even to have led to his death in 1821, although others have attributed the elevated levels of arsenic found in samples of his hair to either intentional or unknowing oral intake of arsenic (Smith et al., 1962; Forshufvud et al., 1964). Scheeles Green was also used for colouring ornamental confectionery in the 19th century. After a banquet in London in 1848, several of the guests took such

decorations home for their families, many of whom died as a result (Emsley, 1985).

Despite its toxicity, arsenic has been used in medicine since the time of Hippocrates (circa 400 B.C.), who recommended a paste of arsenic sulphide (realgar) for the treatment of ulcers. In 1786, Thomas Fowler introduced his solution Liquor of Arsenicals, which contained 1% potassium arsenite and was used during the 18th and 19th centuries for "the cure of agues, remitting fevers and periodic headaches" (Winship, 1984). Since the synthesis of salvarsan by Ehrlich in 1905, many organic arsenicals have been synthesised for use in medicine for the treatment of spirochaetal infections, blood dyscrasias and dermatitis (Merck Index, 9th edition 1982). Since the advent of antibiotics, most of these medicinal products have been rendered obsolete.

Today, arsenic and arsenicals have many diversified industrial, agricultural and medicinal uses, such as the hardening of copper and lead alloys, pigmentation in paints, the active ingredient of wood preservatives, pesticides, herbicides and dessicants, a doping agent in the semiconductor industry, as a decolourising agent in glass manufacture, as arsanilic acid in feed additives to improve the nutritional status of poultry, cattle and pigs and limited use in drugs (Table 1.1).

TABLE 1.1

SOME COMMON ARSENIC COMPOUNDS USED IN INDUSTRY, AGRICULTURE AND MEDICINE *

ARSENIC COMPOUND	SYNONYMS	USES
Arsenic		Alloying additive Electronic devices Veterinary medicines
Arsenic pentoxide	Arsenic oxide Boliden salt	Chemical intermediate Defoliant Wood preservative
Arsenic trioxide	Arsenic Arsenolite White arsenic Arsenious acid	Insecticides Rodenticides Fungicides Glass Chemicals Anti-fouling paints Taxidermy Timber preservation
Arsenic trichloride	Butter of arsenic	Pharmaceuticals and chemicals
Arsanilic acid		Poultry and pig feed additives
Arsphenamine	Salvarsan	Drugs
Arsine		Doping agent in semi-conductors
Copper aceto-arsenite	Paris green	Larvacides
Dimethylarsinic acid (and Na salts)	Cacodylic acid Agent blue	Herbicide Defoliant
Magnesium arsenate	Atoxyl	Trypanicide
Monomethylarsonic acid (and salts)		Grass weed control
Potassium arsenate	Maquer's salt	Taxidermy
Potassium arsenite	Fowlers solution	Veterinary medicine
3-nitro-4-hydroxy phenylarsonic acid		Poultry and pig feed additive

* from Lederer and Fensterheim (1983)

1.2 DISTRIBUTION, CHEMICAL FORMS AND TRANSFORMATIONS OF ARSENIC IN THE ENVIRONMENT

Arsenic is a member of group V in the periodic table with an atomic number of 33 and an atomic weight of 75. It is a shiny grey, brittle element possessing both metallic and non-metallic properties e.g. ductibility, low melting point and high volatility (Fleischer, 1973).

The crustal abundance of arsenic is 1.5 - 2.0mg/kg (Onishi and Sandel, 1955). It is therefore considered to be relatively rare, except where naturally concentrated in sulphide ores in which it occurs as the most common arsenic mineral, arsenopyrite (FeAsS), often associated with ores of copper, tin and iron. As a result, typical concentrations in the atmosphere, lithosphere, hydrosphere and biosphere are relatively low (Table 1.2). For example, atmospheric concentrations in unpolluted areas are generally very low, ranging from a few ng/m^3 in rural areas to an average of $20ng/m^3$ in urban areas (Walsh et al., 1977; Bennett, 1981). However, increased levels of arsenic may be found in the vicinity of point emission sources, e.g. where coal with a high arsenic content is used in power plants, or in the vicinty of smelters, where airborne levels can reach levels of $200ng/m^3$ (Woolson, 1983).

TABLE 1.2

ARSENIC IN THE ENVIRONMENT
SUMMARY OF REPRESENTATIVE VALUES *

COMPARTMENT	CONCENTRATION	MEAN VALUE
ATMOSPHERE		
rural	0.2 - 10ng/m^3	6ng/m^3
urban	10 - 200ng/m^3	20ng/m^3
LITHOSPHERE		
agricultural soil	0.1 - 40mg/kg	7mg/kg
HYDROSPHERE		
freshwater	<10ug/l	
marine	1 - 5ug/l	
BIOSHPERE	Fresh weight	
land plants	<0.4mg/kg	0.1mg/kg
aquatic plants	<6mg/kg	
fish	1 - 100mg/kg	
food	<0.25mg/kg	
Man (body content)	1mg	

* partly from Bennett (1981)

Arsenic concentrations in uncontaminated soils can range from 0.1 - 40mg/kg with an average of 7mg/kg, although soils overlying sulphide ore deposits are naturally enriched with arsenic and can contain several hundred mgAs/kg (Colbourn et al., 1975) (Section 4.3.4). Where the activities of man have released arsenic (e.g. in the mining and smelting of copper ores, or in the treatment of soils with arsenical pesticides), concentrations can rise significantly above the natural background levels, e.g. after pesticide use, soils have been found to contain up to 550mgAs/kg (Walsh and Keeney, 1975; Wauchope, 1983).

The sorption of arsenate ions in the soil by iron and aluminium components greatly restricts the availability of arsenic to land plants (Walsh et al., 1977; Wauchope, 1983). This explains the low average concentration of $<$ 0.4mg/kg (Table 1.2) although some grasses growing on arsenic-contaminated soils have been found to contain elevated levels of arsenic, e.g. 670mg/kg in the leaves (Porter and Peterson, 1975).

In surface waters of the United Kingdom and United States, arsenic is usually found at concentrations of $<$ 10ug/l in freshwater and between 1 - 5ug/l, with an average of 2ug/l, in sea water (Woolson, 1975). In areas of thermal activity, naturally high levels of arsenic can be found in hot springs, e.g. up to 276,000ug/l in the Waiotapu valley

in New Zealand (N.A.S., 1977) and, in wells from areas of rocks with high arsenic content, e.g. in Oregon in the U.S.A., levels of up to 2,150ug/l (Whanger et al., 1977) and of 51 - 123ug/l in Nevada (Valentine et al., 1979). Local water supplies can also become contaminated with the washing of pesticides from agricultural land (Woolson, 1977, Minderhoud et al., 1985).

Freshwater aquatic plants accumulate arsenic to a much lesser extent than do marine plants, e.g. freshwater algae 2 - 12mg/kg (Woolson, 1975), freshwater watercress and duckweed 0.5 - 2.1 and 1 - 3mg/kg (dry weights) respectively (Woolson, 1975; Wauchope, 1983), compared with marine algae 0.1 - 95mg/kg (Woolson, 1975) and marine seaweed 60 - 142mg/kg (Lunde, 1973), 91.7 - 141.4mg/kg (Klump and Peterson, 1979) and marine brown algae 40 - 90mg/kg (Whyte and Englar, 1983). It is only when freshwaters become contaminated or contain naturally elevated levels of arsenic, that freshwater plants accumulate significant amounts of arsenic e.g. 20 - 971mg/kg in submerged weed of the Waikato River New Zealand, with its waters from a geothermal origin (Woolson, 1975). Marine organisms generally accumulate much higher levels than freshwater organisms, e.g. 10 - 100mg/kg in marine algae from the Norwegan coast (Lunde, 1977) and 8.0 - 38.2mg/kg (wet weight) in Crab from the Firth of Forth, Scotland

(Falconer et al., 1983) compared with 0.02 - 2.04mg/kg in freshwater fish (Foley et al., 1978).

Arsenic occurs naturally in the valence states -3, 0, +3 and +5 and has the ability to form bonds with carbon, hydrogen and oxygen. As a result, it can exist in a variety of different inorganic and organic chemical forms. The environmental chemistry of arsenic is essentially that of inorganic arsenic and its simpler methylated forms. Table 1.3 shows some of the common inorganic and organic arsenic compounds in the environment and Table 1.4 the chemical structures of the undissociated forms of the main inorganic and methylated acids of arsenic and their respective acid dissociation constants (pKa). The exact nature of the species present is influenced by the redox potential, especially for As(V)/As(III) oxidation/reduction reactions, and the pH. The latter governs the dissociation of the acids, e.g. at an environmental pH of 4 - 8, arsenous acid (pKa 9.23) is relatively undissociated when compared with arsenic acid, with pKa values of 2.25, 6.7 and 11.3 (Lemmo et al., 1983).

The occurrence of biological methylation reactions can also affect arsenic speciation via production of the organic forms. In an investigation of several poisoning incidents, Challenger and Higginbottom (1935) demonstrated that bread mould _Scropulariopsis brevicaulis_ supplied the methyl groups

TABLE 1.3

SOME COMMON INORGANIC AND ORGANIC ARSENIC COMPOUNDS AND SPECIES OCCURRING IN THE ENVIRONMENT *

NAME	SYNONYMS	FORMULA
INORGANIC ARSENIC		
TRIVALENT		
arsenic (III) oxide	arsenic trioxide arsenous oxide white arsenic	As_2O_3
arsenous acid		H_3AsO_3
arsenenous acid	arsenious acid	$HAsO_2$
arsenites (salts of arsenous acid		$H_2AsO_3^-$, $HAsO_3^{2-}$ AsO_3^{3-}
PENTAVALENT		
arsenic (V) oxide	arsenic pentoxide	As_2O_5
arsenic acid	orthoarsenic acid	H_3AsO_4
arsenenic acid	metaarsenic acid	$HAsO_3$
arsenates (salts of arsenic acid)		$H_2AsO_4^-$, $HAsO_4^{2-}$ AsO_4^{3-}
ORGANIC ARSENIC		
monomethylarsonic acid	methanearsonic acid	$CH_3AsO(OH)_2$
dimethylarsinic acid	cacodylic acid	$(CH_3)_2AsO(OH)$
methylarsine		CH_3AsH_2
dimethylarsine		$(CH_3)_2AsH$
trimethylarsine		$(CH_3)_3As$
arsenobetaine		$(CH_3)_3As^+CH_2COOH$
arsenocholine		$(CH_3)_3As^+CH_2CH_2OH$

* from Table 1 in WHO (1981).

TABLE 1.4

CHEMICAL STRUCTURES AND NOMINAL OXIDATION STATES OF SEVERAL ARSENIC SPECIES

STRUCTURE	SPECIES	OXIDATION STATE	pKa
$O{=}As(OH)_3$ (OH above and below As; O=As-OH)	Arsenic acid	As(V)	2.25, 6.7, 11.3
$As(OH)_3$ (OH above and below As; As-OH)	Arsenous acid	As(III)	9.23
$O{=}As(OH)_2CH_3$ (OH above, CH_3 below As; O=As-OH)	Monomethylarsonic acid	As(III)	4.26, 8.25
$O{=}As(OH)(CH_3)_2$ (CH_3 above and below As; O=As-OH)	Dimethylarsinic acid	As(I)	6.25

for the methylation of the inorganic arsenic salts used as the arsenical pigment Scheele's Green (copper arsenite) and Paris Green (copper aceto-arsenite) of wallpaper, ultimately leading to the synthesis and release of volatile trimethylarsine with a characteristic garlic-like odour. Since then laboratory studies have shown that methanobacterium can reduce and methylate arsenate under aerobic/anaerobic and biotic/abiotic conditions to dimethylarsine (McBride and Wolfe, 1971; Cox and Alexander, 1973). A sequence of reduction/ methylation was later proposed by McBride et al. (1978) and is shown in Figure 1.1. Arsenate (As(V)) is first reduced to arsenite (As(III)) which is then methylated to form MMAA. This, in turn, is reduced and methylated to form DMAA (As(I)), which on further reduction, yields dimethylarsine under anaerobic conditions and trimethylarsine under aerobic conditions.

The biomethylation of arsenic in soils was demonstrated by Braman (1975), with the detection of trimethylarsine in air trapped under glass jars that had been placed over soils treated with sodium arsenite. In water, the presence of both inorganic and organic forms of arsenic has been detected, but typically at much lower levels for MMAA and DMAA than for either As(V) or As(III), e.g. 1.29/0.62/ 0.08/0.29ug/l for As(V), As(III), MMAA and DMAA in

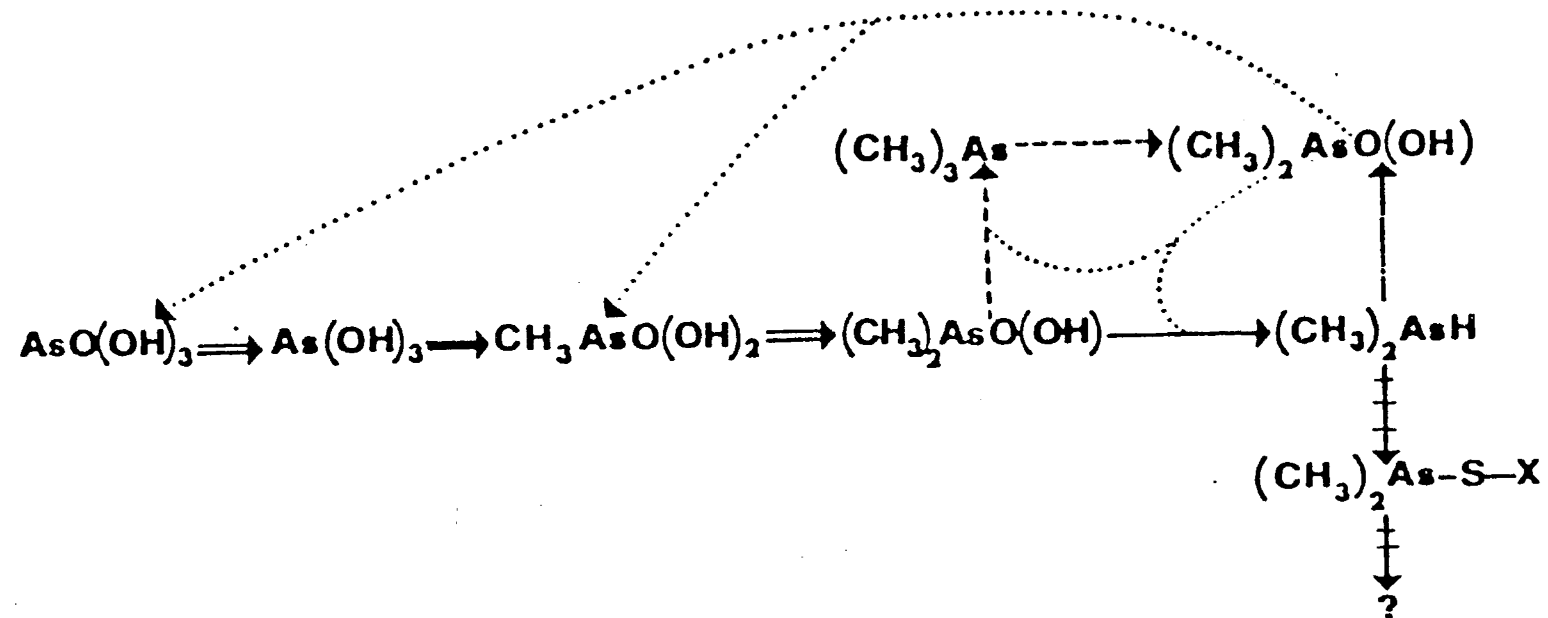


Figure 1.1 Biological arsenic cycle. (═══) aerobic or anaerobic; (· · · ·) aerobic biotic or abiotic; (———) anaerobic; (– – –) aerobic; (–┼–┼–) these reactions are probably abiotic. (From McBride et al., 1978).

Tampa Bay, Florida, (Braman and Foreback, 1973) and 1.95/0.114/0.063/0.051ug/l in the Colorado River, Colorado, (Andreae, 1977). In oxygenated surface waters, arsenic is usually found as the arsenate (As(V)). Under reducing conditions, e.g. as found in deep well waters, it can exist in the lower oxidation state, arsenite (As(III)). Where the methylated forms of arsenic, MMAA and DMAA, have been detected in marine and freshwaters, they are often correlated with the phytoplankton activity (Andreae, 1979; Andreae and Klump, 1979; Section 1.4).

In marine organisms inorganic arsenic is transformed to both lipid-soluble and water-soluble organic arsenic compounds (Irgolic et al., 1977; Lunde, 1977; Whyte and Englar, 1983) and algae in waters with low phosphate content can metabolise arsenate (Edmunds and Francesconi, 1981a). One end product of arsenate metabolism is a membrane phospholipid, O-phosphatidyl- trimethylarsonium lactate, as found in marine diatoms (Cooney et al., 1978; Cooney and Benson, 1980) and in molluscs (Benson and Summon, 1981). In the Australian lobster, dusky shark, whiting, plaice, sole, lemon sole, flounder, dab, crab and shrimps, the principal organoarsenical was identified as arsenobetaine (Edmonds et al., 1977; Cannon et al., 1981; Edmonds and Francesconi, 1981b, 1981c; Norin and Christakopoulos, 1982; Luten et al.,1982, 1983) (Table 1.3). It is believed that the

biota absorb arsenate from the surrounding medium (Section 1.4) and transform it through a sequence of intermediate forms, including As(III), MMAA and DMAA, and trophic levels in the food chain, ultimately to the stable organoarsenical, arsenobetaine (Cannon et al., 1979; Andreae, 1983;). Although the end products are dissimilar, the overall reaction (Zingaro and Bottino, 1983) is analogous to the sequence proposed by McBride and Wolfe (1978) for the fungal and bacterial reduction and methylation of inorganic arsenic.

1.3 ARSENIC IN HUMANS

1.3.1 Exposure and epidemiology

Due to the widespread distribution of arsenic in the environment and its ability to exist in a number of different chemical forms (Section 1.2), human exposure to this potentially toxic element is inevitable. For the general population the most common routes of exposure are the inhalation of air and the ingestion of drinking water and food, for which the arsenic concentrations are usually low (Section 1.2, Table 1.2). It is only when the arsenic concentrations become enriched naturally or through the activities of man that there exists the possibility for increased human exposure to this potentially toxic trace element.

Nelson (1977) found that the monthly average level of arsenic in air reached 1.46ug/m^3 (1460ng/m^3) near a copper smelter in Tacoma, Washington, and a maximum 24-hr concentration of 7.9ug/m^3 (7900ng/m^3) was reported by Roberts et al. (1977). Despite finding increased levels of arsenic in the hair and urine of children living near the Tacoma smelter, Milham (1977) was unable to detect any differences in the hearing abilities and school attendence of the children when compared with an unexposed control group. Bencko and Symon (1977) however, reported the hearing loss in children from an area near a power plant in Czechoslovakia burning coal

with an arsenic content of 1500mg/kg. In the vicinity of a plant emitting arsenic in the processing of non-ferrous metals, Pershagen et al. (1977) found a significant excess mortality due to lung cancer when compared with data for a control group.

The arsenic content of most water supplies in the United Kingdom and the United States rarely exceeds the recommended and maximum limits in drinking water of 10ug/l and 50ug/l respectively (M.A.F.F., 1982; Zielhuis and Wibowo, 1984). However, in other countries, there have been a number of reports of isolated instances of chronic arsenicism caused by a natural increase in the levels of arsenic in the local water supplies. In the Antofogasta region of Chile, a high incidence, particularly among young children, of arsenical dermatoses, i.e. hyperkeratosis and hyperpigmentation, was traced to a water supply containing arsenic at a concentration of 800ug/l (Borgono et al., 1977). In a survey of 114 well waters in the Tainan region of the south west coast of Taiwan, Tseng (1977) found arsenic concentrations to range from 10 - 1820ug/l, with most in the range 400 - 600ug/l. Associated with these levels were 370 cases of Blackfoot disease (a peripheral vascular disorder characterised by gangrene of the extremities, especially the feet) and 428 cases of skin cancer in the area.

A number of beverages have been shown to contain appreciable amounts of arsenic. In Manchester in 1900 - 1901, 70 people died from the drinking of beer contaminated with 15mgAs/l (Pershagen, 1983). Elevated arsenic levels have also been found in some bottled mineral waters, e.g. a mean arsenic concentration of 21ug/l in mineral waters sold in countries within the European community (Zoetman and Brinkmann, 1976), and 150ug/l in Vichy water (Buchet and Lauwerys, 1983). The daily consumption of just 636ml (equivalent to three small cupfuls) of Vichy Celéstins of arsenic concentration 220ug/l (Farmer and Johnson, 1985) by a 70kg man would equal the current maximum provisional tolerable daily intake of inorganic arsenic of 2ug/kg body weight set by FAO/WHO (WHO, 1983).

From Table 1.2, it can be seen that most foodstuffs contain an arsenic concentration of $<$ 0.25mg/kg with the notable exception of seafoods, in which the concentration can exceed 10mg/kg (cf Table 3.1). Most of the arsenic in marine organisms is in the form of the naturally occurring organoarsenical, arsenobetaine (Section 1.2). Due to the stable and unreactive nature of this compound, it is considered non-toxic. Farmers are known to use a manufactured arsenical compound, 4-hydroxy-3-nitrophenylarsonic acid (Roxarsone), to improve the nutritional status of pigs and poultry

(Winship, 1984) although several days are given for clearance from the body before slaughter. The few cases of reported arsenic poisonings from foodstuffs have usually been the result of accidental contamination, e.g. in 1956, in Japan, soy sauce became contaminated with calcium arsenate, while, in 1955, in the Morinaga milk incident, dried milk intended for infants was contaminated with pentavalent arsenic. It was estimated that 1.3 - 3.6mg of arsenic had been ingested daily by the infants and 130 deaths were reported (Pershagen, 1983).

For a small minority of the working population, industry represents the major source of arsenic exposure. Exposure is usually via inhalation and ingestion of dusts contaminated with arsenic trioxide (e.g. in glassworks, in smelters and in the manufacture of arsenical chemicals) or pentavalent arsenic (e.g. in the manufacture and use of wood preservatives as well as in the cutting and sawing of such treated woods), but there also exists a potential for absorption through the skin (especially of As(III)) and the additional transfer of arsenic from the hands to the mouth. Exposure is usually at the subacute level, but often continues over considerable periods of time. Exposure to irritant arsenic compounds such as arsenic trioxide in air can acutely damage the mucous membranes of the respiratory system and exposed skin and can lead to perforation of the

nasal septum and dermatitis after a few weeks of intense exposure (Hine et al., 1977). A number of human studies have linked occupational exposure to arsenic with increased mortality from cancer of the respiratory tract and skin (Section 4.4.4) although many of the animal studies conducted provide conflicting evidence about the identification of arsenic exposure as the unique cause of the malignancy (Pershagen, 1981). In the industrial environment, neither the interaction of arsenic with chemicals such as sulphur dioxide, nor with the smoking habits of the employees, have been fully investigated. Beckman et al. (1977) reported increased chromosomal aberrations in lymphocytes from 9 employees exposed to arsenic at a copper smelter in northern Sweden when compared with a control group. Animal studies have shown that arsenic trioxide can cross the placenta of the rat and, in mouse foetuses, malformations including severe central nervous system and skeletal defects have been produced following intraperitoneal injection of sodium arsenate (WHO, 1981; Pershagen, 1983; Winship, 1984).

1.3.2 Tissue distribution and the mechanism of arsenic toxicity

Absorbed arsenic (ingested or inhaled) is transported by the blood to different organs in the body. Table 1.5 shows the typical concentrations

TABLE 1.5

ARSENIC CONCENTRATIONS IN HUMAN ORGANS AND TISSUES

TISSUE OR ORGAN	ARSENIC CONCENTRATION (mg/kg) DRY WEIGHT (median)	WET WEIGHT (mean)	WET WEIGHT (median)
adrenal	0.03		
aorta	0.04		
whole blood	0.04		
brain	0.01		
hair	0.46		
heart	0.02		
kidney	0.03	0.007	0.004
liver	0.03	0.011	0.003
lung	0.08	0.010	0.008
muscle	0.06	0.004	
nail	0.28		
ovary	0.05		
pancreas	0.05	0.005	
prostate	0.04		
skin	0.08		
spleen	0.02	0.003	
stomach	0.02		
teeth	0.05		
thymus	0.02		
thyroid	0.04		
uterus	0.04		

* from Table 8 in WHO (1981)

found in persons unexposed to elevated levels of arsenic. In the general population by far the highest levels of arsenic are found in the skin, hair and nails. These tissues are rich in keratin, containing sulphydryl groups (SH) to which inorganic trivalent arsenic can readily bind. Smith (1964) found the level of arsenic in hair to be $<$ 1mg/kg in more than 80% of 1000 persons examined, the average level being 0.81mg/kg and the median 0.51mg/kg. Liebscher and Smith (1968) reported arsenic levels ranging from 0.02 - 8.17mg/kg dry weight in over 1200 hair samples from residents in the Glasgow area. As arsenic accumulates in keratin rich material, hair analyses have been used to indicate possible exposure, e.g. levels ranging from 0.6 - 10mg/kg in the hair of boys who lived in the vicinity of a power plant which burnt coal with a high arsenic content (Bencko and Symon, 1977). In theory, periods of exposure to inorganic arsenic can be discerned by the sectioning and analysis of a single hair using neutron activation analysis. In persons occupationally exposed to inorganic arsenic, hair arsenic levels can reach several hundred mg/kg (Smith, 1964; Leslie and Smith, 1978). However, as the exposure is often to elevated airborne levels of inorganic arsenic in the working environment, external contamination of the hair is highly likely. In such situations, hair is of little value as an indicator of the extent of arsenic

exposure and uptake.

It is through its affinity for the sulphydryl groups of enzymes and proteins that trivalent inorganic arsenic exerts its toxic reactions. The toxic nature of trivalent inorganic arsenic was first postulated by Ehrlich (Squibb and Fowler, 1983) and Voegtlin (1925) who concluded that the toxic action occurred via interaction of arsenite with the SH groups of glutathione in cells or with other SH groups in proteins and enzymes to give thioarsenites. As a result, trivalent arsenic has the ability to inhibit the action of many critical enzymes e.g. pyruvate oxidase, an enzyme required to oxidise carbohydrates prior to the synthesis of ATP in the Krebs cycle.

Inorganic arsenate is thought to exert its toxicity through its chemical similarity with phosphate. A number of in-vitro studies have demonstrated that arsenate can substitute for phosphate in enzyme-catalysed reactions. In particular, As(V) is thought to have an inhibitory effect on mitochondrial respiration by uncoupling oxidative phosphorylation (Winship 1984). Inorganic arsenic has been shown to cause impaired tissue respiration in vivo in the liver and kidneys of mice and rats (Fowler et al., 1977). Liver mitochondria from rats or mice exposed to arsenate in drinking water showed mitochondrial swelling accompanied by

changes in mitochondrial function (Fowler et al., 1977; Fowler and Woods, 1979). Following the administration of radiolabelled $^{74}As(III)$ and $^{74}As(V)$ (0.4 - 4.0mg/kg b.w.) to mice, Vahter and Norin (1980) reported higher levels of arsenic in most tissues of animals receiving As(III), especially the liver, kidney, bile and skin. The skeleton, however, had significantly higher levels in the mice given arsenate, which may be explained by the similar chemical properties of arsenate and phosphate and the replacement of the latter in bone.

The low affinity of pentavalent arsenic for the sulphydryl groups is thought to explain its less toxic nature when compared with As(III) (Squibb and Fowler, 1983). The greater toxicity of As(III) has been shown in a number of animal studies, e.g. LD_{50} for arsenate in rats and mice is about 100mg/kg body weight (b.w.) while that for arsenite is about 10mg/kg b.w. (Squibb and Fowler, 1983). Compared with the inorganic forms of arsenic, the methylated forms of arsenic, MMAA and DMAA, have a lower affinity for the tissue components in animals (Vahter and Marafante, 1983) and in man (Buchet et al., 1981a) and are less reactive and therefore less toxic. Penrose (1974) reported the relative toxicity of the different chemical forms of arsenic to be arsenite $>$ arsenate $>$ monomethylarsonic acid, dimethylarsinic-acid $>$ arsenobetaine. The unreactive and non-toxic

nature of the chemical form of arsenic in seafoods, arsenobetaine, is borne out by the absence of cases of arsenic poisoning resulting from the ingestion of seafood. Most cases of arsenic poisoning are related to the ingestion of the inorganic species, as in incidents of attempted suicide or in accidental occupational exposure.

1.3.3 Metabolism

Early work on the metabolism of arsenic by humans centred upon experiments involving the ingestion of high-arsenic seafood. On feeding lobster containing approximately 70mg/kg arsenic to a person, Chapman (1926) observed increased excretion of arsenic in the urine within a comparatively short time of the consumption of the food. Using the high arsenic content of seafood, Coulson et al. (1935) fed a shrimp diet to rats. Only about 4% of the ingested arsenic was recovered in the faeces during the first two days following intake, most being excreted, via the kidneys, in the urine. In addition to experimenting with rats, Coulson et al. (1935) also carried out excretion experiments on two human subjects. Only 5% of the ingested arsenic was recovered in the faeces, with the majority excreted in urine. More recently, with growing interest in the specific identification of seafood organoarsenicals, a number of workers have demonstrated that following the

consumption of seafood, over 70% of the dose is excreted, unchanged, in urine (Crecelius, 1977; Freeman et al., 1979; Luten and Rieweel-Boey, 1983).

It is only in recent years that the development of analytical techniques capable of separating the different chemical forms of arsenic (Section 2.5.3) has enabled metabolic studies to be conducted following the ingestion of the different inorganic and organic forms of arsenic (Chapter 3). The results of numerous animal experiments involving the administration of inorganic pentavalent or trivalent arsenic to cows (Lasko and Peoples, 1975), dogs (Tam et al., 1978, 1979a; Charbonneau et al., 1979) the hamster (Charbonneau et al., 1980), mice (Vahter, 1981; 1983) and rabbits (Marafante et al., 1982; Vahter and Marafante, 1983), indicate that the inorganic form of arsenic is methylated in vivo into metabolites MMAA and DMAA and excreted in the urine. The exceptions to this are the rat, in which arsenic accumulates in the erythrocytes (Klaassen, 1974), and the marmoset monkey, which does not appear to methylate inorganic arsenic (Vahter et al., 1982).

The presence of the methylated forms of arsenic in human urine was first reported by Braman and Foreback (1973). DMAA constituted an average 66% of the total urinary arsenic while MMAA, pentavalent and trivalent arsenic each accounted for 8.0, 17.0 and 8.4% respectively. The in vivo methylation of

inorganic arsenic in humans was later indicated by the work of Crecelius (1977) who measured inorganic arsenic, MMAA and DMAA in urine of a subject who had ingested wine containing inorganic arsenic. Methylated derivatives of arsenic have also been found in urine of copper smelter workers occupationally exposed to arsenic trioxide (Smith et al., 1977). The importance of urine for the removal of inorganic arsenic and its metabolites MMAA and DMAA from humans has been established in a number of metabolic studies in which human volunteers were given an oral dose of inorganic arsenic, typically $<$ 1mg. Yamauchi and Yamamura (1979), following the oral intake of arsenic trioxide by a single volunteer, showed that 70% of the arsenic was excreted in the urine within 72 hours, the majority of which was in the methylated forms. In similar studies, Buchet et al. (1980) and Buchet et al. (1981b) demonstrated the use of urinary arsenic excretion and speciation to indicate in vivo biotransformation and metabolism of ingested inorganic trivalent arsenic, with the methylated derivatives, MMAA and DMAA, becoming the predominant species excreted. In the case of humans acutely intoxicated by much higher levels of arsenic trioxide (e.g. 0.5 - 1.5gAs), initially rapidly excreted inorganic arsenic is replaced as the predominant species by MMAA and DMAA some 3 - 5 days after the intake (Mahieu et al., 1981, Lovell and Farmer, 1985).

As the methylated compounds MMAA and DMAA are known to be less toxic than the inorganic species As(V) and As(III) (Section 1.2), the biotransformation of inorganic arsenic compounds to the methylated forms is thought to act as a natural detoxification mechanism (Vahter, 1983).

Comprehensive investigation of human exposure to arsenic must incorporate the study of populations subject to different types and degrees of exposure (e.g. dietary, environmental and occupational). It is known that urinary arsenic is an important indicator of exposure. Traditional studies of exposure, however, have relied on the non-specific determination of total arsenic concentrations in urine. In view of the different relative toxicities of arsenic in seafood and inorganic arsenic and its metabolites MMAA and DMAA, this approach is clearly inadequate with respect to the identification of the source of the arsenic, assessment of the extent of the exposure and evaluation of the metabolic response of the individuals concerned. The attainment of these goals has now become a realistic proposition as a result of the recent developments in methodology for the analytical speciation of arsenic. Necessary pre-requisites to the full interpretation of urinary arsenic speciation data from potentially exposed groups and populations are the establishment of 'normal' levels and distributions in the general

population and of the uptake, biotransformation and elimination patterns reflected in urinary arsenic following carefully controlled experimental exposure. Information on the former is sparse and only a few of the metabolic studies carried out to date have involved humans, most using animals. Additional deficiencies, of which the following are examples, can be identified in some of the previous investigations. Where the excretion of urinary arsenic has been measured following the ingestion of seafood, the possible presence of inorganic arsenic and its metabolites in urine has usually been ignored. Some studies of inorganic arsenic metabolism have been based upon fortuitous cases of arsenic poisoning, but, with the likely use of chelating agents to counteract the toxicity, it is possible that the 'true' excretion and speciation patterns may have been altered. Where laboratory controlled metabolic studies have been carried out, As(III) has often been used as the source rather than As(V), the species which could well be the most common in 'normal' and some types of additional environmental and occupational exposure. Most have also not distinguished between As(V) and As(III) in the urine, which, certainly after the intake of inorganic pentavalent arsenic, would seem to be an important requirement for the thorough study of reduction/methylation pathways. Thus, direct

experimental investigation of the human metabolism of arsenic can be considered a justifiable fundamental objective as well as a necessary prelude to the survey, via analytical speciation of urinary arsenic, of the actual exposure (e.g. dietary, environmental, occupational) of selected populations to arsenic.

1.4 ARSENIC IN THE AQUATIC ENVIRONMENT

1.4.1 Introduction

Depending on the specific geographical location, attendant human activities and environmetal circumstances, arsenic can be released, in solution or as a constituent of particulate matter, into the freshwater/marine environment via natural erosion, geothermal wells and hot springs, as well as from smelters, the application of herbicides and pesticides to agricultural land, mining operations and the burning of fossil fuels. Although inorganic arsenate is regarded as the principal chemical form of arsenic in seawater and freshwater, the chemical species As(III), MMAA and DMAA have also been detected in both types of water bodies (Braman and Foreback, 1973; Andreae, 1977). Arsenate, because of its chemical similarity with the nutrient phosphate (Andreae, 1978), is taken up by the phytoplankton in the surface water, converted to As(III), MMAA and DMAA and released back into the water column (Andreae, 1977; Andreae and Klumpp, 1979; Sanders and Windom, 1980; Sanders, 1983) (Figure 1.2). Therefore, concentration maxima for As(III), MMAA and DMAA closely follow phytoplankton productivity in the surface layers, decreasing considerably with depth as the penetration of light decreases. While freshwater organisms appear to detoxify inorganic arsenic predominantly by simple methylation for excretion

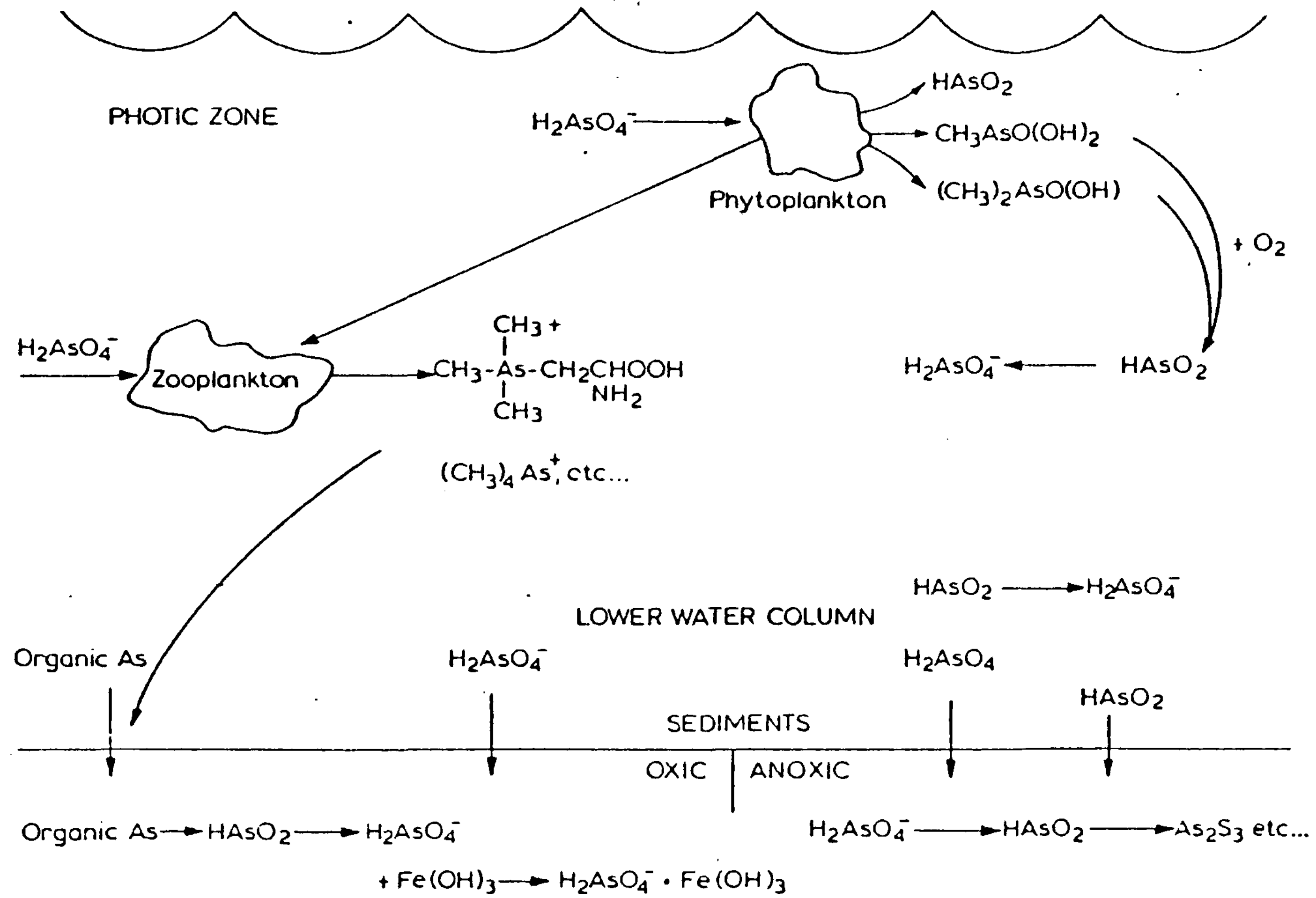


Figure 1.2 Arsenic transformations in aquatic systems. (From Braman, 1983)

(Braman and Foreback, 1973; Lasko and Peoples, 1975; Sanders, 1979), marine organisms produce more complex organoarsenicals (Lunde, 1977; Andreae and Klumpp, 1979; Maher, 1983a) with the potential for passage along the marine food chain (Sanders, 1980).

Arsenic is transported to the sediments via the settling of organic detritus and of inorganic particulate matter with which it is associated. The adsorption of negatively charged arsenate from the water column onto positively charged hydrous iron surfaces is considered to be particularly significant in the removal of arsenic to the sediment column (Pierce and Moore, 1982). Although Ferguson and Gavis (1972) postulated that subsequent biomethylation right through to the generation and liberation of the alkylarsines might occur under certain conditions in bottom sediments (Figures 1.2 and 1.3), this has not been substantiated by the work of Andreae (1977, 1979, 1983) on marine systems.

Enhanced levels of arsenic in freshwater lacustrine surface sediments have usually been attributed to contamination from nearby industrial (e.g. smelting and mining) or agricultural (e.g. pesticide application) activities (Nriagu, 1983; Kobayashi and Lee, 1978). For example, a concentration of 217mg/kg in the surface sediments of Lake Washington has been attributed to the Tacoma copper smelter, Washington (Crecelius, 1975) and a

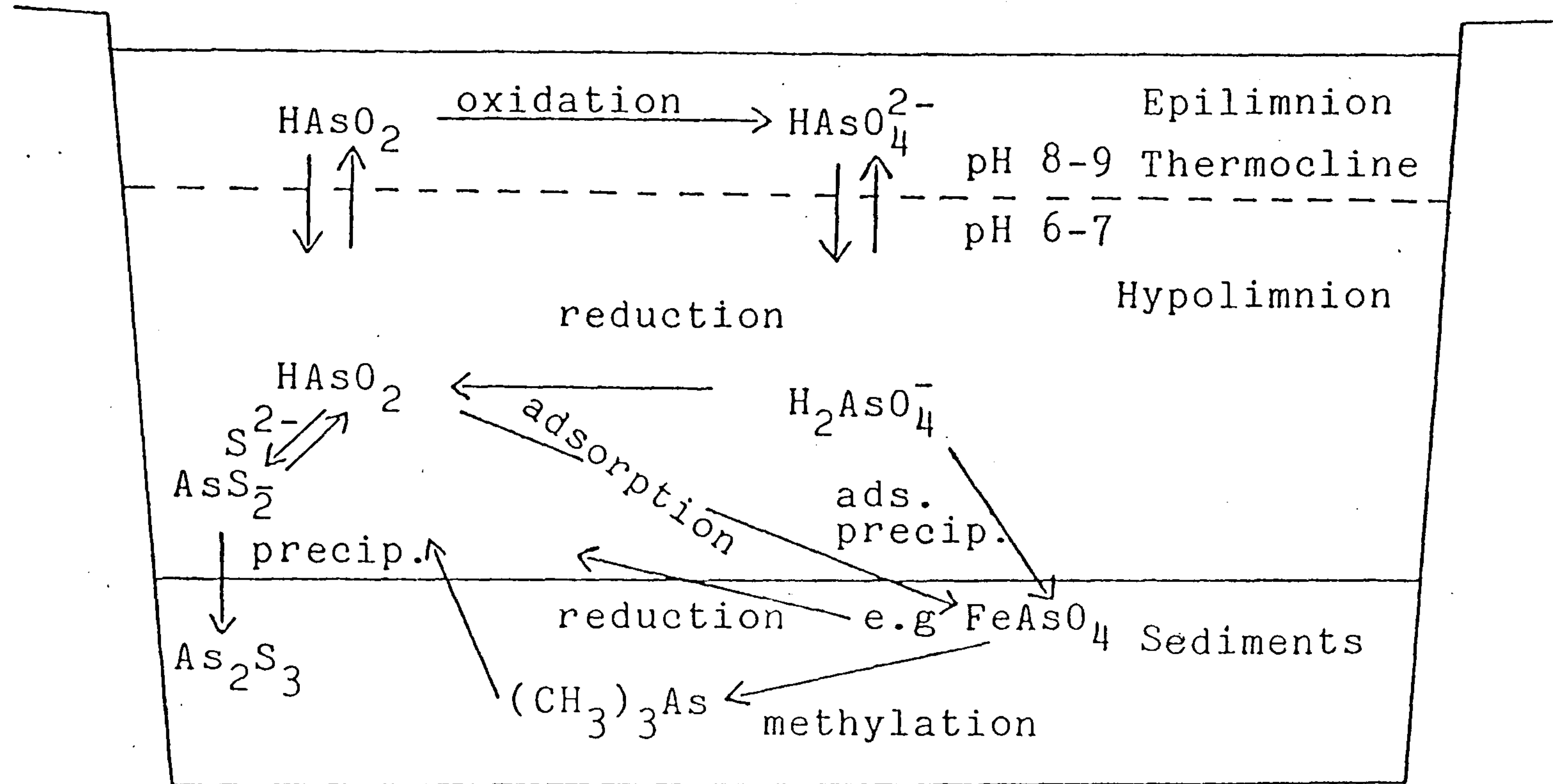


Figure 1.3 Local cycle of arsenic in a stratified lake (Ferguson and Gavis, 1972)

corresponding 3,500mgAs/kg in the lakes in the N.W. territories of Canada to mine tailings (Wageman et al., 1978). However, recent studies conducted on the freshwater system of Loch Lomond, in western Scotland, where there are no local sources of industrial or agricultural contamination, have shown that surface or near surface enhancements in sedimentary arsenic concentrations may well be the result of natural post-depositional enrichment processes (Farmer and Lovell, 1986).

1.4.2 Loch Lomond

Farmer and Cross (1979) reported an elevated level of 474mg/kg arsenic in the 0 - 1cm surface section of a sediment core in the southern basin of Loch Lomond, twenty-five times the mean background level of 18 $\pm$ 5mg/kg. In a more comprehensive investigation, Lovell (1985) found levels of arsenic in the surface or near-surface sediments in 12 of 13 sediment cores taken from the three major basins of Loch Lomond to be substantially enhanced, with levels up to 675mg/kg compared with 'background' values of 15 - 50mg/kg at 20 - 30cm depth. High surface concentrations of arsenic were associated with increased concentrations of iron and manganese (Figure 1.4). The association of arsenic with iron had previously been observed in both iron-enriched sediments and in iron-rich ferromanganese nodules

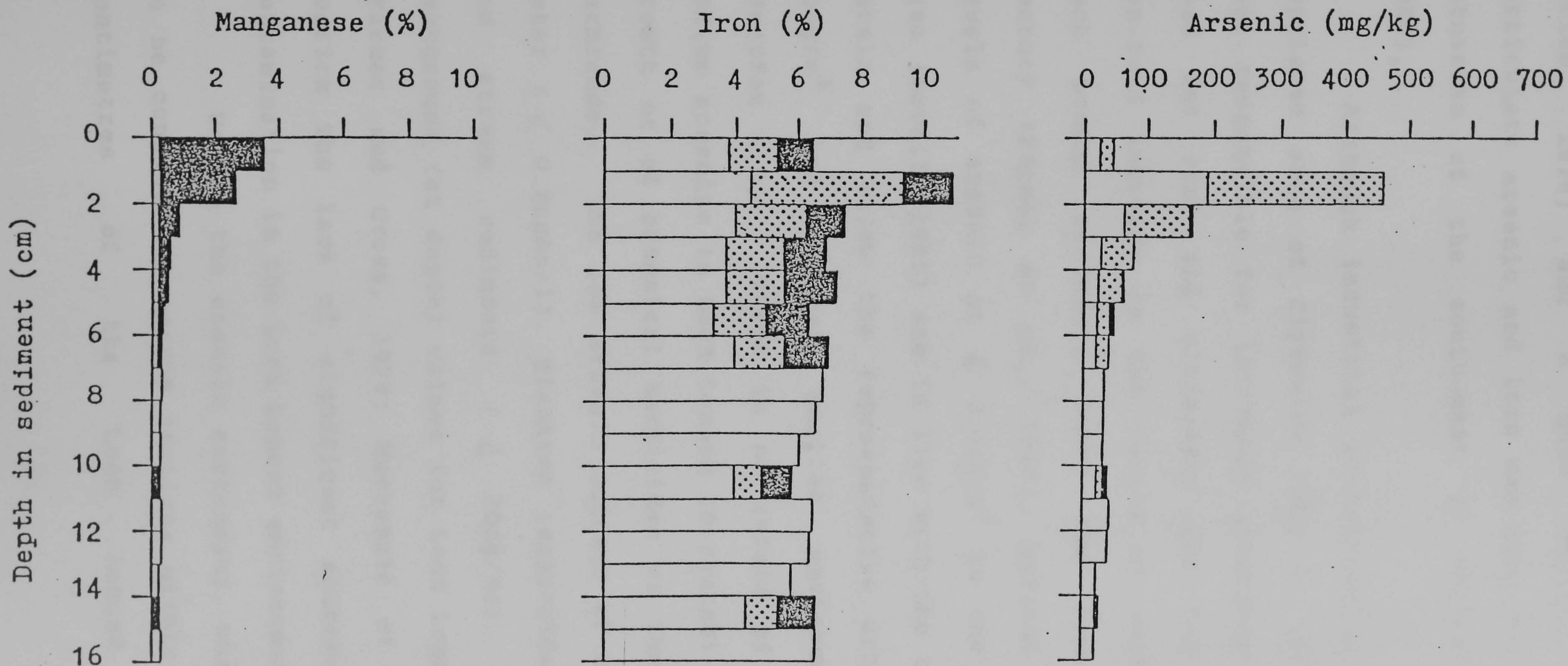


Figure 1.4 Profiles of total manganese, iron and arsenic in a sediment core from the south basin of Loch Lomond (Lovell, 1985)

(Price, 1976) and a good correlation between particulate arsenic and iron was also reported in the estuaries of the south-west of England (Langston, 1980).

Although industrial activities in the heavily populated area of Clydeside 30km to the south have been responsible for increased atmospheric levels of lead and zinc and ultimately for the approximate ten-fold increase in the levels of those metals in Loch Lomond sediments since the late eighteenth century (Farmer et al., 1980), current atmospheric levels of arsenic at $\leqslant$ 0.1ng/m^3 in the Loch Lomond area (Lovell, 1985) are in line with the typical rural levels and below the representative urban level of 20ng/m^3 and typical smelter environment values (Section 1.2). There is no record of the use of sodium arsenite in Loch Lomond to control rooted plant growth or of arsenical herbicides on the surrounding farmlands. The low arsenic content of filtered loch water ($<$ 0.2ugAs/l), plankton (approximately 3mg/kg) and stream sediments ($\leqslant$ 20mg/kg), similar to background (at depth) values for Loch Lomond sediments (Farmer and Cross, 1979; Mackenzie et al., 1983), confirm the lack of significant sources of arsenic contamination in the Loch Lomond environment today.

Unlike the arsenic enrichment, which was found to be confined to narrow sections within the top few centimetres of the Loch Lomond sediments,

concentration gradients in the sedimentary profiles of lead and zinc enhancement profiles from a previous core taken from the southern basin of Loch Lomond, were much more gradual and extended over 14 - 30cm of the sediment column (Figure 1.5, Farmer et al., 1983). Arsenic profiles were found to be much more closely related to those of manganese and iron (Figure 1.4), suggesting that the major influences on the vertical distribution of arsenic, manganese and iron are distinct from those controlling lead and zinc.

It is well known that sedimentary profiles of manganese and iron are largely controlled by post-depositional diagenetic processes. After burial of the surface sediment, iron and manganese compounds dissolve under the reducing conditions produced at depth by microbiological decomposition of organic matter in the sediment column. Once mobilised, they migrate upwards as the divalent cations through the interstitial porewaters to be reoxidised (first iron, then manganese) and fixed in the aerobic surface layer of the sediments (Farmer and Cross, 1979; Berner, 1980; Farmer and Lovell, 1984).

A similar diagenetic mechanism for the control of arsenic in Loch Lomond sediments has been postulated (Lovell, 1985). In the oxic surface sediments, arsenic is strongly associated with iron, probably either as arsenate adsorbed on ferric oxides and hydroxides, or as a precipitated iron arsenate

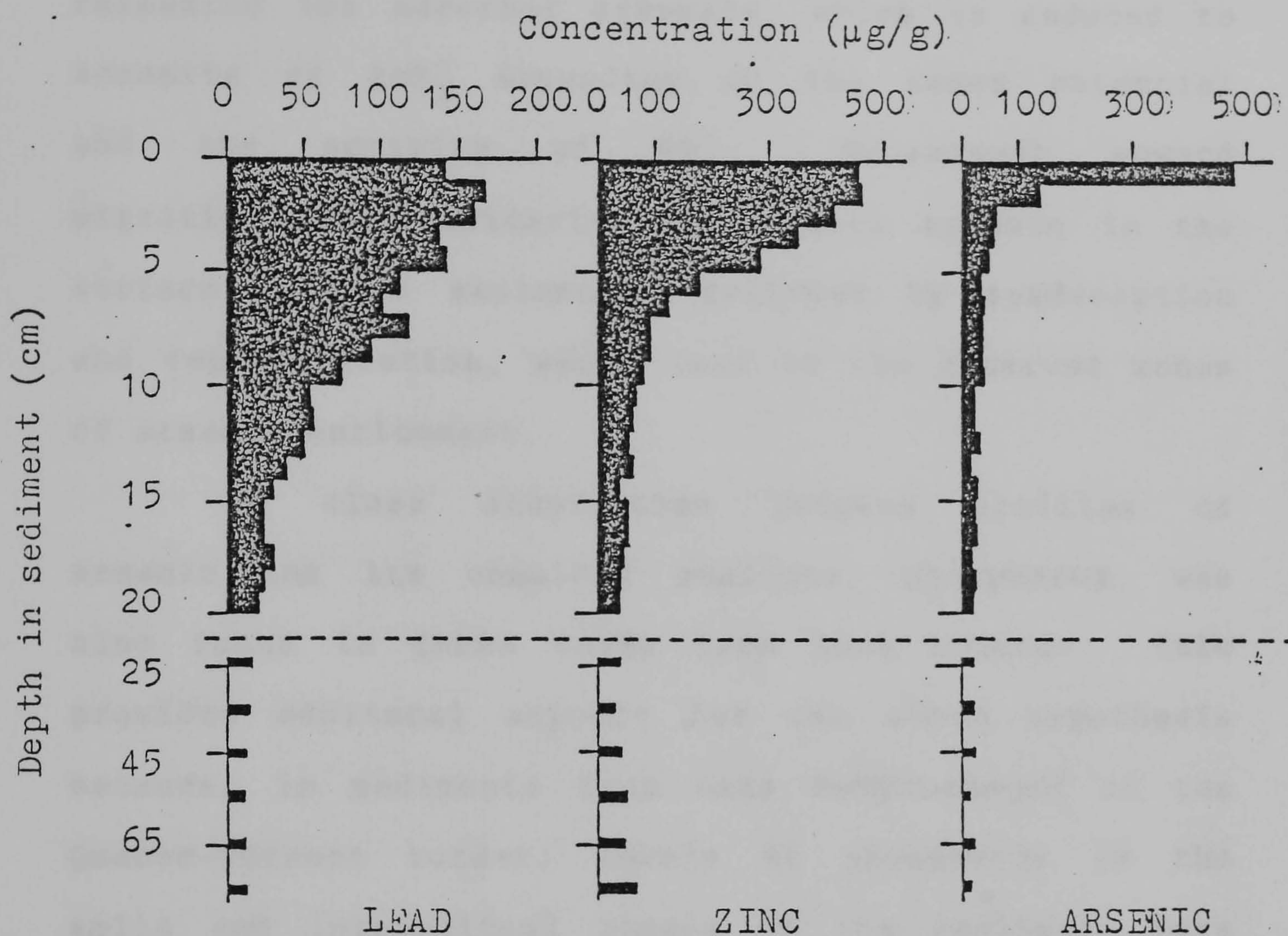


Figure 1.5 The concentration profiles of Pb, Zn and As in sediment core LLRPM 1 from the southern basin of Loch Lomond. (Farmer et al., 1983)

$(Fe(III)AsO_4)$ (Ferguson and Gavis, 1972; Crecelius, 1975; Price, 1976). Lower in the sediment column, the anoxic and hence reducing environment leads to the solubilisation of the ferric oxides and hydroxides, releasing the adsorbed arsenate, which is reduced to arsenite or AsS_2^- depending on the redox potential and the activity of HS^-. Subsequent upward migration and reoxidation of soluble arsenic in the surface aerobic sediments, followed by readsorption and reprecipitation, would lead to the observed zones of arsenic enrichment.

A close association between profiles of arsenic and its chemical analogue, phosphorus, was also found in three cores from Loch Lomond. This provides additonal support for the above hypothesis because, in sediments from Lake Memphremagog on the Quebec-Vermont border, levels of phosphorus in the solid and interstitial phases of the sediments were shown to be controlled by the dissolution/migration/ precipitation cycle of manganese and iron (Carignan and Flett, 1981).

Thus, most of the evidence for the post-depositional remobilisation of arsenic in Loch Lomond has been obtained indirectly by comparison with the sedimentary profiles of iron, manganese and phosphorus. Direct evidence for the upward migration of solubilised arsenic is limited to a porewater profile of total arsenic concentrations from a single

sediment core (Lovell, 1985). Identification and quantification of processes and parameters controlling the behaviour and distribution of sedimentary arsenic are hindered by the absence of information on the chemical speciation of porewater arsenic. So, too, is resolution of the question of whether or not methylation of arsenic occurs in bottom sediments of freshwater aquatic systems such as Loch Lomond. In view of the nature of the Loch Lomond system, the associated body of recent work on sedimentary arsenic and the postulated post-depositional diagenetic remobilisation mechanisms, there is a clear need (and, indeed, an excellent opportunity) for the practical application of speciation techniques to the analysis of porewaters.

It should also be of interest to look at the environmental chemistry of arsenic in other systems, e.g. the much smaller freshwater neighbouring Dubh Lochan, with both oxic and periodically anoxic sites, and the marine environment of the sealochs immediately to the west, for which previous sedimentary trace element data, excluding arsenic, are available. Farmer (1983) reported enhanced levels of lead, zinc and chromium in a number of sealoch sediment cores from the Clyde Sea Area, reflecting anthropogenic metal pollution via the Clyde Estuary and the River Clyde. Surface sediment enhancement of manganese was also found in a number of the sealoch cores and was

attributed to the natural diagenetic mechanisms previously described. The measurement of arsenic, iron and manganese in the sections of remaining library sediment core material would help clarify whether similar enrichments of arsenic can occur in a coastal marine system.

1.5 ARSENIC ANALYSIS

Any method used for arsenic determination must be adaptable for use on a variety of human and environmental samples and have the ability to detect the low concentrations typical of many of these samples.

Many methods have been developed to measure the total arsenic concentration in a wide range of samples. Neutron activation analysis is a non-destructive, but costly, method which has been employed for the analysis of hair (Smith, 1964) and sediments (Crecelius et al., 1975; Farmer and Cross, 1979). All other methods require initial degradation and digestion of the sample matrix either in a furnace (Pahlavanpour et al., 1984) or with hot acid (Lunde, 1973; Arafat and Glooschenko, 1981) or alkali (Luten et al., 1982). The Marsh test, which was the first traditional method of forensic analysis for arsenic, involved the heating of the suspect tissue with acid, converting the arsenic in solution to arsine gas (AsH_3) via reaction with zinc-liberated hydrogen and

condensing the gas to form a mirror for comparison with standard mirrors of known weight. Generation of the hydride either by potassium iodide/tin chloride/zinc or sodium borohydride has proved a popular preliminary step in arsenic analysis. The liberated gas may be complexed for Gutzeit analysis (Aston et al., 1975) or colorimetry, via complexing with molybdenum blue (Talmi and Feldman, 1975; Livesey and Huang, 1981) or silver diethyldithiocarbamate (Colbourn et al., 1975; Whyte and Englar, 1983), introduced into an argon/hydrogen flame (Terashima, 1976), quartz tube (Thompson and Thomerson, 1974; Wauchope, 1976) or graphite furnace (Thompson and Thoresby, 1977; Langston, 1980) for the commonly used technique of atomic absorption spectrometry (AAS), to an inductively coupled plasma (Oatey and Thornton, 1984) for emission spectrometry (Feldman, 1977) or to an argon/hydrogen flame for atomic fluorescence spectrometry (Azad et al., 1980).

The discussion of Sections 1.2, 1.3, 1.4 on the relative toxicities, behaviour, interconversions and biotransformations of the different chemical forms of arsenic in humans and in the environment has shown how important it is that the measurement of arsenic is not restricted to the determination of total arsenic concentrations. Although specific identification of the complex organoarsenicals present in biological material may not always be necessary, it is important

that a distinction be made between inorganic arsenic and the more stable organic, nontoxic forms of arsenic, e.g. by distillation of the inorganic arsenic from the solid sample with hydrochloric acid (Lunde, 1973). Methods for the quantitative analytical speciation of As(V), As(III), MMAA and DMAA in liquid samples are essential. While a number of methods based on hydride generation employ differential volatilisation (Braman and Foreback, 1973) or pH control (Aggett and Aspell, 1976) to separate some of the species, chromatographic methods using simple ion-exchange (Yamamoto, 1975; Iverson et al., 1979) or more sophisticated high performance liquid chromatography are becoming increasingly popular (Stockton and Irgolic, 1979; Brinckman et al., 1980; Woolson and Aharanson, 1980; Morita et al., 1981).

In this particular laboratory, limited past experience has shown, on the grounds of cost, availability of technology, sample handling capability and sensitivity, that the most promising technique for the analytical speciation of arsenic in urine and porewaters in this study would probably be that based on separation by combined cation-anion exchange chromatography, fraction collection of individual arsenic species and subsequent element-specific detection by hydride generation atomic absorption spectrometry (Lovell and Farmer, 1983, 1985; cf Chapter 2).

Finally, it should be noted that, in the absence of certified reference materials for the concentrations of As(V), As(III), MMAA and DMAA in water and urine samples, corroboration of speciation data via intercomparison exercises with other laboratories, preferably employing alternative analytical methods, is an important requirement in analytical arsenic speciation studies.

1.6 OBJECTIVES

The principal objectives of this work can be grouped under three main headings:

1.6.1 Analytical requirements

The development of reliable methods for:

1) the analytical speciation of As(V), As(III), MMAA and DMAA in human urine, water and the porewaters of aquatic sediments.
2) the differentiation of inorganic and organic arsenic in biological tissues.
3) the preparation of human urine, biological tissues and aquatic sediments for total arsenic measurement by atomic absorption spectrometry.

1.6.2 Arsenic in humans

1) using analytical speciation techniques, the establishment of the major patterns of human metabolism, biotransformation and urinary excretion of arsenic following the oral administration of single doses of the seafood organoarsenical and inorganic arsenic to volunteers in laboratory-controlled experiments.

2) the investigation of relationships between the intake, transformations and urinary output of arsenic in a further laboratory-controlled experiment designed to simulate regular exposure to elevated levels of inorganic arsenic in the workplace or in a contaminated environment.

3) the determination and comparison of the urinary concentrations of the different chemical forms of arsenic in the general population and in various groups liable to enhanced environmental or occupational exposure to inorganic arsenic.

4) an assessment of the intakes, retention, elimination and possible health effects of arsenic for U.K. inhabitants as a result of typical and elevated exposures to inorganic arsenic under a variety of dietary, environmental and occupational conditions.

1.6.3 Arsenic in the aquatic environment

1) the elucidation of processes responsible for enrichment of arsenic in the upper layers of sediment from freshwater Loch Lomond, with particular emphasis on direct investigation, via appropriate analytical speciation of arsenic in porewater from the sediment column, of the postulated post-depositional diagenetic mechanism.

2) a concomitant study of arsenic behaviour in bottom sediments at oxic and periodically anoxic sites in the shallow, neighbouring, freshwater Dubh Lochan.

3) further investigation of the nature and extent of the phenomenon of arsenic enrichment via the determination of arsenic profiles in marine sediment cores previously collected at nearby and distant sites off the west coast of Scotland.

CHAPTER 2

THE DETERMINATION OF ARSENIC IN BIOLOGICAL, SEDIMENT, WATER AND HUMAN URINE SAMPLES BY ATOMIC ABSORPTION SPECTROMETRY

2.1 ATOMIC ABSORPTION SPECTROMETRY

2.1.1 Principles

Atomic absorption is the process by which the free ground state atoms of an element are converted into an excited state by the absorbance of discrete quantities of light energy at a wavelength (resonance line), specific to that element. Each element has its own characteristic spectra over a range of wavelengths. At the resonance line selected for the element to be analysed, the amount of light absorbed is a function of the elemental number of atoms of the analyte element in the light path. Thus measurement of the amount of light absorbed via the relationship below, enables a quantitative determination of the amount of the element present.

$$A = \log_{10} \frac{I_o}{I}$$

Where A = Absorbance

I_o = Light intensity source

I = Light intensity following absorption

2.1.2 Instrumentation

Figure 2.1 shows the basic instrumental layout for atomic absorption spectrometry (AAS). The element of interest is introduced by appropriate means into the atom cell, which may take the form of a flame, a graphite tube or a heated quartz cell. Here, the element is converted into an atomic vapour which can then absorb light from the source. The standard light source for AAS is usually a hollow cathode lamp, but for arsenic analysis a more efficient electrodeless discharge lamp (EDL) is used (Section 2.2.1). Light from the source can be divided into a sample beam and a reference beam directed around the sample cell so that fluctuations in the source intensity do not effect the measurement. Alternatively this latter beam can be replaced by continuum radiation from a deuterium arc to correct for the effects of any non-specific absorption and light scattering in the atom cell. A monochromator (a diffraction grating) is used to select the wavelength of interest, and the appropriate slit setting selected to eliminate any extraneous bands of light, before entering the photomultiplier detector where the light intensity is measured and the signal amplified. An electric current is produced depending upon the light intensity and processed by the instrument electronics. The output signal can be displayed in various ways e.g. digital display, paper

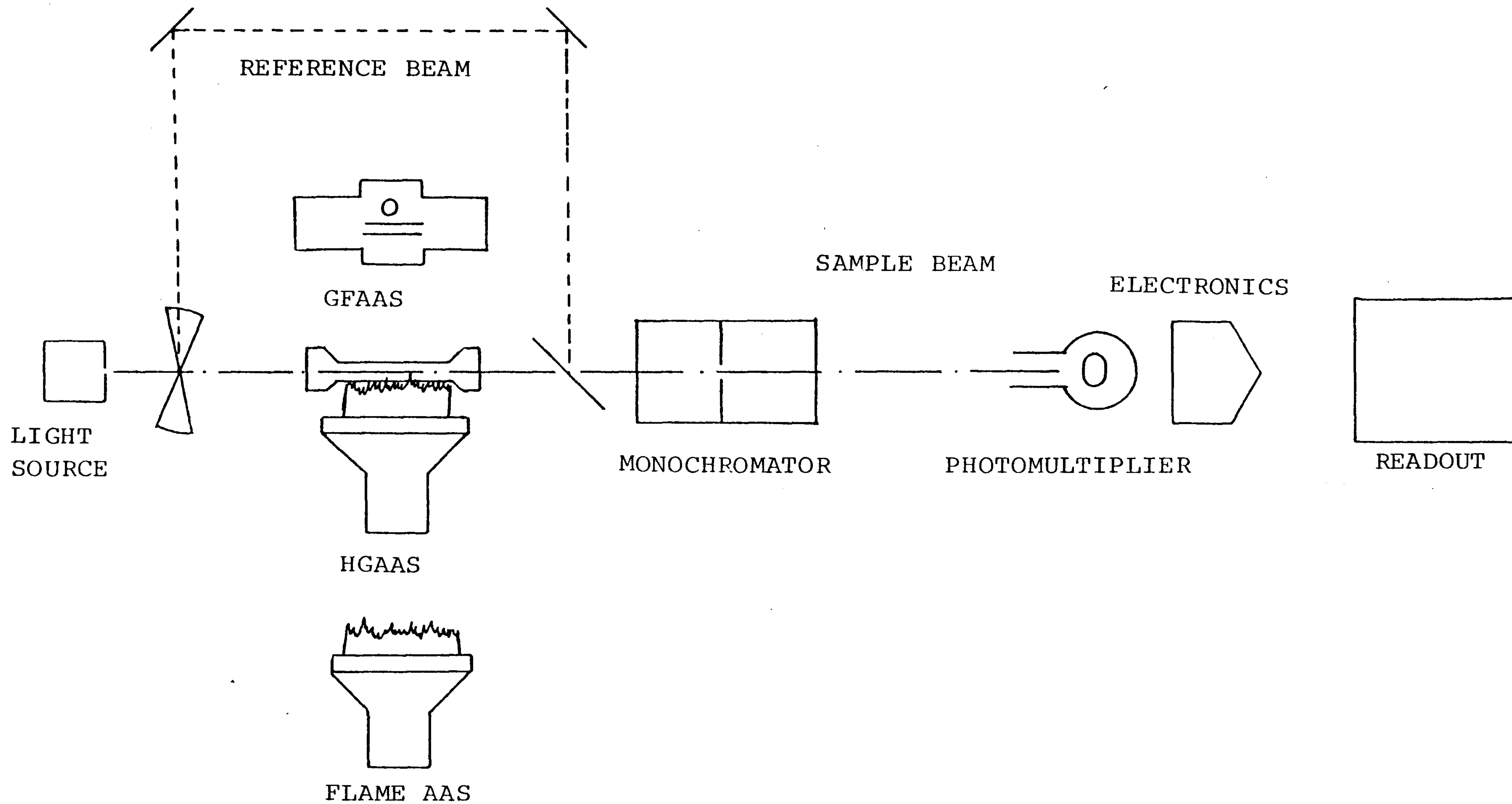


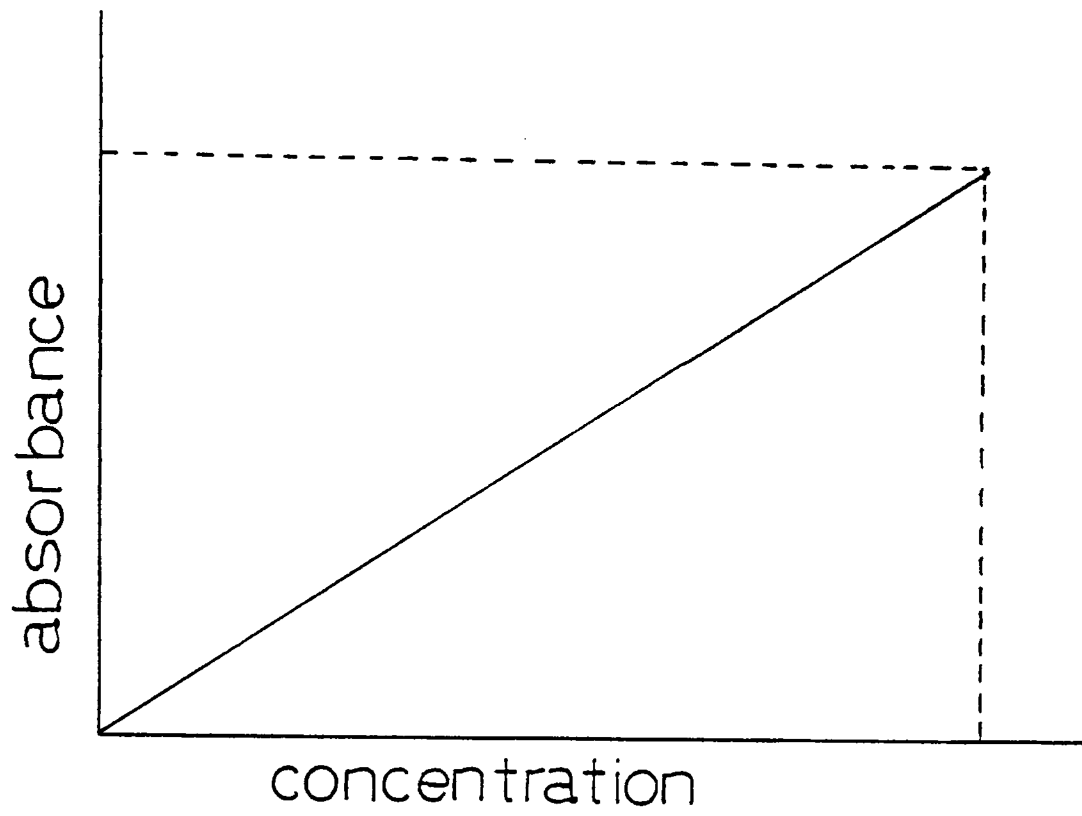
Figure 2.1 Basic Atomic Absorption Instrumentation

tape printout, or peak height on a chart recorder.

2.1.3 Calibration and calculation of results

Standard solutions of known arsenic concentration are used to construct a calibration plot of absorbance vs concentration (Figure 2.2). Once the absorbance of the analyte solution of unknown arsenic content is measured the arsenic concentration can be readily obtained from the calibration plot. Where possible, the non-linear regions of the calibration curve, typically obtained at higher concentrations, should be avoided. In atomic absorption spectrometry, in general, there are several well-known types of interferences (e.g. spectral, non-specific molecular absorption and light scattering, ionisation, chemical and matrix) which can adversely affect the accuracy of analytical determinations (Ebdon, 1982). Where these are of relevance to arsenic determination in this work they are discussed, along with remedial measures, in subsequent sections.

When dealing with trace analysis it is essential that sample contamination be minimised. Thus during chemical manipulation of the samples, Milli-Q-water and Aristar grade acids were utilised, while AAS standard solutions of 1000mg/l as supplied by BDH were used for all subsequent standard solution preparations.



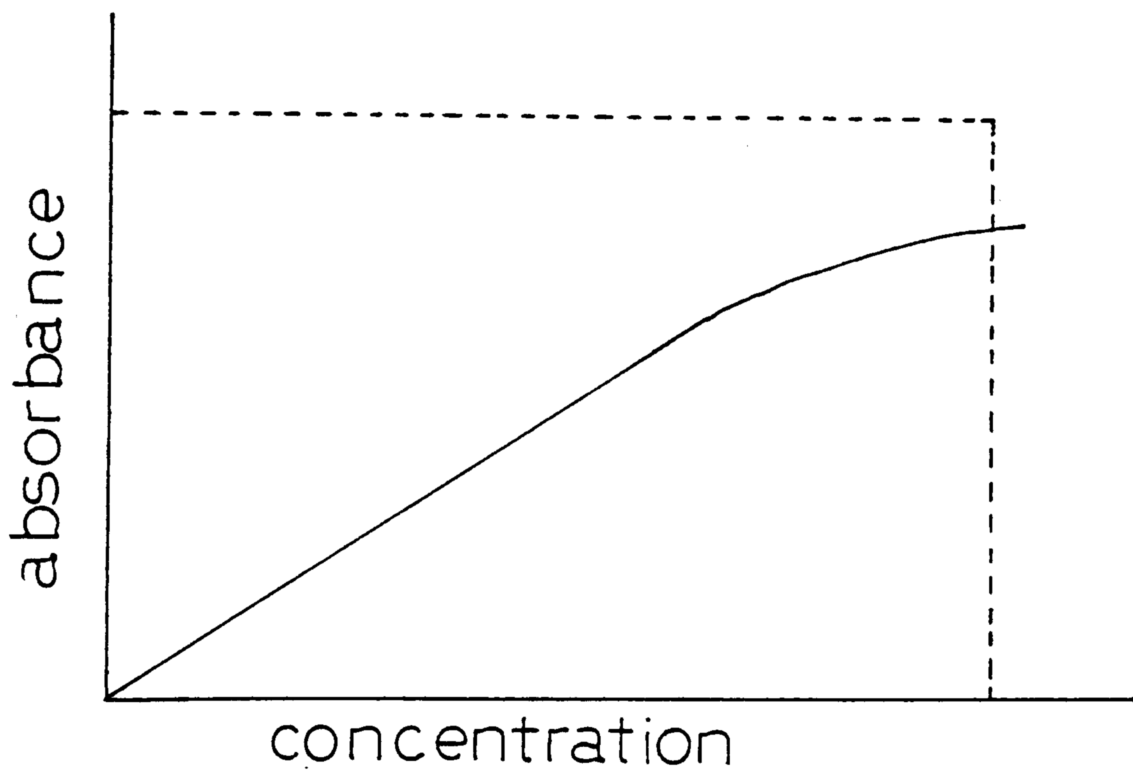


Figure 2.2 Calibration curves (a) Ideal (b) Normal

2.2 ARSENIC DETERMINATION BY ATOMIC ABSORPTION SPECTROMETRY

2.2.1 Difficulties encountered in arsenic analysis

The arsenic resonance lines lie in the far ultraviolet region of the spectrum (below 200nm), where non-specific absorption can be severe, making spectrometric determinations difficult (Talmi and Feldman, 1975). Thus background correction with a deuterium arc (Section 2.1.2) was employed for all arsenic analyses carried out in this study.

Atomisation systems have been developed in place of the flame, where most of the sample is lost to waste via droplet formation, in an attempt to increase detection sensitivity, by extending the residence time of the analyte in the optical path of the atomic spectrometer, and to enable all of the sample aliquot to be used in the measurement. The most commonly used methods for arsenic are an electrically heated graphite furnace, or a quartz tube heated electrically or by flame in combination with hydride generation (Figure 2.1).

In graphite furnace atomic absorption spectrometry (GFAAS), a sample aliquot (solid or liquid) is dispensed into a small graphite tube which is heated electrically. Drying, ashing and atomisation of the sample occur at pre-determined temperatures in succession with an inert purging gas stream passing through the tube to remove any solvent

and/or matrix vapours. During atomisation, the gas stream can be partially or completely shut off so that free atoms remain in the path of the light beam up to a thousand times longer than with the conventional flame to increase detection sensitivity. GFAAS has been the technique chosen by many workers for the determination of arsenic in a variety of samples, e.g. soil and plant material (Thompson and Thoresby, 1977), siliceous materials (Lovell and Farmer, 1983), and both freshwater and seawater (Chakraborti et al., 1980). Matrix modification with nickel nitrate can be used to increase the ashing temperatures and improve the sensitivity (Iverson et al., 1979; Edgar and Lum, 1983; Lovell and Farmer, 1983). Although generation and retention of sample atoms within the graphite tube can lead to enhanced sensitivity (e.g. over flame determination), confined vaporisation can result in severe non-specific interferences, especially with samples containing high concentrations of dissolved salts.

Atomisation via the generation of the hydride has improved analytical sensitivity and given a greater freedom from interferences (Welz, 1986). Hydride generation atomic absorption spectrometry (HGAAS) exploits the ability of elements (e.g. within group 5 of the periodic table) to form covalent hydrides readily. The accepted procedure by which hydride generation is performed is to reduce the

analyte of interest to its volatile hydride and to sweep the generated gas into the heated quartz tube atomiser, where quantitative spectrochemical measurement can be made (Snook, 1981).

2.2.2 A review of hydride generation atomic absorption spectrometric methods and instrumentation.

Modifications of the classical Gutziet method, utilising generation of arsine from the addition of stannous chloride, HCl and zinc granules to sample solutions (Aston et al., 1975) have been applied to AAS to overcome interference problems encountered when analysing an element with resonance lines in the far UV region of the spectrum. Spectrophotometric methods involving the reaction of arsine with silver diethyldithiocarbamate to form a red complex suffer from the disadvantage of requiring large amounts of sample in order to obtain sufficient sensitivity. Early attempts at arsine generation used mixtures such as potassium iodide/zinc powder/tin II chloride (Chu et al., 1972), or titanium II chloride/magnesium powder (Dalton and Malanoski, 1971; Fernandez and Manning, 1971). However, generation of the hydride was often slow and erratic. This was improved by the reaction of sodium tetrahydroborate III ($NaBH_4$) with an acidified sample (usually in dilute hydrochloric acid (HCl)), to provide efficient and rapid reduction

(Schmidt and Royer, 1973), and is now the method most frequently used in hydride generation techniques.

As only a few elements form gaseous compounds with hydrogen under the conditions used, hydride generation is a superior method for arsenic determination because arsenic is separated from the sample matrix prior to its introduction into the atomiser, thus minimising matrix interferences typical of flame analysis. The complete transport of the element into the atomiser, compared with the 2 - 5% transport efficiency of pneumatic nebulisation, and the discrete nature of the sample introduction technique also mean that higher powers of detection are possible (Ward and Stockwell, 1983; Snook, 1981). As the peak height of the arsenic signal is commonly measured, it is advantageous to transport the liberated hydride to the detection system in the shortest possible time. In the past it was common practice to collect the hydrides in a collection reservoir e.g. a balloon, (Fernandez and Manning, 1971; Manning, 1971; Chu et al., 1972; Fiorino et al., 1976; Cox, 1980). However, when sodium borohydride is used as the reducing agent, the need to collect the liberated hydride is unecessary. Dalton and Malanoski (1971) were the first to report the direct aspiration of arsine into a flame without the use of a collecting device. This work utilised an argon/hydrogen flame, the liberated arsine being carried to the nebuliser by

the released hydrogen.

As outlined in Section 2.1.3, flame atomisation can often lead to significant non-specific absorption and scattering, especially at the low resonance lines for arsenic (193.7nm). Chu et al. (1972) first reported the use of an electrically-heated tube as an alternative to the flame. The liberated hydride was swept into the tube by a stream of argon carrier gas and, as no flame was employed, the background absorption was reduced. Thompson and Thomerson (1974) used a silica tube heated by an air/acetylene flame. The advantages claimed for the technique were that no collection vessel was required, background absorption was virtually eliminated, better sensitivity was achieved than with the argon/hydrogen flame and one sample could be analysed every 40 seconds. Atomisers in the form of a quartz (silica) tube heated electrothermally (Chu et al., 1972; Thompson and Thoresby, 1977; Cox, 1980; Subramanian and Meranger, 1982), or with an air/acetylene flame (Thompson and Thomerson, 1974; Peats, 1979; Sinemus et al., 1981; Arbab-Zavar and Howard, 1980), have improved sensitivity by increasing the residence time of the atoms in the light path of the detector.

The mechanism of atomisation in the quartz tube is still not fully understood. Thompson and Thoresby (1977) proposed that the hydride is thermally decomposed on contact with the hot walls of the tube

yielding a concentration of ground state atoms for atomic absorption measurement. However, Welz and Melcher (1983) believed atomisation not to be the result of thermal decomposition, but to be caused by collision of the gaseous hydride and free hydrogen radicals formed in reaction with oxygen at temperatures greater than 600°C.

HGAAS has been applied to the analysis of arsenic from a variety of samples e.g. soil and plant materials (Thompson and Thomerson, 1974; Thompson and Thoresby, 1977), seaweed (Peats, 1979), water and urine samples (Cox, 1980), lakewater samples (Sinemus et al., 1981), and human liver and kidney samples (Subramanian and Meranger, 1982). Commercially available hydride generation units have been widely used with atomic absorption spectrometry e.g. the MHS-10 unit (Mercury Hydride System-10) (Peats, 1979) and (Section 2.2.3) and the MHS-20 unit (Sinemus et al., 1981) which was found to be "quick, simple to use and sensitive". Although the absolute limit of detection for GFAAS at 0.1ng As is superior to the 1ng typical of the hydride generation technique, the fact that the MHS-10 reaction vessel can hold a sample volume of 50ml means that its detection limit per ml of sample is greatly improved (Table 2.1). Hydride generation, using the MHS-10 system and heated quartz tube atomic absorption spectrometry using the PE-306, with an arsenic electrodeless discharge lamp at a

TABLE 2.1

COMPARISON OF GFAAS VS HGAAS DETECTION LIMITS FOR ARSENIC DETERMINATION

	Absolute detection limit	sample aliquot	detection limit
GFAAS	0.1ng	20ul	5.00ng/ml
		200ul	0.50ng/ml
HGAAS	1.0ng	1ml	1.00ng/ml
		10ml	0.10ng/ml
		50ml	0.02ng/ml

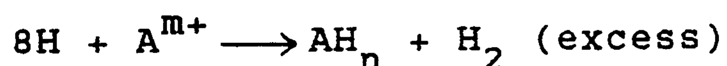
resonance line of 193.7nm, was the method chosen for the determination of arsenic in this study.

2.2.3 Arsenic determination by hydride generation-atomic absorption spectrometry using the MHS-10 system

The MHS-10 unit is mechanically very simple and can be quickly and easily fitted to an atomic absorption spectrometer. (Figure 2.3)

By increasing the pressure in the reservoir vessel containing the sodium borohydride reducing agent (on activation of the pneumatic plunger), the reductant is dispersed into the acidified (HCl) sample solution in a stream of argon gas, contained within the reaction vessel. The reactions involved are:

$$NaBH_4 + 3H_2O + HCl \longrightarrow H_3BO_3 + NaCl + 8H$$



$$8H + A^{m+} \longrightarrow AH_n + H_2 \text{ (excess)}$$

(Welz, 1986)

where A = analyte element and m may or may not = n.

The reductant is added continuously until in the case of arsenic, the arsine absorbance begins to decline. The arsine formed is then stripped from the solution in the stream of carrier gas and conveyed to the heated (air/acetylene flame) quartz tube where it is atomised and the absorption of light, according to the concentration of arsenic atoms, is measured.

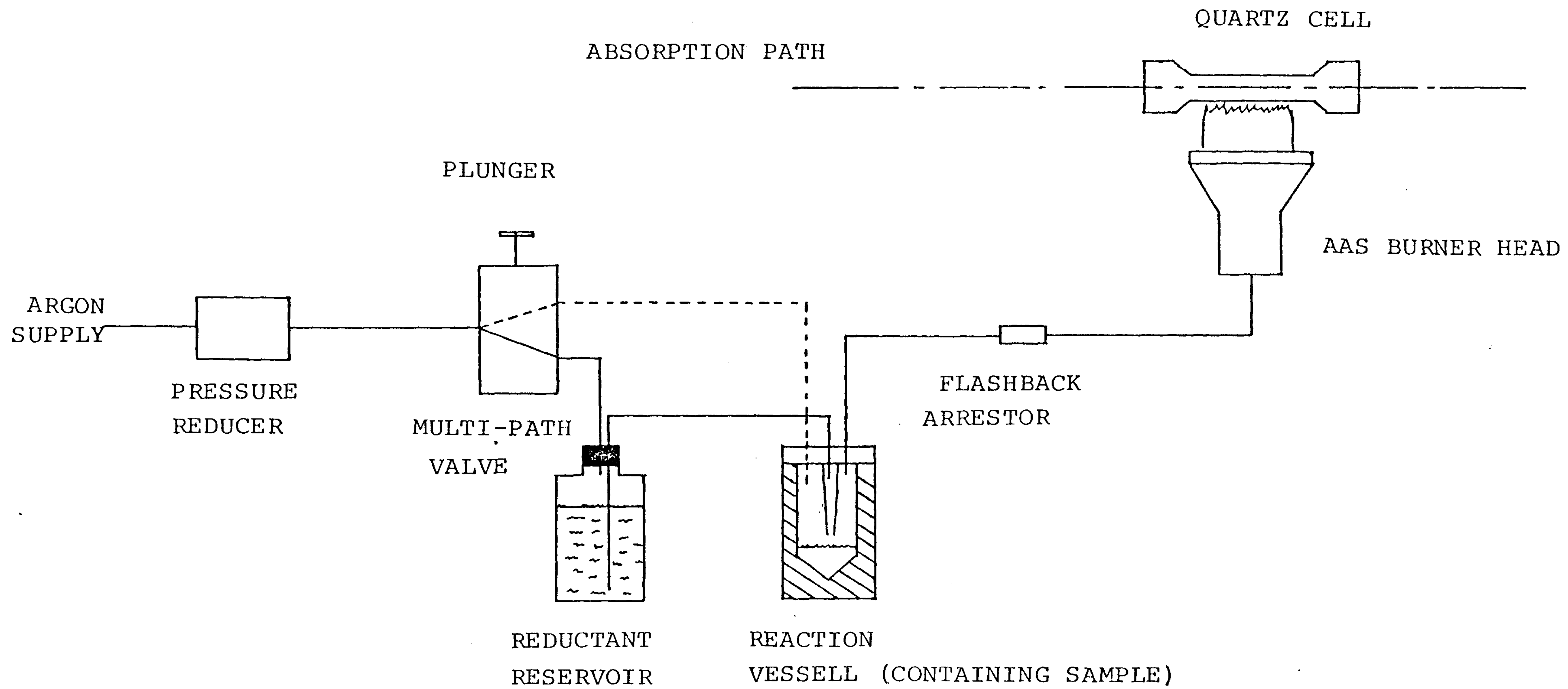


Figure 2.3 Simplified diagram of an MHS-10

Such a system ensures complete arsenic reduction and conversion to the arsine gas with 100% transport efficiency into the atomiser.

Typically there are several components of such a system that can be varied and ultimately optmised. Thompson and Thoresby (1977) stated that cell dimensions, carrier gas flow rate and borohydride concentration are inter-related in that they dictate the rate at which arsine is released to the atomiser and hence the response produced. As later corroborated by Howard and Arbab Zavar (1981), they found that 2% w/v $NaBH_4$ gave optimum signal response. Yamamoto et al. (1984) were able to show that a 5% $NaBH_4$ solution resulted in severe interferences but that such effects were eliminated using a 1% $NaBH_4$ solution. Using the MHS units, both Peats (1979) and Sinemus et al. (1981) used a 3% w/v $NaBH_4$ solution stabilised with 1% NaOH. The manufacturers of the MHS-10 unit, Perkin Elmer, recommend the use of a 3% w/v $NaBH_4$ solution stabilised with 1% w/v NaOH. (note that sodium borohydride solution is unstable to direct uv light and has to be used within 2 - 3 days even after filtering).

However, HGAAS is not completely free from analytical difficulties. Problems can arise as a result of changes in the hydride generation rate and/or by a decreased fraction of the analyte reduced

or released from the sample solution, (Section 2.4.2). The quartz tube has a finite lifetime as severe contamination can be caused by excess foaming in the reaction vessel with carry over of the sample solution into the quartz tube. The tube can be cleaned with extreme care using concentrated hydrofluoric acid, but usually weakens the tube to the extent that a replacement is required. Once a new tube is fitted, it must be conditioned by repeated measurements of the most concentrated standard solution to be used until a consistent absorbance value is obtained.

2.3 SIGNIFICANT PARAMETERS IN THE DETERMINATION OF ARSENIC BY HYDRIDE GENERATION ATOMIC ABSORPTION SPECTROMETRY

A number of factors which are known to effect the detection and measurement of arsenic by HGAAS are discussed below.

2.3.1 Hydrochloric acid concentration

Although Perkin Elmer recommend an HCl concentration of 1.5% w/v, the variation in acid concentrations used by a number of workers (e.g. Peats, 1979; Hobbins, 1982) and the pH dependence of hydride generation (Section 2.5.2), made it necessary to find the optimum acid concentration for this system using simple acidified aqueous solutions. Using a 10ml sample volume the arsine signal response was

measured for 10, 30 and 50ng of an As(III) standard in solutions of HCl concentration ranging from 1.5% w/v (4.3% v/v) to 15% w/v (43% v/v). The results in Figure 2.4 show that very little change in sensitivity is produced by the higher HCl concentrations until a 15% w/v (43% v/v) HCl concentration is used. Accordingly a 1.5% w/v HCl matrix was used for samples, standards and blanks unless otherwise stated.

2.3.2 Sample volume

As the MHS-10 unit allows the use of a range of sample volumes in the reaction vessel, it is important to determine what effect this has upon the resultant arsenic signal. Thus As(III) standards of 10, 30 and 50ng in volumes of 1.5% w/v HCl ranging from 10 to 25ml were tested. Figure 2.5 shows that maximum sensitivity was obtained with a 10ml sample volume and that increasing the sample volume caused a reduction in the sensitivity at arsenic levels ranging from 10 - 50ng.

Thus, throughout the following work sample solutions in 10ml of 1.5% w/v HCl were used except where samples of very low arsenic concentration required a greater sample volume to increase the detection sensitivity. Unless otherwise stated the conditions employed for HGAAS were as outlined in Table 2.2

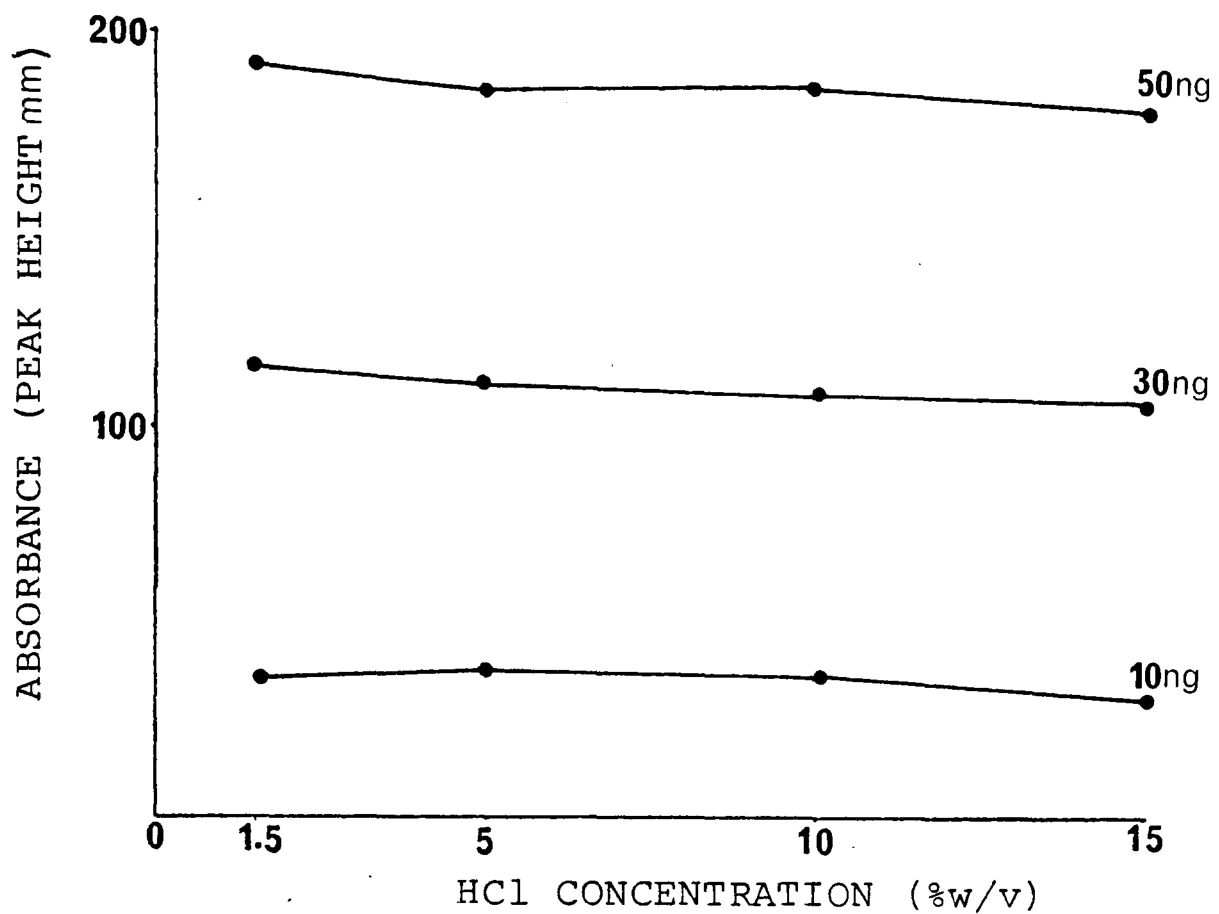


Figure 2.4 The effect of HCl concentration on signal response in the determination of As(III) by HGAAS

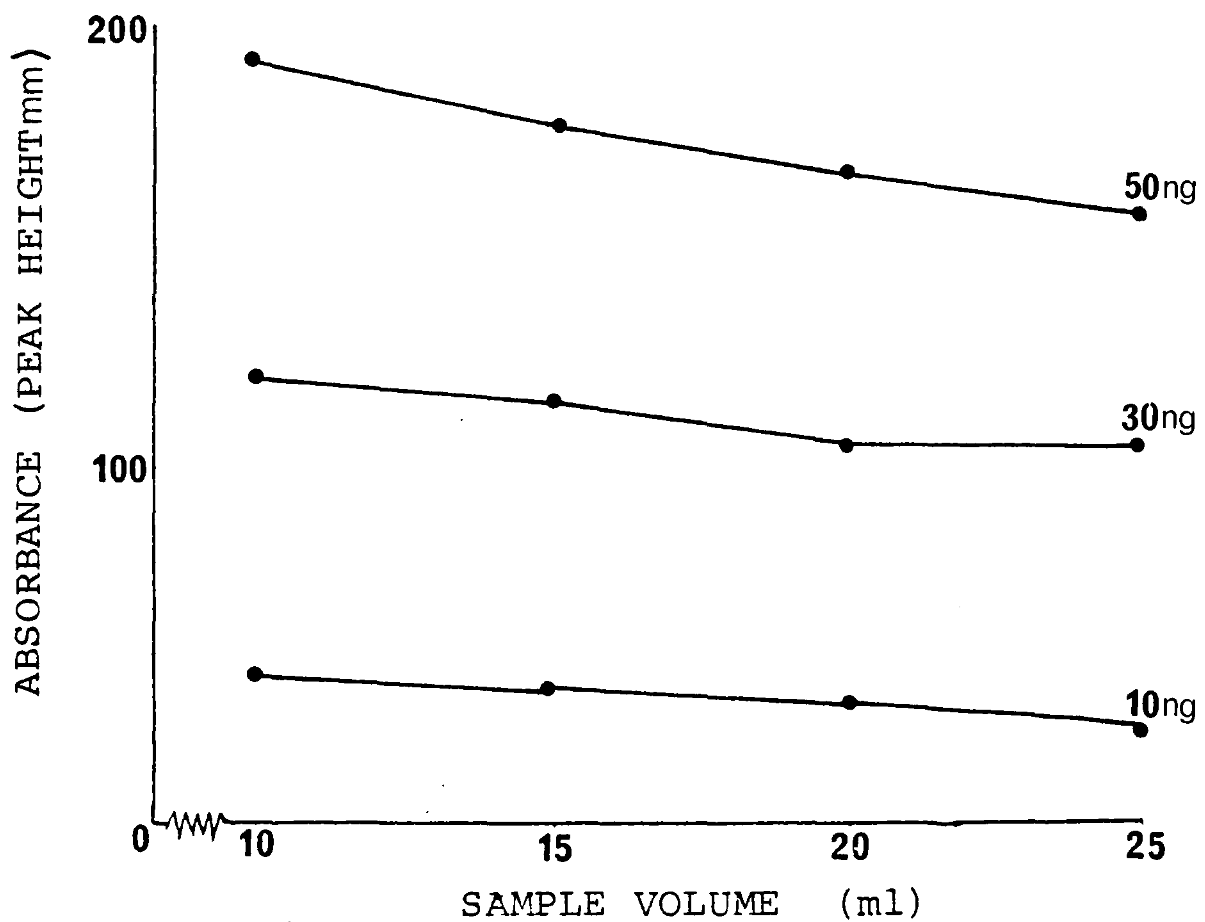


Figure 2.5 The effect of sample volume on signal response in the determination of As(III) by HGAAS

TABLE 2.2

STANDARD CONDITIONS USED FOR THE DETERMINATION OF ARSENIC BY HGAAS

ATOMIC ABSORPTION SPECTROMETER	PERKIN ELMER 306
Wavelength	193.7nm
Slit width	0.7nm
Mode	Absorption
Lamp	Electrodeless discharge lamp
Lamp power	7.5W
Background correction	Deuterium lamp
Flame	Air/Acetylene (lean)
Flow rates	air : 11.5 l/min acetylene : 2.5 l/min

HYDRIDE GENERATION	MHS - 10
Reaction solution	1.5% w/v HCl
Sample volume	10 - 50ml
Sample aliquot	10 - 50ul
Reductant	3% w/v $NaBH_4$ in 1% w/v NaOH
Atom cell	Quartz tube (@ 800 - 900°C)
Purge gas	High purity Argon

Absorbances as peak heights were recorded on a Kipp and Zonen BD8 chart recorder.

2.3.3 Chemical form of the dissolved arsenic

The four arsenic species of particular significance to this work were inorganic As(V) and As(III) and organic MMAA and DMAA, (Section 1.2). Standard solutions of each species were made up from the following arsenic salts.

As (V)	Sodium arsenate	$NaHAsO_4.7H_2O$
As (III)	Sodium arsenite	$NaAsO_2$
	Arsenic trichloride	$AsCl_3$
MMAA	Monomethylarsonic acid	$CH_3AsO(OH)_2$
DMAA	Dimethylarsinic acid	$(CH_3)_2AsO.OH$

Each of the four species was pipetted separately as 10 - 50ng sample aliquots into 10ml of 1.5% w/v HCl in the reaction vessel and, after hydride generation, the peak heights for each noted. The results were plotted as calibration graphs for each of the four species (Figure 2.6). From Figure 2.6 it can be seen that the signal response was of the order As(III) $>$ MMAA $>$ As(V) $>$ DMAA and, that the two As(III) salts yielded identical responses. Within the linear range the response of MMAA, was on average, 75% of the As(III) signal, while As(V) and DMAA resonses were, on average, 60 - 65% of the As(III) signal. Sinemus et al. (1981) reported that the signal response of As(V) standards was 25% smaller than As(III) standards at the same concentrations, while Peats (1979) noted that

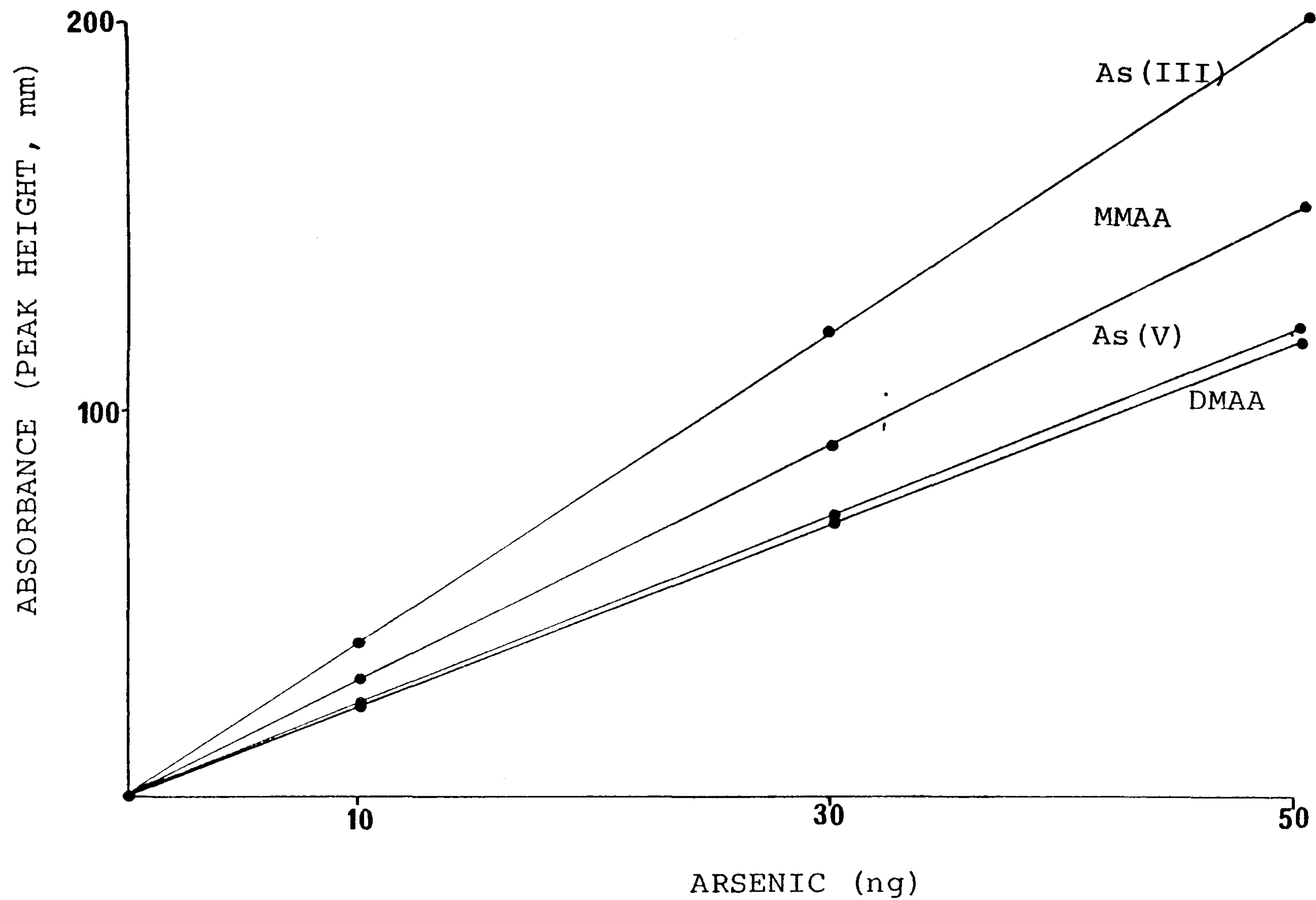


Figure 2.6 Comparison of As(V), As(III), MMAA, and DMAA signal response by HGAAS

As(V) standard peak heights were 30% lower than those of the As(III) standards. During measurement of the four species it was noted that generation of the hydride from the As(V) and the DMAA standards was much slower than from the other two species. Thompson and Thoresby (1977) suggested that this behaviour could be the result of differences in reaction kinetics for the reduction mechanism, i.e. As(V) has firstly to be reduced to As(III) and then converted to the hydride, thus causing a much slower response than for As(III), while MMAA and DMAA conversions could be hindered by the presence of the methyl groups (steric hindrance). The fact that the different arsenic species at the same concentration give a different signal response has important implications for the analysis of arsenic in many samples of human and environmental origin. It means that measurement of the total arsenic concentration in a sample where a mixture of the dissolved species is present cannot readily be made by a single direct analysis of the sample.

2.3.4 Sample pre-reduction

In Section 2.3.3 it was noted that the lower oxidation state As(III) gave a greater detection sensitivity than the higher oxidation state As(V). It follows that detection sensitivity could be increased if all the arsenic present in a sample

solution were reduced to the As(III) oxidation state prior to analysis. Acidified potassium iodide solutions are commonly used to perform this reduction (Peats, 1979; Sinemus et al., 1981; Hobbins, 1982).

A range of pre-reduction solutions were made up (Table 2.3) containing 1mg/l of each of the arsenic species and left to stand for a minimum of one hour to allow the reduction to take place. Sample aliquots of 10, 30 and 50ul corresponding to 10, 30 and 50ng were abstracted from the reduced solutions and analysed in 10ml of 1.5% w/v HCl. From Figures 2.7 (a - d), it can be seen that pre-reduction solutions (b), (c) and (d) increased the As(V) signal to equal the As(III) signal, while solution (a) had no effect. None of the solutions could bring the MMAA and DMAA response in line with that of As(III), so that, even with pre-reduction, the presence of MMAA and/or DMAA in sample solutions would result in an underestimate of the total arsenic concentration present when evaluated against inorganic As(III) standards. Figures 2.7(a) and 2.7(b) show that 6% w/v HCL is required, while (b) and (d) show a 10% w/v HCl solution to be unnecessary. Figures 2.7(b) and (c) show that a 1% w/v KI solution is adequate to bring about the reduction of As(V) to As(III). Thus, for samples containing just the inorganic arsenic species, the total arsenic concentration could be obtained by reducing all the arsenic present to the

TABLE 2.3

POTASSIUM IODIDE PRE-REDUCTION SOLUTIONS TESTED FOR ARSENIC ANALYSIS BY HGAAS

a). 1.0% w/v KI
1.0% w/v asc
1.5% w/v HCl

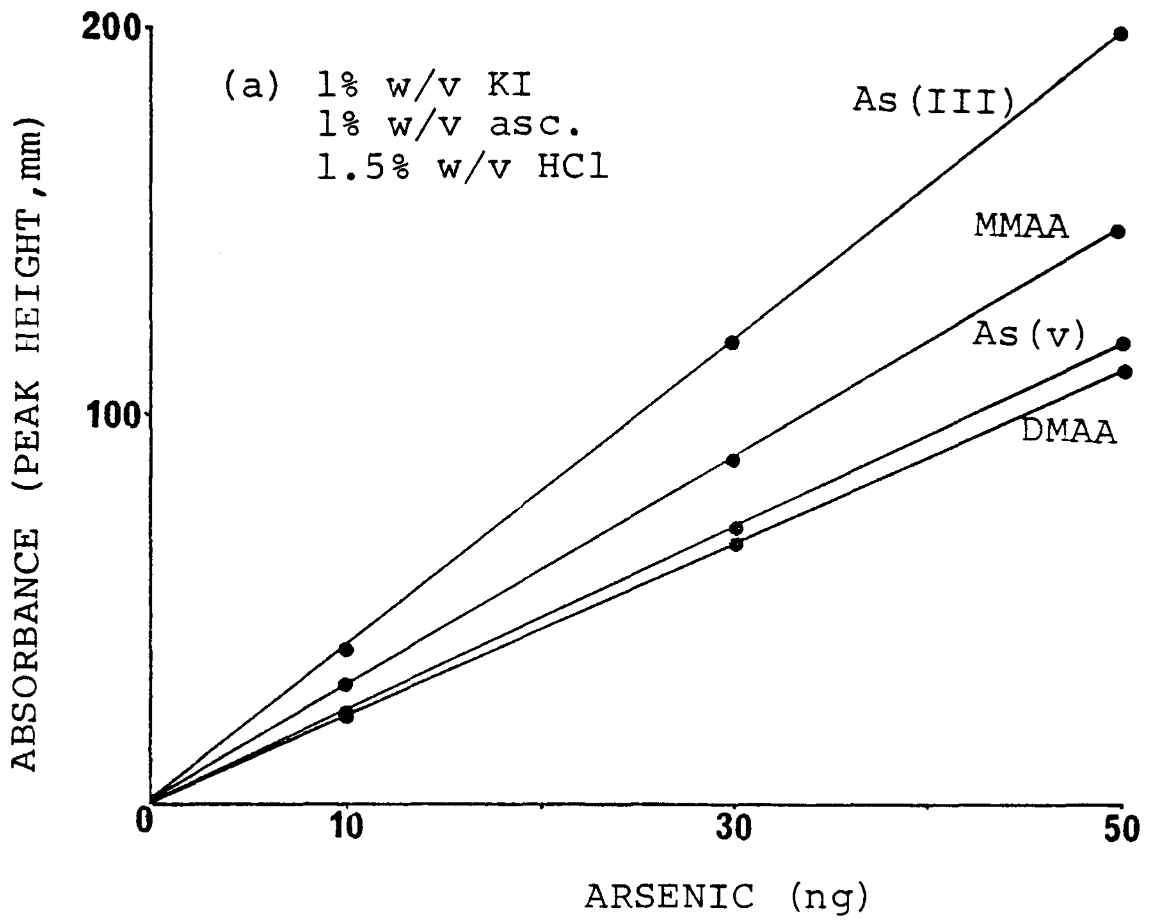
b). 1.0% w/v KI
1.0% w/v asc
6.0% w/v HCl

c). 10.0% w/v KI
1.0% w/v asc
6.0% w/v HCl

d). 1.0% w/v KI
1.0% w/v asc
10.0% w/v HCl

where KI = potassium iodide
asc = ascorbic acid ($C_6H_8O_6$) (to stabilise the solutions)

HCl = hydrochloric acid



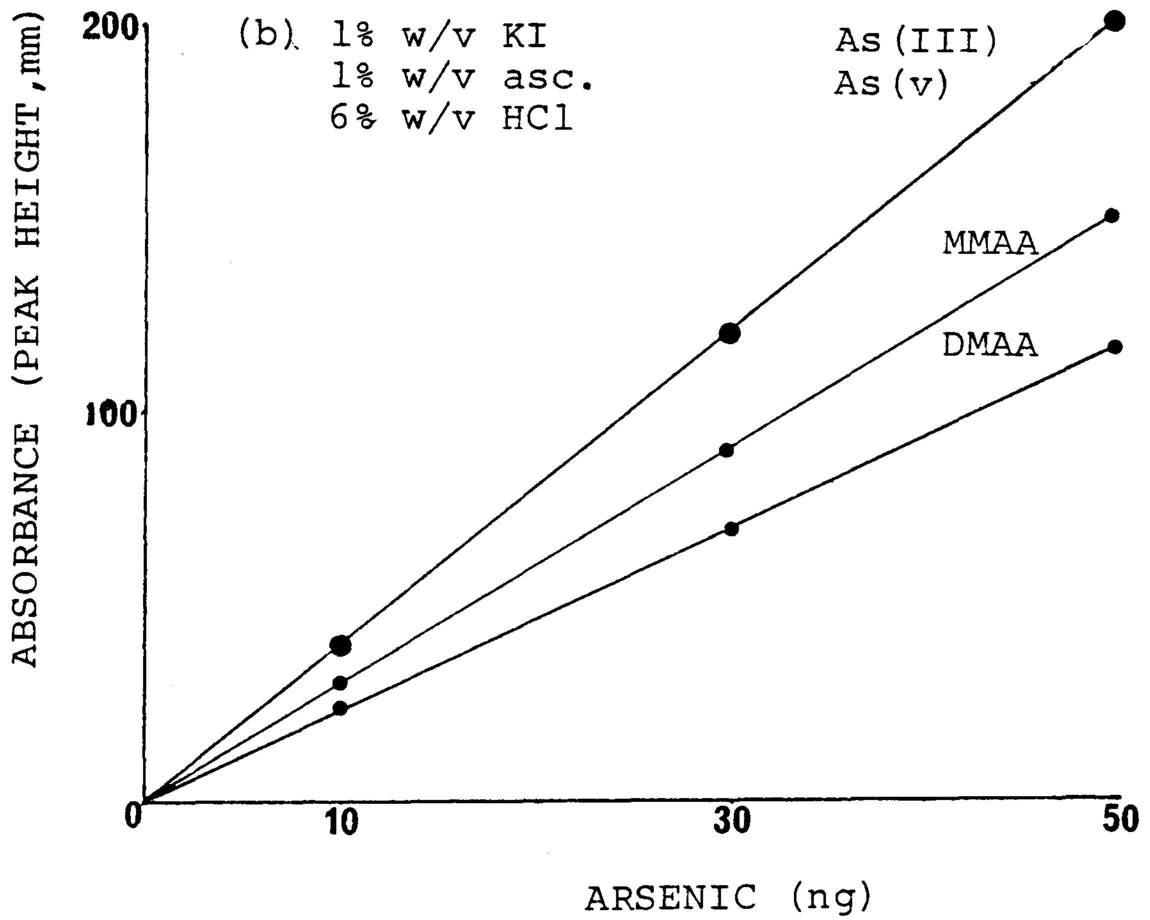
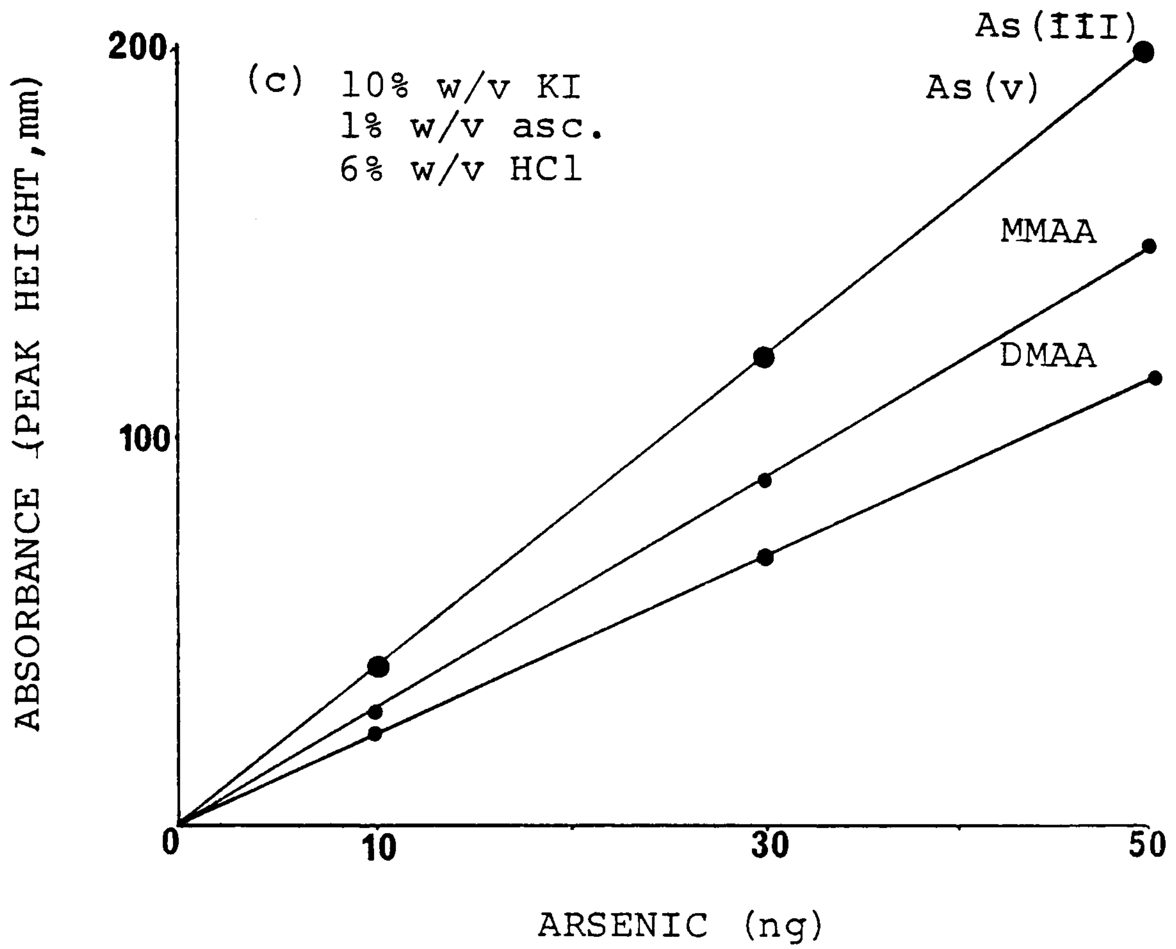


Figure 2.7 The effect of pre-reduction solutions (a) and (b) on As(III), As(V), MMAA and DMAA signal response



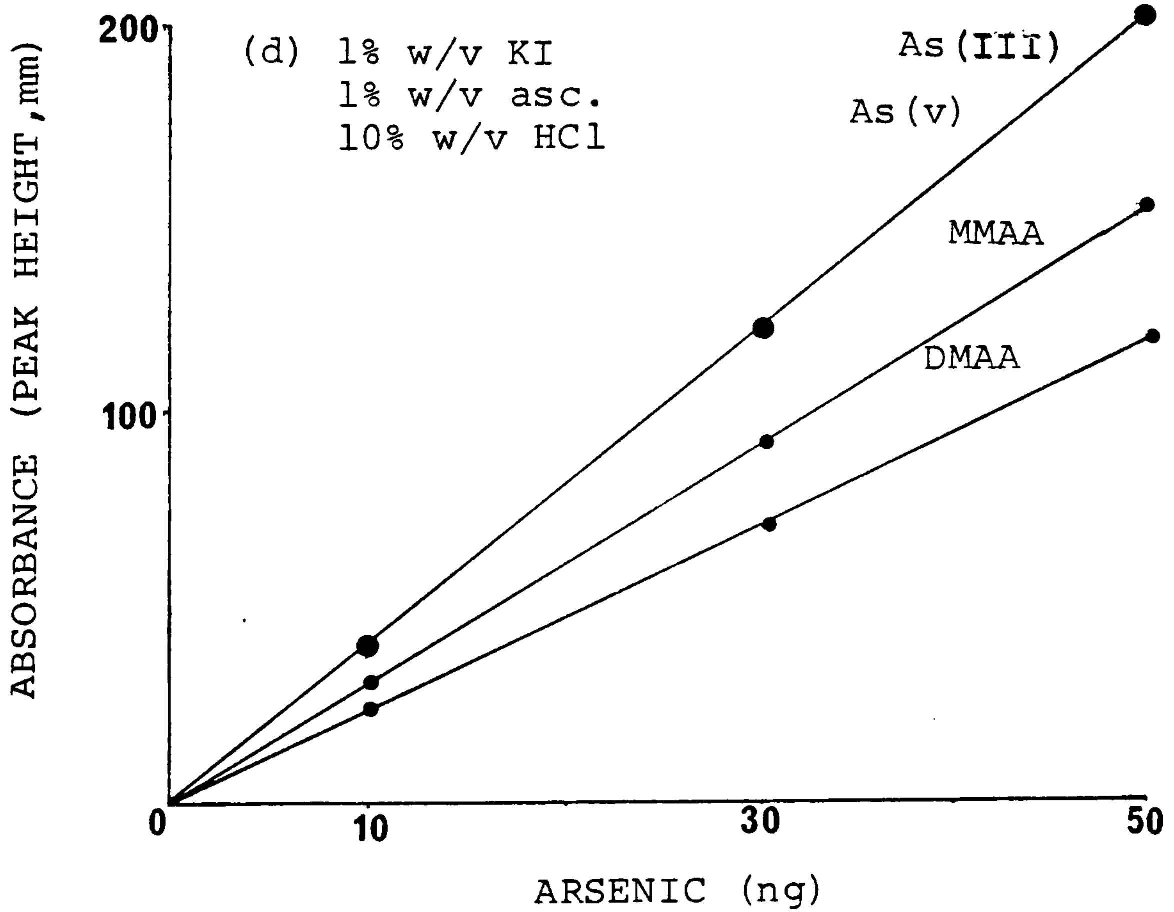


Figure 2.7 contd. The effect of pre-reduction solutions (c) and (d) on As(III), As(V), MMAA and DMAA signal response

As(III) oxidation state in a solution made 1% w/v with respect to KI, 1% w/v w.r.t. ascorbic acid and 6% w/v w.r.t. HCl.

The validity of the above method was tested by the determination of arsenic in an IAEA/W-4 freshwater interlaboratory comparison standard. When pre-reduced and analysed against As(III) standards, an arsenic concentration of 23.4 ± 1.8ug/l (n = 5) was obtained, and of 25.5 ± 1.2ug/l (n = 9), when analysed non pre-reduced against As(V) standards, in good agreement with the certified value of 25.0ug/l (Pszonicki et al., 1985).

2.3.5 Sensitivity and detection limits

Sensitivity is defined as the quantity of an element required to produce a signal of 1% absorption, i.e. 0.0044 absorbance units. As long as measurements are made within the linear range, sensitivity can be determined by reading the absorption produced by a known quantity of the element and solving:

$$\frac{\text{quantity of the element}}{\text{measured absorption}} = \frac{\text{sensitivity}}{0.0044}$$

$$\text{sensitivity} = \frac{\text{quantity of element} \times 0.0044}{\text{measured absorption}}$$

Using the MHS-10 unit, it was noted that a 50ng As(III) standard produced, on average, an absorbance of 0.4 units,

thus:

$$\text{sensitivity} = \frac{\text{As standard} \times 0.0044}{\text{measured absorbance}}$$

$$= \frac{50 \times 0.0044}{0.4}$$

$$= \underline{0.55\text{ng}}$$

This compares favourably with a sensitivity of 0.63ng and 0.47ng obtained for a 50ng standard by Peats (1979) and Welz and Melcher (1983) respectively and 0.95ng quoted by Perkin Elmer.

The power of detection for AAS is conventionally expressed as the lower limit of detection of the elment of interest. A theoretical detection limit can be calculated from the mean and standard deviation of 10 successive 'blank' determinations (i.e. 10ml of 1.5% w/v HCl), from the following equation (Ebdon 1982):

$$\text{Detection limit} = X_{bl} + 2S_{bl}$$

X_{bl} = mean of blank determinations
S_{bl} = mean of standard deviation on blank determinations.

Such a calculation yielded a detection limit of 0.94ng for the set up in this laboratory. Practically a detection limit can also be quoted as being equal to a signal:noise ratio of 2:1 taking into account the 'background noise' produced.

For the analysis of water samples with very low arsenic concentrations a larger sample volume can be used, care being taken that blanks and standards are in the same volume.

2.4 DETERMINATION OF THE TOTAL ARSENIC CONCENTRATION IN BIOLOGICAL AND SEDIMENT SAMPLES

For arsenic determination by HGAAS, the arsenic species have to be in a dissolved form. Thus solid material such as animal tissues, herbage and sediment samples and human urine samples must be decomposed to release the chemically bound arsenic into solution. The pre-treatment method chosen e.g. wet or dry ashing, depends upon the type of sample material to be analysed.

2.4.1 Determination of the total arsenic concentration in biological material

The decomposition method preceding the determination of the total arsenic concentration in material of biological origin must be sufficiently vigorous to break down any stable organoarsenical compounds present (Section 1.2), but at the same time avoid the loss of such a volatile element (Talmi and Feldman, 1975; Agemian and Thomson, 1980). The method should also be easy to use, safe and sufficiently rapid for the routine laboratory analysis of a large number of samples. The procedure favoured by many authors is to digest the sample by heating with a combination of the strong oxidising acids, nitric acid HNO_3, sulphuric acid H_2SO_4, perchloric acid $HClO_4$ and hydrogen peroxide

H_2O_2. HCl cannot be used because of the formation of volatile arsenic trichloride when the solutions are heated (Arafat and Glooschenko, 1981). Thompson and Thoresby (1977) found that nitric and sulphuric acids were sufficient to decompose plant material, whereas Lunde (1973) and Whyte and Englar (1983) found it necessary to digest algae with nitric acid and hydrogen peroxide. Arafat and Glooschenko (1981) utilised a combination of nitric& sulphuric acid + hydrogen peroxide on plant material and these were the acids used for the decomposition of food samples by Evans et al. (1979). Nitric, sulphuric and perchloric acids were found to be necessary for the digestion of biological material of marine origin, especially fish and shellfish samples (Agemian and Thompson, 1980; Edmunds and Francesconi, 1981c; Maher, 1983a, 1983b; Welz and Melcher, 1985), and for urine samples (Norin and Vahter, 1981). In view of the range of possible acid combinations available, it was necessary to find a single method that could be used in this laboratory for both human and environmental samples.

2.4.1.1 Acid digestion experiments

Five different methods were tested on a number of National Bureau of Standards (NBS) Standard Reference Materials (SRM's), for which the arsenic content is known. All involved the addition of 10ml

of the appropriate acid mixture (Aristar grade) to 0.25g (dry weight) of the standard in 125ml Erlenmeyer flasks to which air condensors could be fixed. The air condensors were necessary during the subsequent heating stage to promote refluxing of the acids and hence maintain and maximise the oxidation process, thereby preventing loss of arsenic. All samples were allowed to stand overnight in the acid mixture at ambient temperature (previous tests had shown that this improved the results), and the following day the flasks were heated on a hot plate until it was thought the digestion was complete. For each digested sample, aliquots (volume dependent upon probable arsenic concentration) were abstracted and made up to 10ml with 1.5% w/v HCl and analysed for the total arsenic concentration by HGAAS. The use of strong oxidising acids ensured that all the arsenic present was of the higher As(V) oxidation state, so that the arsenic concentration of the digested solutions could be obtained by comparison with As(V) standard solutions in HGAAS. Table 2.4 shows the results of initial tests using National Bureau of Standards orchard leaves SRM 1571 and oyster tissue SRM 1566. The values obtained for the orchard leaves were closer to the certified results than for the oyster tissue, but both suffered poor recoveries.

TABLE 2.4

RESULTS OF INITIAL ACID DIGESTION EXPERIMENTS USING NBS ORCHARD LEAVES SRM 1571 AND OYSTER TISSUE SRM 1566.

	ARSENIC CONCENTRATION mg/kg*	
	oyster tissue	orchard leaves
SRM certified value	13.4±1.9	10.3±2.0
Digestion mixture		
$HNO_3:H_2SO_4$		
2:1	3.5	9.3
$HNO_3:HClO_4$		
4:1	2.8	7.7
$HNO_3:H_2SO_4:HClO_4$		
7:1:2	3.4	9.4
$HNO_3:H_2SO_4:HClO_4$		
5:1:3	1.7	4.9
$HNO_3:H_2SO_4:H_2O_2$		
3:2:drops	4.5	8.6

* all values corrected for moisture content.

Possible causes considered were:

a) Loss of volatile arsenic compounds

b) Interference in arsenic determination by the remaining concentrated acids.

c) Incomplete digestion of the stable organoarsenic compounds especially for the marine biological material.

Each of these possibilities is discussed below:

a) <u>Possible loss of analyte</u>

To determine whether the poor recoveries were due to the loss of any volatile arsenic compounds, each of the reagent mixtures was 'spiked' with 3ug of As(V) standard and taken through the whole digestion procedure. A 3ug spike, once made up to a final volume of 25ml, corresponds to 120ng/ml in the 25ml. When 1ml of the 25ml is abstracted for arsenic determination and diluted 1 in 2 , there should be 60ng arsenic per ml of final solution for measurement.

The results of such a spiking experiment proved that there was insignificant loss of arsenic during the digestion process.

b) <u>Acid interferences</u>

Kang and Valentine (1977) reported that the strong oxidising acids in a sample solution may inhibit the reduction of As(V) (the arsenic species present as a result of the oxidation) to arsine after the addition

of sodium borohydride in the hydride generation stage. To assess the effect of possible acid interference, a series of simple tests were carried out with a range of the acid concentrations (1 - 20% v/v) in the 10ml sample volume in the reaction vessel using As(V) standard solutions. Figure 2.8 shows that with acid concentrations above 4% v/v, (i.e. 0.4ml concentrated acid per 10ml of solution) there was a significant depression of the As(V) peak height. Sulphuric acid had the greatest effect with a 23% reduction at an acid concentration of 10% v/v, confirming the results of Kang and Valentine (1977), who found that acid interference decreased in the order $H_2SO_4 > HNO_3 > HClO_4$. The results obtained from the above experiments show that if the acid concentration is 4% v/v or less in the final solution for arsenic determination (25ml), then there is minimal effect on arsine generation. Thus the final acid volume after digestion is reduced to 1 - 2ml by continued heating. If 1ml of the 25ml is abstracted and made up to the required 10ml for arsenic determination, then the final acid concentration in the sample matrix is only 0.4 - 0.8% v/v with respect to these acids.

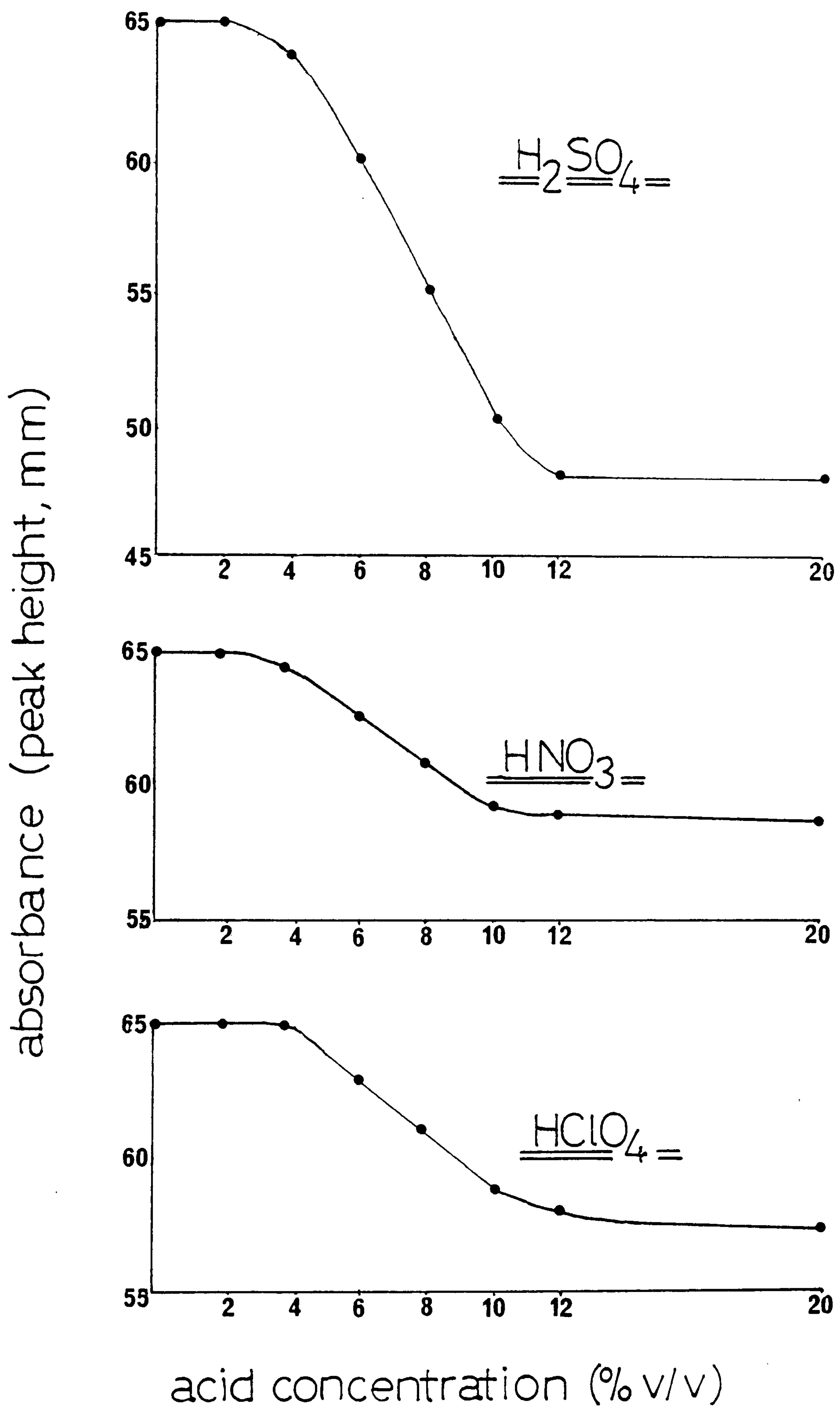


Figure 2.8 The effect of oxidising acid concentration on As(V) signal response by HGAAS

c) <u>Incomplete digestion</u>

It was noted that, by continued heating in an effort to reduce the acid volume, the digests passed through a vigorous effervescence stage in which dense white fumes of sulphur trioxide and perchloric acid were produced. Table 2.5 shows the results obtained by allowing each of the methods to pass through such a stage in their digestion. By allowing the digestion procedure to go to completion with the production of the dense white fumes, the binary and ternary acid mixtures were sufficient for the complete oxidation of the NBS orchard leaves, but the ternary mixture with either perchloric or hydrogen peroxide was necessary for the complete digestion of the oyster tissue. Most of the arsenic within the orchard leaves is later shown to be of an inorganic form (Section 2.5.1), which can be readily digested. It is thought that, during the vigorous digestion stage, the highly oxidising environment of the perchloric acid or hydrogen peroxide is necessary for the oxidation of the resistant fatty tissue and organoarsenic species typical of marine biological material (Fiorino et al., 1976; Agemian and Thomson, 1980; Subramanian and Meranger, 1982). Digestion mixtures using perchloric acid were preferred to that with hydrogen peroxide as this was added in the latter stages of the digestion procedure and was considered to be dangerous when using hot acids.

TABLE 2.5

ARSENIC CONCENTRATION OF NBS ORCHARD LEAVES SRM 1571 AND OYSTER TISSUE SRM 1566 BY HGAAS FOLLOWING THE VIGOROUS ACID DIGESTION PRE-TREATMENT

	ARSENIC CONCENTRATION mg/kg*	
	oyster tissue	orchard leaves
SRM certified value	13.4 ± 1.9	10.3 ± 2.0
Digestion mixture		
1) $HNO_3:H_2SO_4$		
2:1	6.8	9.8
2) $HNO_3:HClO_4$		
4:1	6.5	10.4
3) $HNO_3:H_2SO_4:HClO_4$		
7:1:2	13.1	10.9
4) $HNO_3:H_2SO_4:HClO_4$		
5:1:3	13.7	11.4
5) $HNO_3:H_2SO_4:H_2O_2$		
3:2:drops	12.4	10.1

* = all values corrected for the moisture content.

Perchloric acid was used in both methods 3) and 4) with similar results, but 4) was chosen in preference to 3) as a smaller proportion of nitric acid was required. Thus to obtain the total arsenic concentration in all biological materials in this study (i.e. vegetation, plankton, fish, shellfish and human urine), samples were digested with a mixture of nitric, sulphuric and perchloric acids in the following manner:

1) Approximately 0.25g sample (freeze-dried) was weighed (or 10ml urine sample was pipetted) into a 125ml Erlenmeyer flask.
2) 10ml of the acid mixture $HNO_3:H_2SO_4:HClO_4$ in the ratio - 5:1:3 was added, the flasks stoppered and allowed to stand overnight at room temperature.
3) The following day the stoppers were replaced with air condensors and the flasks heated gently (approximately 100 - 130°C) on a hot plate for half an hour.
4) The temperature was increased to approximately 170°C and continued until brown fumes of nitrogen dioxide ceased to evolve (approximately two hours) and a colourless solution was obtained.

5) When cooled, the contents from the walls of the air condensors were washed into the flasks with purified water (Section 2.1.3), the air condensors were then removed.

6) The flasks were re-heated rapidly until dense white fumes appeared. Heating was continued to reduce the acid volume to 1 - 2ml, care being taken not to char the samples.

7) When cooled, the digests were made up to a final volume of 25ml in volumetric flasks with purified water.

8) Sample aliquots (usually 1ml from the 25ml) were made up to 10ml with 1.5% w/v HCl and analysed against As(V) standards by HGAAS.

9) The samples were stored in HNO_3 rinsed 30ml sterilin containers.

2.4.1.2 <u>Pre-reduction of digested sample solutions</u>

For samples with very low arsenic concentrations, detection sensitivity could be improved by the reduction of the As(V) present to the As(III) oxidation state with the acidified potassium iodide solution (Section 2.3.4) and measurement against As(III) standards. It was found that the 1% w/v KI, 1% w/v ascorbic acid and 6% w/v HCl pre-reduction solution used on the simple aqueous standards in Section 2.3.4 was insufficient to complete the reduction process in the digested

solutions in the hitherto satisfactory 60 minute period. The solutions had to be left standing for 3.5 hours before the reduction was complete (Table 2.6). It was thought that the 1% w/v KI solution was not sufficiently concentrated to bring about the reduction in the presence of the strong oxidising acids. Reduction with higher potassium iodide concentrations (5 and 10% w/v KI) was tested on the digested sample solutions. Table 2.6 shows that a 10% w/v KI pre-reduction solution was necessary to convert the arsenic in the digests to the As(III) oxidation state prior to analysis. Thus, once the sample digests were made up to 25ml in the volumetric flasks, aliquots were abstracted (depending on estimated arsenic concentration) and made up to 10ml (10% w/v with respect to KI, 1% w.r.t. ascorbic acid and 6% w/v w.r.t. HCl) from which aliquots were abstracted (usually 1ml) for arsenic analysis by HGAAS.

2.4.1.3 International atomic energy authority (IAEA) inter-laboratory comparison material

The digestion procedure established with nitric, sulphuric and perchloric acids was used in the analysis of samples of unknown arsenic concentration distributed by the IAEA for the purpose of inter-laboratory comparison.

TABLE 2.6

PRE-REDUCTION OF DIGESTED SAMPLE SOLUTIONS WITH ACIDIFIED POTASSIUM IODIDE SOLUTIONS

	ARSENIC CONCENTRATION mg/kg*					
	oyster tissue SRM 1566			orchard leaves SRM 1571		
Certified value	13.4 + 1.9			10.4 + 2.8		
1% KI solution	left to stand for x hours					
	1	1.75	3.5	1	1.75	3.5
	8.6	10.8	12.9	9.0	9.5	10.2
5% KI solution	left to stand for 1 hour					
	11.9 ± 0.8 (n = 4)			9.9 ± 1.1 (n = 4)		
10% KI solution	left to stand for 1 hour					
	13.3 ± 0.5 (n = 4)			11.3 ± 0.8 (n = 4)		

* = all values corrected for the moisture content

IAEA Mussel tissue MA-M-2/TM (1983)

Six separate determinations were made using 0.1 - 0.25g (dry weight), along with certified standard reference materials and blank solutions (Table 2.7). The moisture content, determined on a separate sub-sample after drying for 48 hours at 85°C as recommended, was found to be 6.6% by weight. The certified result was obtained at a later date in report No. 26 of the IAEA intercalibration on marine samples and was "certified with satisfactory degree of confidence" at a value of 12.8mg/kg (dry weight), with a confidence interval at the 0.05 significance level of 11.8 - 14.4mg/kg, in exellent agreement with the mean value of 13.2 ± 0.5mg/kg obtained here.

IAEA shrimp tissue MA-A-3/TM

Again six separate determinations (mean 32.4 ± 1.1mg/kg) were made along with the digestion of albacore tuna, oyster tissue, mussel tissue and blank solutions (Table 2.8). The moisture content was found to be 6.4% by weight. The results of this intercomparison exercise are awaited.

TABLE 2.7

DETERMINATION OF THE TOTAL ARSENIC CONCENTRATION IN IAEA MUSSEL TISSUE MA-M-2/TM

	ARSENIC CONCENTRATION mg/kg*		
	Oyster tissue SRM 1566	Mussel tissue IAEA MA-M-2/TM	Orchard leaves SRM 1577
Pre-reduced	13.9	13.5	11.7
	12.5	13.4	10.9
	13.0	13.7	11.0
		12.6	
		12.0	
		12.8	
Non pre-reduced	13.3	13.9	12.6
	14.0	13.2	11.6
	12.6	13.3	11.7
		13.3	
		13.5	
		12.9	
Mean obtained	13.2±0.6	13.2±0.5	11.6±0.6
certified	13.4±1.9		10.3±2.0

* = all values corrected for the moisture content

TABLE 2.8

DETERMINATION OF THE TOTAL ARSENIC CONCENTRATION IN IAEA SHRIMP TISSUE MA-A-3/TM

SAMPLE			ARSENIC CONCENTRATION mg/kg*
Shrimp MA-A-3/TM	1		31.8
	2		31.4
	3		33.0
	4		31.1
	5		33.1
	6		33.7
		mean	32.4 ± 1.1
Albacore tuna SRM 50			2.9 (cert = 3.3 ± 0.4)
Oyster tissue SRM 1566			12.7 (cert = 13.3 ± 1.9)
Mussel tissue IAEA MA-M-2/TM			12.8 (cert = 12.8 ± 1.3)

* = corrected for the moisture content

2.4.2 <u>Determination of the total arsenic concentration in human urine samples</u>

As discussed above, the ternary acid digestion technique decomposes the stable organoarsenicals, such as arsenobetaine, present in biological samples e.g. fish and shellfish (Section 1.2). Accordingly, the total arsenic concentration can be readily determined in urine samples containing these stable forms of arsenic as a result of the recent consumption of fish.

Analysis was made of the total arsenic concentration in NBS freeze-dried urine sample SRM 2670. The freeze-dried sample could be stored at a temperature between 4 - 8°C, but once constituted with 20ml of purified water it was considered as fresh urine and had to be used immediately. The sample provided had an arsenic concentration certified at 0.48ug/ml (480ug/l) and was considered to have an elevated arsenic concentration with respect to a normal urine sample (Section 4.2.3, Table 4.3). Using the digestion procedure as outlined in Section 2.4.1.1 the total arsenic concentration was found to be 0.52ug/ml, which was considered to be in satisfactory agreement with the certified value.

However, in the assessment of possible occupational or environmental exposure to inorganic arsenic, it is important to distinguish between the above dietary forms and those likely to result from exposure to inorganic arsenic (e.g. As(V), As(III) and

metabolites MMAA and DMAA) (Sections 1.3.1, 4.3.7 and 4.4.3). Whereas the latter can form hydrides directly from an acidified sample solution, the stable organoarsenicals (e.g. arsenobetaine) do not. This affords an opportunity for the rapid screening of urine samples to estimate a range for the <u>sum</u> of the four species (As(V), As(III), MMAA and DMAA), free from interference by arsenic of dietary origin, using the following procedure.

1) 1ml of the urine sample (diluted if required with 1.5% w/v HCl), was added to 9ml of the 1.5% w/v HCl (plus 1 - 2 drops of the anti-foaming agent Tri-n-butyl phosphate), (see below) in the reaction vessel
2) Standard determinations were made with 10 - 50ng As(III) or DMAA in 10ml of 1.5% w/v HCl (plus 1 - 2 drops of the anti-foaming agent.
3) Blank determinations were made with 10ml of 1.5% w/v HCl plus 1 - 2 drops of the anti-foaming agent.

If no precaution against sample foaming is taken, then the addition of sodium borohydride to an acidified urine sample can result in the transfer of the solution into the quartz tube, making analysis impossible and causing severe contamination of the quartz cell. Foam formation can also result in

signal suppression by retention in the foam of some of the hydride generated (Weltz and Melcher, 1979). As shown in Section 2.3.3, DMAA gave the lowest response at approximately 60% of the As(III) signal (greatest response); thus arsenic concentrations can only be quoted between these two extremes, as measured against the corresponding standards. During analysis, it was noted that the sensitivity of both standards often deteriorated, making it necessary to carry out frequent standard determinations. It was thought that a decline in the sensitivity was the result of slight contamination of the inner surface of the quartz tube from the untreated urine samples.

2.4.3 Determination of the total arsenic concentration in sediment samples

Many methods used to decompose soil and sediment samples involve either the use of hydrogen fluoride (HF) in acid mixtures to completely digest the silicate material (Feldman, 1977; Lovell and Farmer, 1983; Thompson and Thoresby, 1977) or acid digestion under pressure (Van der Veen et al., 1985). Dry ashing with magnesiun nitrate ($Mg(NO_3)_2$) in a muffle furnace has been used by a number of authors for the determination of the total arsenic content of such materials (Pahlavanpour et al., 1980, 1984; Oatey and Thornton, 1984) and was the technique employed in a previous study on the arsenic

content of garden soils and housedusts from the south west of England (Johnson, 1983). The method was shown to be simple, rapid and easily applicable to the analysis of a large number of samples on a routine basis. The method combines well with hydride generation as the ashed residue quickly dissolves in the reaction medium - hydrochloric acid, and the production of arsine is unaffected by high concentrations of the magnesium ions (Pahlavanpour et al., 1984). The method involves a two part sample decomposition:

1) an ashing stage for the destruction of the organic matter in the presence of magnesium nitrate at 450°C, followed by,
2) an acid attack (concentrated hydrochloric acid) at ambient temperature for the dissolution of the ash and solubilisation of the inorganically bound analytes.

Digestion procedure used

1) Approximately 0.25g (dry weight) of sediment sample (dried in a drying cabinet at $\leqslant$ 30°C) was weighed into a marked pyrex beaker.
2) Four ml of a 25% w/v $Mg(NO_3)_2$ solution were added to each beaker, care being taken that no 'blowing' of the dry sample occured A few drops of a soap solution were used to break the surface tension, if required, to ensure complete wetting

of the sample.

3) Once the samples were thoroughly mixed (to prevent uneven heating), the beakers were placed in a muffle furnace pre-heated to 100°C. (Pre-heating was necessary to prevent the temperature rising above 100°C). This temperature was maintained for a minimum of four hours or overnight as convenient.

4) After that the temperature was increased to 450°C, and maintained at that temperature for a minimum of four hours.

5) When ashing was complete, the beakers were removed from the furnace to aid their rapid cooling. When cooled, 5ml of concentrated HCl were added. The beakers were covered with plastic film and allowed to shake on an orbital shaker overnight.

6) After removal of the plastic film the samples were filtered through Whatman filter papers (ashless, standard grade 40, 4.5cm) into 25ml volumetric flasks, rinsing through with purified water, and made up to 25ml.

7) Sample aliquots were abstracted and pre-reduced in a 10ml solution made 1% w/v with respect to KI, 1% w/v w.r.t. ascorbic acid and 6% w/v w.r.t. HCl from which aliquots were abstracted (usually 1ml) for arsenic analysis by HGAAS.

8) The same procedure was applied to standard reference materials and to blank solutions.

Table 2.9 shows the results obtained for the digestion of several standard reference materials as well as some Loch Lomond sediment samples previously analysed by an alternative method incorporating treatment with hydrogen fluoride (Lovell and Farmer, 1983). From the table it can be seen that the measured arsenic concentrations agreed with the certified concentrations showing that there had been complete digestion of the sample with magnesium nitrate in the muffle furnace. This digestion procedure was employed for all sediment samples prior to arsenic determination by HGAAS.

2.5 ANALYTICAL SPECIATION OF ARSENIC

It has been established that inorganic arsenic is more toxic than the organic forms and that biomethylation of inorganic to organic arsenic in the food chain acts as a detoxification process (Lunde, 1975; Edmunds and Francesconi, 1977; Flanjak, 1982), (Section 1.2). It is therefore necessary to establish the chemical form of the arsenic compounds in both human and environmental samples in view of their differing toxicities and the need to understand the processes involved in the interconversions of such forms.

TABLE 2.9

DETERMINATION OF THE TOTAL ARSENIC CONCENTRATIOM IN A NUMBER OF STANDARD REFERENCE MATERIALS BY HGAAS AFTER ASHING WITH MAGNESIUM NITRATE IN A MUFFLE FURNACE

Sample	ARSENIC CONCENTRATION mg/kg measured concentration	certified concentration
NBS River sediment SRM 1645	66.9 ± 0.3 (n = 2)	66
IAEA Lake sediment SL-1	25.3 ± 1.7 (n = 9)	27.5±2.9
IAEA Soil-5	87.9 ± 3.9 (n = 5)	93.9±7.5
IAEA SD-N-1	52.4 ± 3.0 (n = 2)	52

Loch Lomond core 3	HF alternative method	$Mg(NO_3)_2$ method
0 - 1 cm section	48	46
1 - 2 cm section	424	442
15 - 16 cm section	22	27

2.5.1 Biological samples of environmental origin: inorganic/organic arsenic speciation

The total arsenic concentration in biological material can be readily established by HGAAS after the acid digestion method described in Section 2.4.1. As stated by Whyte and Englar (1983), there have been few detailed analyses on the proportions of inorganic (As(V) + As(III)) and organic arsenic (MMAA + DMAA + the stable organoarsenicals) in environmental samples. However, Lunde (1973) developed a method that can separate inorganic from organic arsenic in marine organisms based upon the stability of the organoarsenic compounds in the presence of 6.6M HCl at 100°C. Under such treatment, inorganic arsenic forms volatile arsenic trichloride ($AsCl_3$). Thus inorganic arsenic can be removed by distillation as $AsCl_3$ under conditions where stable organoarsenic compounds are not volatile. Such a method has been applied to the speciation of arsenic in marine brown kelp (Whyte and Englar, 1983) and in fish tissue (Brook and Evans, 1981; Flanjak, 1982). The same method was adopted in this laboratory to establish the inorganic/organic arsenic concentrations in freshwater plant and marine tissue, using the distillation apparatus shown in Figure 2.9 and the procedure outlined below:

1) Approximately 2g of the freeze-dried sample was weighed into a round bottomed distillation flask.

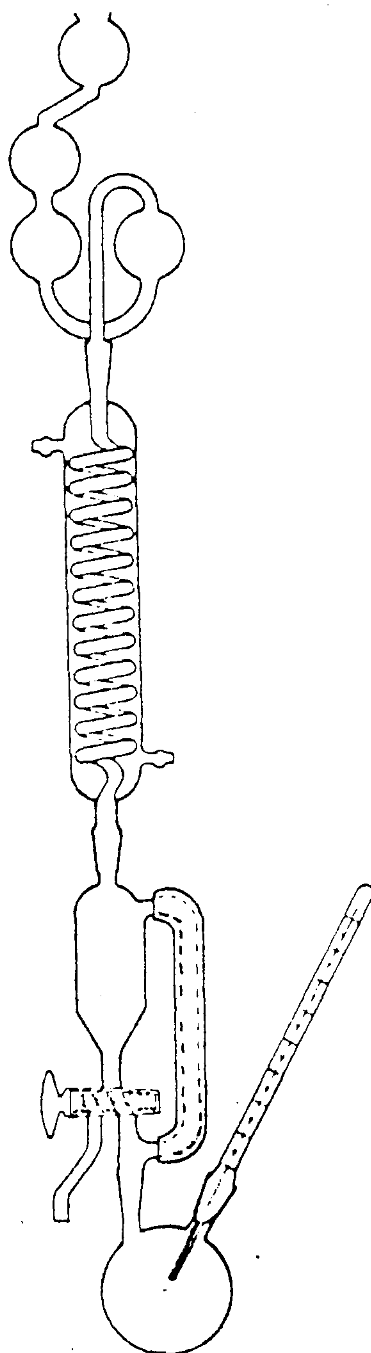


Figure 2.9 Apparatus used for the distillation of inorganic arsenic (Lunde, 1973).

2) 50ml of acidified ferrous chloride solution (2g Fe^{2+} in 50ml of 6.6M HCl) was added to the flask + a few boiling granules to assist even heating (Fe^{2+} is necessary as a reducing agent) (Brook and Evans, 1981).

3) With the flask connected to the condensing unit (making sure that all the joints were well greased and that the splash trap was just under half filled with purified water), a heating mantle was used to heat the flask with the tap in the receiver open.

4) The solution was allowed to boil and reflux for approximately 30 minutes with the temperature maintained between 100 - 108°C.

5) With the tap in the receiver closed, the fraction that distilled over between 108 - 110°C was collected and drained off when approximately two thirds (33ml) had been collected. (The temperature must be maintained below 114°C to prevent distillation of the organic material).

6) A further 50ml of acidified ferrous solution was added through the thermometer opening and the distillation procedure repeated to provide a further 30 - 35ml of condensate.

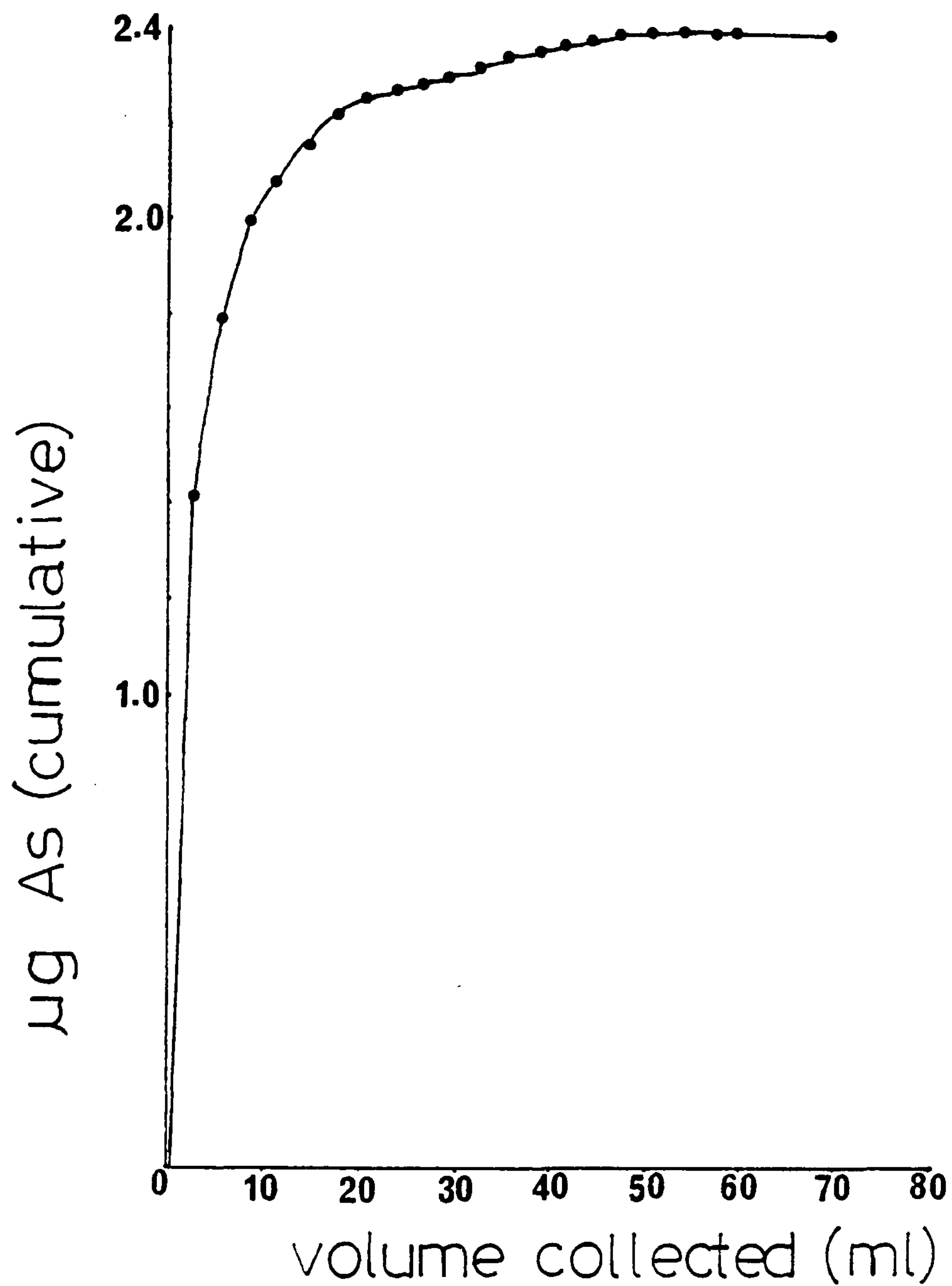
7) Once the distillation had finished and the unit cooled down, the two condensates were combined together with the contents of the splash trap and washings from the condensing unit.

8) Aliquots were abstracted and pre-reduced in a 10ml solution made to be 10% w/v with respect to KI for arsenic determination by HGAAS against As(III) standards

Initially the distillate was collected in 3ml fractions and analysed separately so that the course of distillation could be determined. Two examples of this are shown for NBS orchard leaves SRM 1571, and for prawn tissue purchased from a fishmongers. (Appendix 1.1 and 1.2 , and Figures 2.10 and 2.11 respectively).

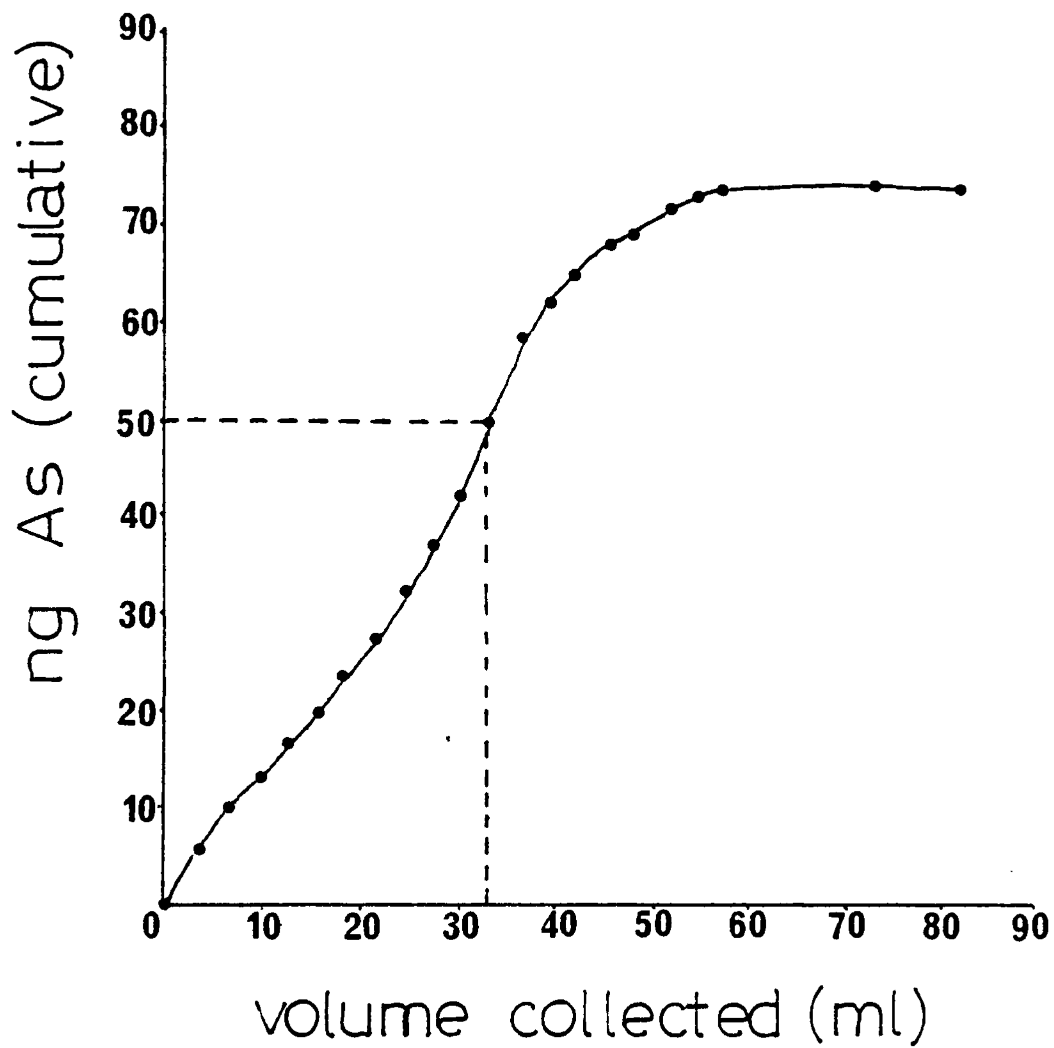
From Figures 2.10 and 2.11 it can be seen that most of the inorganic arsenic had distilled over in the first 50ml (96.7% for the orchard leaves in 33ml and 66.8% for the prawn tissue), but that a second distillation was necessary to complete the recovery of the inorganic arsenic. Appendix 1.1 and 1.2 show that very little arsenic was left remaining in the condensation unit and splash trap. Thus, for samples with a high percentage of inorganic arsenic, the distillation was almost complete after the addition of the first 50ml, but for samples with very little inorganic arsenic a more lengthy distillation was required. The results of such a distillation on a number of samples are presented in Table 2.10.

From the above it can be seen that the inorganic arsenic content as a percentage of the total (after digestion, Section 2.4.1) can be determined in a



Inorganic arsenic	=	2.398ug
sample weight	=	0.2058g
∴ Inorganic arsenic concentration	=	11.65ug/g

Figure 2.10 The cumulative inorganic arsenic collected from NBS orchard leaves (SRM 1571) in 3ml fractions after distillation with 6.6M HCl



Inorganic arsenic	=	76.6ng
Sample weight	=	0.3348g
∴ Inorganic arsenic concentration	=	228.9ng/g
	=	0.229 ug/g

Figure 2.11 The cumulative inorganic arsenic collected from prawn tissue in 3ml fractions after distillation with 6.6M HCl

TABLE 2.10

DETERMINATION OF THE INORGANIC ARSENIC CONTENT OF A SELECTED NUMBER OF BIOLOGICAL MATERIALS BY HGAAS AFTER DISTILLATION WITH 6.6M HCl

Sample	ARSENIC CONCENTRATION mg/kg* Inorganic arsenic	Total arsenic	As_i % of total
Prawn 1	0.20	13.9	1.4
2	0.23	13.9	1.7
NBS Oyster tissue	2.60	13.4	19.4
Cod	0.07	4.8	0.3
Haddock	0.02	3.7	0.1
Whiting	0.02	2.4	0.1
Loch Lomond plankton			
South basin	0.50	2.2	22.7
Mid basin	0.40	1.8	22.2
North basin	0.32	1.6	20.0
Pətamegetan Natens (leaf) (Loch Lomond sample)	0.12	0.7	17.1
NBS Orchard leaves	11.7	cert. 10.3 ± 2.0	100

Where As_i = inorganic arsenic concentration
* = corrected for the moisture content

variety of biological materials after distillation with 6.6M HCl. Under these conditions, organoarsenical compounds are stable, while inorganic arsenic forms the volatile trichloride which is distilled over and collected for arsenic determination. Of the materials analysed, fish samples of marine origin have the least inorganic arsenic content (except oyster tissue with 19.4%), while freshwater plankton have a significant 20 - 23%. The high inorganic arsenic content of NBS orchard leaves is thought to be the result of spraying with arsenical pesticides.

2.5.2 Water samples : As(V)/As(III) speciation

As shown in Section 2.3.3, the arsenic signal in arsine generation varies with the oxidation state of the dissolved inorganic species, the As(V) signal being 60 - 65% of the As(III) signal (Figure 2.6). Such a characteristic can be exploited in the analysis of water samples for inorganic arsenic but only where no other species capable of forming hydrides are present (Hinners, 1980). In Section 2.3.4, it was shown that the response from As(V) standard solutions was equal to As(III) standard solutions at the same arsenic concentration if the former were reduced with an acidified potassium iodide solution prior to analysis. Thus one sample aliquot can be pre-reduced (and allowed to stand for a minimum of one hour) and

analysed against As(III) standards, while a second aliquot is analysed against As(V) standards in a non pre-reduced form. If the arsenic concentration from the two separate determinations is the same, it is deduced that all the arsenic in the sample is in the As(V) form. Where the results differ, the sodium acetate/acetic acid pH buffering method of Aggett and Aspell (1976) can be used to prevent the release of arsine from any As(V) present, the resultant signal being attributable to As(III). These authors were able to show that generation of arsine from As(III) is essentially independent of pH in the region studied (pH 1 - 5), but that As(V) generation falls with increasing pH, becoming negligible at pH $>$ 3.5. The explanation for this behaviour appears to be that as the pH is raised the rate of the reduction reaction (As(V) to As(III)) decreases until eventually it becomes insignificant. To ensure the quantitative conversion of As(III) to arsine it is necessary to maintain the pH at a value no greater than 5.5. An acetate buffer (0.5M) was found to have sufficient buffering capacity (to within 0.25 pH units of the initial value), even on the addition of the alkaline sodium borohydride solution.

The method was used in the determination of the As(V)/As(III) concentrations in a number of natural mineral waters using a combination of the As(V) pre-reduction and pH suppression methods, in the

following manner: A 20ml sample aliquot (20ml used because of the low arsenic concentrations present in natural waters), was pre-reduced with 1ml of a 25% w/v KI, 2.5% w/v ascorbic acid and 4ml of concentrated HCl (thus making a final solution concentration of 1% w/v KI, 1% w/v ascorbic acid and 6% w/v HCl in 25ml), to convert all the arsenic to As(III) oxidation state. Calibration standards were prepared from sodium arsenite and were treated in the same way i.e. made up to 25ml, as were the blanks. A separate 20ml aliquot from the same sample was analysed non pre-reduced, but acidified with 1ml of concentrated HCl (to make a 1.5% w/v HCl matrix), against pentavalent arsenic As(V) standards (21ml) prepared from sodium arsenate. Aliquots (5ml) of the sodium acetate/acetic acid buffer solution (7ml aristar acetic acid, 17g sodium acetate trihydrate made up to 250ml) were added to a 20ml sample aliquot to give the correct pH. Standards and blank solutions were treated in the same way i.e. to give a final volume of 25ml.

MMAA and DMAA also form volatile hydrides but Farmer and Johnson (1985) were able to conclude that a comparison of the results obtained from the analysis of 23 bottled mineral waters using the above methods showed their contribution to be negligible. Complex organoarsenicals do not yield volatile hydrides under the above conditions; thus, even if present, they would not contribute to the arsenic concentration of

the sample. Appendix 2 shows the arsenic content of the bottled mineral waters analysed. Only seven exceeded 1ug/l, of which three were $>$ 10ug/l but only one (Vichy Célestins) $>$ 100ug/l. Arsenic was present as As(V) in 19 of the 23 mineral waters and predominantly as As(III) (determined from the suppression of As(V) at pH 5), in the other four (Champney's Pure Chiltern, Sainsbury's Cwm Dale, Perrier and Spa Marie Henriette), all $<$ 0.3ug/l.

When the pH dependent suppression method was applied to Vichy St Yorre, which was known to contain a relatively high arsenic concentration, no signal was produced. It was concluded that all the arsenic was in the oxidised As(V) state.

2.5.3 Water and urine samples : As(V), As(III), MMAA and DMAA speciation

The speciation methods described above were limited to the analysis of water samples for inorganic pentavalent and trivalent arsenic only. A speciation method was required to separate the four species of human and environmental significance (i.e. As(V), As(III), MMAA and DMAA), from each other and their surrounding medium.

Braman and Foreback (1973) trapped a mixture of the arsines in a liquid nitrogen cooled trap, which was then heated to selectively volatilise each of the arsines into an electric discharge emission

detector. The technique was later used by Howard and Arbab Zavar (1981) but using a quartz atomiser in the flame of an AAS for the detection. Separation based upon the differing volatility of the species was also used by Talmi and Bostick (1975) in a gas chromatographic technique.

Chakraborti and Irgolic (1983) utilised solvent extraction followed by an ion-exchange method to eventually achieve separation of As(V), As(III), MMAA and DMAA. In recent years the development of ion-exchange chromatography with HGAAS or GFAAS as the element-specific detection systems has proved to be a powerful technique for arsenic speciation in the liquid phase. A single cation-exchange column was used to separate arsenic species at low (ug/l) levels in natural water samples by Yamamoto (1975), Iverson et al. (1979), Wauchope and Yamamoto (1980) and Persson and Irgum (1982). Although these methods achieved separation of inorganic arsenic, MMAA and DMAA, no distinction was made between As(V) and As(III) in the samples. Pacey and Ford (1981) had to use a cation-exchange column followed by an anion-exchange column to complete the separation of the four species. A cation-exchange column was used to retain and then elute DMAA in 1.0M ammonium hydroxide (NH_4OH), while MMAA and As(V) were eluted from an anion exchange column with 0.5M and 1.0M acetate buffers respectively. As(III) concentration

was calculated by difference. This method was improved upon by Grabinski (1981), using a cation-exchange resin followed by an anion-exchange resin in series in the same column to achieve separation of all four species from a single sample aliquot placed on the column. A two-stage anion-exchange method was developed by Aggett and Kadwani (1983), but involved a complicated elution procedure with carbon dioxide.

The necessity to speciate the different chemical forms of arsenic has prompted the development of a technique that is relatively simple to use, rapid and highly sensitive. Based upon the separation by ion-exchange resins, high perfomance liquid chromatographic (HPLC) methods have been developed which have better resolution than conventional ion-exchange methods and can be linked, directly or indirectly, to GFAAS (Stockton and Irgolic, 1979; Brinckman et al., 1980; Woolson and Aharanson, 1980), to ICP (Morita et al., 1981; Irgolic et al., 1983; McCarthy et al., 1983) and to HGAAS (Tye et al., 1985). The ability to use high pressures (over 3000 psi) to elute the species from the column means that good separation can be achieved fairly rapidly (e.g. within 20 minutes) and with distinct separation of the species. Brinckman et al. (1980) separated the four species in drinking water and soil solution samples using a C_{18} reversed phase column, a method which

Woolson and Aharonson (1980) also applied to soil extracts and pesticide residues.

In view of the available technology, the method chosen in this laboratory for the separation of the four compounds was based on the ion-exchange method of Grabinski (1981), an adaptation of which had previously been used successfully in this laboratory for arsenic speciation in water and urine samples (Lovell and Farmer, 1985).

2.5.3.1 Column packing and conditioning

10cm of the anion-exchange resin AG1-X8 (100 - 200 mesh) was slurry packed into the base of a 36cm X 1cm glass column fitted with a 100ml reservoir and allowed to settle. With approximately 1cm of water remaining on top of the resin, the cation-exchange resin AG 50W-X8 (100 - 200 mesh) was similarly packed into the remaining 26cm of the column. Care was taken a) not to allow air bubbles to become trapped at the junction of the two resins, and b) that there was minimal mixing of the two resins. Using increased pressures supplied by an external argon source, 70ml each of 1.0M NH_4OH, 1.0M HCl and 0.48M HCl were passed through the column in succession. The passage of these conditioning solvents through the column caused a re-settling of the packing material. To prevent irreversible adsorption of the arsenic species from a sample onto the column, the resins were first

exposed to 50ug of each of the species. The column was then washed through three times with the series of conditioning solutions. These solutions were also used to regenerate the column after every sample run and if the column became dried out through non-use for a number of days.

2.5.3.2 Chromatographic procedure

The sample, in a volume not exceeding 4ml, was gravity loaded onto the column. The column was then eluted with 55ml 0.006M trichloroacetic acid (TCA) and then 8ml 0.2M TCA at a flow rate of 2ml/min, followed by 55ml 1.0M NH_4OH and 50ml 0.2M TCA at a flow rate of 6ml/min. The eluent was collected in 3ml fractions using a fraction collector. To determine which fractions contained which species, 500ng of each were placed on the column separately and all the fractions analysed.

2.5.3.3 Arsenic determination in fractions collected from the ion-exchange column

Each of the 3ml fractions containing As(V), MMAA and DMAA species was made up to 10ml with 1.5% w/v HCl for arsenic determination by HGAAS against the corresponding arsenic standards. A few drops of the antifoaming reagent tri-n-butyl phosphate were added to the fractions containing the DMAA species to prevent excessive foam formation. DMAA standards

were similarly treated. The sample fractions thought to contain the As(III) species were pre-reduced in 10ml of an acidified KI solution (i.e made 1% w/v with respect to KI, 1% w/v w.r.t. ascorbic acid and 6% w/v w.r.t. HCl), to prevent an underestimation of the As(III) concentration through possible oxidation of the species during storage of the separated fractions. Tests had shown that the presence of the eluting solvent in the sample matrix did not significantly effect the arsenic signal produced. By placing each of the species separately on the column, the following order of elution was established:

As(III):	fractions 3-10	equivalent to	9- 30ml
MMAA :	11-15		33- 45ml
As(V) :	21-26		63- 78ml
DMAA :	40-50		120-150ml

When a mixture containing 500ng of each of the species (in an initial 4ml sample volume) was eluted through the column, the separation achieved was as shown in Figure 2.12. It was found that by keeping the initial flow rate to a minimum of 1 - 2ml/min, thereby giving the species sufficient time to equilibrate with the resins, very good recoveries could be achieved. Once equilibrated, a faster flow rate could be used to elute the species from the

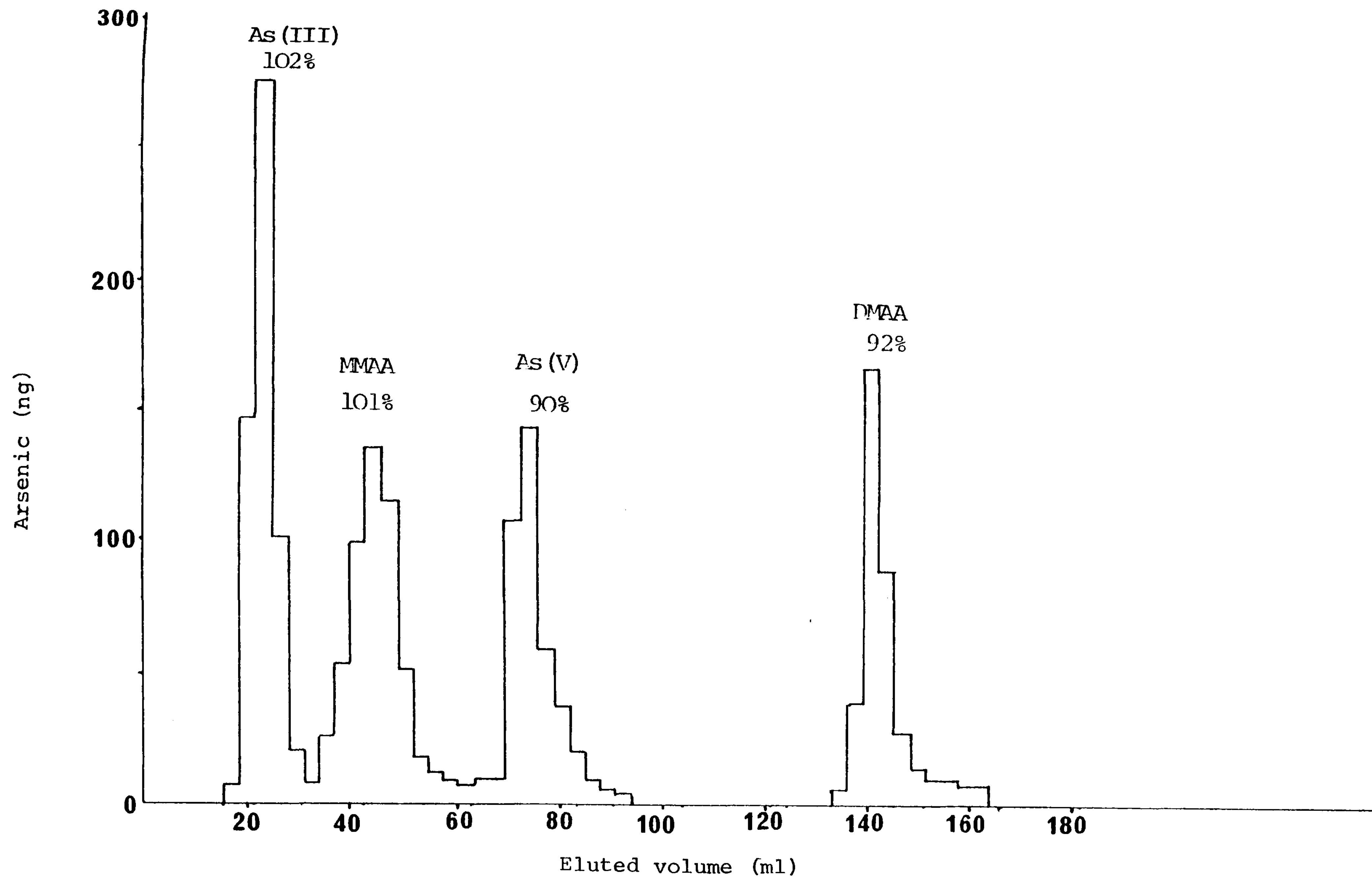


Figure 2.12 Chromatographic speciation of As(III), As(V), MMAA and DMAA by ion-exchange using a 4ml sample solution spiked with 500ng of each species

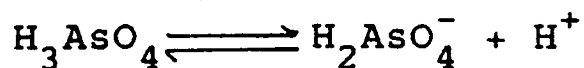
column, but care was taken not to exceed 6ml/min (4ml/min was preferred), to prevent poor resolution and consequential low recoveries. A 'blank', a 4ml purified water sample, was also eluted through the column. None of the arsenic species could be detected.

2.5.3.4 Theory of separation

The separation of the four species is based upon their differing first dissociation constants.

		pKa_1
Arsenous acid	As(III): $HAsO_2$	9.23
Arsenic acid	As(V) : H_3AsO_4	2.25
Monomethylarsonic acid	MMAA : $CH_3AsO(OH)_2$	4.26
Dimethylarsinic acid	DMAA : $(CH_3)_2AsO.OH$	6.25

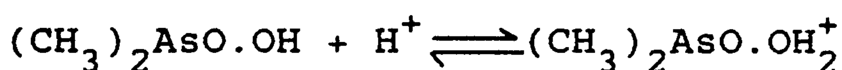
Thus, in the initial elution of the sample through the column with 0.006M TCA, arsenous acid, with a high pKa value of 9.23, remains undissociated and is not retained by either resin, but elutes rapidly from the column to be collected in the first 30ml. The low first pKa value of 2.25 for arsenic acid indicates that it is comparatively dissociated according to the following equilibrium reaction:



$$H_3AsO_4 \rightleftharpoons H_2AsO_4^- + H^+$$

and, as the anion species, is not retained by the

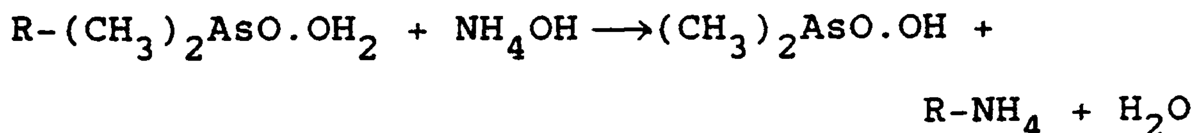
cation-exchange resin but by the anion-exchange resin. MMAA, with a pKa between As(III) and As(V), is weakly retained on the cation-exchange resin (probably as a result of its amphoteric nature (Dietz and Perez, 1976)), to be eluted from the column with 0.006M TCA in the 33 - 45ml fractions, while DMAA despite its higher pKa, is strongly retained on the cation-exchange resin, probably as the protonated species as a result of the following reaction:



$$(CH_3)_2AsO.OH + H^+ \rightleftharpoons (CH_3)_2AsO.OH_2^+$$

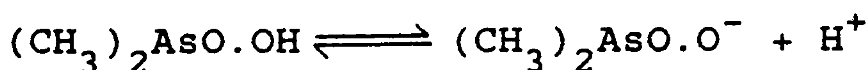
(Dietz and Perez, 1976).

The more concentrated 0.2M TCA is needed to provide sufficient H^+ to release arsenic acid from the anion exchange resin, eluting in the 63 - 78ml fractions. DMAA is stripped from the cation-exchange resin by the base 1.0M NH_4OH because of the strong affinity of the NH_4 ions for the cation resin. It was noted that elution with NH_4OH generates noticable heating of the resin because of the neutralisation reaction:



$$R\text{-}(CH_3)_2AsO.OH_2 + NH_4OH \longrightarrow (CH_3)_2AsO.OH + R\text{-}NH_4 + H_2O$$

DMAA dissociates:



$$(CH_3)_2AsO.OH \rightleftharpoons (CH_3)_2AsO.O^- + H^+$$

and attaches to the anion-exchange resin from which it is eluted with 0.2M TCA and collected in fractions 120 - 150ml.

2.5.3.5 Arsenic speciation in urine samples

To test the applicability of the method to the separation of arsenic in urine samples, a 4ml sample aliquot from a person who was known not to have been exposed to inorganic arsenic, MMAA or DMAA, was spiked with 50ng of each of the species to simulate the levels that could occur in the urine as a result of occupational exposure (Section 4.4.3). The resulting chromatogram is shown in Figure 2.13. Good recoveries were obtained. As there was no certified standard reference material for the four species in urine, comparison of the data obtained from samples supplied by the Health and Safety Executive (HSE) in London, where speciation is carried out using an HPLC/HGAAS technique, provided a means of validating both methods. Because As(V) concentrations in urine samples were often low, it was necessary to use 4ml of the sample even when the concentrations of the other species were very much higher. Table 2.11 shows the close agreement between the results and confirms the ability of the ion-exchange method to separate the four species satisfactorily.

2.5.3.6 The arsenic detection limit in ion-exchange chromatography/HGAAS

A detection limit of 0.5ug/l arsenic was obtained using the above system with a 4ml sample on

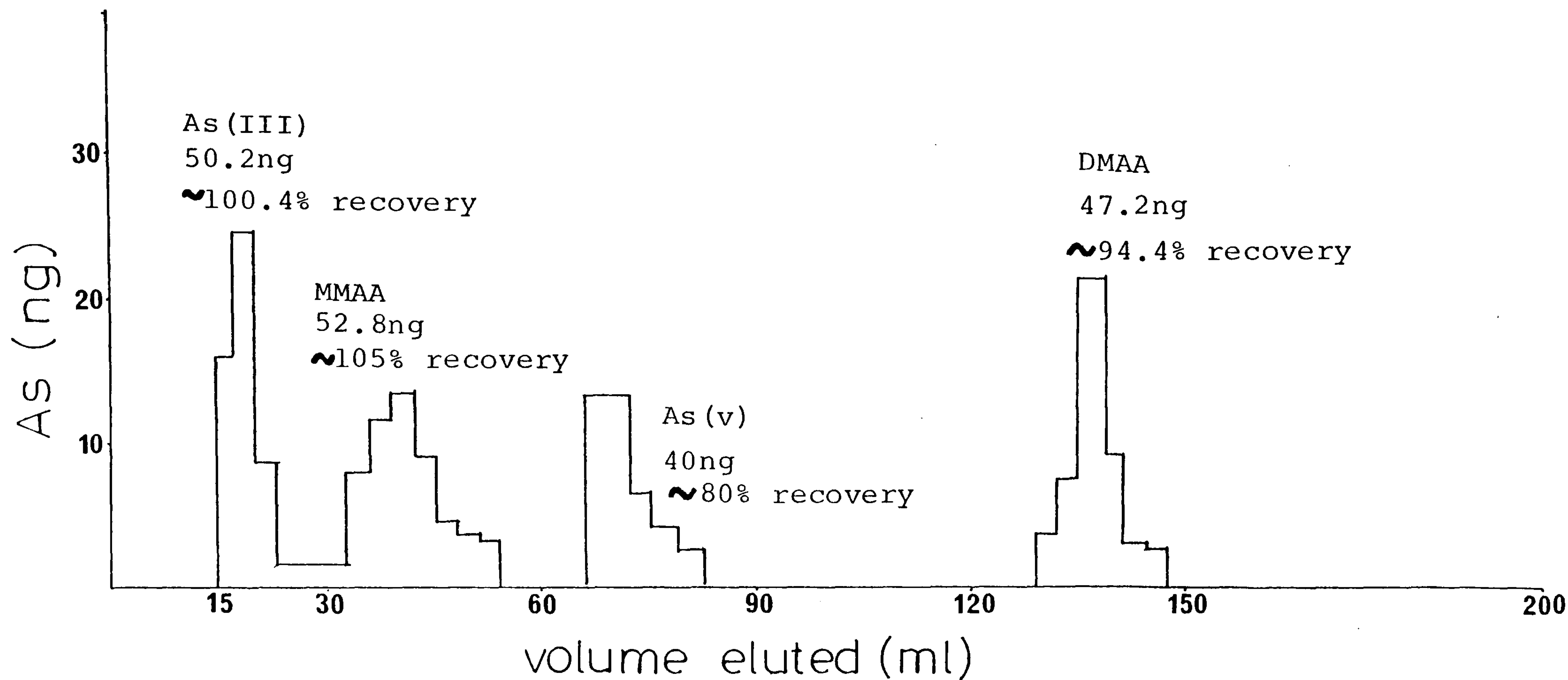


Figure 2.13 Speciation of As(V), As(III), MMAA and DMAA in a 4ml urine sample spiked with 50ng of each species.

TABLE 2.11

COMPARISON OF ARSENIC SPECIATION IN URINE SAMPLES BY HPLC AND ION-EXCHANGE CHROMATOGRAPHY (HGAAS DETECTION)

Sample		Total**	ARSENIC CONCENTRATION (ug/l) Speciation				
			As(V)	As(III)	MMAA	DMAA	SUM
HSE 6	*	305	4.1	32.0	36.8	198.8	271.7
		320	3.9	25.7	51.4	209.0	290.0
HSE 7	*	195	2.3	25.9	31.4	126.7	186.3
		178	2.6	22.9	35.1	113.2	173.8
		D	2.3	19.2	27.0	115.2	163.7
HSE 8	*	226	5.6	28.8	30.8	146.1	211.3
		207	5.0	25.4	36.0	134.4	200.8
		D	4.5	25.3	36.8	142.7	209.3
HSE 12	*	35	N.D	N.D	0.9	9.2	10.1
		45	0.5	0.5	0.5	9.0	9.0
HSE 14	*	98	N.D	12.8	17.6	40.8	71.2
		98	0.5	8.0	13.7	39.9	61.6

** = after acid digestion
* = analysed by the Health and Safety Executive (HSE)
D = analysed by ion exchange in duplicate.
N.D = non detectable i.e < 0.5ug/l (Section 2.5.3.6)

the column. From Figure 2.13 it can be seen that most of each arsenic species was eluted in three fractions (i.e. 98% for As(III), 64% for MMAA, 81% for As(V) and DMAA). Typically, about 50% of the arsenic is eluted in one fraction (the largest peak) and, with 1ng as the absolute limit of detection, 25% (or 0.5ng) arsenic would theoretically be eluted in each of the two fractions either side of the largest peak. Thus, a minimum of 2ng arsenic could be detected from a 4ml sample, equivalent to a detection limit of 0.5ng/ml.

2.6 ION CHROMATOGRAPHY

To investigate the possible influence of diagenetic mechanisms on arsenic speciation and distribution between the sediments and associated porewaters of Loch Lomond and the Dubh Lochan (Chapter 5), it was necessary to determine the concentration of other inorganic anions in the porewaters as well as the concentrations of the arsenic species. A measure of anions such as sulphate in each sample should give an indication of the change in redox conditions with sediment depth at a particular location.

Such analyses were carried out by ion chromatography using a Wescan model 268 ion analyser fitted with a 25cm resin-based anion-exchange column and employing electrical conductivity detection. 100ul sample aliquots were injected onto the column

(via a guard column protecting the separator column from damage caused by particulate matter) and eluted with 5 millimolar para-hydroxybenzoic acid at pH 8.6 at a flow rate of 2.0 ml/min. Separation of the anions is achieved by the exchange of ions between the mobile phase and the stationary phase, which means that the concentration of ions in the effluent remains constant but that their identity changes. Thus, when using electrical conductivity detection, sensitivity depends on the difference in equivalent conductance between the eluent and sample ions. Because sample ions are likely to be intermediate in equivalent conductance, the most useful eluent ions have either very high or very low equivalent conductance. In anion analysis this usually means large complex organic acids or their salts (low equivalent conductance), or hydroxide ion (high equivalent conductance) (Jupille 1985). Para-hydroxybenzoic acid has pKa values of 3.4 - 9.4 which makes it a good eluent in the pH range of 4.0 - 10.0, and allows control of retention as a function of pH, (Figure 2.14). Wescan recommend a pH of 8.6 for the resolution of the seven commonly encountered inorganic anions. In a single run the anions can be resolved from one another in the following order: fluoride, chloride, nitrite, bromide, nitrate, phosphate, sulphate, and a system peak at approximately 15

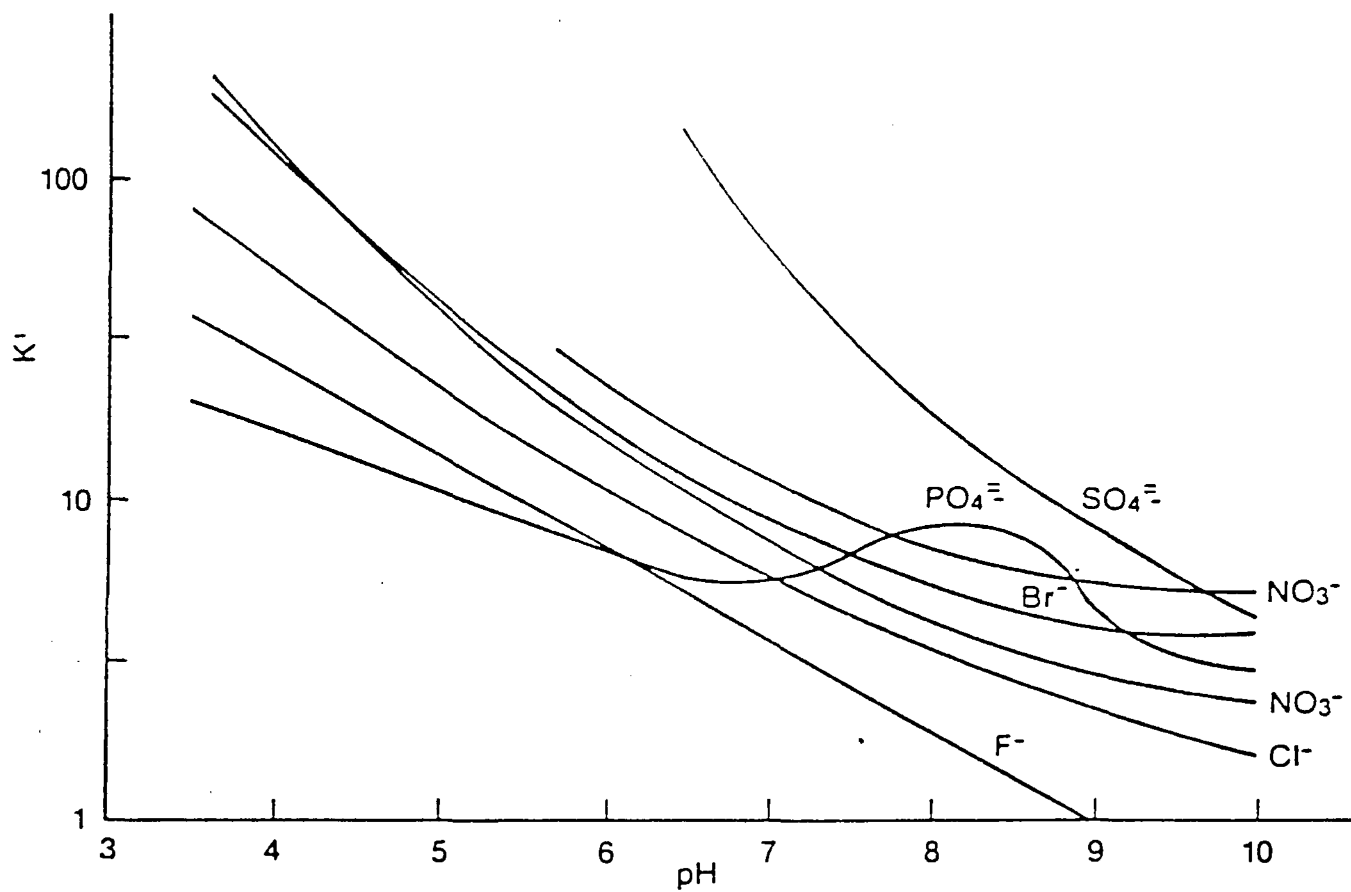


Figure 2.14 Retention as a function of pH for 5mM p-hydroxybenzoate on Wescan anion/R column.

minutes (Figure 2.15). 100ul of a solution containing 10mg/l (from a stock solution of 100mg/l) of each of the anions was used to provide 10mg/l standard from which the concentration of the sample anions could be calculated. Using this method, sulphate concentrations in the porewaters from Loch Lomond and the neighbouring Dubh Lochan were measured at concentrations ranging from 1.2 - 12.3mg/l, with a detection limit of 0.2mg/l (Sections 5.6.1, 5.7.1).

1 fluoride (F^-)
2 chloride (Cl^-)
3 nitrite (NO_2^-)
4 bromide (Br^-)
5 nitrate (NO_3^-)
6 phosphate (PO_4^{3-})
7 sulphate (SO_4^{2-})

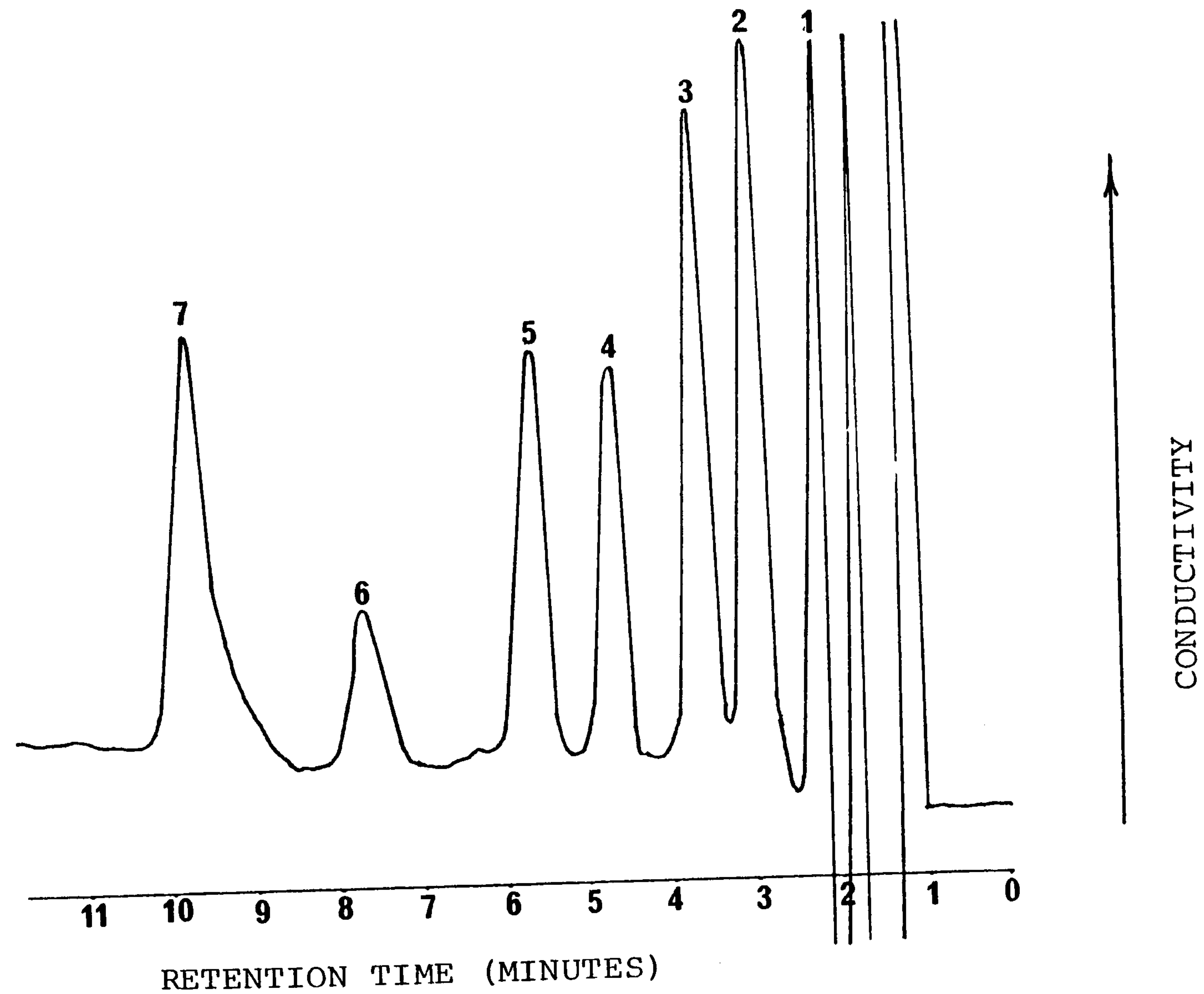


Figure 2.15 Simultaneous chromatographic analysis of seven common anions (10mg/l)

CHAPTER 3

THE HUMAN METABOLISM OF ARSENIC

3.1 INTRODUCTION

Humans can be exposed to a variety of chemical forms of arsenic, of differing toxicity, in the diet and as a consequence of occupational and environmental exposure. In the body, some of these forms may be subject to biotransformation, as subsequently reflected in the composition of urine, the main excretion route (Section 1.3.3). Not all analytical techniques available for the estimation of arsenic in urine, however, are necessarily adequate for an understanding of the fundamental metabolism of arsenic or for an assessment of the extent of potential harmful exposure. With these latter objectives in mind and in preparation for a detailed study of exposed groups of people (Chapter 4), three experiments were carried out in which volunteers ingested known quantities of specific chemical forms of arsenic, metabolism being monitored via the analysis of urinary arsenic using the techniques developed and described in Sections 2.4 and 2.5.

The arsenic intakes were as follows:

I Ingestion of a single meal of seafood, characteristically rich in the stable non-toxic organoarsenical form, by three volunteers.

II Ingestion of a single aqueous dose of pentavalent inorganic arsenic by two volunteers.

III Ingestion of an aqueous dose of pentavalent inorganic arsenic at regular intervals by one volunteer.

3.2 METABOLIC STUDY I: URINARY ARSENIC EXCRETION FOLLOWING THE INGESTION OF A SINGLE MEAL OF SEAFOOD

3.2.1 Objectives

1) To determine the rate at which arsenic is excreted in the urine following the consumption of a single meal of seafood.
2) To determine if there is any biotransformation, after ingestion, of the complex organoarsenical present in seafood.
3) To assess the analytical techniques necessary for the measurement of the different chemical forms of arsenic found in human urine as a result of exposure to arsenic compounds.

3.2.2 Experimental design

From a preliminary survey of the total arsenic content of a range of fish and shellfish, which yielded levels in line with those typically found by M.A.F.F. (1982) (Table 3.1), prawns (13.9mg/kg wet weight) were selected. Distillation of a portion of the prawn tissue (Section 2.5.1) showed that less than 0.4% of the total arsenic was in an inorganic form, confirming that virtually all of the arsenic was present in one of the stable organoarsenical forms characteristic of seafood. For the purposes of the metabolic experiment, the prawns also possessed the

TABLE 3.1

THE TOTAL ARSENIC CONCENTRATION IN A SELECTION OF FISH AND SHELLFISH

FISH TYPE	Total arsenic concentration (mg/kg wet weight)		
		M.A.F.F. (1982)	
	This study	National range	National average
Cod	5.0 ± 0.1	0.3 - 45.0	4.8
Haddock	3.7 ± 0.1	1.0 - 6.0	3.0
Whiting	2.5 ± 0.1	0.4 - 3.9	2.4
Herring	3.3 ± 0.4	0.8 - 12.5	3.7
Prawns	13.9 ± 0.1	0.5 - 17.0	8.8
Whelks	8.0 ± 0.4	1.0 - 40.0	9.0
NBS Albacore Tuna			
(Dry weight)	3.4 ± 0.5		
Certified value	3.3 ± 0.4		

added advantage that they could be consumed without cooking, thus avoiding any possible changes to the arsenic content and form introduced by such preparation.

Three volunteers, two females (A) and (B) weighing approximately 54kg and one male (C) of approximately 80kg, ingested portions of wet prawn tissue weighing 38.87g (A), 38.67g (B) and 38.62g (C) and corresponding to a total arsenic consumption of 541ug (A), 538ug (B) and 537ug (C) respectively. Each had been requested not to eat fish for 3 - 4 days prior to the experiment, nor for its duration. Each had collected a bulk urine sample over the 24 hours preceding the consumption of the seafood and another was collected immediately before commencement of the experiment. The prawns were then consumed in a single meal.

Complete urine samples were collected in acid washed polypropylene bottles, the total volume measured and an aliquot removed for analysis. As it was suspected that initial arsenic excretion would be rapid, samples were collected at two hourly intervals for the first 14 hours and then bulk eight hourly samples were collected over the next 88 hours. The samples were not subjected to any chemical preservation treatment but were kept under refrigeration at $4^{o}C$ or frozen at $-20^{o}C$ if longer

storage time was required.

3.2.3 Analytical methods

A 10ml aliquot of each sample was analysed for the total arsenic concentration after acid digestion (Section 2.4.1). All samples were digested and analysed in duplicate and all analyses included standard reference materials and reagent blanks (Section 2.4.1).

An estimate of the sum concentration of any hydride forming species present was made by analysing all the samples by direct hydride as described in Section 2.4.2.

A number of samples were selected for speciation by ion-exchange chromatography/HGAAS to determine the concentrations of As(V), As(III), MMAA and DMAA excreted (Section 2.5.3).

3.2.4 Results

Tables 3.2, 3.3 and 3.4 and Figures 3.1 and 3.2 show the concentrations of total urinary arsenic excreted by each of the volunteers (A), (B) and (C). It can be seen that the concentration of urinary arsenic, when expressed in ug/l, was influenced by the volume of the sample, the relationship being approximately inverse. To correct for the effects of sample volume, the urinary excretion of creatinine has been used by a number of workers when examining the output of other compounds (Appendix 3.1). In this study, urinary creatinine concentrations were measured using the Jaffe reaction (Appendix 3.2). Thus when urine volumes are small (large), giving high (low) arsenic concentrations, the corresponding creatinine concentrations can be used to 'normalise' the arsenic output. By comparing Figure 3.1 (in which the concentrations are in ug/l) with Figure 3.2 (in which the concentrations have been converted into ugAs/g creatinine) it can indeed be seen that many of the fluctuations in the concentration of urinary arsenic were eliminated.

Figure 3.2 shows that from the low concentrations of urinary arsenic excreted in the 24-hour period prior to consuming the seafood, there was a rapid increase in the concentration of arsenic excreted in the urine just two hours after ingestion of the prawns, reaching maximum concentrations of

TABLE 3.2

URINARY ARSENIC CONCENTRATION FOLLOWING THE CONSUMPTION OF PRAWNS BY VOLUNTEER (A)

SAMPLE	TIME INTERVAL(hr)	VOL (ml)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	CREAT. (g/l)	TOTAL As /g creat
24-hour	24-hour	1710	1 - 2	5	0.26	19
09.00	0 hour	309	1 - 2	6	0.20	30
DAY 1						
11.00	0 - 2	240	1 - 2	162	0.34	477
13.00	2 - 4	391	1 - 2	159	0.20	795
15.00	4 - 6	294	1 - 2	146	0.19	768
17.00	6 - 8	108	4 - 6	255	0.52	490
19.00	8 - 10	133	2 - 3	166	0.36	461
21.00	10 - 12	229	2 - 3	84	0.19	442
23.00	12 - 14	62	4 - 6	305	0.62	492
DAY 2						
07.00	14 - 22	783	1 - 2	81	0.21	386
15.00	22 - 30	1190	2 - 3	34	0.18	189
23.00	30 - 38	730	2 - 3	34	0.32	106
DAY 3						
07.00	38 - 46	422	3 - 5	66	0.77	86
15.00	46 - 54	1056	1 - 2	25	0.41	61
23.00	54 - 62	788	1 - 2	23	0.27	85
DAY 4						
07.00	62 - 70	210	4 - 6	62	0.71	87
15.00	70 - 78	613	2 - 3	19	0.24	79
23.00	78 - 86	930	1 - 2	11	0.26	42
DAY 5						
07.00	86 - 94	882	1 - 2	12	0.08	150
15.00	94 - 102	1370	1 - 2	8	0.09	89

TABLE 3.3

URINARY ARSENIC CONCENTRATION FOLLOWING THE CONSUMPTION OF PRAWNS BY VOLUNTEER (B)

SAMPLE	TIME INTERVAL(hr)	VOL (ml)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	CREAT. (g/l)	TOTAL As /g creat.
24-hour	24-hour	2340	2 - 3	8	0.14	57
09.00	0 hour	53	8 - 12	43	1.59	27
DAY 1						
11.00	0 - 2	92	5 - 8	369	0.72	513
13.00	2 - 4	326	1 - 2	145	0.12	1208
15.00	4 - 6	255	1 - 2	189	0.18	1050
17.00	6 - 8	319	1 - 2	91	0.29	314
19.00	8 - 10	90	5 - 8	192	0.37	519
21.00	10 - 12	21	10 - 15	463	1.79	259
23.00	12 - 14	87	6 - 9	342	1.04	329
DAY 2						
07.00	14 - 22	477	3 - 5	99	0.46	215
15.00	22 - 30	943	2 - 3	46	0.28	164
23.00	30 - 38	1069	2 - 3	28	0.26	108
DAY 3						
07.00	38 - 46	388	1 - 2	54	0.29	186
15.00	46 - 54	1325	1 - 2	19	0.27	70
23.00	54 - 62	1300	1 - 2	16	0.18	89
DAY 4						
07.00	62 - 70	157	6 - 9	64	1.16	55
15.00	70 - 78	652	2 - 3	22	0.53	42
23.00	78 - 86	985	1 - 2	11	0.43	26
DAY 5						
07.00	86 - 94	248	1 - 2	32	0.38	84
15.00	94 - 102	940	2 - 3	11	0.23	48

TABLE 3.4

URINARY ARSENIC CONCENTRATION FOLLOWING THE CONSUMPTION OF PRAWNS BY VOLUNTEER (C)

			ARSENIC CONCENTRATION (ug/l)			
SAMPLE	TIME INTERVAL(hr)	VOL (ml)	DIRECT HYDRIDE	TOTAL	CREAT. (g/l)	TOTAL As /g creat.
24-hour	24-hour	1464	2 - 3	7	0.29	24
09.00	0 hour	63	5 - 7	18	0.85	21
DAY 1						
11.00	0 - 2	170	2 - 3	265	0.25	1060
13.00	2 - 4	290	2 - 3	197	0.14	1407
15.00	4 - 6	144	4 - 6	348	0.31	1123
17.00	6 - 8	103	5 - 7	274	0.62	442
19.00	8 - 10	114	2 - 3	203	0.54	376
21.00	10 - 12	89	7 - 11	303	0.78	388
23.00	12 - 14	216	3 - 4	129	0.45	287
DAY 2						
07.00	14 - 22	550	3 - 4	76	0.35	217
15.00	22 - 30	240	6 - 10	157	0.94	167
23.00	30 - 38	3015	-	10	-	-
DAY 3						
07.00	38 - 46	186	8 - 13	80	0.91	88
15.00	46 - 54	275	4 - 6	63	0.44	143
23.00	54 - 62	330	8 - 13	47	0.83	57
DAY 4						
07.00	62 - 70	355	4 - 6	26	0.44	59
15.00	70 - 78	677	4 - 6	22	0.76	29
23.00	78 - 86	590	5 - 8	25	0.61	41
DAY 5						
07.00	86 - 94	528	3 - 4	17	0.46	37
15.00	94 - 102	382	3 - 4	29	0.47	62

\- no data available

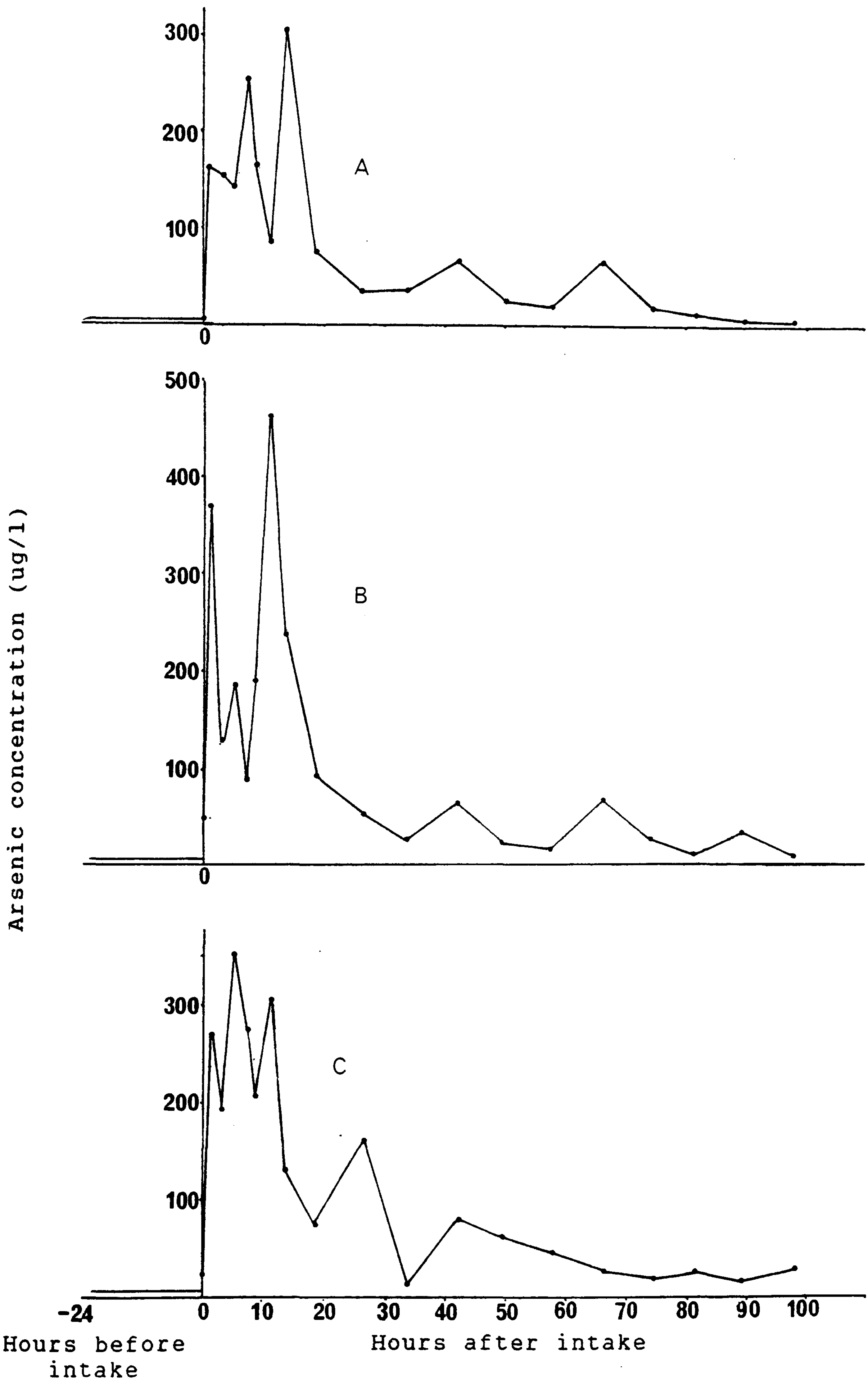


Figure 3.1 Total urinary arsenic concentration (ug/l) in three volunteers A,B & C following the consumption of prawns.

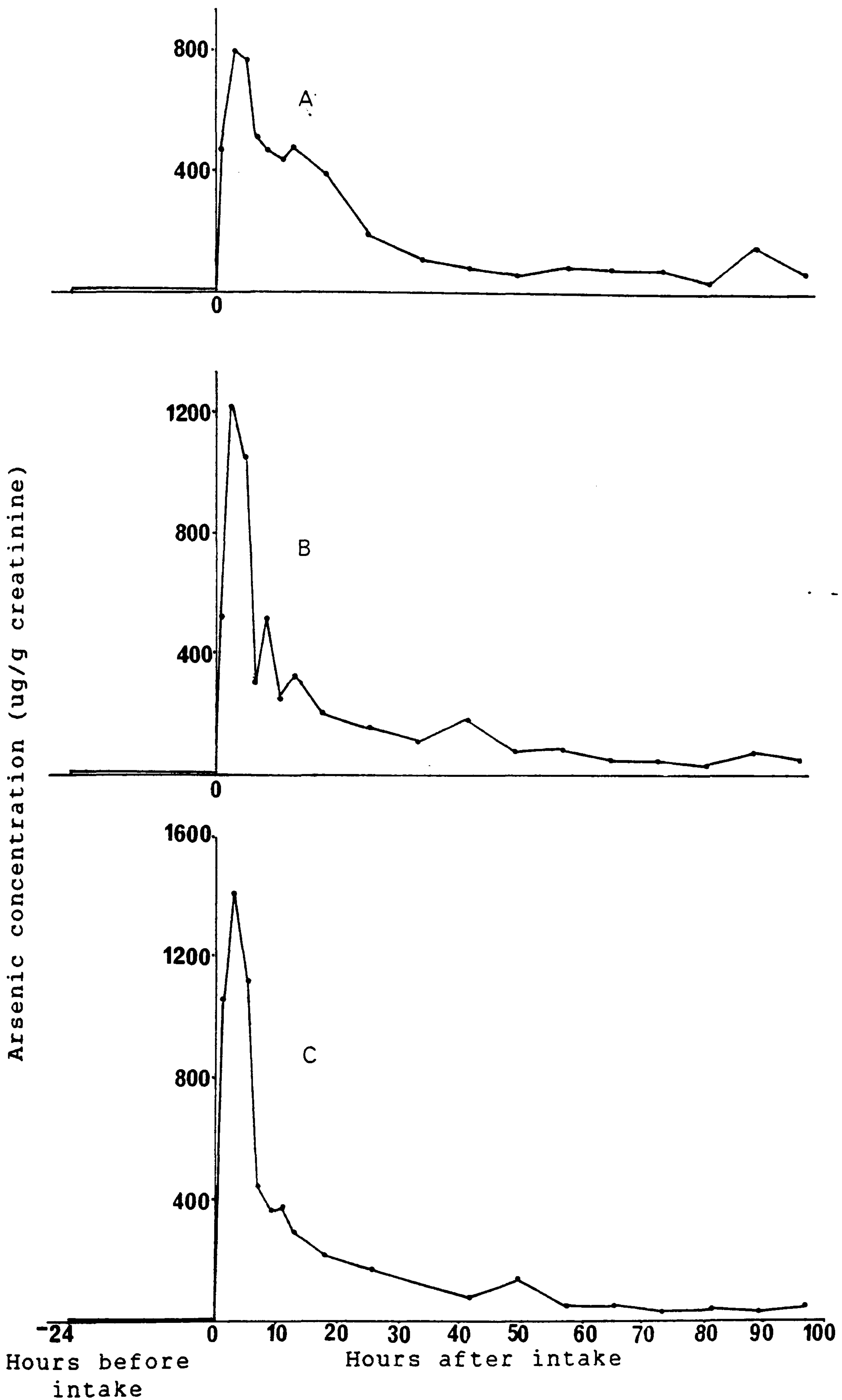


Figure 3.2 Total urinary arsenic concentration (ug/g creatinine) in three volunteers A,B & C following the consumption of prawns.

795ug/g creatinine for (A), 1208ug/g creatinine for (B) and 1407ug/g creatinine for (C) 2 - 4 hours after the intake (Tables 3.2, 3.3 and 3.4). Thereafter there was a rapid decline in the concentration of arsenic excreted in the urine by all three volunteers over the next 36 hours or so, ultimately approaching background levels by the fifth day.

Tables 3.5, 3.6 and 3.7 show the excreted amount, cumulative excreted amount and excretion rate of arsenic for each volunteer after consumption of the prawns. As shown in Figure 3.3, there was a rapid rate of arsenic excretion in the urine over the first 6 hours, reaching maximum rates of 31.1 and 28.6ug/hr for (A) and (C), respectively, only 2 - 4 hours after intake and of 24.1ug/hr for (B) 4 - 6 hours after ingestion of the seafood. Thus, after only 6 hours, each had excreted similarly significant amounts - 26.6%, 24.1% and 28.4% of the total arsenic ingested.

From Figure 3.4, showing arsenic excreted as a cumulative percentage of the intake, it can be seen that for each volunteer almost 50% of the ingested arsenic was excreted within the first 20 hours (i.e. 19, 23 and 17 hrs for (A), (B) and (C) respectively).

For the remainder of the experiment, there was a marked overall decline in the rate at which arsenic was excreted, with average rates of 4.2, 3.9 and 3.3 ug/hr for (A), (B) and (C), respectively, between 14 - 62 hours and of only 1.4 - 1.6ug/hr for the final 40

TABLE 3.5

THE EXCRETED AMOUNT, CUMULATIVE EXCRETED AMOUNT AND EXCRETION RATE OF URINARY ARSENIC BY VOLUNTEER (A) FOLLOWING THE CONSUMPTION OF PRAWNS

Hours after intake	Total As excreted (ug)	Cumulative As excreted (ug)	Cumulative As excreted as a % of intake	Excretion rate of As (ug/hr)
Pre 24 hours	8.6			0.4
0 - 2	38.9	38.9	7.2	19.5
2 - 4	62.2	101.1	18.7	31.1
4 - 6	42.9	144.0	26.6	21.5
6 - 8	27.5	171.5	31.7	13.8
8 - 10	22.1	193.6	35.8	11.1
10 - 12	19.2	212.8	39.3	9.6
12 - 14	18.9	231.7	42.8	9.5
14 - 22	63.4	295.1	54.5	7.9
22 - 30	40.5	335.6	62.0	5.1
30 - 38	24.8	360.4	66.7	3.1
38 - 46	27.9	388.3	71.8	3.5
46 - 54	26.4	414.7	76.7	3.3
54 - 62	18.1	432.8	80.0	2.3
62 - 70	13.0	445.8	82.4	1.6
70 - 78	11.6	457.4	84.5	1.5
78 - 86	10.2	467.6	86.4	1.3
86 - 94	10.6	478.2	88.4	1.3
94 - 102	11.0	489.2	90.4	1.4

TABLE 3.6

THE EXCRETED AMOUNT, CUMULATIVE EXCRETED AMOUNT AND EXCRETION RATE OF URINARY ARSENIC FOR VOLUNTEER (B) FOLLOWING THE CONSUMPTION OF PRAWNS

Hours after intake	Total As excreted (ug)	Cumulative As excreted (ug)	Cumulative As excreted as a % of intake	Excretion rate of As (ug/hr)
Pre 24 hours	18.7			0.8
0 - 2	33.9	33.9	6.3	17.0
2 - 4	47.3	81.2	15.1	23.7
4 - 6	48.2	129.4	24.1	24.1
6 - 8	29.0	158.4	29.4	14.5
8 - 10	17.3	175.7	32.7	8.7
10 - 12	9.7	185.4	34.5	4.9
12 - 14	29.8	215.2	40.0	14.9
14 - 22	47.2	262.4	48.8	5.9
22 - 30	43.4	305.8	56.9	5.4
30 - 38	29.9	335.7	62.4	3.7
38 - 46	21.0	356.7	66.3	2.6
46 - 54	25.2	381.9	71.0	3.2
54 - 62	20.8	402.7	74.9	2.6
62 - 70	10.0	412.7	76.7	1.3
70 - 78	14.3	427.0	79.4	1.8
78 - 86	10.8	437.8	81.4	1.4
86 - 94	7.9	445.7	82.9	1.0
94 - 102	10.3	456.0	84.8	1.3

TABLE 3.7

THE EXCRETED AMOUNT, CUMULATIVE EXCRETED AMOUNT AND EXCRETION RATE OF URINARY ARSENIC BY VOLUNTEER (C) FOLLOWING THE CONSUMPTION OF PRAWNS

Hours after intake	Total As excreted (ug)	Cumulative As excreted (ug)	Cumulative As excreted as a % of intake	Excretion rate of As (ug/hr)
Pre 24 hours	10.2			0.5
0 - 2	45.1	45.1	8.4	22.6
2 - 4	57.1	102.2	19.0	28.6
4 - 6	50.1	152.3	28.4	25.1
6 - 8	28.2	180.5	33.6	14.1
8 - 10	23.1	203.6	37.9	11.6
10 - 12	27.0	230.6	42.9	13.5
12 - 14	27.9	258.5	48.1	14.0
14 - 22	41.8	300.3	55.9	5.2
22 - 30	37.7	338.0	62.9	4.7
30 - 38	30.2	368.2	68.6	3.8
38 - 46	14.9	383.1	71.3	1.9
46 - 54	17.3	400.4	74.6	2.2
54 - 62	15.5	415.9	77.4	1.9
62 - 70	9.2	425.1	79.2	1.2
70 - 78	14.9	440.0	81.9	1.9
78 - 86	14.8	454.8	84.7	1.9
86 - 94	10.0	464.8	86.6	1.3
94 - 102	11.1	475.9	88.6	1.4

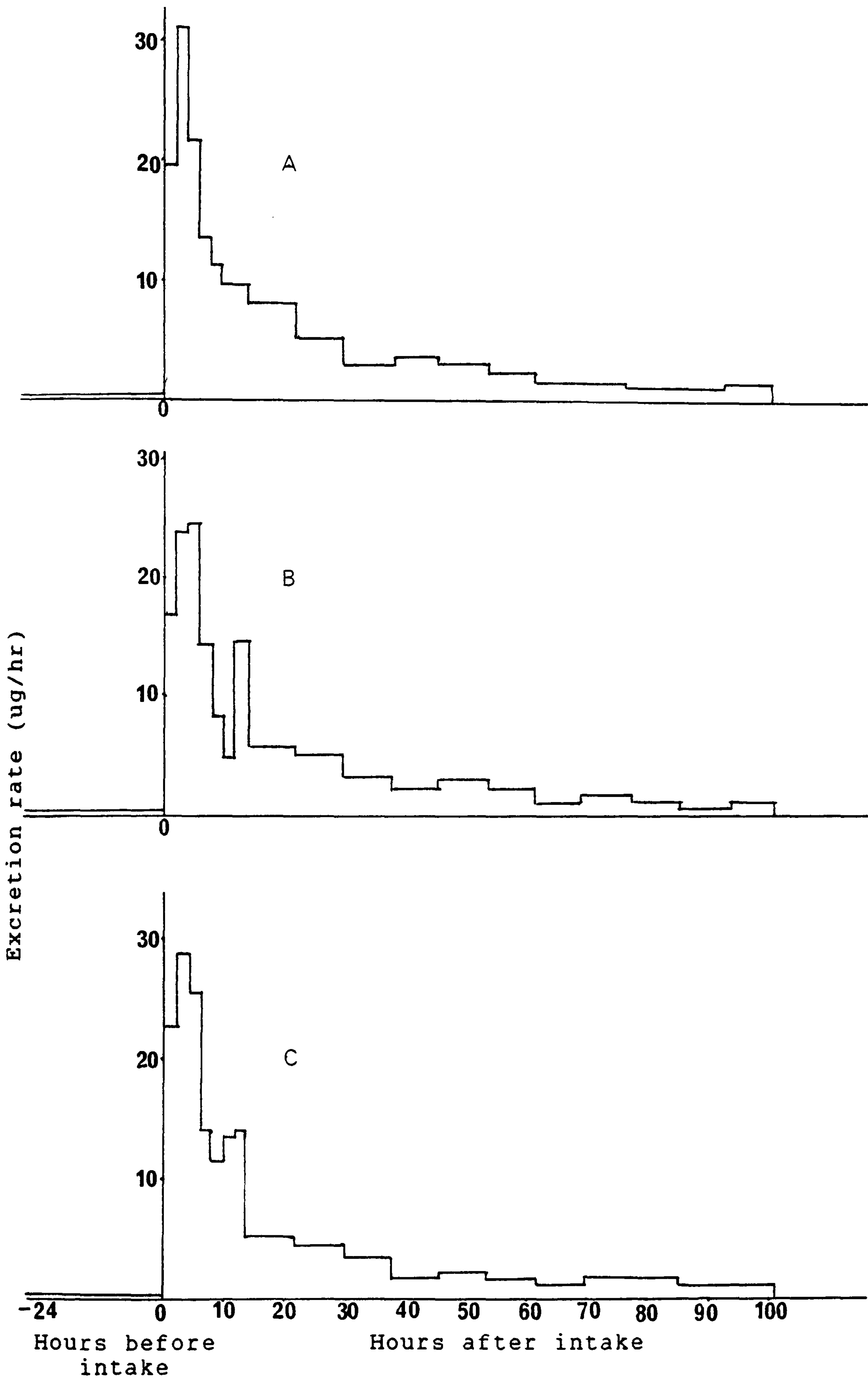


Figure 3.3 Excretion rate of total urinary arsenic by three volunteers A,B & C, following the consumption of prawns.

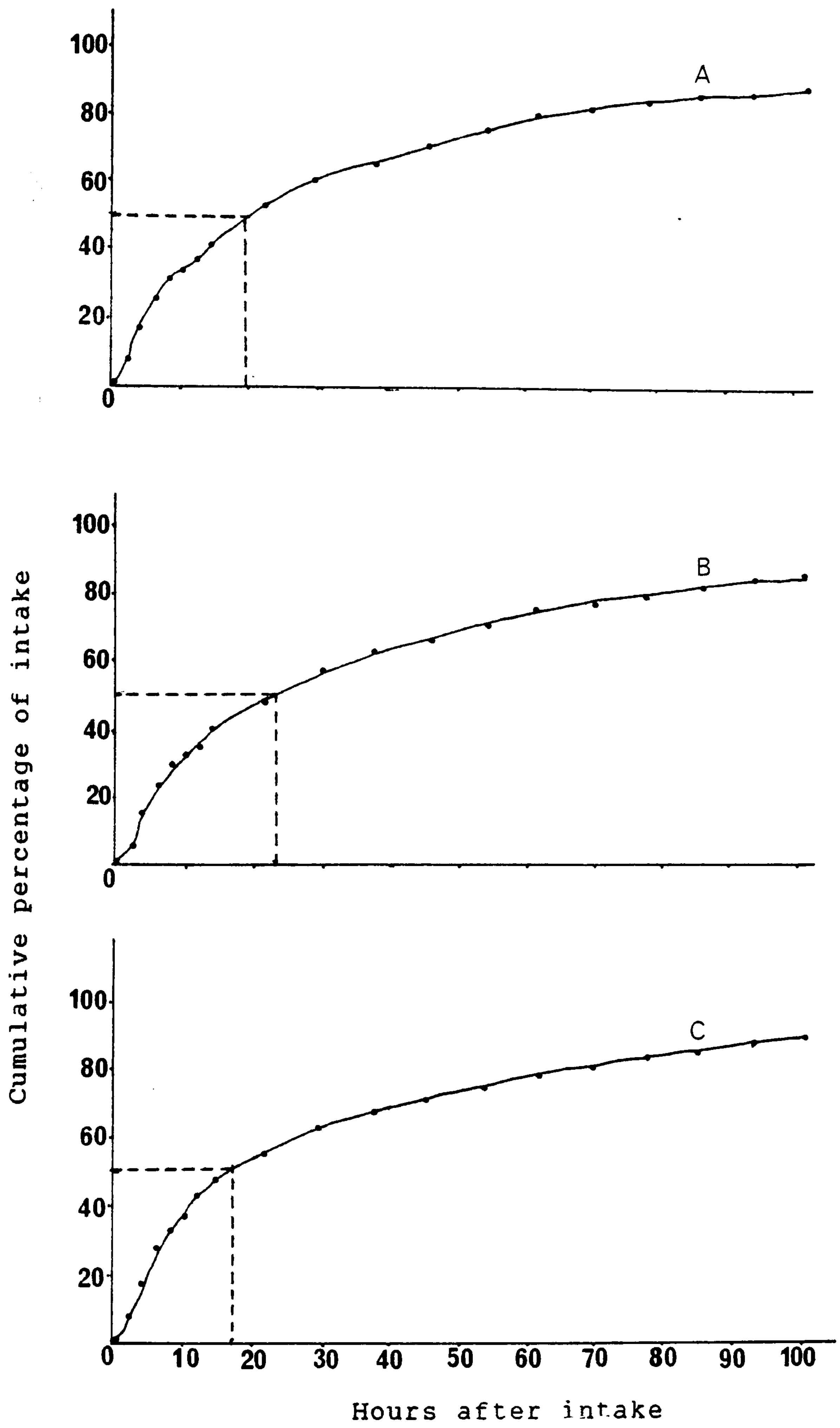


Figure 3.4 Total urinary arsenic excreted as a cumulative percentage of the intake by three volunteers A, B & C following the consumption of prawns.

hours for all three volunteers. Therefore, over the final stages of the experiment there was very little increase in the cumulative percentage of ingested arsenic that was excreted (Figure 3.4).

After 102 hours each of the volunteers had excreted most of the ingested arsenic i.e. 90% by (A), 85% by (B) and 89% of the dose by (C). Correction for normal background excretion would reduce these figures to 83.7%, 70.0% and 80.5% for (A), (B) and (C), respectively.

The rapid scan of all urine samples using the direct hydride method (Section 2.4.2) showed that the concentrations of excreted hydride-forming species As(V), As(III), MMAA and DMAA, were consistently low (Tables 3.2, 3.3 and 3.4). This was substantiated by the detailed speciation results for a selected number of samples (Table 3.8). While the total arsenic concentrations were high, the sum of the species concentrations were very low with As(V), As(III) and MMAA often below the detection limit of 0.5ug/l (Section 2.5.3.6). DMAA was detected consistently in all the samples, but only at levels comparable to those normally found in urine from humans unexposed to either organoarsenic compounds in seafood or inorganic arsenic (Section 4.2.3).

TABLE 3.8

ARSENIC SPECIATION IN A SELECTED NUMBER OF URINE SAMPLES FROM THREE VOLUNTEERS A, B and C FOLLOWING THE CONSUMPTION OF PRAWNS

		ARSENIC CONCENTRATION (ug/l)					
		Total	Speciation				
			As(V)	As(III)	MMAA	DMAA	SUM
DAY 1 11.00 volunteer	(A)	162	*	*	*	1.8	1.8
	(B)	369	*	0.9	*	3.8	4.7
15.00	(B)	189	*	*	*	1.9	1.9
	(C)	348	*	*	*	5.1	5.1
17.00	(C)	274	*	*	*	6.9	6.9
19.00	(C)	203	*	*	*	3.0	3.0
23.00	(C)	129	*	*	*	3.0	3.0
DAY 2 07.00	(C)	76	*	*	*	2.6	2.6
DAY 3 07.00	(C)	80	*	2.7	2.0	6.3	11.0
DAY 4 07.00	(C)	26	*	1.2	1.1	6.0	8.3
DAY 5 07.00	(C)	17	*	*	*	2.8	2.8

* corresponds to a species concentration (ug/l) below the detection limit of 0.5ug/l

3.2.5 Discussion

The consumption of prawns with a high arsenic concentration of 13.9mg/kg (wet weight), corresponding to intakes of about 540ug arsenic resulted in the rapid excretion of arsenic in urine by all three volunteers. Peak concentrations were reached just four hours after the intake and an amount equivalent to 50% of the ingested arsenic was eliminated, on average, after about the first 20 hours. After 102 hours each of the volunteers had eliminated 90%, 85% and 89%, respectively, of the ingested amount.

The rapid elimination of arsenic in the urine of man after the consumption of seafood has been reported elsewhere. Crecelius (1977) found that 50% of the arsenic ingested from the consumption of 340g of crab meat (equivalent to about 2000ug arsenic) was eliminated within the first 20 hours of a 70 hour experiment. The ingestion of 100g shrimps (equivalent to about 1100ug arsenic), in a similar experiment conducted by Foa et al. (1984), resulted in a peak in the urinary arsenic concentration 5 - 10 hours later. The marked increase in the urinary excretion of total arsenic lasted 24 - 48 hours and then declined rapidly to background concentrations. Although much greater amounts of arsenic, 5000ug and 8000ug respectively, were ingested in metabolic studies carried out by Freeman et al. (1979) and by Luten et al. (1982, 1983), 74 - 90% of the arsenic was

eliminated after 10 days in the former and 69 - 85% within five days for the latter.

In an experiment in which 6 human volunteers ingested radiolabelled inorganic arsenic, Pomroy et al. (1980) measured the amount of arsenic retained in the body with time. They were able to describe the decrease in arsenic retention by a three-component exponential curve which they suggested was equivalent to the retention of arsenic in three body compartments with increasing biological half-life from the first to the third. Similar curves were constructed for the retention of total arsenic by the three volunteers in this study following the consumption of the seafood. The data fitted a two-component exponential model for each with 53.0% of the ingested dose excreted with a biological half-life of 10.3hrs for (A), for (B) 44.3% (11.0hrs) and for (C) 50.7% (6.9hrs) (Figure 3.5). The remaining arsenic was excreted with an average biological half-life of 75.7hrs (3.15 days). A longer first component half-life of 18 hours was measured by Buchet et al. (1980) while a shorter biological half-life of only 6 hours was recorded by Yamauchi and Yamamura (1984), but, in accordance with the study carried out in this laboratory, they found that 86.8 - 89.2% of the ingested arsenic (747 and 750ug As in prawns) had been excreted after 72 hours, with very low excretion rates after the first 24 hours and only trace amounts detected after 60 hours.

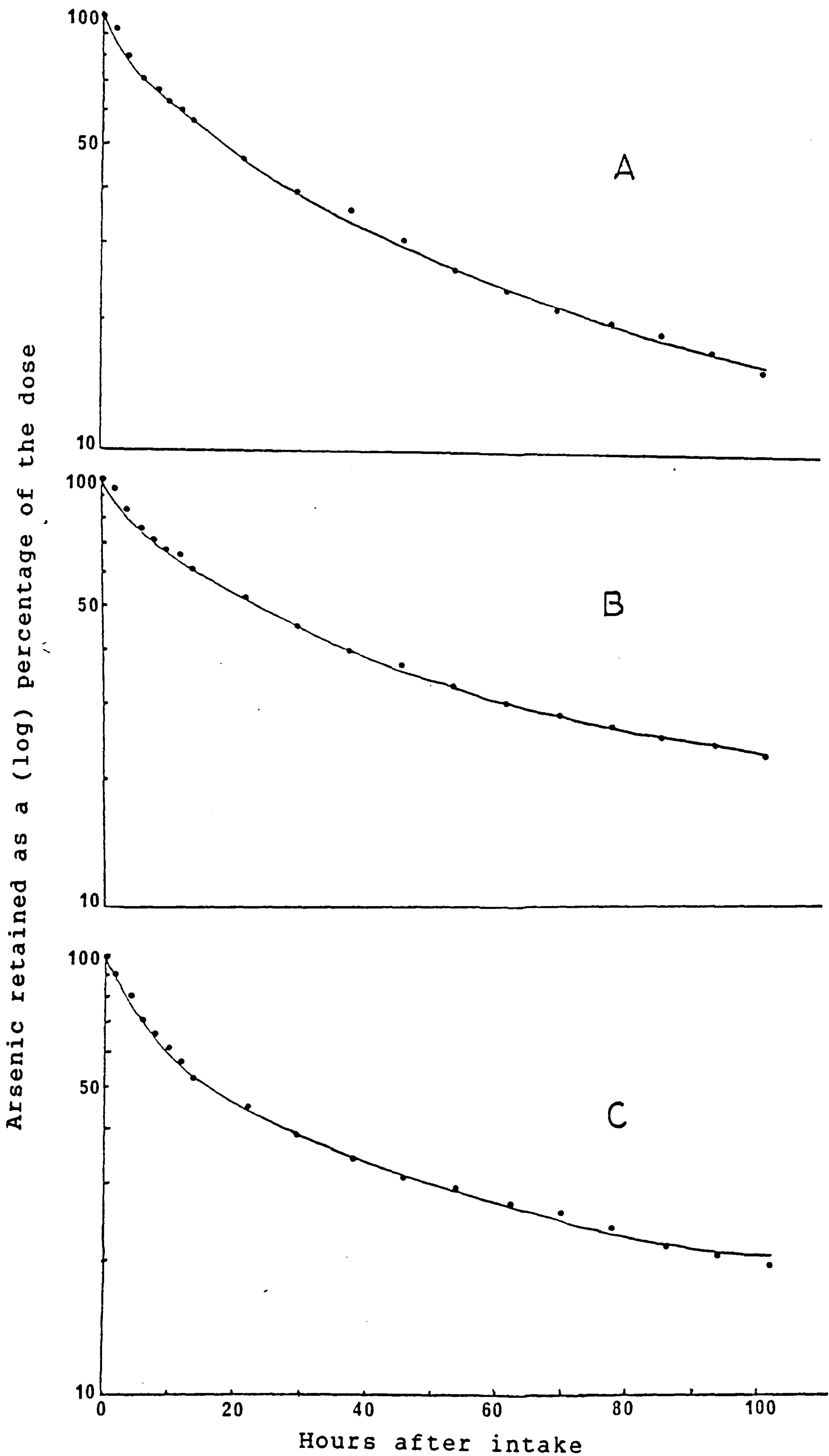


Figure 3.5 Arsenic retention curves for three volunteers (A), (B) and (C) following the consumption of prawns.

In all of the above metabolic studies, the highly stable nature of the arsenic excreted was shown by the fact that the total arsenic concentration in the urine could <u>only</u> be obtained after vigorous digestion of the samples, e.g. dry ashing (Buchet et al., 1980; Foa et al., 1984), hot alkaline digestion of an aqueous fraction with potassium hydroxide (Luten et al., 1982, 1983) or sodium hydroxide (Yamauchi and Yamamura, 1984), or, as in this study, an acid digestion with a combination of strong oxidizing acids (Crecelius, 1977).

In only three of the studies referenced was measurement made of the concentrations of the species As(V), As(III), MMAA and DMAA excreted in the urine following the consumption of seafood. Yamauchi and Yamamura (1984) suggested that the slight increases in the urinary excretion of inorganic arsenic (no differentiation made between As(V) and As(III)), MMAA and DMAA following the consumption of prawns could be due to a partial in vivo demethylation of the stable organoarsenical. However, Crecelius (1977) found that there was no significant change in the levels of As(V), As(III), MMAA and DMAA excreted during the 60hrs following ingestion of crab meat and, similarly, Foa et al. (1984) noted that the elimination of inorganic arsenic (i.e. As(V) + As(III)), MMAA and DMAA remained at background levels after the consumption of the seafood. In the metabolic study

conducted in this laboratory, the speciated urine samples (Table 3.8) contained very low levels of As(V), As(III), MMAA and DMAA, present only at concentrations expected of persons unexposed occupationally or environmentally to these chemical forms of arsenic (Section 4.2.3). Thus, it is unlikely that there is any biotransformation of the ingested organoarsenical into any of the hydride forming species As(V), As(III), MMAA and DMAA.

A number of workers have isolated and identified the stable organoarsenical present in fish and shellfish as arsenobetaine (Section 1.2), found to be the major form of arsenic excreted in the urine after the consumption of lobster by Cannon et al. (1981), of plaice (Luten et al., 1983) and of prawns (Yamauchi and Yamamura, 1984).

Cannon et al. (1983) went on to show that mice injected intraperitoneally with an aqueous solution of synthetic arsenobetaine hydrate rapidly eliminated the substrate unchanged in the urine. There were no differences in behaviour or mortality when compared with control animals injected with water alone, the arsenic levels in carcasses of the treated animals were the same as in the controls and no metabolism to other chemical forms of arsenic was observed. Similarly, Vahter et al. (1983) were able to show that following intraveneous administration of synthesized ^{73}As-Arsenobetaine to mice, rats and rabbits and

oral administration to mice, this was the only labelled arsenic compound detected in the urine and soluble extracts of tissues, indicating that no biotransformation had occurred.

Although arsenobetaine was not specifically identified in the urine of the three volunteers in the metabolic study conducted here, the available evidence suggests that it was the major form of eliminated arsenic. The excreted arsenic could only be measured after a vigorous acid digestion of the urine samples and none of the hydride-forming species (As(V), As(III), MMAA and DMAA) were detected above normal levels.

3.2.6 Conclusions

1) Seafood arsenic, usually in the form of arsenobetaine, is absorbed from the gastrointestinal tract and rapidly excreted via the kidneys. The ingestion of approximately 540ug arsenic in prawns resulted in almost 50% of the dose eliminated in the first 20hrs with some 80 - 90% of the dose (70 - 84% after correction for background arsenic concentrations) being excreted in the urine after 4.25 days. The urinary elimination of arsenic can be described by a two-component exponential model, with approximately half of the ingested arsenic excreted due to a short

lived component (biological half-life of 6.9 - 11.0 hours) and half excreted with a longer lived component (biological half-life of about 3 days).

2) Seafood arsenic is not transformed by the human body to As(V), As(III), MMAA or DMAA. Thus urinary concentrations of these four species are not normally influenced by the consumption of seafood.

3) Analytical techniques for the determination of total urinary arsenic are unable to discriminate between seafood arsenic and the forms likely to result from exposure to inorganic arsenic. Analytical speciation methods are required.

3.3 METABOLIC STUDY II: ARSENIC METABOLISM FOLLOWING A SINGLE ORAL DOSE OF AQUEOUS PENTAVALENT INORGANIC ARSENIC.

3.3.1 Objectives

1) To determine the rate of urinary arsenic excretion following a single oral dose of inorganic As(V).
2) To investigate the biotransformation of inorganic arsenic within the human body.
3) To assess the relative merits of analytical procedures available for the individual or collective determination of urinary arsenic species following the ingestion of inorganic arsenic.

3.3.2 Experimental design

Two volunteers were used, a 54kg female (A) and an 80kg male (B). Both were asked to abstain from the consumption of any fish during the week prior to the commencement of the experiment and for its duration. Each collected a bulk urine sample 24 hours prior to the start of the experiment and was required to give another sample just before intake of the inorganic arsenic. The mineral water Vichy Célestins, previously shown to contain a high concentration of inorganic As(V) (Appendix 2), was used as the source of inorganic arsenic exposure.

Each volunteer ingested 1-litre of the mineral water, corresponding to an intake of 220 $\pm$ 15ug of inorganic As(V).

Urine samples were collected every 2 hours for the first 14 hours in anticipation of an initial rapid excretion of arsenic in the urine. From day 2 until day 5, urine samples were collected at 8-hour intervals and, for days 6 and 7, 24-hour bulk urine samples were collected.

For each urine sample collected, the total volume was measured and an aliquot stored in an acid-washed polypropylene container. The samples were not treated in any other way. Samples not analysed immediately were stored at -20°C and when required for analysis, were thawed overnight at 4°C.

3.3.3 Analytical methods

All samples were rapidly scanned for the concentration of the hydride-forming species by the direct hydride method (Section 2.4.2) and all were speciated by the ion-exchange/HGAAS method (Section 2.5.3). For each, the total arsenic concentration was obtained by digestion of a 10ml aliquot as in Section 2.4.1.

3.3.4 Results

Urinary concentrations, expressed in ug/l, of individual arsenic species, As(V), As(III), MMAA and DMAA, the sum of these four species, the corresponding direct-hydride estimate of the sum and the total arsenic concentration are listed for (A) and (B) in Tables 3.9 and 3.10, respectively. Concentrations (ug/l) of individual arsenic species and the sum of the species are plotted for (A) and (B) in Figures 3.6 and 3.7, respectively. It is clear from the irregular patterns in the concentrations of urinary arsenic species in Figures 3.6 and 3.7 and from the inverse relationship between urine volume and creatinine content (Figures 3.8, 3.9) that, as in metabolic study I, normalisation of concentrations to creatinine is desirable (Tables 3.11, 3.12; Figures 3.10, 3.11). Notable examples of the effects of this correction procedure are provided by changes in the magnitude, relative to adjacent samples, of the sum of the concentrations of the four arsenic species for the high-creatinine 6 - 8 hr and 8 - 10 hr samples for (A) (Tables 3.9, 3.11; Figures 3.6, 3.8, 3.10) and the low-creatinine 8 - 10 hr and 10 - 12 hr samples for (B) (Tables 3.10, 3.12; Figures 3.7, 3.9, 3.11).

Prior to the consumption of the mineral water, urinary arsenic concentrations were very low, only DMAA of the four hydride-forming species being detected. After oral ingestion of 220 ± 15 ug As(V),

TABLE 3.9

THE CONCENTRATION OF URINARY ARSENIC EXCRETED FOLLOWING THE INGESTION OF A SINGLE ORAL DOSE OF 220ug INORGANIC As(V) BY VOLUNTEER (A)

				ARSENIC CONCENTRATION (ug/l)								
						SPECIATION						
SAMPLE	TIME INTERVAL	VOL (ml)	CREAT (g/l)	DIRECT HYDRIDE	TOTAL	As(V)	As(III)	MMAA	DMAA	SUM	(/g creat.) TOTAL	SUM
Pre expt.	24-hr	1700	0.44	2-4	6	*	*	*	2.5	2.5	14	5.7
10.00	2 hr	150	0.55	3-4	6	*	*	*	2.7	2.7	11	4.9
DAY 1												
11.00	0-1	400	0.10	1-2	3	*	*	*	*	*	30	*
12.00	1-2	400	0.06	3-4	3	*	*	*	*	*	50	*
14.00	2-4	410	0.24	9-14	22	6.0	7.9	0.9	4.7	19.5	92	81.3
16.00	4-6	65	1.00	54-88	110	10.0	24.0	11.6	34.9	80.5	110	80.5
18.00	6-8	33	2.56	120-180	215	11.3	35.6	52.5	70.4	169.8	84	66.3
20.00	8-10	38	2.91	112-172	200	6.6	31.3	29.5	78.1	145.5	69	50.0
22.00	10-12	80	1.46	64-105	96	6.4	14.5	16.3	47.9	85.1	66	58.3
24.00	12-14	180	0.48	24-36	33	5.8	4.6	5.3	16.0	31.7	69	66.0
DAY 2												
08.00	14-22	820	0.23	11-18	17	1.4	1.8	2.4	10.8	16.4	74	71.3
16.00	22-30	500	0.42	20-39	30	2.4	3.7	4.0	19.7	29.8	71	71.0
24.00	30-38	1312	0.18	7-13	14	3.2	1.3	0.9	8.7	14.1	78	78.3

TABLE 3.9 cont.

SAMPLE	TIME INTERVAL	VOL (ml)	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	SPECIATION TOTAL	As(V)	As(III)	MMAA	DMAA	SUM	(/g creat.) TOTAL	SUM
DAY 3												
08.00	38-46	280	0.84	30-58	56	4.5	4.1	3.0	42.7	54.3	67	64.6
16.00	46-54	448	0.44	13-20	23	2.6	2.3	1.3	18.9	25.1	52	57.0
24.00	54-62	422	0.96	19-30	32	3.8	2.5	2.3	30.2	38.8	33	40.4
DAY 4												
08.00	62-70	215	1.45	22-37	33	2.3	2.8	2.3	27.0	34.4	23	23.7
16.00	70-78	528	0.42	9-16	16	*	1.5	1.0	11.2	13.7	38	32.6
24.00	78-86	362	0.44	8-14	14	*	1.2	*	10.0	11.2	32	25.5
DAY 5												
08.00	86-94	255	1.80	14-23	25	*	1.8	1.9	15.4	19.1	14	10.6
16.00	94-102	473	0.29	4-7	10	*	1.1	0.9	7.8	9.8	34	33.8
24.00	102-110	615	0.49	5-8	11	*	0.8	0.9	8.0	9.7	22	19.8
DAY 6												
08.00	110-118	365	0.95	7-11	8	*	1.1	*	7.7	8.8	8	9.3
DAY 6-7												
16.00	118-142	1470	0.42	3-5	5	*	0.8	*	4.8	5.6	12	13.3
DAY 7-8												
16.00	142-166	900	1.31	7-11	10	*	1.2	*	8.6	9.8	8	7.5

* corresponds to a species concentration below the detection limit of 0.5ug/l

TABLE 3.10

THE CONCENTRATION OF URINARY ARSENIC EXCRETED FOLLOWING THE INGESTION OF A SINGLE ORAL DOSE OF 220ug INORGANIC As(V) BY VOLUNTEER (B)

				ARSENIC CONCENTRATION (ug/l)								
						SPECIATION						
SAMPLE	TIME INTERVAL	VOL (ml)	CREAT (g/l)	DIRECT HYDRIDE	TOTAL	As(V)	As(III)	MMAA	DMAA	SUM	(/g creat.) TOTAL	(/g creat.) SUM
Pre expt.	24-hr	2140	0.29	4-8	14	*	*	*	4.5	4.5	48	15.5
10.00	0	73	2.02	6-9	28	*	*	*	5.4	5.4	14	2.7
DAY 1												
11.00	0-1	390	0.18	2-5	8	*	*	*	*	*	44	*
12.00	1-2	360	0.07	4-6	8	*	*	*	*	*	114	*
14.00	2-4	106	1.20	29-44	50	16.3	18.5	3.5	5.7	44.0	42	36.7
16.00	4-6	107	0.76	36-48	55	8.6	25.7	7.3	7.3	48.9	72	64.3
18.00	6-8	240	0.42	13-20	36	4.5	6.2	6.3	5.9	22.9	86	54.5
20.00	8-10	1015	0.10	4-6	6	4.7	1.7	1.3	3.5	11.2	60	112.0
22.00	10-12	460	0.26	6-9	30	5.8	2.4	3.1	4.2	15.5	115	59.6
24.00	12-14	168	1.11	22-39	38	9.1	7.2	9.0	11.4	36.7	34	33.1
DAY 2												
08.00	14-22	220	1.66	36-54	54	4.8	8.9	14.0	20.3	48.0	33	28.9
16.00	22-30	290	1.40	23-45	35	4.3	1.5	11.3	17.9	35.0	25	25.0
24.00	30-38	325	1.11	25-49	53	*	3.4	8.2	21.0	32.6	48	29.4

TABLE 3.10 cont.

SAMPLE	TIME INTERVAL	VOL (ml)	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIATION As(V)	As(III)	MMAA	DMAA	SUM	(/g creat.) TOTAL	SUM
DAY 3												
08.00	38-46	205	1.81	29-57	50	0.8	3.4	8.8	34.0	47.0	28	26.0
16.00	46-54	243	1.98	24-39	64	*	4.3	8.3	29.5	42.1	32	21.3
24.00	54-62	890	0.57	8-13	17	0.9	1.3	2.6	8.2	13.0	30	22.8
DAY 4												
08.00	62-70	508	0.52	9-16	18	*	1.0	2.3	9.9	13.2	35	25.4
16.00	70-78	325	1.50	16-28	35	*	1.8	4.8	14.7	21.3	23	14.2
24.00	78-86	1084	0.21	5-9	8	*	*	1.2	5.3	6.5	38	31.0
DAY 5												
08.00	86-94	235	1.79	14-23	37	*	*	4.0	14.9	18.9	21	10.6
16.00	94-102	325	1.89	13-21	26	*	2.0	3.5	9.3	14.8	14	7.8
24.00	102-110	1052	0.23	3-5	8	*	*	1.2	2.4	3.6	33	15.7
DAY 6												
08.00	110-118	910	0.80	5-9	18	*	*	*	7.3	7.3	23	9.1
DAY 6-7												
16.00	118-142	1970	0.70	5-8	11	*	1.4	*	4.5	5.9	16	8.4
DAY 7-8												
16.00	142-166	1970	0.77	4-6	8	*	2.0	*	3.4	5.4	10	7.0

* corresponds to a species concentration below the detection limit of 0.5ug/l

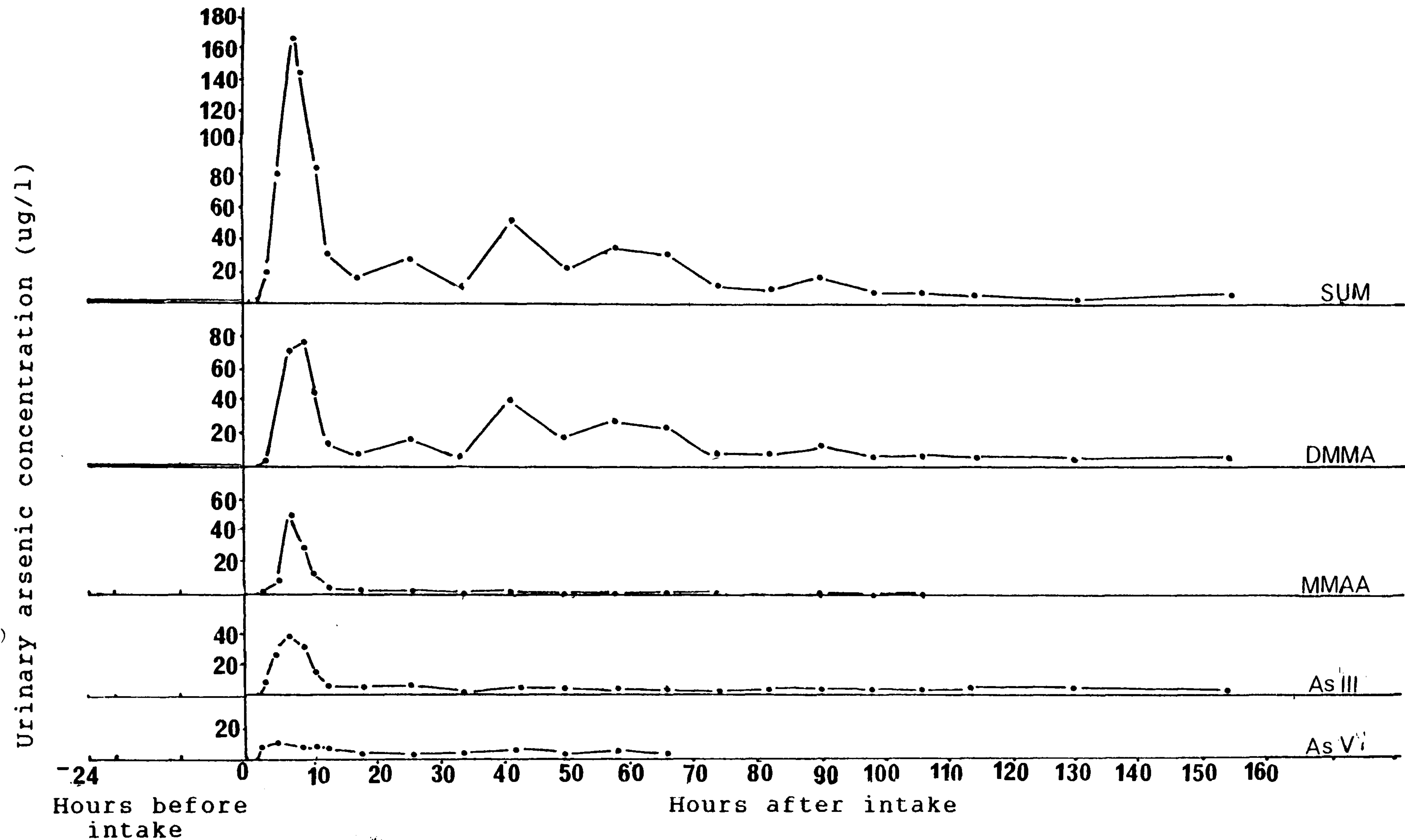


Figure 3.6 Urinary arsenic excreted (ug/l) following the ingestion of a single oral dose of 220ug of inorganic As (V) by volunteer A.

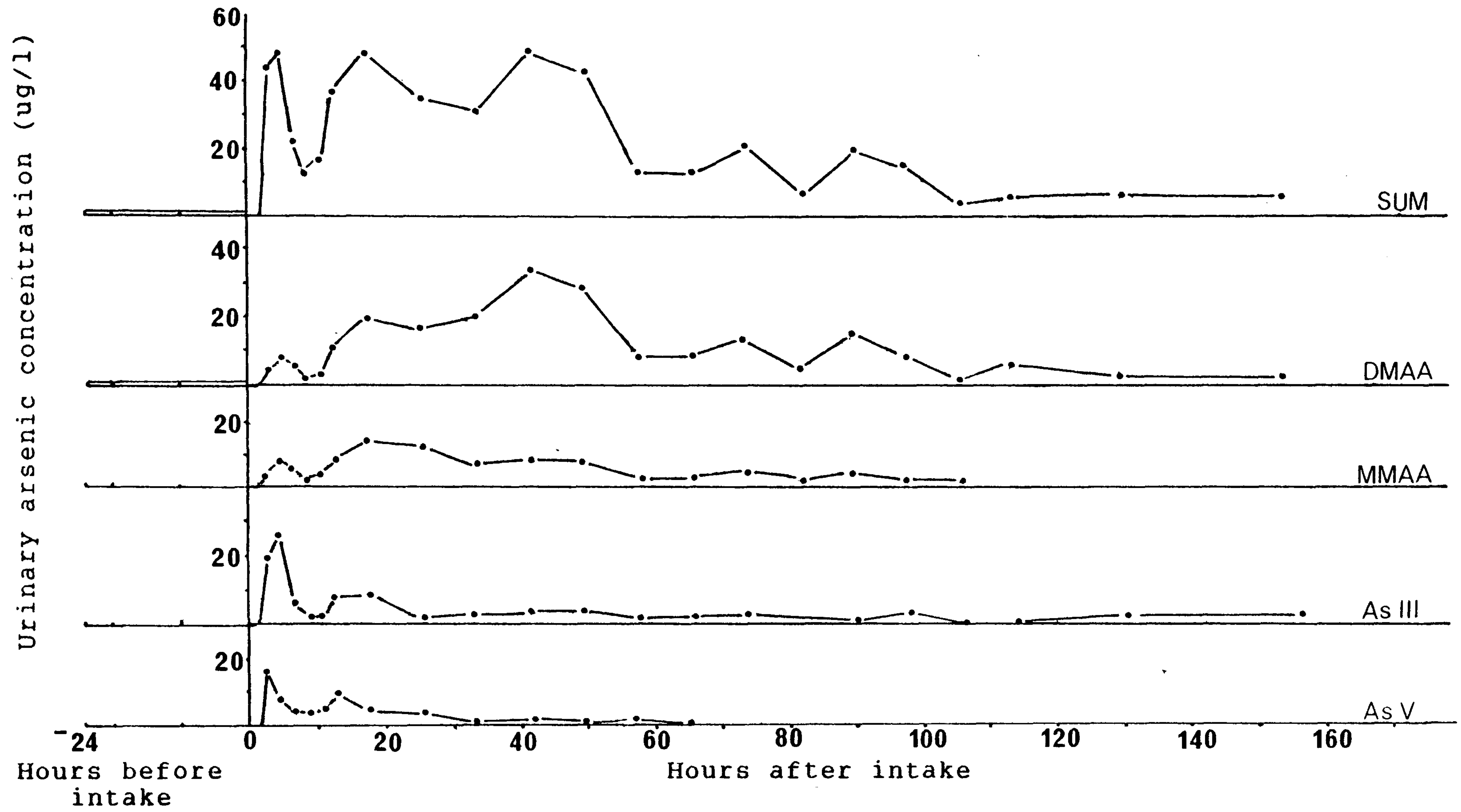
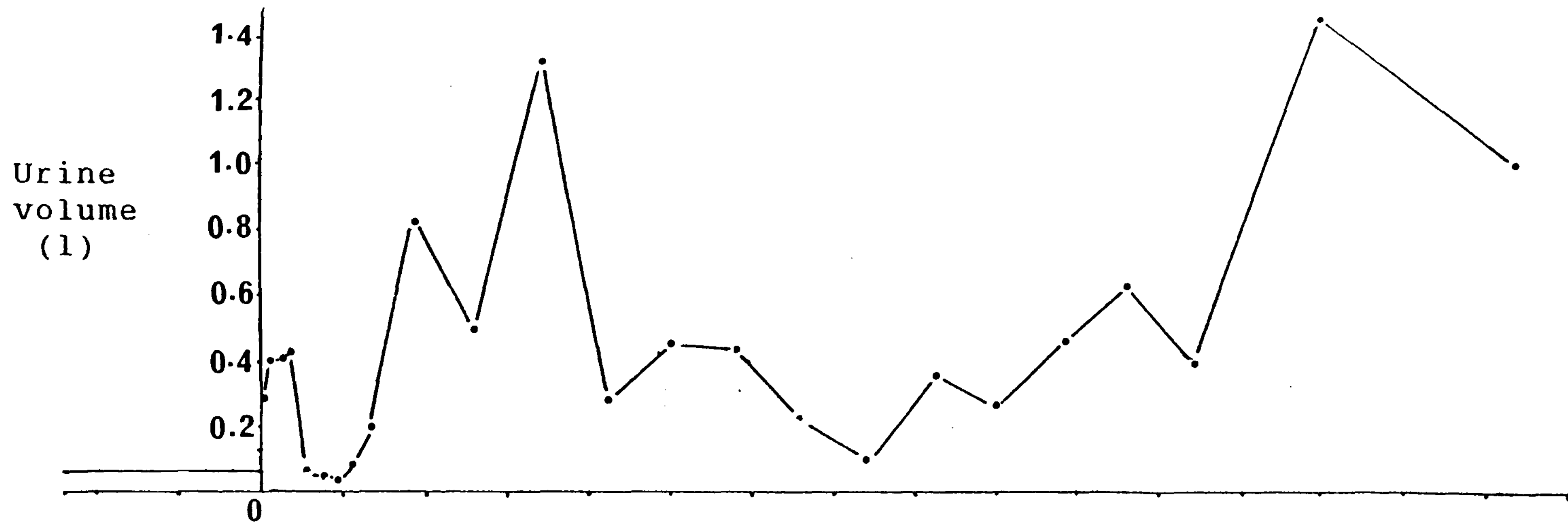


Figure 3.7 Urinary arsenic excreted (ug/l) following the ingestion of a single oral dose of 220ug inorganic As(V) by volunteer B.



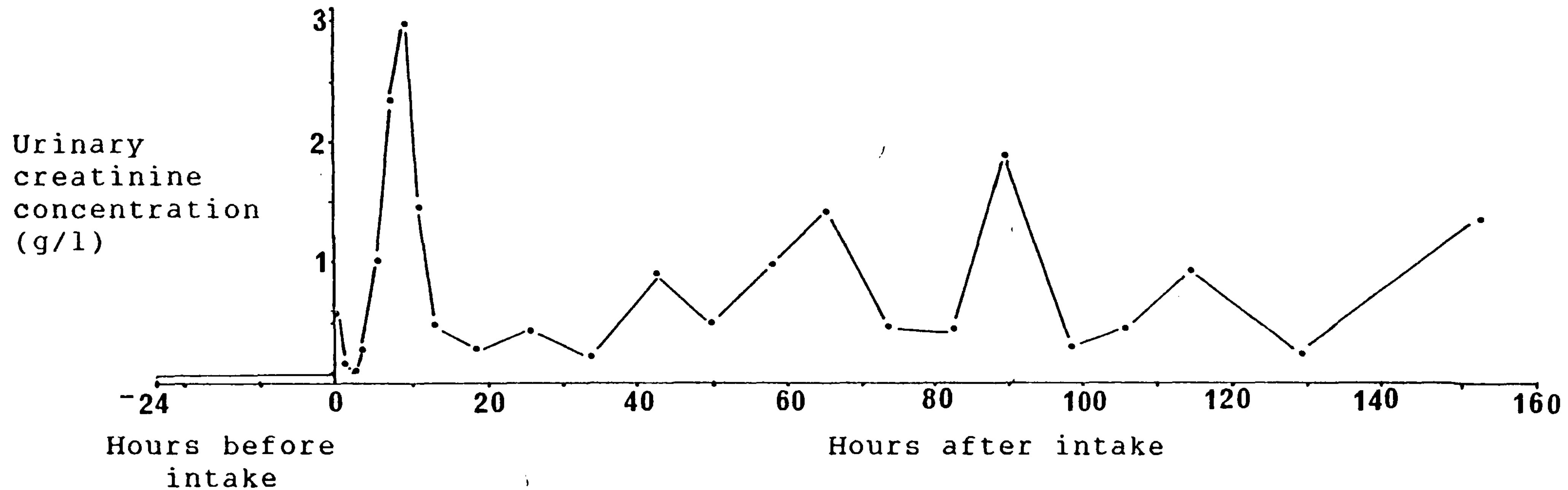
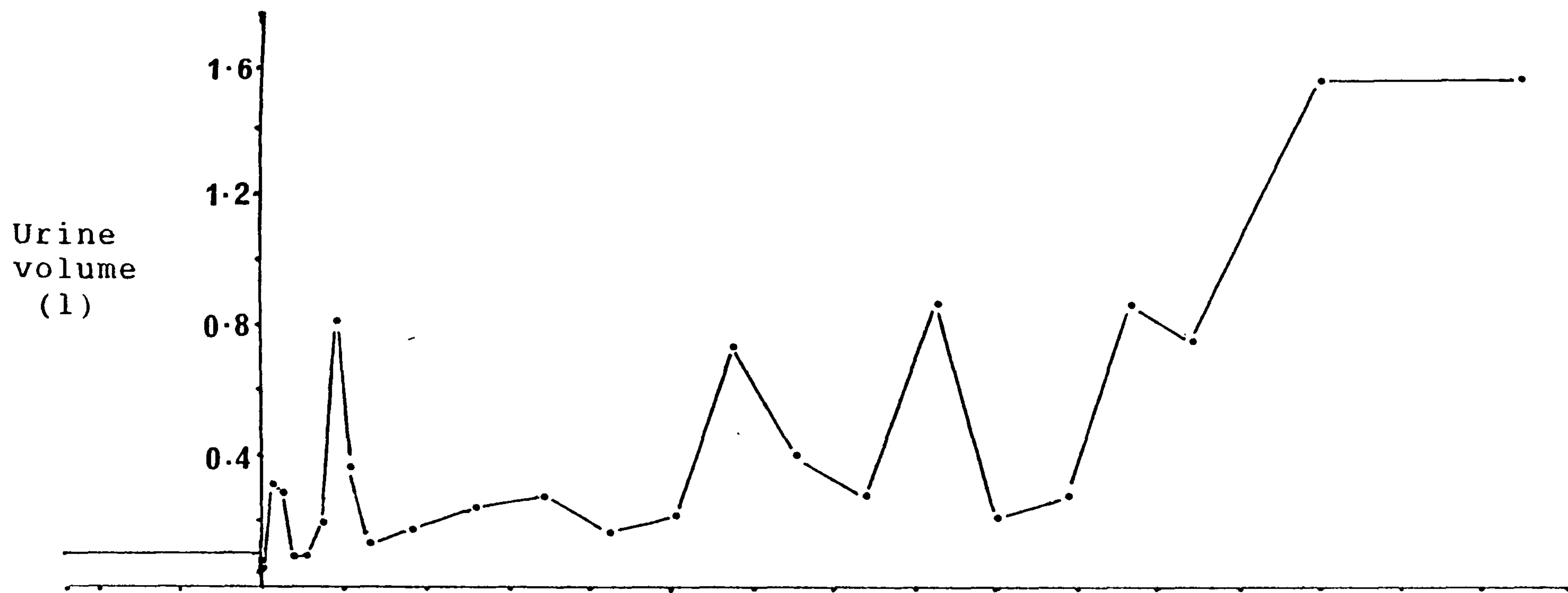


Figure 3.8 relationship between urine volume and creatinine concentration for volunteer A. (Correlation coefficient Rs = -0.5)



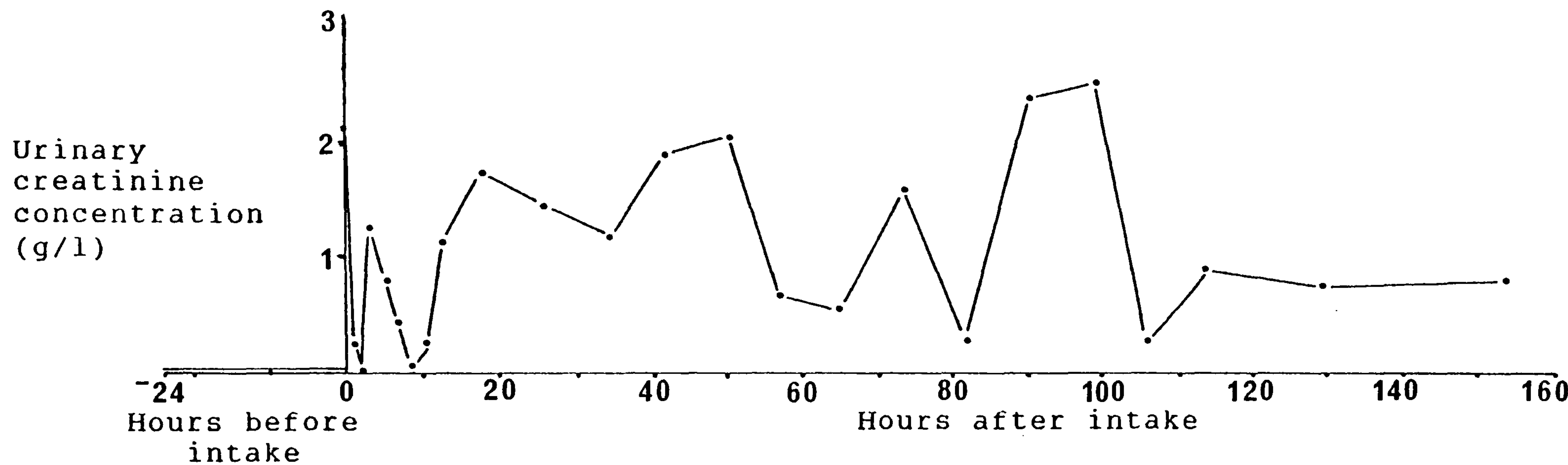


Figure 3.9 relationship between urine volume and creatinine concentration for volunteer B. (Correlation coefficient Rs = -0.62)

TABLE 3.11

EXCRETION OF URINARY ARSENIC (ug/g creatinine) FOLLOWING THE SINGLE ORAL DOSE OF 220ug INORGANIC As(V) BY VOLUNTEER (A)

	ARSENIC CONCENTRATION (ug/g creatinine)				
TIME INTERVAL(hr)	As(V)	As(III)	MMAA	DMAA	SUM OF SPECIES
Pre-24 hr	*	*	*	5.7	5.7
0 hr	*	*	*	4.9	4.9
0-1	*	*	*	*	*
1-2	*	*	*	*	*
2-4	25.0	33.0	3.8	19.6	81.4
4-6	10.0	24.0	11.6	34.9	80.5
6-8	4.4	13.9	20.5	27.5	66.3
8-10	2.3	10.8	10.1	26.8	50.0
10-12	4.4	9.9	11.2	32.8	58.3
12-14	12.1	9.6	11.0	33.3	66.0
14-22	6.1	7.8	10.4	47.0	71.3
22-30	5.7	8.8	9.5	47.0	71.0
30-38	17.8	7.2	5.0	48.3	78.3
38-46	5.4	4.9	3.6	50.8	64.7
46-54	5.9	5.2	3.0	43.0	57.1
54-62	4.0	2.6	2.4	31.5	40.5
62-70	1.6	1.9	1.6	18.6	23.7
70-78	*	3.6	2.4	26.7	32.6
78-86	*	2.7	*	22.7	25.4
86-94	*	1.0	1.1	8.6	10.7
94-102	*	3.8	3.1	26.9	33.8
102-110	*	1.6	1.8	16.3	19.7
110-118	*	1.1	*	8.1	9.2
118-142	*	1.9	*	11.4	13.3
142-166	*	0.9	*	6.6	7.5

* corresponds to species concentrations (ug/l) of less than the detection limit of 0.5ug/l.

TABLE 3.12

EXCRETION OF URINARY ARSENIC (ug/g creatinine) FOLLOWING THE SINGLE ORAL DOSE OF 220ug INORGANIC As(V) BY VOLUNTEER (B)

	ARSENIC CONCENTRATION (ug/g creatinine)				
TIME INTERVAL(hr)	As(V)	As(III)	MMAA	DMAA	SUM OF SPECIES
Pre-24 hr	*	*	*	15.5	15.5
0 hr	*	*	*	2.7	2.7
0-1	*	*	*	*	*
1-2	*	*	*	*	*
2-4	13.6	15.4	2.9	4.8	36.7
4-6	11.3	33.8	9.6	9.6	64.3
6-8	10.7	14.8	15.0	14.0	54.5
8-10	47.0	17.0	13.0	35.0	112.0
10-12	22.3	9.2	11.9	16.2	59.6
12-14	8.2	6.5	8.1	10.3	33.1
14-22	2.9	5.4	8.4	12.2	28.9
22-30	3.1	1.1	8.1	12.8	25.1
30-38	*	3.1	7.4	18.9	29.4
38-46	0.4	1.9	4.9	18.8	26.0
46-54	*	2.2	4.2	14.9	21.3
54-62	1.6	2.3	4.6	14.4	22.9
62-70	*	1.9	4.4	19.0	25.3
70-78	*	1.2	3.2	9.8	14.2
78-86	*	*	5.7	25.2	30.9
86-94	*	*	2.2	8.3	10.5
94-102	*	1.1	1.9	4.9	7.9
102-110	*	*	5.2	10.4	15.6
110-118	*	*	*	9.1	9.1
118-142	*	2.0	*	6.4	8.4
142-166	*	2.6	*	4.4	7.0

* corresponds to species concentrations (ug/l) of less than the detection limit of 0.5ug/l.

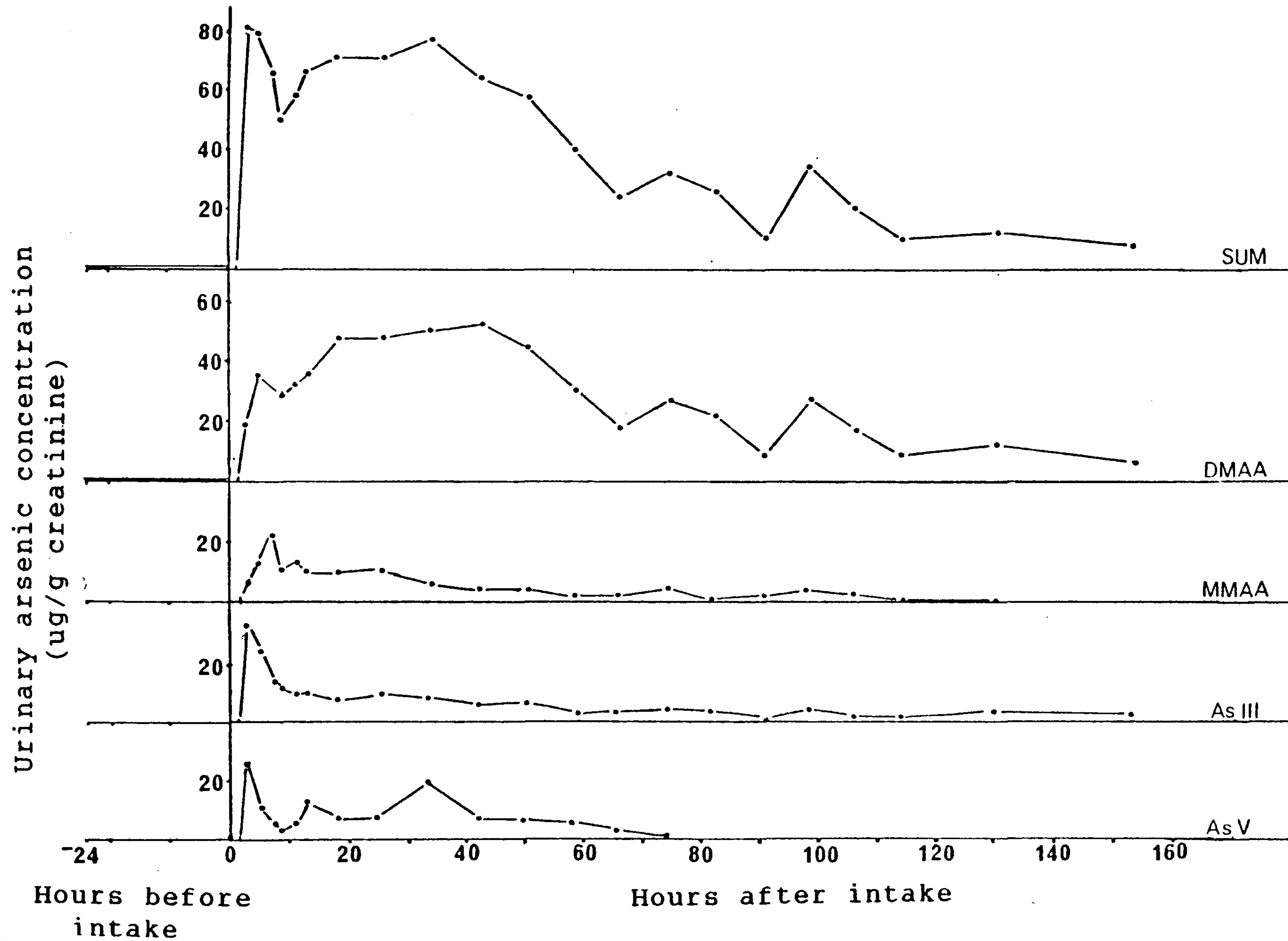


Figure 3.10 Urinary arsenic excreted (ug/g creatinine) following the single oral dose of 220ug inorganic As(V) by volunteer (A).

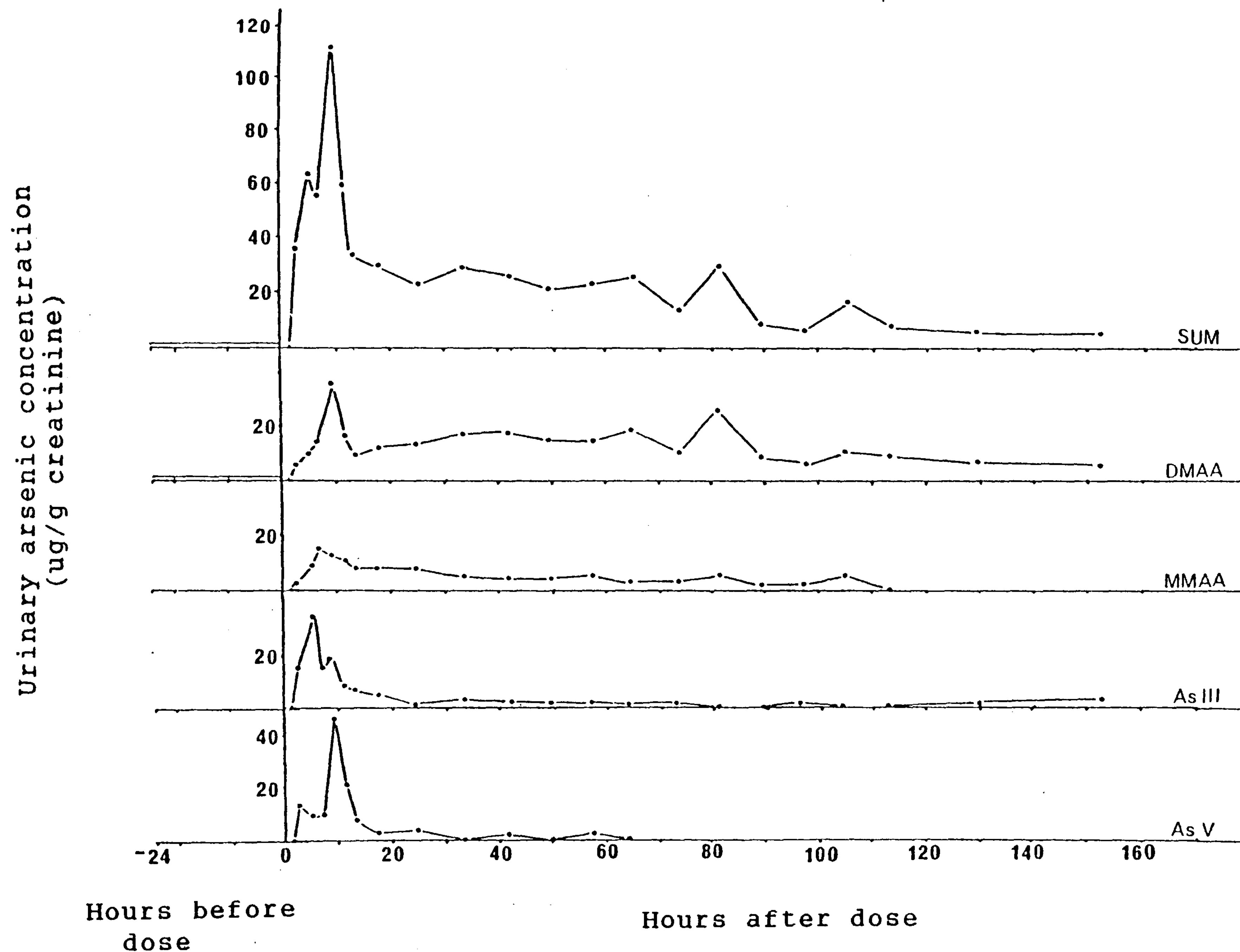


Figure 3.11 Urinary arsenic excreted (ug/g creatinine) following the single oral dose of 220ug inorganic As(V) by volunteer (B).

none of the four arsenic species was detected in the urine of (A) or (B) until the 2 - 4 hr sample, when all were present at significantly elevated concentrations, with inorganic forms, As(V) and As(III), as the major species. The rapid rise in urinary arsenic concentrations was reflected in the sum of the four species being greatest for (A) in the 2 - 4 hr and 4 - 6 hr samples and for (B) in the 8 - 10 hr sample. Whereas the sum of the species' levels for (A) (50 - 81ug/g creatinine) remained relatively constant until the onset of a marked decline after 34 hours, concentrations for (B) fell sharply from the peak of 112ug/g creatinine (8 - 10 hr) to 33.1ug/g creatinine by 12 - 14 hr, followed by a gradual decline. Eventually, sum concentrations for both (A) and (B) reached general "background" levels by about 110 hr. By this time, only DMAA, the predominant individual species since 4 hr for (A) and 12 hr for (B), and low levels of As(III) remained, As(V) having fallen below detection limits at 70 hr for (A) and 62 hr for (B) and MMAA likewise at 110 hr for both.

Concentrations of inorganic arsenic species, As(V) and As(III), were generally at their highest in the first six hours but a few unexpectedly high As(V) levels appeared later - at 12 - 14 hr and 30 - 38 hr for (A) and 8 - 10 hr and 10 - 12 hr for (B). Indeed, the 8 - 10 hr As(V) concentration of 47.0ug/g creatinine for (B) was the highest observed. It is

interesting to note that these elevated As(V) concentrations were associated with unusually large urinary volumes, either in absolute terms (1312ml for (A) 30 - 38 hr, 1015ml and 460ml for (B) 8 - 10 hr and 10 - 12 hr, respectively) or relative to those immediately preceding (180ml for (A) 12 - 14 hr against a total of 216ml for the previous eight hours). Of the methylated forms, MMAA peaked at 6 - 8 hr for (A) and (B) while DMAA exhibited near-constant maximum concentrations from 14 - 46 hr for (A) in contrast to the clearly discernible peak at 8 - 10 hr for (B).

The amounts and associated rates of excretion for each arsenic species (and the sum of the species) for each urine sampling interval are shown in Tables 3.13 (A) and 3.14 (B), and Figures 3.12 (A) and 3.13 (B). The relative proportions of excreted arsenic species are expressed as percentages for each individual sampling interval in Tables 3.15 (A) and 3.16 (B) and Figures 3.14 (A) and 3.15 (B) and as the cumulative amounts and percentages of the cumulative sums excreted from the time of As(V) intake to the end of each sampling interval in Tables 3.17 (A) and 3.18 (B) and as the cumulative amount in Figures 3.16 (A) and 3.17 (B).

Over the 166 hours following As(V) intake, (A) excreted 181ug arsenic as As(V), As(III), MMAA and DMAA in the urine, corresponding to 82.3% of the 220ug

TABLE 3.13

THE AMOUNT OF ARSENIC ELIMINATED AND RATE OF EXCRETION BY VOLUNTEER (A) FOLLOWING THE SINGLE ORAL DOSE OF 220ug INORGANIC As(V)

TIME INTERVAL	As(V)		As(III)		MMAA		DMAA		SUM/hr	
(hr)	ug	ug/hr	ug	ug/hr	ug	ug/hr	ug	ug/hr	ug	ug/hr
24-hr	-	-	-	-	-	-	4.30	0.18	4.30	0.18
0-1	-	-	-	-	-	-	-	-	-	-
1-2	-	-	-	-	-	-	-	-	-	-
2-4	2.46	1.23	3.24	1.62	0.37	0.19	1.93	0.97	8.00	4.00
4-6	0.65	0.33	1.56	0.78	0.75	0.38	2.27	1.14	5.23	2.62
6-8	0.37	0.19	1.17	0.59	1.73	0.87	2.32	1.16	5.59	2.80
8-10	0.25	0.13	1.19	0.60	1.12	0.56	2.97	1.48	5.53	2.77
10-12	0.51	0.26	1.16	0.58	1.30	0.65	3.83	1.92	6.80	3.40
12-14	1.04	0.52	0.83	0.41	0.95	0.48	2.88	1.44	5.70	2.85
14-22	1.15	0.14	1.48	0.18	1.97	0.25	8.86	1.11	13.46	1.68
22-30	1.20	0.15	1.85	0.23	2.00	0.25	9.85	1.23	14.90	1.86
30-38	4.20	0.52	1.71	0.21	1.18	0.15	11.41	1.43	18.50	2.31
38-46	1.26	0.16	1.15	0.14	0.84	0.11	11.96	1.49	15.21	1.90
46-54	1.16	0.15	1.03	0.13	0.58	0.07	8.47	1.06	11.24	1.41
54-62	1.60	0.20	1.06	0.13	0.97	0.12	12.74	1.59	16.37	2.05
62-70	0.49	0.06	0.60	0.08	0.49	0.06	5.81	0.73	7.39	0.92
70-78	-	-	0.79	0.10	0.53	0.07	5.91	0.74	7.23	0.90
78-86	-	-	0.43	0.05	-	-	3.62	0.45	4.05	0.51
86-94	-	-	0.46	0.06	0.48	0.06	3.93	0.49	4.87	0.61
94-102	-	-	0.52	0.07	0.43	0.05	3.69	0.46	4.64	0.58
102-110	-	-	0.49	0.06	0.55	0.08	4.92	0.62	5.96	0.75
110-118	-	-	0.40	0.05	-	-	2.81	0.35	3.21	0.40
118-142	-	-	1.18	0.05	-	-	7.06	0.29	8.24	0.34
142-166	-	-	1.08	0.05	-	-	7.74	0.32	8.82	0.37

\- corresponds to less than 0.3ug excreted over 8hrs or less than 0.7ug excreted over 24 hrs.

TABLE 3.14

THE AMOUNT OF ARSENIC ELIMINATED AND RATE OF EXCRETION BY VOLUNTEER (B) FOLLOWING THE SINGLE ORAL DOSE OF 220ug INORGANIC As(V)

TIME INTERVAL (hr)	As(V) ug	As(V) ug/hr	As(III) ug	As(III) ug/hr	MMAA ug	MMAA ug/hr	DMAA ug	DMAA ug/hr	SUM ug	SUM ug/hr
24-hr	-	-	-	-	-	-	9.63	0.40	9.63	0.40
0-1	-	-	-	-	-	-	-	-	-	-
1-2	-	-	-	-	-	-	-	-	-	-
2-4	1.73	0.86	1.96	0.98	0.37	0.19	0.60	0.30	4.66	2.33
4-6	0.92	0.46	2.75	1.37	0.78	0.39	0,78	0.39	5.23	2.62
6-8	1.08	0.54	1.49	0.74	1.51	0.76	1.42	0.71	5.50	2.75
8-10	4.77	2.39	1.73	0.86	1.32	0.66	3.55	1.78	11.37	5.69
10-12	2.67	1.33	1.10	0.55	1.43	0.71	1.93	0.97	7.13	3.57
12-14	1.53	0.76	1.21	0.60	1.51	0.76	1.92	0.96	6.17	3.09
14-22	1.06	0.13	1.96	0.24	3.08	0.39	4.47	0.56	10.57	1.32
22-30	1.25	0.16	0.44	0.05	3.28	0.41	5.19	0.65	10.16	1.27
30-38	-	-	1.11	0.14	2.67	0.33	6.83	0.85	10.61	1.33
38-46	0.16	0.02	0.70	0.09	1.80	0.23	6.97	0.87	9.63	1.20
46-54	-	-	1.04	0.13	2.02	0.25	7.17	0.90	10.23	1.28
54-62	0.80	0.10	1.16	0.14	2.31	0.29	7.30	0.91	11.57	1.45
62-70	-	-	0.51	0.06	1.17	0.15	5.03	0.63	6.71	0.84
70-78	-	-	0.59	0.07	1.56	0.20	4.78	0.60	6.93	0.87
78-86	-	-	-	-	1.30	0.16	5.75	0.72	7.05	0.88
86-94	-	-	-	-	0.94	0.12	3.50	0.44	4.44	0.56
94-102	-	-	0.65	0.08	1.14	0.14	3.02	0.38	4.81	0.60
102-110	-	-	-	-	1.26	0.16	2.52	0.32	3.78	0.47
110-118	-	-	-	-	-	-	6.64	0.83	6.64	0.83
118-142	-	-	2.76	0.12	-	-	8.86	0.37	11.62	0.48
142-166	-	-	3.94	0.16	-	-	6.70	0.28	10.64	0.44

\- corresponds to less than 0.3ug excreted over 8 hrs and less than 0.7ug excreted over 24 hrs.

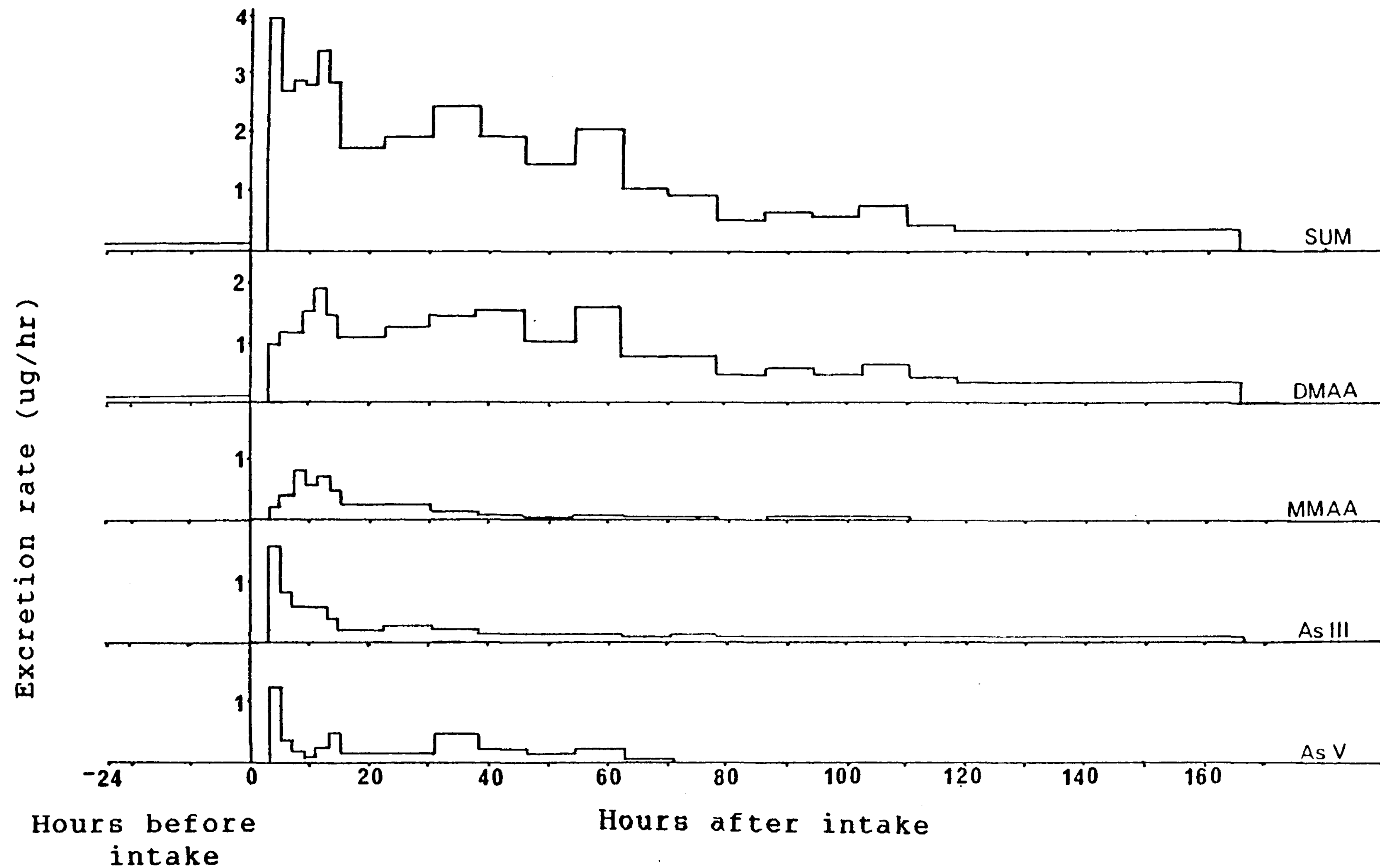


Figure 3.12 Rate of urinary arsenic excretion by volunteer (A) following the single oral dose of 220ug inorganic As(V).

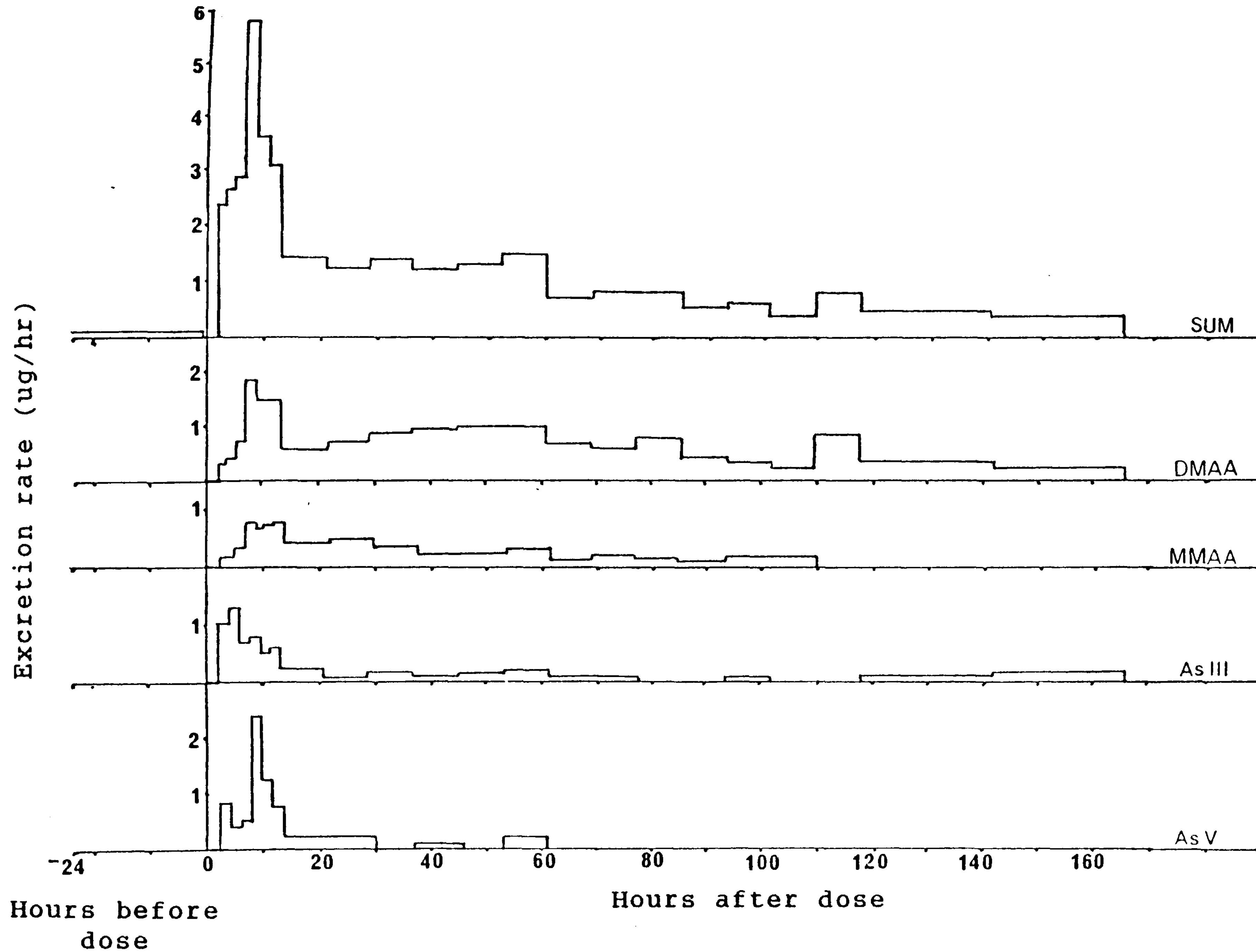


Figure 3.13 Rate of urinary arsenic excretion by volunteer (B) following the single oral dose of 220ug inorganic As(V).

TABLE 3.15

THE RELATIVE PROPORTIONS OF EACH SPECIES EXCRETED BY VOLUNTEER (A) FOLLOWING THE SINGLE ORAL DOSE OF 220ug INORGANIC As(V)

TIME INTERVAL(hr)	As(V) %	As(III) %	MMAA %	DMAA %
0-1	-	-	-	-
1-2	-	-	-	-
2-4	30.8	40.5	4.6	24.1
4-6	12.4	29.8	14.4	43.4
6-8	6.7	21.0	30.9	41.5
8-10	4.5	21.5	20.3	53.7
10-12	7.5	17.0	19.2	56.3
12-14	18.3	14.5	16.7	50.5
14-22	8.5	11.0	14.6	65.9
22-30	8.1	12.4	13.4	66.1
30-38	22.7	9.2	6.4	61.7
38-46	8.3	7.6	5.5	78.6
46-54	10.4	9.2	5.2	75.3
54-62	9.8	6.4	5.9	77.8
62-70	6.7	8.1	6.7	78.5
70-78	-	10.9	7.3	81.8
78-86	-	10.7	-	89.3
86-94	-	9.4	9.9	80.6
94-102	-	11.2	9.2	79.6
102-110	-	8.2	9.3	82.5
110-118	-	12.5	-	87.5
118-142	-	14.3	-	85.7
142-166	-	12.2	-	87.8

\- corresponds to species concentrations below the detection limit of 0.5ug/l (Table 3.9).

TABLE 3.16

THE RELATIVE PROPORTIONS OF EACH SPECIES EXCRETED BY VOLUNTEER (B) FOLLOWING THE SINGLE ORAL DOSE OF 220ug INORGANIC As(V)

TIME INTERVAL(hr)	As(V) %	As(III) %	MMAA %	DMAA %
0-1	-	-	-	-
1-2	-	-	-	-
2-4	37.0	42.0	8.0	13.0
4-6	17.6	52.6	14.9	14.9
6-8	19.7	27.1	27.5	25.8
8-10	42.0	15.2	11.6	31.3
10-12	37.4	15.5	20.0	27.1
12-14	24.8	19.6	24.5	31.1
14-22	10.0	18.5	29.2	42.3
22-30	12.3	4.3	32.3	51.1
30-38	-	10.4	25.2	64.4
38-46	1.7	7.2	18.7	72.3
46-54	-	10.2	19.7	70.1
54-62	6.9	10.0	20.0	63.1
62-70	-	7.6	17.4	75.0
70-78	-	8.5	22.5	69.0
78-86	-	-	18.5	81.5
86-94	-	-	21.2	78.8
94-102	-	13.5	23.6	62.8
102-110	-	-	33.3	66.7
110-118	-	-	-	100.0
118-142	-	23.7	-	76.3
142-166	-	37.0	-	63.0

\- corresponds to species concentration below the detection limit of 0.5ug/l (Table 3.10)

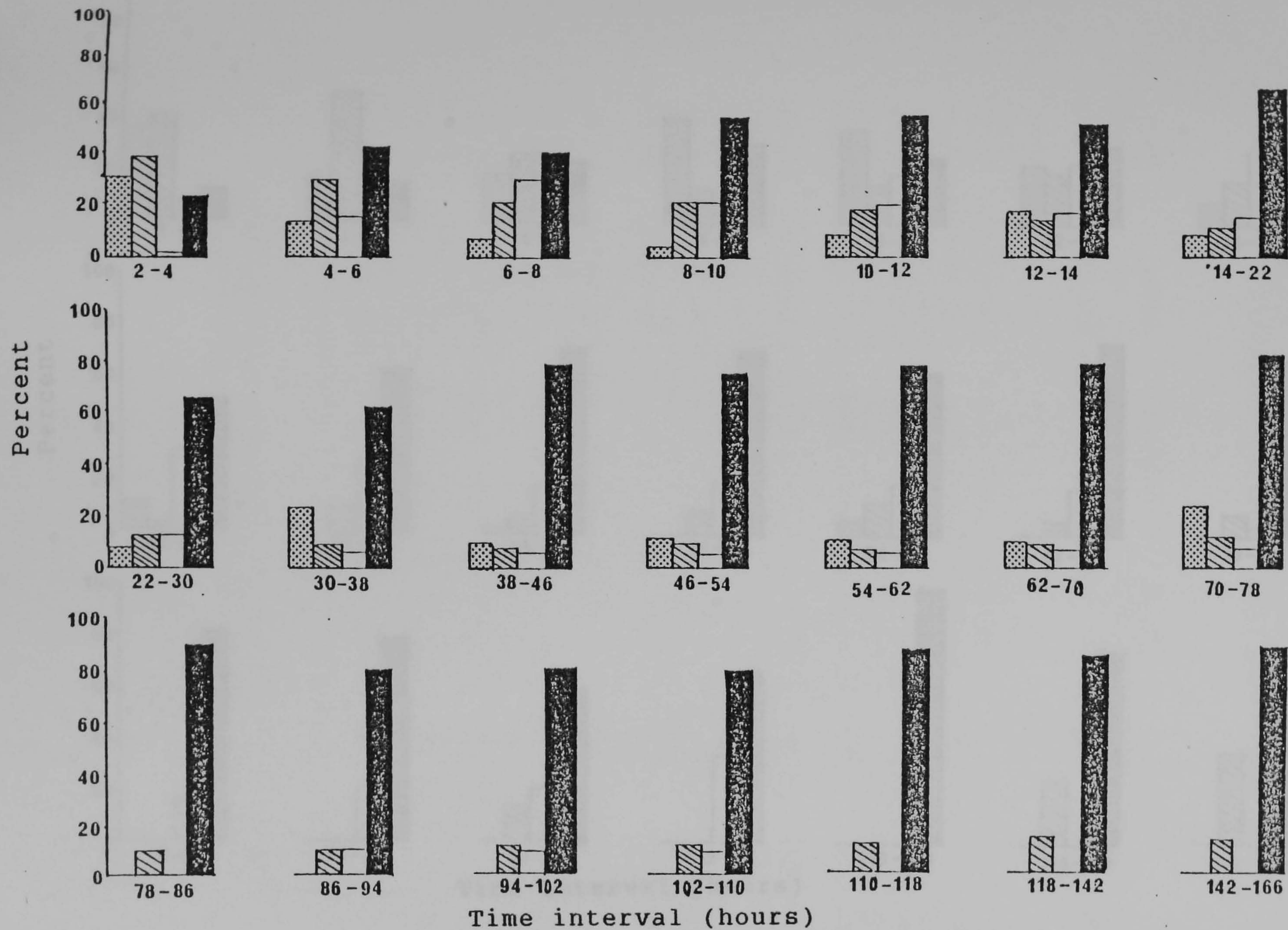


Figure 3.14 Relative proportions of each species excreted by volunteer (A) following a single oral dose of 220ug inorganic As(V).

As(V) As(III) MMAA DMAA

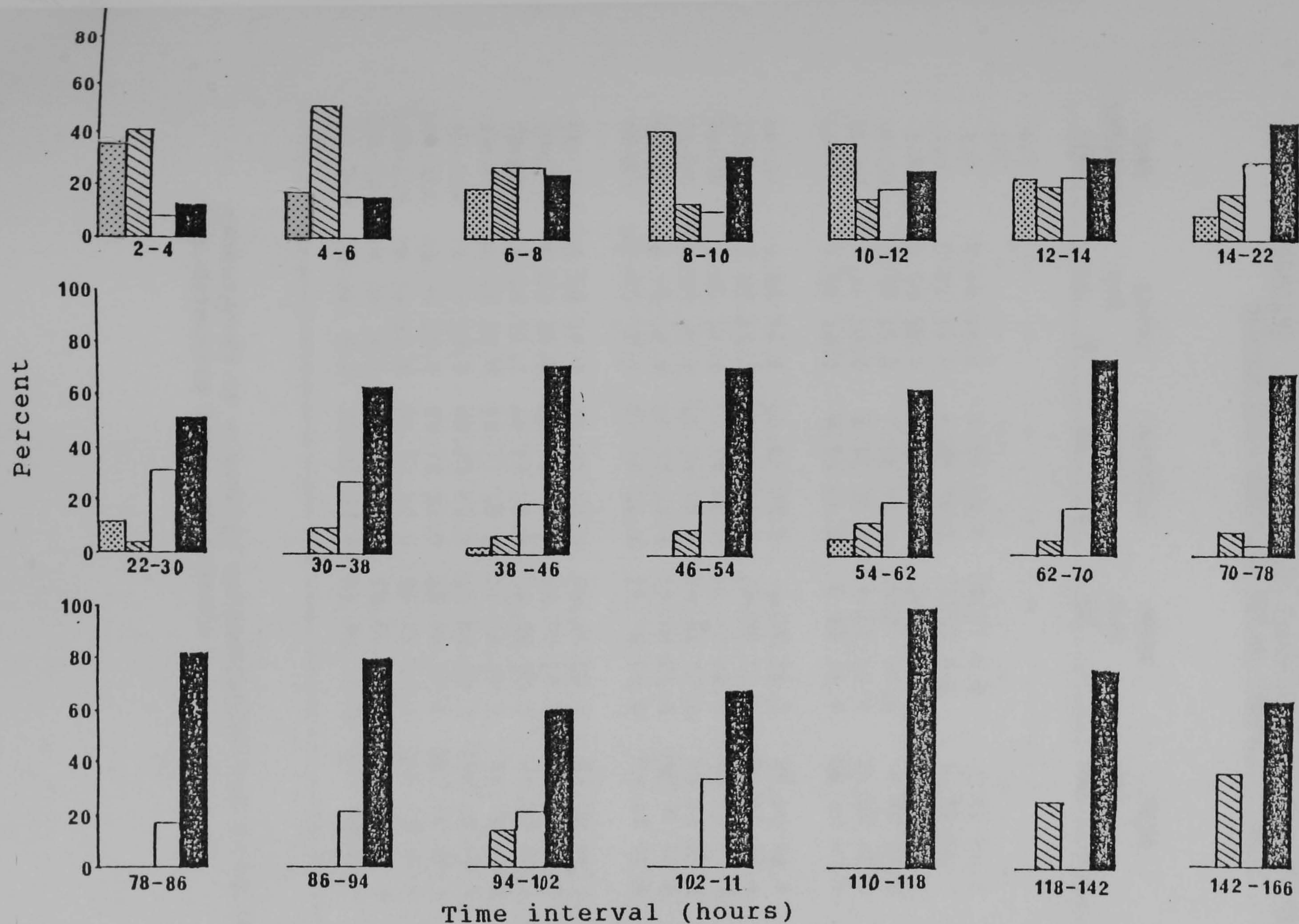


Figure 3.15 Relative proportions of each species excreted by volunteer (B) following a single oral dose of 220ug inorganic As(V).

As(V) As(III) MMAA DMAA

TABLE 3.17

THE CUMULATIVE AMOUNT OF ARSENIC AND PERCENTAGE OF THE CUMULATIVE SUMS EXCRETED BY VOLUNTEER (A) FOLLOWING THE SINGLE ORAL DOSE OF 220ug INORGANIC As(V)

TIME INTERVAL (hr)	As(V) cum ug	As(V) %	As(III) cum ug	As(III) %	MMAA cum ug	MMAA %	DMAA cum ug	DMAA %	SUM cum ug	SUM % of dose
0-1	-	-	-	-	-	-	-	-	-	-
1-2	-	-	-	-	-	-	-	-	-	-
2-4	2.46	30.8	3.24	40.5	0.37	4.6	1.93	24.1	8.00	3.6
4-6	3.11	23.5	4.80	36.3	1.12	8.5	4.20	31.7	13.23	6.0
6-8	3.48	18.5	5.97	31.7	2.85	15.1	6.52	34.6	18.82	8.6
8-10	3.73	15.3	7.16	29.4	3.97	16.3	9.49	39.0	24.35	11.1
10-12	4.24	13.6	8.32	26.7	5.27	16.9	13.32	42.7	31.19	14.2
12-14	5.28	14.3	9.15	24.8	6.22	16.9	16.20	43.9	36.89	16.8
14-22	6.43	12.8	10.63	21.1	8.19	16.3	25.06	49.8	50.35	22.9
22-30	7.63	11.7	12.48	19.1	10.19	15.6	34.91	52.4	65.25	29.7
30-38	11.83	14.1	14.19	16.9	11.37	13.6	46.32	55.3	83.75	38.1
38-46	13.09	13.2	15.34	15.5	12.21	12.3	58.28	58.9	98.96	45.0
46-54	14.25	12.9	16.37	14.9	12.79	11.6	66.75	60.6	110.20	50.1
54-62	15.85	12.5	17.43	13.8	13.76	10.9	79.49	62.8	126.57	57.5
62-70	16.34	12.3	18.03	13.5	14.25	10.7	85.30	64.1	133.16	60.9
70-78	16.34	11.6	18.82	13.3	14.78	10.5	91.21	64.6	141.19	64.2
78-86	16.34	11.3	19.25	13.3	14.78	10.2	94.83	65.3	145.24	66.0
86-94	16.34	10.9	19.71	13.1	15.26	10.2	98.76	65.8	150.11	68.2
94-102	16.34	10.6	20.23	13.1	15.69	10.1	102.45	66.2	154.75	70.3
102-110	16.34	10.2	20.72	12.9	16.24	10.1	107.37	66.8	160.71	73.1
110-118	16.34	10.0	21.12	12.9	16.24	10.0	110.18	67.2	163.92	74.5
118-142	16.34	9.6	22.30	12.9	16.24	9.4	117.24	68.1	172.16	78.3
142-166	16.34	9.0	23.38	12.9	16.24	9.0	124.98	69.1	180.98	82.3

\- corresponds to a species concentration (ug/l) below the detection limit of 0.5ug/l

TABLE 3.18

THE CUMULATIVE AMOUNT OF ARSENIC AND PERCENTAGE OF THE CUMULATIVE SUMS EXCRETED BY VOLUNTEER (B) FOLLOWING THE SINGLE ORAL DOSE OF 220ug INORGANIC As(V)

TIME INTERVAL (hr)	As(V) cum ug	%	As(III) cum ug	%	MMAA cum ug	%	DMAA cum ug	%	SUM cum ug	% of dose
0-1	-	-	-	-	-	-	-	-	-	-
1-2	-	-	-	-	-	-	-	-	-	-
2-4	1.73	37.1	1.96	42.1	0.37	7.9	0.60	12.9	4.66	2.1
4-6	2.65	26.8	4.71	47.6	1.15	11.6	1.38	14.0	9.89	4.5
6-8	3.73	24.2	6.20	40.3	2.66	17.3	2.80	18.2	15.39	7.0
8-10	8.50	31.8	7.93	29.6	3.98	14.9	6.35	23.7	26.76	12.2
10-12	11.17	33.0	9.03	26.6	5.41	16.0	8.28	24.4	33.89	15.4
12-14	12.70	31.7	10.24	25.6	6.92	17.3	10.20	25.5	40.06	18.2
14-22	13.76	27.2	12.20	24.1	10.00	19.8	14.67	29.0	50.63	23.0
22-30	15.01	24.7	12.64	20.8	13.28	21.8	19.86	32.7	60.79	27.6
30-38	15.01	21.0	13.75	19.3	15.95	22.3	26.69	37.4	71.40	32.5
38-46	15.17	18.7	14.45	17.8	17.75	21.9	33.66	41.5	81.03	36.8
46-54	15.17	16.6	15.49	17.0	19.77	21.7	40.83	44.7	91.26	41.5
54-62	15.97	15.5	16.65	16.2	22.08	21.5	48.13	46.8	102.83	46.7
62-70	15.97	14.6	17.16	15.7	23.25	21.2	53.16	48.5	109.54	49.8
70-78	15.97	13.7	17.75	15.2	24.81	21.3	57.94	49.7	116.47	52.9
78-86	15.97	12.9	17.75	14.4	26.11	21.1	63.69	51.6	123.52	56.1
86-94	15.97	12.5	17.75	13.9	27.05	21.1	67.19	52.5	127.96	58.2
94-102	15.97	12.0	18.40	13.9	28.19	21.2	70.21	42.9	132.77	60.4
102-110	15.97	11.7	18.40	13.5	29.45	21.6	72.73	53.3	136.55	62.1
110-118	15.97	11.1	18.40	12.9	29.45	20.6	79.37	55.4	143.19	65.1
118-142	15.97	10.3	21.16	13.7	29.45	19.0	88.23	57.0	154.81	70.4
142-166	15.97	9.7	25.10	15.2	29.45	17.8	94.93	57.4	165.45	75.2

\- corresponds to a species concentration (ug/l) below the detection limit of 0.5ug/l

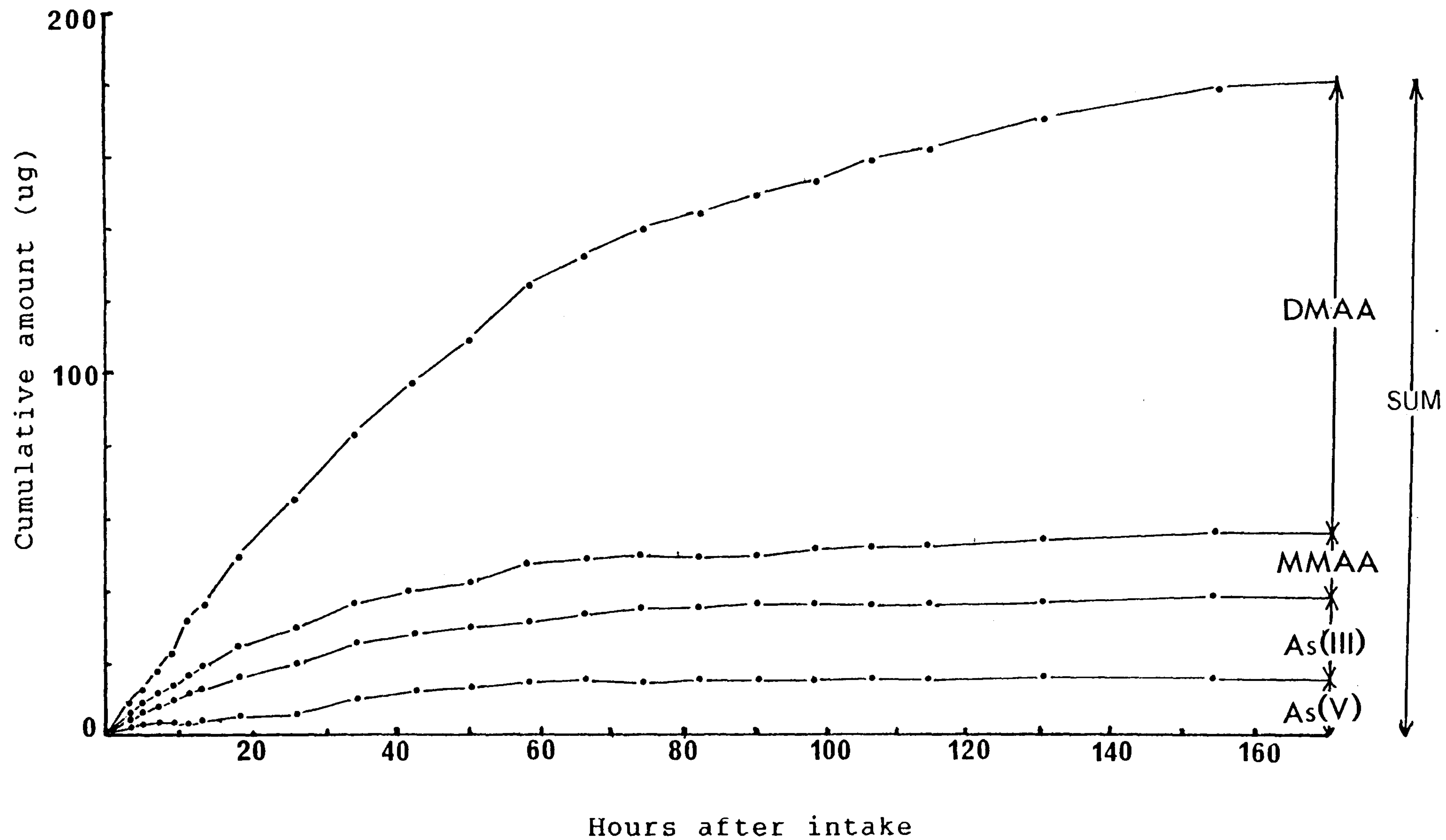


Figure 3.16 The cumulative amount of arsenic excreted by volunteer A, following the single oral dose of 220ug of inorganic As(V).

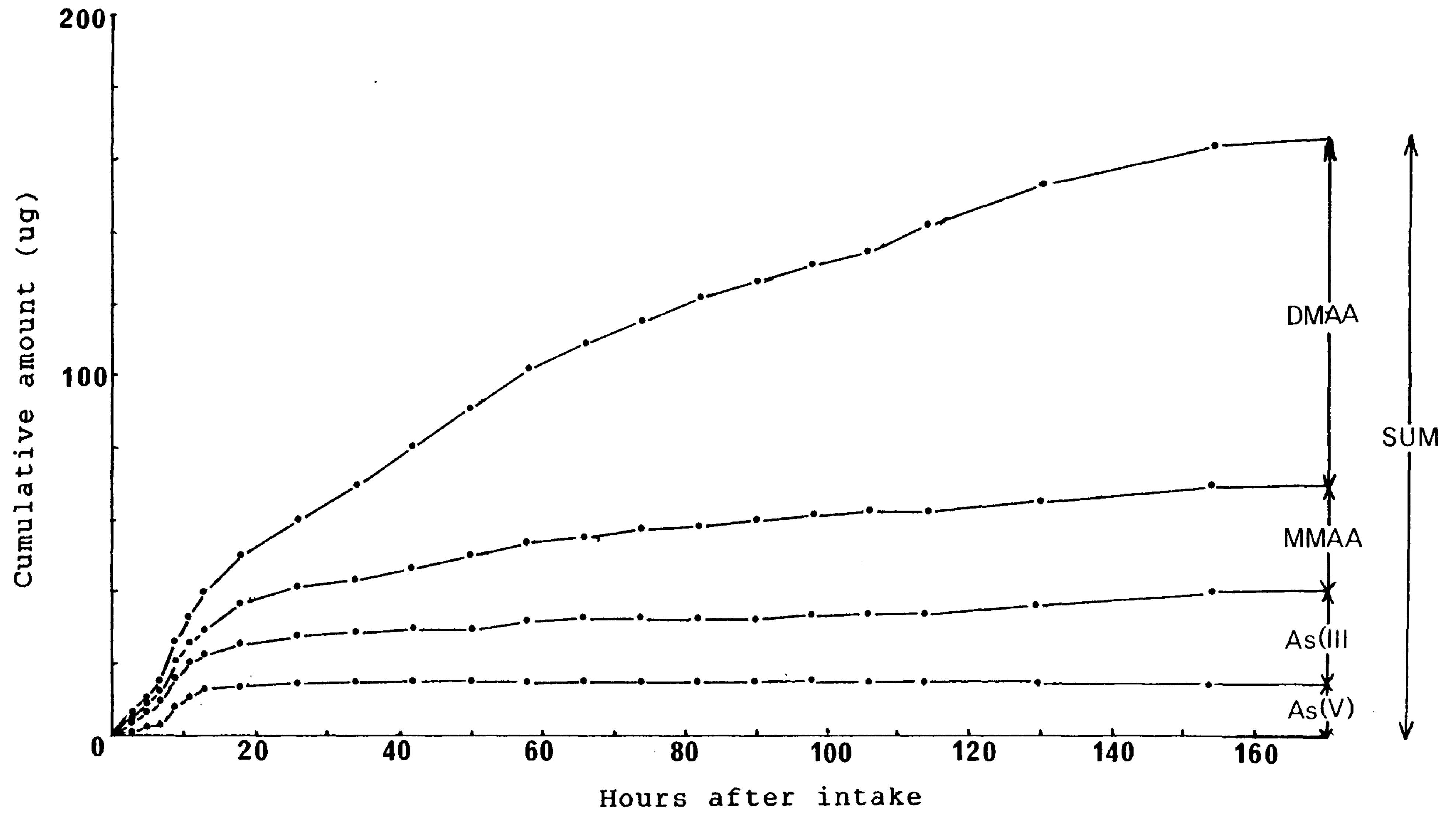


Figure 3.17 The cumulative amount of arsenic excreted by volunteer B, following the single oral dose of 220ug of inorganic As(V).

dose (68.9% after correction for 'background' excretion using the pre-24hr concentration in Table 3.9). Of the excreted amount, DMAA comprised 69.1%, As(III) 12.9%, MMAA 9.0% and As(V) 9.0%. The other volunteer, (B), excreted 165.4ug, equivalent to 75.2% (63.9% after correction for 'background' excretion, using the pre-24hr data in Table 3.10, but then modifying the pre-24hr output to a 'normal' urine concentration of 5.0ug/g creatinine - Table 4.3) of the As(V) dose, of which DMAA contributed 57.4%, MMAA 17.8%, As(III) 15.2% and As(V) 9.7%.

The two volunteers excreted similar amounts of arsenic (as As(V), As(III), MMAA and DMAA) in urine over the first 22 hours, corresponding to 22.9% and 23% of the intake for (A) and (B), respectively. In contrast, the speciation patterns for this period were rather different: (A) - 12.9% As(V), 21.1% As(III), 16.3% MMAA and 49.8% DMAA; (B) - 27.2% As(V), 24.1% As(III), 19.8% MMAA and 29.0% DMAA. The times to excrete amounts equivalent to 50% and 75% of the dose were approximately 54 hr and 118 hours, respectively, for (A) and 70 hr and 166 hours for (B).

For (A), the maximum rate of arsenic excretion (4.00ug/hr) was attained just 2 - 4 hours after intake and remained fairly constant at 2.62 - 3.40 ug/hr thereafter until 14 hrs. The average rate for the 2 - 14 hr period was 3.07 ug/hr. Between 14 and 62 hours, the excretion rate (1.41 - 2.31ug/hr) averaged

1.87ug/hr, while for 62 - 166 hrs the rate (0.34 - 0.92ug/hr) dropped to a mean of 0.52ug/hr. A similar pattern emerged for (B), although the maximum excretion rate was both greater in magnitude (5.69ug/hr) and occurred later (8 - 10 hrs). Over the same three time intervals, however, the average rates of excretion for (B) were 3.34ug/hr (2 - 14 hr), 1.31ug/hr (14 - 62 hr) and 0.60ug/hr (62 - 166 hr).

The predominance of the two inorganic species, As(V) and As(III), in the individual 2 - hour samples was very brief for (A) (2 - 4 hr, 71.3% of the sum of the species) and, for (B), after exceeding 70% from 2 - 6 hr, fell into the range 44.4 - 57.2% for only the next eight hours. Only once, in the 4 - 6 hr sample for (B), did either of the two inorganic species exceed 50% of the sum of the four species. Dimethylarsinic acid, which became the major species for (A) in the 4 - 6hr sample, exceeded 50% of the sum for the first time in the 8 - 10 hr sample and thereafter steadily increased in relative importance, consistently representing over 80% of the excreted arsenic in post - 70 hr samples. In contrast, for (B), only in the 12 - 14 hr sample did DMAA first become the major species, passing 50% of the sum in the 22 - 30 hr sample and generally representing 60 - 80% of the excreted arsenic in the remaining samples. These features are reflected in the relative proportions of excreted species for the time

intervals 2 - 14 hr, 14 - 62 hr and 62 - 166 hr, corresponding to the three zones previously delineated on the basis of excretion rate, in Table 3.19. The relatively greater prominence of MMAA in the urine of (B) after 14 hr, and especially after 30 hr, (Tables 3.15, 3.16) and the continuing presence from 62 - 166 hr of As(III) at about 10% of the sum for (A) and (B), while As(V) and, to a lesser extent, MMAA decreased markedly, should also be noted.

The calculated sum of species concentrations for (A) were generally in good agreement with the measured total arsenic concentrations, the mean ratio (sum/total) averaging 0.94 ± 0.13. For (B) the mean ratio was much lower, 0.76 ± 0.11, probably as a result of differences after 30 hours. From 2 - 30 hrs, the ratios for (A) and (B) were similar, 0.87 ± 0.11 and 0.89 ± 0.13 (once somewhat erratic 8 - 10 hr and 10 - 12 hr ratios for (B) were excluded), respectively, but from 30 - 166 hrs, 0.98 ± 0.13 (A) and only 0.64 ± 0.15 (B). Whereas small deviations from unity, especially at high concentrations, may well be experimental in origin, the large discrepancy for (B) is probably due to unexpected excretion, or more likely, unplanned consumption of a small amount of seafood in the latter stages of the study. Indeed, (B) excreted a total of 240.9ug arsenic, a figure actually in excess of the As(V) intake, whereas the total amount excreted in urine by (A), 191ug, was

TABLE 3.19

SUMMARY OF RELATIVE PROPORTIONS OF ARSENIC SPECIES EXCRETED IN URINE FOLLOWING THE SINGLE ORAL DOSE OF 220ug INORGANIC As(V) BY VOLUNTEERS (A) AND (B)

TIME INTERVAL		PERCENTAGE As(V)	As(III)	MMAA	DMAA	SUM as a % of amount excreted
2-14	A	14.3	24.8	16.9	43.9	20.4
	B	31.7	25.6	17.3	25.5	24.2
14-62	A	11.7	9.2	8.4	70.6	49.6
	B	5.2	10.2	24.2	60.4	37.9
62-166	A	0.9	10.9	4.6	83.6	30.1
	B	-	13.5	11.8	74.9	37.9

\- corresponds to a species concentration (ug/l) below the detection limit of 0.5ug/l

less than the intake and only slightly exceeded the integrated sum of the species excreted. Furthermore, in cases where the total arsenic concentration was appreciably greater than the sum of the species concentration for (B) (e.g. 46 - 54 hr, 70 - 78 hr, 86 - 94 hr), the latter was found to be in reasonably good agreement with the range estimated for the hydride-forming species by the direct scan. This relationship generally prevailed for all samples analysed in the study.

3.3.5 Discussion

In the seven days following the oral ingestion of 220ug As(V), volunteers (A) and (B) excreted at least 69% and 64%, respectively, of the dose via the kidneys. Analytical losses (e.g. incomplete recovery during ion-exchange separation), incomplete absorption and other elimination mechanisms (e.g perspiration, faeces) might account for part of the apparent shortfall, but some of the ingested arsenic could also have been retained in the body to be eliminated at a later stage. Pomroy et al. (1980), using the three compartment model for excretion described in Section 3.2.5, calculated biological half-lives of 2.1 days (66% of the dose) 9.5 days (30% of the dose) and 38.4 days (3.7% of the dose), suggesting that a prolonged, but somewhat impractical, period of monitoring at levels close to normal background levels might be necessary.

The results of the seafood and single-dose inorganic arsenic metabolic studies show that, while the uptake of arsenic is high in both cases, the rate of arsenic excretion is much slower in the latter (e.g. 44 - 53% of the arsenic eliminated with a biological half-life of 6.9 - 11.0 hours after the ingestion of seafood compared with a first component half-life of 24.1hrs (49% of the dose) and 17.7hrs (32% of the dose) for (A) and (B) respectively following inorganic arsenic ingestion (second component half-lives of 8.6 days and 7.1 days respectively) (Figure 3.18). Whereas the complex organoarsenical absorbed in the seafood experiment was excreted unchanged, however, the ingested pentavalent inorganic arsenic, As(V), was largely converted to As(III) and the methylated metabolites MMAA and DMAA before excretion in the urine (Tables 3.15 - 3.18, Figures 3.14 - 3.17). Although differences were observed in the urinary arsenic speciation pattern of the two participants in the second experiment, DMAA quite rapidly became the major individual species (4 - 6 hr (A); 12 - 14hr (B)). Similar excretory and reduction/methylation trends have been observed in the few previously published papers on the speciation of urinary arsenic after the controlled oral administration of a single dose of inorganic arsenic to human volunteers.

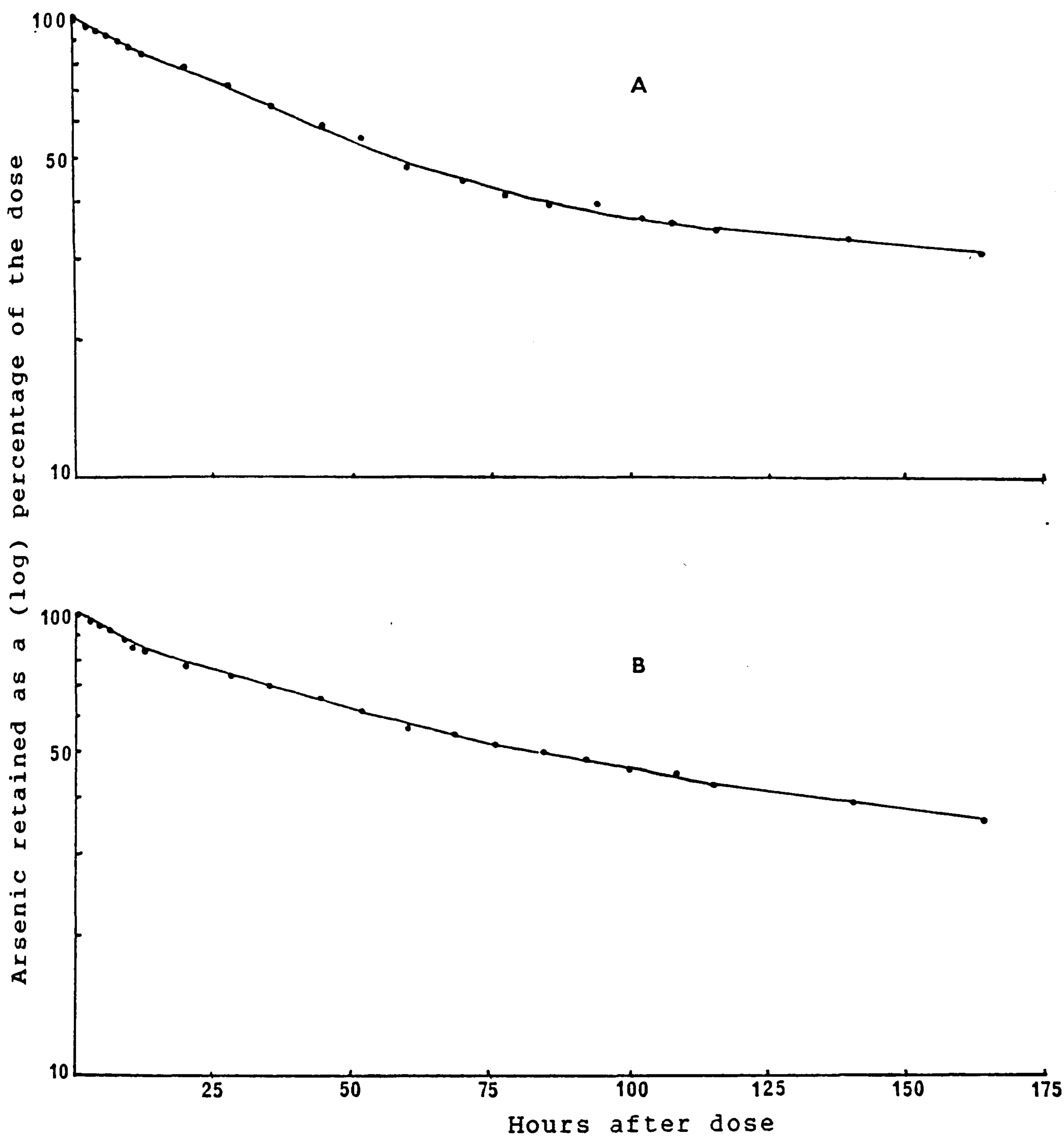


Figure 3.18 Arsenic retention curves for volunteers (A) and (B) following the single oral dose of 220ug inorganic As(V).

Crecelius (1977) was the first to report on the methylation of ingested inorganic arsenic by human volunteers. After the ingestion of 200ug As(V) in well water, urinary concentrations of As(V) and, to a lesser extent, As(III) rose rapidly within the first few hours but thereafter fell rapidly, especially As(V), to typical background levels by about 10 hours. Both MMAA and DMAA concentrations increased more slowly until, by 10 hours, DMAA had become the major species, increasing steadily and maintaining an elevated concentration over the next 50 - 60 hours. However, less than 50% of the ingested dose was excreted in the urine during the 70 hours following ingestion. After the oral administration of 0.01ug radiolabelled As(V), Tam et al. (1979b) found that 22.4% of the dose was excreted in urine over the first 24 hours and 66% of the dose over five days. Of this, 27% had been excreted as inorganic arsenic (no differentiation made between As(V) and As(III)), 21% as MMAA and 51% as DMAA. They noted that inorganic arsenic excretion was predominant for the first day only, the relative proportion rapidly decreasing with time as DMAA increased. Similarly Pomroy et al. (1980) in an experiment in which 6 volunteers took oral doses of inorganic ^{74}As, found that, on average, only 62% of the dose was recovered in the urine 7 days after intake of the dose.

Most previous studies of inorganic arsenic

metabolism, however, have been based on the ingestion of inorganic trivalent arsenic. The intake of 63ug inorganic arsenic in wine (50ug As(III) and 13ug As(V)) gave rise to a five fold increase in As(V), As(III), MMAA and DMAA production in the urine with 80% of the dose excreted within 61 hours (2.5 days), 50% as DMAA, 14% as MMAA and 36% as inorganic arsenic (Crecelius, 1977). Yamauchi and Yamamura (1979) noted that the As(III) excretion was greater than MMAA and DMAA during the first 12 hours following the intake of 700ug As(III) as As_2O_3. Thereafter, the urinary concentrations of both MMAA and DMAA exceeded that of As(III), still remaining above background levels at 72 hours by which time 70% of the ingested dose had been excreted. Buchet et al. (1980, 1981a) found that after the administration of a single oral dose of 500ug inorganic As(III) to three human volunteers, almost 50% of the intake was recovered in urine in 4 days. Of this, 75% was methylated (1/3 as MMAA, 2/3 as DMAA). While excretion in the inorganic form largely occurred during the first few hours following the exposure, the methylation process was rapidly triggered and led to a preponderance of the methylated forms after eight hours.

Methylated metabolites MMAA and DMAA, have also been identified in human urine after the ingestion of much larger amounts (e.g. approximately 1

- 2.5mgAs/kg body weight) of inorganic arsenic in cases of reported arsenic poisoning. In two cases involving the acute intoxication of human subjects by arsenic trioxide ($As(III)_2O_3$) and sodium orthoarsenate ($Na_2HAs(V)O_4.7H_2O$), respectively, in unsuccessful suicide attempts (Lovell and Farmer, 1985), MMAA and DMAA steadily increased as a proportion of the total, constituting approximately 50% of the total arsenic excreted in the urine three days after ingestion. Mahieu et al. (1981), in a study of five humans acutely intoxicated by arsenic trioxide with ingested amounts of inorganic arsenic exceeding 500mg, found that the organic methylated forms approached 50% of the total at 4 days and 90% between 6 and 9 days after ingestion, the dimethylated derivative becoming increasingly preponderant. Similarly, Foa et al. (1984), in a case of acute poisoning by ingested arsenic trioxide (approx. 2300mg arsenic), found that methylated species accounted for 70 - 90% of total arsenic in urine 9 to 20 days later, with DMAA increasing from 30% (8 days) to 82% (20 days) of the total. In all the cases of acute arsenic intoxication, several days elapsed before the dimethylated form predominated in urine (e.g. 5 days Buchet et al., 1980; 4 days Mahieu et al., 1981; 3 days Farmer and Lovell, 1985; 9 days Foa et al., 1984;), a much longer time interval than the 4 - 6hrs (A) and 12 - 14hrs (B) of the metabolic study

conducted here. Formerly, such delays were attributed to possible saturation of the body's methylating capacity at such high doses (Buchet et al., 1981b). Buchet and Lauwreys (1985), however, have recently demonstrated that it is the methylation of MMAA to DMAA which is inhibited by high concentrations of As(III), so that there is a latent period before both the appearance and preponderance of DMAA in urine. In general, the increasing predominance of the less reactive and hence more readily excreted methylated species in both low-dose experimental studies and high-dose poisoning incidents supports the concept of methylation as a natural detoxification mechanism through the biotransformation of the more toxic inorganic arsenic by the human body following the general mechanistic sequence As(V)$\longrightarrow$ As(III)$\longrightarrow$MMAA$\longrightarrow$DMAA.

It should be noted that the use of dimercaptan chelating agents to aid the excretion of arsenic (e.g. as in British Anti-Lewisite (BAL) therapy of many of the inorganic arsenic poisoning cases above) may not only increase the rate of arsenic excretion in urine, but also influence the relative amounts of the different arsenic metabolites (Maiorino and Aposhian, 1985). Such considerations do not, of course, affect the detailed interpretation of the excretion rates and speciation patterns observed in this metabolic study,

where the general reduction/methylation trend is in evidence.

In this study, as distinct from most other studies, the inorganic species As(V) and As(III) were determined separately. Lauwreys and Buchet (1985) have questioned the validity of As(V)/As(III) speciation in human urine. They believe that the relative proportion of both inorganic species found in collected urine samples is not necessarily representative of the true situation in vivo. It is their view that a variable proportion of As(III) may be chemically transformed (i.e. oxidised) into As(V), depending on the urine pH, the duration of its retention in the bladder and the duration of sample storage before analysis. These findings, however, were based on a study restricted to the storage of samples of 100ug/l As(III) concentration at differing pH for 8 days at room temperature only (Buchet et al., 1980). A more comprehensive study was carried out in this laboratory on samples of varying arsenic concentration at a pH of 5, 7 and 9, with storage temperatures of $^{-}20^{o}C$, $4^{o}C$ and room temperature, and storage times of 7, 14 and 28 days. From this it was concluded that:

1) Urine samples (10 - 1000ug/l As(III)) can be stored at $^{-}20^{o}C$ and $4^{o}C$ for 28 days with $<$ 10% oxidation of As(III) to As(V) provided the pH is $\leqslant$ 7.

2) Urine samples can be stored at room temperature for 7 days with $<$ 10% oxidation of As(III) to As(V) (25% if $\leqslant$ 10ug/l at pH 7) provided pH$\leqslant$ 7. (Buchet et al. (1980) similarly found that only 11 - 15% of As(III) at a concentration of 100ug/l was oxidised to As(V) in urine of pH 4.8 - 7.0 after 8 days at room temperature).

3) If urine is at pH 9, then $>$ 50% oxidation of As(III) to As(V) occurs after 7 days storage even at $^{-}20^{o}$C.

Therefore the potential for oxidation of As(III) to As(V) in urine is clearly more dependent upon sample pH than on the storage temperature or time.

The pH of human urine lies typically between 5 and 6 and, indeed, no more than 3% of all urine samples investigated in this work were found to be of pH $\geqslant$ 7, the probable critical level. Under the sampling, storage and analytical conditions employed in this particular metabolic study (Sections 3.3.2, 3.3.3), it therefore seems reasonable to conclude that the measured As(V)/As(III) speciation is representative of the in vivo situation.

Initial urinary As(III)/As(V) ratios, over the first 8 hours for both volunteers (A) and (B), for example, exceeded 1.0 (Tables 3.11, 3.12). As the intake was in the form of As(V) and there is a large difference in pKa between As(V) (2.23 - H_3AsO_4 and As(III) (9.23 - $HAsO_2$), direct excretion, via the

kidney, of unchanged As(V) might have been expected to predominate at this stage. Clearly the reduction of As(V) to As(III) was extremely rapid. The continuing output of small quantities of As(III) in the later stages of the experiment (Tables 3.11, 3.12) when As(V) could not be detected, reflects the differences in the interactions of the two inorganic species with cellular constituents (Section 1.3.2). Essentially unionised, As(III) is more readily assimilated by cells through diffusion-controlled processes (Lerman et al., 1983), the concentration gradient being maintained by further metabolism to MMAA, and exhibits greater affinity than As(V) for the sulphydryl groups of proteins and enzymes (Knowles and Benson, 1983; Lerman et al., 1983; Vahter, 1983; Vahter and Marafantę, 1983; Fischer et al., 1985). At intermediate stages in the experiment, the link between high volumes of urinary output and the unexpected occasional surges in release of As(V) relative to As(III) (Section 3.3.4) may possibly be a result of enhanced flushing of As(V) from preferred retention sites in the kidney itself (Marafante et al., 1985).

After the initial prominence of the inorganic species, the methylated metabolites, MMAA and DMAA, soon constituted most of the arsenic excreted in the urine samples as methylation proceeded (Tables 3.15, 3.16 and Figures 3.14, 3.15). While only 9.0% and

17.8% of the eliminated arsenic was excreted as MMAA, 69.1% and 57.4% was excreted as DMAA by (A) and (B) respectively (Tables 3.17, 3.18). It was noticeable that the MMAA/DMAA ratio, after peaking in the 6 - 8 hour sample, decreased for both (A) and (B) during the course of the experiment. This trend is reflected in Table 3.19, the MMAA/DMAA ratio declining from 0.38 (2 - 14hr) to 0.12 (14 - 62hr) to 0.06 (62 - 166hr) for (A), with corresponding values of 0.68, 0.40 and 0.16 for (B). The reduction in the MMAA/DMAA ratio with time is attributable to the transformation of MMAA to DMAA along with the drop in production of MMAA from remaining As(III) and, also, in the early stages, faster direct excretion of MMAA by the kidney due to differences in pKa (MMAA 4.26; DMAA 6.25). This interpretation is supported by the results of human metabolic experiments, involving the direct ingestion of monomethylarsonate and dimethylarsinate, in which it was also found that no demethylation reaction occurs in vivo (Buchet et al., 1981a).

This metabolic study clearly demonstrates variation between individuals (A) and (B) in the speciation pattern of excreted urinary arsenic produced in response to an identical oral dose of inorganic As(V) (Tables 3.15 - 3.18 and Figures 3.14, 3.15). Two recent studies (Buchet et al., 1984; Buchet and Lauwerys, 1985) suggest possible explanations for the remarkable speciation differences

observed over the first 22 hours of this experiment, when both excreted approximately 23% of the arsenic intake but with As(V)/As(III)/MMAA/DMAA speciation patterns (as percentage of the cumulative sums) of 12.9/21.1/16.3/49.8(%) for (A) (Table 3.17) and 27.2/24.1/19.8/29.0(%) for (B) (Table 3.18). Buchet et al. (1984), after injecting healthy volunteers and patients with different types of liver disease with 7.14ug/kg inorganic As(III), found that liver disease does not affect the percentage of the injected dose excreted within 24 hours but has striking and opposite effects on the proportions of MMAA and DMAA excreted. The speciation pattern for the controls was 62.9% inorganic arsenic, 12.8% MMAA and 24.3% DMAA in contrast with 53.1% inorganic arsenic, 6.1% MMAA and 40.7% DMAA for liver disease patients. On the basis of a subsequent study of inorganic arsenic methylation by rat liver in vitro, Buchet and Lauwerys (1985) concluded that, in the patients with liver disease , As(III) uptake by liver parenchymal cells is depressed, the reduction in substrate (i.e. As(III)) availability leading to a reduction in MMAA production. At the same time, however, further methylation of MMAA into DMAA will be stimulated due to the reduction of an inhibitory effect of As(III) on the MMAA to DMAA reaction. (It is this latter effect which causes the delay of several days before DMAA becomes the preponderant metabolite in patients

acutely intoxicated with inorganic arsenic). Therefore, the different speciation patterns for (A) and (B) during the first 22 hours, in particular the vastly differing MMAA/DMAA ratios of 0.33 (A) and 0.68 (B), may well arise as a consequence of comparatively lower uptake of inorganic arsenic by the liver of (A), reduced MMAA production but enhanced conversion of MMAA to DMAA. The higher initial (2 - 4 hour) excretion of inorganic arsenic by (A) (5.70ug), compared with that for (B) (3.69ug), tends to support this hypothesis, although it must be recognised that other fundamental physical, dietary and behavioural differences may also influence the metabolic response.

The results of this metabolic study, one of the few involving the detailed analytical speciation of arsenic in human urine, appear to be in line with an emerging consensus, based largely on animal and in vitro experiments, of the mammalian metabolism of arsenic (Vahter and Marafante, 1983; Marafante and Vahter, 1984; Fischer et al., 1985; Marafante et al., 1985). Methylation of arsenic is a detoxification mechanism rendering arsenic less reactive and facilitating excretion via the kidneys (Buchet et al., 1981a,b; Buchet and Lauwerys, 1983; Peoples, 1983, Vahter, 1983). The appropriate overall reaction sequence, starting with pentavalent inorganic arsenic, As(V), is As(V) $\longrightarrow$ As(III) $\longrightarrow$ MMAA $\longrightarrow$ DMAA, i.e. intracellular in vivo reduction of As(V) to As(III) is

required before methylation occurs (Vahter and Envall, 1983; Foa et al., 1985). The methylation of As(III) to DMAA is a two-step process which appears to involve two different enzymatic activities for the reactions As(III) ⟶ MMAA and MMAA ⟶ DMAA (Buchet and Lauwreys, 1985).

The specific mechanism and location of methylation are not known with certainty but S-adenosylmethionine and liver have been suggested as methyl-donor and site respectively (Tam et al., 1979b; Vahter, 1981; Lerman et al.,1983, Vahter and Envall, 1983). Strong supporting evidence has been forthcoming in three recent publications. In an in vitro study of the methylation of inorganic arsenic by rat liver, Buchet and Lauwreys (1985) established that the methylating activity was localised in the cytosol fraction and accepted only As(III) as substrate. S-adenosylmethionine was found to be the essential methyl group donor, corrinoid derivatives (e.g.two vitamin B_{12} analogues, methylcobalamin - MeB12 and 5'-deoxyadenosylcobalamin - coenz B12) acting synergistically. In the first of two animal studies, Marafante and Vahter (1984) found that periodate-oxidised adenosine (PAD), an inhibitor of certain methyltransferases, significantly reduced the production of DMAA in mice and rabbits injected with ^{74}As(III). Injection of PAD in mice causes a pronounced increase in liver S-adenosyl-

homocysteine. As transmethylation reactions involving S-adenosylmethionine result in the formation of S-adenosylhomocysteine, the accumulation of the latter strongly inhibits the transmethylation from S-adenosylmethionine. Due to interaction of the unmethylated As(III) with tissue constituents, the PAD treated animals had significantly higher tissue concentrations of administered ^{74}As than did the controls. This effect was first observed in the liver, indicating that this organ is the main site of the methylation of arsenic. Marafante et al. (1985) also conducted a similar study, this time administering $^{74}As(V)$, on the rabbit, considered one of the most suitable animal models for the study of arsenic metabolism since it has a degree of methylation similar to that of man, although, like most animals, it does not produce MMAA. Again, inhibition of methyltransferase activity by injection of PAD into some rabbits caused a marked decrease in the formation of DMAA and gave rise to increased tissue levels of ^{74}As. In rabbits with normal methylating capacity, the liver was the only organ in which DMAA was found one hour after the administration, again indicating this to be the main site of the methylation. In the PAD/As(V) treated rabbits, increased tissue levels of ^{74}As, as found previously after administration of $^{74}As(III)$, and the excretion unchanged of only 30% of administered

As(V) indicated a rapid reduction of As(V) to As(III), as in the human metabolic study presented here. Therefore, methylation to DMAA seems to be almost as important for detoxification following exposure to As(V) as that following exposure to As(III).

Beyond its role in fundamental investigations of the human metabolism of arsenic, analytical speciation of urinary arsenic is clearly of potential practical value in several important areas. In the clinical realm, the extent of methylation, the natural detoxifying mechanism, may assist the clinician in deciding when to modify or cease the somewhat drastic chelation therapy commonly employed (Mahieu et al., 1981). It could also assist in the diagnosis of liver disease (Buchet et al., 1984). In the monitoring of exposure to occupational, dietary and environmental sources of inorganic arsenic, analytical arsenic speciation is necessary to separate the products of exposure from the predominant forms of dietary exposure, inert unchanged organoarsenicals such as arsenobetaine. It may also assist in the identification of the extent and nature of the exposure (Chapter 4).

3.3.6 Conclusions

Although some differences were observed between results for the two participants in this metabolic experiment, the following conclusions can be drawn:

1) Concentrations of urinary arsenic rose rapidly in the first 10 hours after the ingestion of 220ug pentavalent inorganic arsenic. Subsequent decline led to a resumption of values close to normal background levels about 4 - 5 days after the intake.

2) Inorganic arsenic excretion predominated during the first few hours after intake, but As(V) was clearly rapidly reduced to As(III). Further biotransformations quickly converted As(III) into the relatively less toxic methylated form MMAA and thereafter DMAA, which became the predominant form after about 0.5 days and, from 1 - 1.5 days onwards, typically represented 60 - 90% of excreted arsenic.

3) In the 7 days after the intake of 220ug As(V), amounts corresponding to 75 - 82% of ingested arsenic were excreted in urine (64 - 69% after correction for likely background contributions) of which As(V) constituted 9 - 10%, As(III) 12 - 15%, MMAA 9 - 18% and DMAA 57 - 69%

4) The urinary excretion of arsenic after 220ug As(V) intake was much slower than after the consumption of seafood containing 540ug total arsenic, the time to eliminate amounts corresponding to 50% of the ingested dose ranging from 54 - 70 hours. Biological half-lives for the more rapidly eliminated component (equivalent to 32 - 49% of the dose) ranged from 17 - 24 hours, with much longer second component half-lives of 7.1 - 8.6 days.

5) The observed pattern of urinary elimination of individual arsenic species, in particular the increasing predominance of the less reactive and more rapidly excreted methylated species, is in line with the concept of methylation as a natural detoxification mechanism through the biotransformation of the more toxic inorganic arsenic by the human body via the sequence As(V) $\longrightarrow$ As(III) $\longrightarrow$ MMAA $\longrightarrow$ DMAA.

6) Observed differences between individuals in urinary arsenic speciation patterns, especially the relative prominence of inorganic and methylated species (temporal changes in the MMAA/DMAA ratio) can be reasonably interpreted in terms of current theories on the role of the liver as the site of methylation and the contrasting dependence of two reactions controlling the production

and conversion of MMAA on the extent of the uptake of inorganic As(III) by the liver.

7) The ability of the analytical speciation technique employed to distinguish between As(V) and As(III) enabled the observation of:

(a) rapid reduction of As(V) to As(III)

(b) the enhancing effects of increased liquid intake and urinary output on the "flushing" of As(V) from preferred retention sites in the kidneys soon after intake, and

(c) the continuing release of small amount of As(III), in the absence of As(V), several days after arsenic intake as a result of the comparatively greater tissue retention of As(III).

8) The ability of the analytical speciation technique to separate and measure As(V), As(III), MMAA and DMAA enables a distinction to be made between arsenic acquired as a result of seafood ingestion and that resulting from exposure to the more toxic forms of inorganic arsenic. The rapid-scan direct hydride technique for the estimation of the sum concentration of these hydride-forming species provides a quicker but less accurate and considerably less informative picture of urinary arsenic status and arsenic exposure.

3.4 METABOLIC STUDY III: ARSENIC METABOLISM FOLLOWING REPEATED ORAL DOSES OF AQUEOUS PENTAVALENT INORGANIC ARSENIC

3.4.1 Objectives

1) To simulate an arsenic intake corresponding to regular occupational, environmental or dietary exposure to inorganic arsenic.

2) To determine the rate and speciation pattern of urinary arsenic excretion following regularly repeated oral doses of aqueous inorganic As(V).

3) To examine the use of urinary arsenic excretion and speciation as an indicator, both qualitative and quantitative, of exposure to inorganic arsenic.

3.4.2 Experimental design

The mineral water Vichy Célestins, 220 ± 15ug As(V)/l, was again chosen as the source of inorganic arsenic. While clearly an acceptable form of dietary exposure, it was also considered, for the purposes of this study, the best practical means of simulating the types of exposure (e.g. occupational and, possibly, environmental) where inhalation of arsenic-containing particles might be significant. It is known that larger particles are deposited in the upper respiratory system and can be brought to the

gastro-intestinal tract by mucociliary clearance mechanisms (WHO, 1981) (Section 4.4.4).

In view of the large number of samples anticipated over a three week period, only one subject took part in the experiment. A 54kg female, she was asked to abstain from the consumption of any fish products prior to and during the experiment. A bulk urine sample was collected over the 24-hour period preceding the study and another spot sample taken just before commencement of the experiment. The volunteer consumed 100ml of Vichy Celestins three times a day (i.e. at 07.30, 15.30 and 23.30) for ten days. The 07.30 sample was taken with the first meal of the day, (usually cereal and milk) and a smaller intake of food (e.g. bread or fruit) was taken with the 15.30 and 23.30 dose. Thus, three 1-litre bottles of Vichy Célestins were consumed in all, corresponding to a total of 660 $\pm$ 45ug As(V) ingested at a rate of 66 $\pm$ 4.5ug per day.

Urine samples were collected every four hours during the first day and then over eight-hour periods (07.30 - 15.30, 15.30 - 23.30, 23.30 - 07.30,) for the following 17 days. As before, the total urine sample was collected, the volume measured, and an aliquot stored for analysis in acid-washed polypropylene containers (Section 3.2.2.).

3.4.3 Analytical methods

Each sample was subjected to arsenic determination by direct-hydride analysis (Section 2.4.2) and the concentration of the different chemical forms was measured in all samples after separation of the four hydride-forming species from a 4ml sample aliquot by ion-exchange chromatography (Section 2.5.3). The total arsenic concentration was determined in a selected number of samples after acid digestion of a 10ml aliquot (Section 2.4.1.).

3.4.4 Results

Urinary arsenic excretion prior to the first intake of the mineral water was low at 12ug/l (total) and 2.6ug/l (sum of As(V), As(III), MMAA and DMAA species) (Table 3.20, Figure 3.19).

As in metabolic studies I and II, the concentration of urinary arsenic over the course of the experiment was influenced by the volume of the individual samples. While the subject, from 0 - 424hr, excreted creatinine at fairly constant average rates of 0.35g/8hr (23.30 - 07.30), 0.29g/8hr (07.30 - 15.30) and 0.36g/8hr (15.30 - 23.30), average urine volumes and creatinine concentrations for these eight hour periods ranged from 337ml and 1.31g/l (23.30 - 07.30) to 567ml and 0.64g/l (07.30 - 15.30) and 478ml and 0.94g/l (15.30 - 23.30). Thus, when urinary arsenic concentrations were normalised to creatinine

TABLE 3.20

EXCRETION OF URINARY ARSENIC (ug/l) FOLLOWING THE REPEATED ORAL INTAKE OF 22ug INORGANIC As(V) THREE TIMES A DAY FOR TEN DAYS BY A SINGLE VOLUNTEER

				ARSENIC CONCENTRATION (ug/l)								
						SPECIATION						
SAMPLE TIME	TIME INTERVAL	VOL (ml)	CREAT (g/l)	DIRECT HYDRIDE	TOTAL	As(V)	As(III)	MMAA	DMAA	SUM	(/g creat.) TOTAL	(/g creat.) SUM
Pre expt.	24hr	1955	0.75	3-5	12	*	*	*	2.6	2.6	16	3.5
DAY 1												
11.30	0-4	305	0.38	3-5	4	*	*	*	1.8	1.8	9	4.7
15.30	4-8	260	1.15	10-15	17	*	3.7	*	7.9	11.6	15	10.1
19.30	8-12	130	0.90	12-17	22	1.6	3.2	*	6.4	11.2	24	12.4
23.30	12-16	330	0.67	10-14	22	*	5.1	1.2	8.4	14.7	33	21.9
07.30	16-24	880	0.38	6-9	11	*	3.0	0.5	4.4	7.9	29	20.8
DAY 2												
15.30	24-32	320	0.94	22-30	40	1.3	7.3	4.5	13.7	26.8	43	28.5
23.30	32-40	255	1.15	24-34	40	1.8	6.6	4.8	23.5	36.7	35	31.9
07.30	40-48	350	0.87	21-30	31	1.0	6.6	5.4	17.9	30.9	36	35.5
DAY 3												
15.30	48-56	463	0.51	11-16	19	0.9	1.9	2.5	9.6	14.9	37	29.2
23.30	56-64	455	1.10	25-36	30	3.1	3.7	3.3	15.8	25.9	27	23.5
07.30	64-72	225	1.31	27-40	50	1.8	4.7	6.5	22.3	35.3	38	26.9
DAY 4												
15.30	72-80	475	0.57	16-24	31	1.7	2.9	3.4	13.8	21.8	54	38.2
23.30	80-88	770	0.37	11-16	16	1.6	1.8	2.3	8.3	14.0	43	37.8
07.30	88-96	760	0.34	10-14	12	1.0	1.8	2.2	7.4	12.4	35	36.5

TABLE 3.20 cont.

SAMPLE TIME	TIME INTERVAL	VOL (ml)	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l): DIRECT HYDRIDE	TOTAL	SPECIATION: As(V)	As(III)	MMAA	DMAA	SUM	(/g creat.) TOTAL	(/g creat.) SUM
DAY 5												
15.30	96-104	560	0.45	12-18	19	1.2	1.8	2.0	10.1	15.1	42	33.6
23.30	104-112	250	1.57	36-53	56	2.8	6.0	6.4	26.9	42.1	36	26.8
07.30	112-120	205	1.72	37-55	46	2.0	6.5	7.6	27.8	43.9	27	25.5
DAY 6												
15.30	120-128	725	0.52	9-14	7	0.6	2.9	1.6	8.4	13.5	13	25.9
23.30	128-136	485	0.69	21-30	29	0.6	5.8	3.4	17.9	27.7	42	40.1
07.30	136-144	405	1.14	29-42	37	2.3	5.6	5.0	24.0	36.9	32	32.4
DAY 7												
15.30	144-152	900	0.18	7-10	4	0.7	1.3	1.0	4.9	7.9	19	43.9
23.30	152-160	448	0.87	27-40	28	1.0	5.7	4.8	23.1	34.6	32	39.7
07.30	160-168	278	1.36	32-47	42	2.3	8.0	6.3	25.3	41.9	31	30.8
DAY 8												
15.30	168-176	530	0.62	19-28	20	1.6	2.9	3.0	17.0	24.5	32	39.5
23.30	176-184	570	0.58	15-23	17	0.8	2.2	2.2	15.7	20.9	29	36.0
07.30	184-192	270	1.70	35-51	50	2.0	7.0	7.6	26.4	43.0	29	25.3
DAY 9												
15.30	192-200	175	2.01	50-74	64	1.9	9.1	9.6	33.5	54.1	32	26.9
23.30	200-208	498	0.81	25-37	21	1.5	4.5	3.8	18.4	28.2	26	34.8
07.30	208-216	210	1.44	34-49	36	1.6	6.4	5.8	26.1	39.9	25	27.7

TABLE 3.20 cont.

SAMPLE TIME	TIME INTERVAL	VOL (ml)	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIATION As(V)	As(III)	MMAA	DMAA	SUM	(/g creat.) TOTAL	SUM
DAY 10												
15.30	216-224	630	0.61	11-16	16	1.1	2.2	1.7	7.8	12.8	26	21.0
23.30	224-232	410	1.33	32-46	43	1.5	4.9	4.4	26.4	37.2	32	28.0
07.30	232-240	345	1.56	26-39	-	1.0	4.4	4.5	22.5	32.4	-	20.8
DAY 11												
15.30	240-248	940	0.21	6-9	-	*	0.5	*	5.8	6.3	-	30.0
23.30	248-256	340	1.54	30-45	37	*	3.0	5.1	34.9	43.0	24	27.9
07.30	256-264	180	1.11	28-41	16	*	2.8	4.0	28.2	35.0	14	31.5
DAY 12												
15.30	264-272	700	0.59	8-12	-	*	0.8	*	9.9	10.7	-	18.1
23.30	272-280	570	0.32	10-14	-	*	*	*	4.8	4.8	-	15.0
07.30	280-288	177	1.45	22-33	23	*	1.5	2.4	19.9	23.8	16	16.4
DAY 13												
15.30	288-296	710	0.40	5-7	5	*	*	*	6.3	6.3	13	15.8
23.30	296-304	580	1.04	11-16	15	*	0.6	0.9	14.4	15.9	14	15.3
07.30	304-312	225	1.54	15-21	-	*	0.7	1.8	20.1	22.6	-	14.7
DAY 14												
15.30	312-320	735	0.21	5-7	-	*	*	*	3.2	3.2	-	15.2
23.30	320-328	590	0.32	9-13	-	*	*	*	8.1	8.1	-	25.3
07.30	328-336	425	0.87	10-15	-	*	*	*	9.9	9.9	-	11.4

TABLE 3.20 cont.

				ARSENIC CONCENTRATION (ug/l)								
						SPECIATION						
SAMPLE TIME	TIME INTERVAL	VOL (ml)	CREAT (g/l)	DIRECT HYDRIDE	TOTAL	As(V)	As(III)	MMAA	DMAA	SUM	(/g creat.) TOTAL	SUM
DAY 15												
15.30	336-344	400	0.64	9-13	-	*	*	*	9.4	9.4	-	14.7
23.30	344-352	158	2.17	21-30	-	*	1.1	*	23.1	24.2	-	11.2
07.30	352-360	190	2.52	16-24	-	*	1.0	*	18.3	19.3	-	7.7
DAY 16												
15.30	360-368	363	1.19	8-12	-	*	*	*	8.8	8.8	-	7.4
23.30	368-376	415	0.69	6-9	-	*	*	*	6.8	6.8	-	9.9
07.30	376-384	168	2.20	13-19	-	*	*	*	14.4	14.4	-	6.5
DAY 17												
15.30	384-392	297	0.92	9-12	-	*	*	*	7.3	7.3	-	7.9
23.30	392-400	200	1.32	9-13	-	*	*	*	9.7	9.7	-	7.3
07.30	400-408	438	0.71	3-5	-	*	*	*	5.5	5.5	-	7.7
DAY 18												
15.30	408-416	710	0.20	1-2	-	*	*	*	1.9	1.9	-	9.5
23.30	416-424	1150	0.31	1-2	-	*	*	*	2.0	2.0	-	6.5
07.30	424-432	300	-	6-8	-	-	-	-	8.7	8.7	-	-

* corresponds to a species concentration below the detection limit of 0.5ug/l

\- no data available

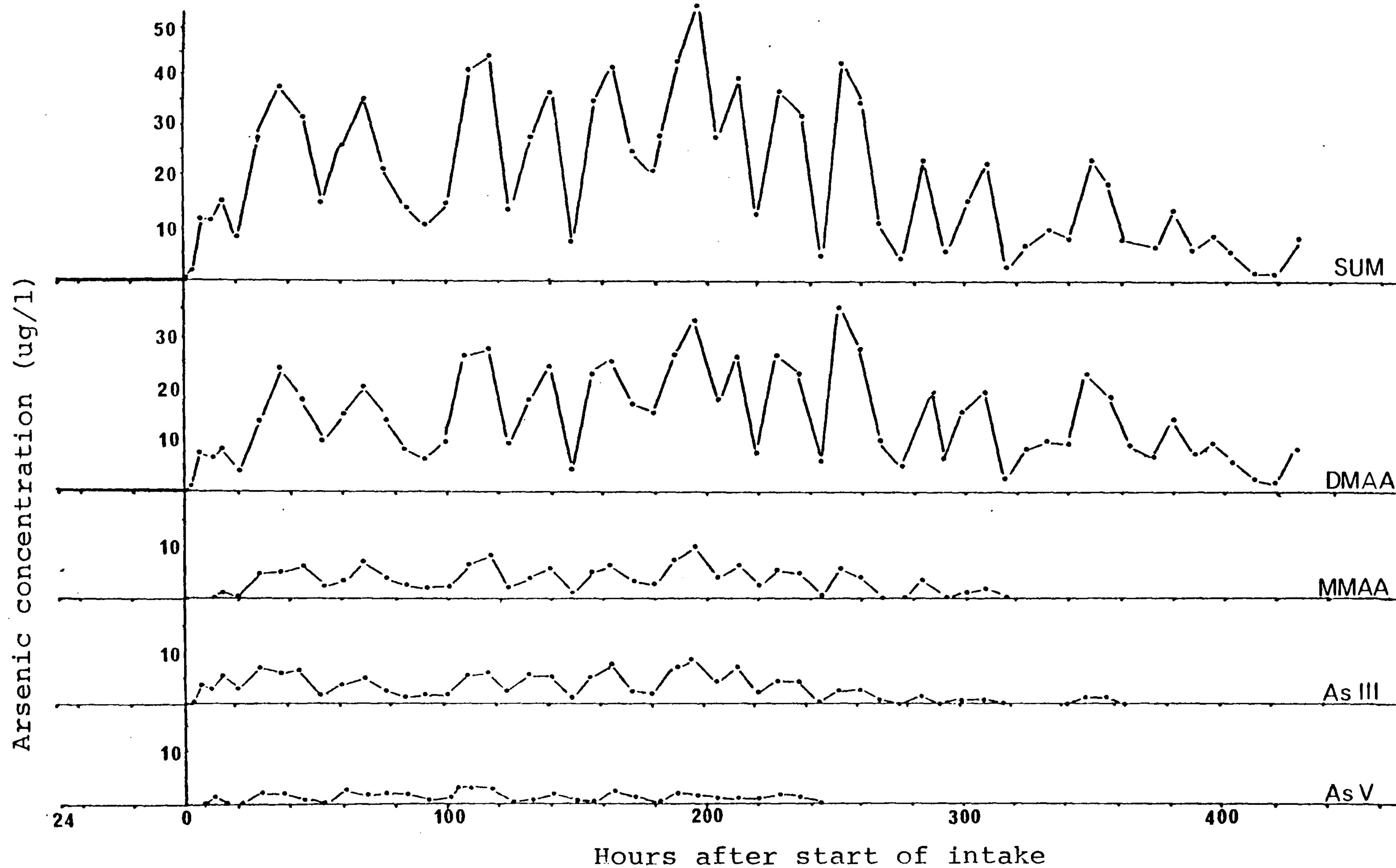


Figure 3.19 Urinary arsenic excreted (ug/l) following the repeated oral intake of 22ug of inorganic As(V) three times a day for ten days by a single volunteer

content in Figure 3.20 (Table 3.21), much of the irregular structure in the corresponding concentration curve (ug/l) of Figure 3.19 (Table 3.20) was removed.

The first significant increase in arsenic concentration was observed in the urine sample corresponding to 4 - 8hrs after the administration of the first As(V) dose. Both DMAA and As(III) were present in this sample but not until 8 - 12hr and 12 - 16hr, respectively, were As(V) and MMAA detected (Table 3.21, Figure 3.20). With DMAA the predominant species throughout, the sum of species concentration exceeded 20ug/g creatinine in the 12 - 16hr sample and thereafter stayed in the range 20.8 - 43.9ug/g creatinine up to and including the 256 - 264hr sample i.e. 24 - 32hr after intake of the final As(V) dose. Maximum individual concentrations attained were 4.3ug/g (As(V)), 8.4ug/g (As(III), 6.5ug/g (MMAA) and 27.6ug/g (DMAA). With As(V), As(III) and MMAA effectively falling below detection limits (except for two isolated samples of modest As(III) concentration) after 240hrs, 304hrs and 312hrs respectively, urinary arsenic concentrations slowly declined after 264hrs, falling below 10ug/g creatinine after 352hr, i.e. 120 - 128hr after the final dose.

The amount of each species and the corresponding sum of the four species excreted in each urine sample are listed in Table 3.22. These results are further summarised for 24-hr periods, commencing

TABLE 3.21

EXCRETION OF URINARY ARSENIC (ug/g creatinine) FOLLOWING THE REPEATED ORAL INTAKE OF 22ug INORGANIC As(V) THREE TIMES A DAY FOR TEN DAYS BY A SINGLE VOLUNTEER

TIME	ARSENIC CONCENTRATION (ug/g creatinine)				
INTERVAL	As(V)	As(III)	MMAA	DMAA	SUM
24-hr	*	*	*	3.5	3.5
0-4	*	*	*	4.7	4.7
4-8	*	3.2	*	6.9	10.1
8-12	1.7	3.6	*	7.1	12.4
12-16	*	7.6	1.8	12.5	21.9
16-24	*	7.9	1.3	11.6	20.8
24-32	1.4	7.8	4.8	14.6	28.6
32-40	1.6	5.7	4.2	20.4	31.9
40-48	1.2	7.6	6.2	20.6	35.6
48-56	1.8	3.7	4.9	18.8	29.2
56-64	2.8	3.4	3.0	14.4	23.6
64-72	1.4	3.6	5.0	17.0	27.0
72-80	3.0	5.1	6.0	24.2	38.2
80-88	4.3	4.9	6.2	22.4	37.8
88-96	2.9	5.3	6.5	21.8	36.5
96-104	2.7	4.0	4.4	22.4	33.6
104-112	1.8	3.8	4.1	17.1	26.8
112-120	1.2	3.8	4.4	16.2	25.5
120-128	1.2	5.6	3.1	16.2	26.0
128-136	0.9	8.4	4.9	25.9	40.1
136-144	2.0	4.9	4.4	21.1	32.4
144-152	3.9	7.2	5.6	27.2	43.9
152-160	1.2	6.6	5.5	26.6	39.8
160-168	1.7	5.9	4.6	18.6	30.8
168-176	2.6	4.7	4.8	27.4	39.5
176-184	1.4	3.8	3.8	27.1	36.0
184-192	1.2	4.1	4.5	15.5	25.3
192-200	0.9	4.5	4.8	16.7	26.9
200-208	1.9	5.6	4.7	22.7	34.8
208-216	1.1	4.4	4.0	18.1	27.7
216-224	1.8	3.6	2.8	12.8	21.0
224-232	1.1	3.7	3.3	19.8	28.0
232-240	0.6	2.8	2.9	14.4	20.8

TABLE 3.21 cont

TIME INTERVAL	ARSENIC CONCENTRATION (ug/g creatinine) As(V)	As(III)	MMAA	DMAA	SUM
240-248	*	2.3	*	27.6	30.0
248-256	*	1.9	3.3	22.7	27.9
256-264	*	2.5	3.6	25.4	31.5
264-272	*	1.4	0.5	16.8	18.1
272-280	*	*	0.5	15.0	15.0
280-288	*	1.0	1.7	13.7	16.4
288-296	*	*	0.5	15.8	15.8
296-304	*	0.6	0.9	13.8	15.3
304-312	*	*	1.2	13.1	14.8
312-320	*	*	*	15.2	15.2
320-328	*	*	*	25.3	25.3
328-336	*	*	*	11.4	11.4
336-344	*	*	*	14.7	14.7
344-352	*	0.5	*	10.6	11.1
352-360	*	0.4	*	7.3	7.7
360-368	*	*	*	7.4	7.4
368-376	*	*	*	9.9	9.9
376-384	*	*	*	6.5	6.5
384-392	*	*	*	7.9	7.9
392-400	*	*	*	7.3	7.3
400-408	*	*	*	7.7	7.7
408-416	*	*	*	9.5	9.5
416-424	*	*	*	6.5	6.5
424-432	-	-	-	-	-

* corresponds to a species concentration (ug/l) below the detection limit of 0.5ug/l

\- no creatinine value available

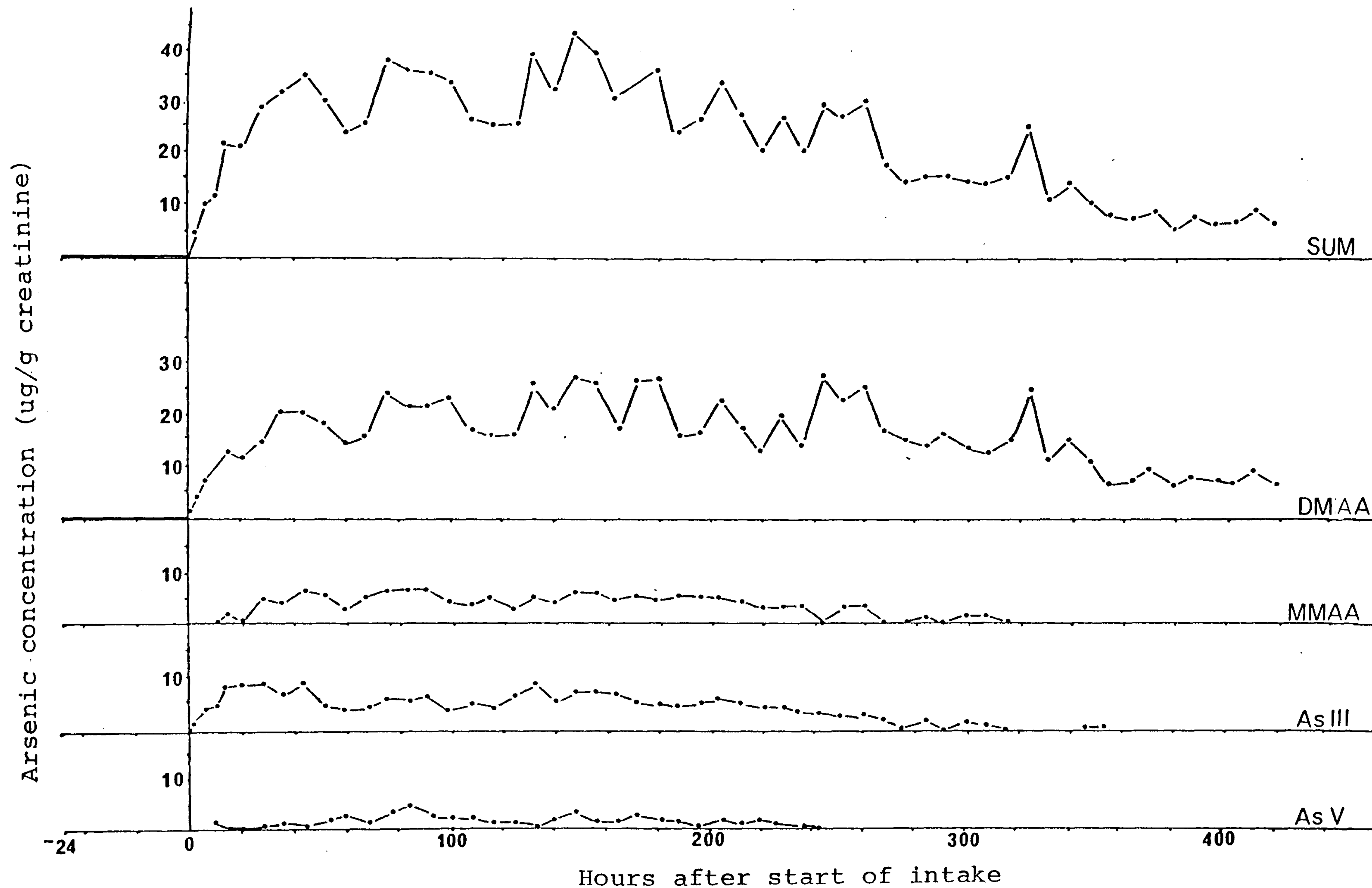


Figure 3.20 Urinary arsenic excretion (ug/g creatinine) following the oral intake of 22ug of inorganic As(V) three times a day for ten days by a single volunteer.

TABLE 3.22

THE AMOUNT OF ARSENIC EXCRETED FOLLOWING THE REPEATED ORAL INTAKE OF 22ug INORGANIC As(V) THREE TIMES A DAY FOR TEN DAYS

SAMPLE TIME	AMOUNT (ug)				
	As(V)	As(III)	MMAA	DMAA	SUM
DAY 1					
0-4	*	*	*	0.55	0.55
4-8	*	0.96	*	2.05	3.01
8-12	0.21	0.42	*	0.83	1.46
12-16	*	1.68	0.40	2.77	4.85
16-24	*	2.64	0.44	3.87	6.95
DAY 2					
24-32	0.42	2.34	1.44	4.38	8.58
32-40	0.46	1.68	1.22	5.99	9.35
40-48	0.35	2.31	1.89	6.27	10.82
DAY 3					
48-56	0.42	0.88	1.16	4.44	6.90
56-64	1.41	1.68	1.50	7.19	11.78
64-72	0.41	1.06	1.46	5.02	7.95
DAY 4					
72-80	0.81	1.38	1.62	6.56	10.37
80-88	1.23	1.39	1.77	6.39	10.78
88-96	0.76	1.37	1.67	5.62	9.42
DAY 5					
96-104	0.67	1.00	1.12	5.66	8.45
104-112	0.70	1.50	1.60	6.73	10.53
112-120	0.41	1.33	1.56	5.70	9.00
DAY 6					
120-128	0.44	2.10	1.16	6.09	9.79
128-136	0.29	2.81	1.65	8.68	13.43
136-144	0.93	2.27	2.03	9.72	14.95
DAY 7					
144-152	0.63	1.17	0.90	4.41	7.11
152-160	0.45	2.55	2.15	10.35	15.50
160-168	0.64	2.22	1.75	7.03	11.64
DAY 8					
168-176	0.85	1.54	1.59	9.01	12.99
176-184	0.46	1.25	1.25	8.95	11.91
184-192	0.54	1.89	2.05	7.13	11.61

TABLE 3.22 cont.

SAMPLE TIME	AMOUNT (ug)				
	As(V)	As(III)	MMAA	DMAA	SUM
DAY 9					
192-200	0.33	1.59	1.68	5.86	9.46
200-208	0.75	2.24	1.89	9.16	14.04
208-216	0.34	1.34	1.22	5.48	8.38
DAY 10					
216-224	0.69	1.39	1.07	4.91	8.06
224-232	0.62	2.01	1.80	10.82	15.25
232-240	0.35	1.52	1.55	7.76	11.18
DAY 11					
240-248	*	0.47	*	5.45	5.92
248-256	*	1.02	1.73	11.87	14.62
256-264	*	0.50	0.72	5.08	6.30
DAY 12					
264-272	*	0.56	*	6.93	7.49
272-280	*	*	*	2.74	2.74
280-288	*	0.27	0.42	3.52	4.21
DAY 13					
288-296	*	*	*	4.47	4.47
296-304	*	0.35	0.52	8.35	9.22
304-312	*	0.16	0.41	4.52	5.09
DAY 14					
312-320	*	*	*	2.35	2.35
320-328	*	*	*	4.78	4.78
328-336	*	*	*	4.21	4.21
DAY 15					
336-344	*	*	*	3.76	3.76
344-352	*	0.17	*	3.65	3.76
352-360	*	0.19	*	3.48	3.67
DAY 16					
360-368	*	*	*	3.19	3.19
368-376	*	*	*	2.82	2.82
376-384	*	*	*	2.42	2.42
DAY 17					
384-392	*	*	*	2.17	2.17
392-400	*	*	*	1.94	1.94
400-408	*	*	*	2.41	2.41
DAY 18					
408-416	*	*	*	1.35	1.35
416-424	*	*	*	2.30	2.30
424-432	*	*	*	2.61	2.61

* corresponds to a species concentration (ug/l) below the detection limit of 0.5ug/l

from the time of the first intake of As(V), with the sum of the 07.30 - 15.30, 15.30 - 23.30 and 23.30 - 07.30 periods as Day 1 etc. in Table 3.23. On Day 1, 16.82ug were excreted, equivalent to 25.5% of the first 3 x 22ug (i.e. 66ug) doses. From Day 2 to Day 11, i.e. from one day after commencement to one day after cessation of intake, the daily amount excreted in urine was relatively constant at 26.63 - 38.17ug (mean 31.6 $\pm$ 4.1ug), corresponding to 40.3 - 57.8% (mean 47.9%) of the daily dose (Note, if correction for background excretion is made, this figure drops to 40.2%). From Day 12 to Day 15, the average amount excreted dropped to 14ug/day and, from Day 16, when the excreted amount fell below 10ug for the first time, to Day 18, an average of 7.1ug/day.

The total amount excreted as As(V), As(III), MMAA and DMAA over 18 days was 409.91ug, equivalent to 62.1% of the total As(V) dose (48.2% after correction for background contributions) (Table 3.26). It is interesting to note that 161.1ug were excreted between 15.30 and 23.30 compared with 132.8ug from 23.30 to 07.30 and 116.0ug from 07.30 to 15.30. In the first 11 days, 332.9ug, 50.4% of the As(V) intake, were excreted, of which 5.0% was present as As(V), 16.1% As(III), 13.5% MMAA and 65.4% DMAA. Over the following seven days, a further 77.0ug were excreted, with 0% as As(V), 2.2% As(III), 1.8% MMAA and 96.0% DMAA.

TABLE 3.23

THE AMOUNT OF ARSENIC EXCRETED PER 24 HOURS FOLLOWING THE DAILY ORAL INTAKE OF 66ug INORGANIC As(V) FOR TEN DAYS

DAY	TIME INTERVAL	AMOUNT (ug)				
		As(V)	As(III)	MMAA	DMAA	SUM
1	0-24	0.21	5.70	0.84	10.07	16.82
2	24-48	1.23	6.33	4.55	16.64	28.75
3	48-72	2.24	3,62	4.12	16.65	26.63
4	72-96	2.80	4.14	5.06	18.57	30.57
5	96-120	1.78	3.83	4.28	18.09	27.98
6	120-144	1.66	7.18	4.84	24.49	38.17
7	144-168	1.72	5.94	4.80	21.79	34.25
8	168-192	1.85	4.68	4.89	25.09	36.51
9	192-216	1.42	5.17	4.79	20.50	31.88
10	216-240	1.66	4.92	4.42	23.49	34.49
11	240-264	*	1.99	2.45	22.40	26.84
12	264-288	*	0.83	0.42	13.19	14.44
13	288-312	*	0.51	0.93	17.34	18.78
14	312-336	*	*	*	11.34	11.34
15	336-360	*	0.36	*	10.89	11.25
16	360-384	*	*	*	8.43	8.43
17	384-408	*	*	*	6.52	6.52
18	408-432	*	*	*	6.26	6.26
TOTAL		16.57	55.20	46.39	291.75	409.91

* corresponds to a species concentration (ug/l) below the detection limit of 0.5ug/l

The predominance of DMAA is reflected in Table 3.24, which shows the relative proportions of each species for the individual urine samples, and in Table 3.25 and Figure 3.21, which summarise these values for 24-hr periods over 18 days. Over the first two days As(III) and DMAA were clearly the major species in all samples but from Day 3 to Day 10 the speciation pattern became firmly established, As(V) ranging from 4.3 - 8.4% (mean 5.8%), As(III) 12.8 - 18.8% (mean 15.2%), MMAA 12.7 - 16.6% (mean 14.3%) and DMAA 60.7 - 68.7% (mean 64.7%). After Day 10, DMAA rose rapidly to in excess of 80% for all remaining samples.

The trends outlined above are also manifested in Table 3.26 and Figure 3.22 which present the cumulative amounts of each species as percentages of the cumulative intake over the course of the experiment.

As in metabolic study II, the direct-hydride scan usually provided a reasonable estimate of the sum of the individual species concentrations. Furthermore, agreement between the sum and the measured total was good, with the calculated mean ratio (excluding a few values, largely for concentrations $< 10ug/l$) of 0.93 ± 0.19 for sum/total.

TABLE 3.24

THE RELATIVE PROPORTIONS OF THE ARSENIC SPECIES EXCRETED FOR EACH SAMPLE COLLECTED FOLLOWING THE REPEATED ORAL INTAKE OF 22ug INORGANIC As(V) THREE TIMES A DAY FOR TEN DAYS

DAY	SAMPLE TIME	PERCENTAGE EXCRETED			
		As(V)	As(III)	MMAA	DMAA
1	0-4	-	-	-	100
	4-8	-	31.9	-	68.1
	8-12	14.3	28.6	-	57.1
	12-16	-	34.7	8.2	57.1
	16-24	-	38.0	6.3	55.7
2	24-32	4.9	27.2	16.8	51.0
	32-40	4.9	18.0	13.1	64.0
	40-48	3.2	21.4	17.5	57.9
3	48-56	6.0	12.8	16.8	64.4
	56-64	12.0	14.3	12.7	61.0
	64-72	5.1	13.3	18.4	63.2
4	72-80	7.8	13.3	15.6	63.3
	80-88	11.4	12.9	16.4	59.3
	88-96	8.1	14.5	17.7	59.7
5	96-104	7.9	11.9	13.2	66.9
	104-112	6.7	14.3	15.2	63.9
	112-120	4.6	14.8	17.3	63.3
6	120-128	4.4	21.5	11.9	62.2
	128-136	2.2	20.9	12.3	64.6
	136-144	6.2	15.2	13.6	65.0
7	144-152	8.9	16.5	12.7	62.0
	152-160	2.9	16.5	13.9	66.8
	160-168	5.5	19.1	15.0	60.4
8	168-176	6.5	11.8	12.2	69.4
	176-184	3.8	10.5	10.5	75.1
	184-192	4.7	16.3	17.7	61.4
9	192-200	3.5	16.8	17.8	61.9
	200-208	5.3	16.0	13.5	65.2
	208-216	4.0	16.0	14.5	65.4
10	216-224	8.6	17.2	13.3	60.9
	224-232	4.0	13.2	11.8	71.0
	232-240	3.1	13.6	13.9	69.4

TABLE 3.24 cont.

DAY	SAMPLE TIME	PERCENTAGE EXCRETED As(V)	As(III)	MMAA	DMAA
11	240-248	-	7.9	-	92.1
	248-256	-	7.0	11.9	81.2
	256-264	-	8.0	11.4	80.6
12	264-272	-	7.5	-	92.5
	272-280	-	-	-	100
	280-288	-	6.3	10.1	83.6
13	288-296	-	-	-	100
	296-304	-	3.8	5.7	90.6
	304-312	-	3.1	8.0	88.9
14	312-320	-	-	-	100
	320-328	-	-	-	100
	328-336	-	-	-	100
15	336-344	-	-	-	100
	344-352	-	4.5	-	95.5
	352-360	-	5.2	-	94.8
16	360-368	-	-	-	100
	368-376	-	-	-	100
	376-384	-	-	-	100
17	384-392	-	-	-	100
	392-400	-	-	-	100
	400-408	-	-	-	100
18	408-416	-	-	-	100
	416-424	-	-	-	100
	424-432	-	-	-	100

\- corresponds to a species concentration (ug/l) below the detection limit of 0.5ug/l (Table 3.20)

TABLE 3.25

THE RELATIVE PROPORTIONS OF THE ARSENIC SPECIES EXCRETED PER 24 HOURS FOLLOWING THE DAILY ORAL INTAKE OF INORGANIC As(V) FOR TEN DAYS

DAY	TIME INTERVAL	PERCENTAGE EXCRETED			
		As(V)	As(III)	MMAA	DMAA
1	0-24	1.2	33.9	5.0	59.9
2	24-48	4.3	22.0	15.8	57.9
3	48-72	8.4	13.6	15.5	62.5
4	72-96	9.2	13.5	16.6	60.7
5	96-120	6.4	13.7	15.3	64.7
6	120-144	4.3	18.8	12.7	64.2
7	144-168	5.0	17.3	14.0	63.6
8	168-192	5.1	12.8	13.4	68.7
9	192-216	4.5	16.2	15.0	64.3
10	216-240	4.8	14.3	12.8	68.1
11	240-264	-	7.4	9.1	83.5
12	264-288	-	5.7	2.9	91.3
13	288-312	-	2.7	5.0	92.3
14	312-336	-	-	-	100
15	336-360	-	3.2	-	96.8
16	360-384	-	-	-	100
17	384-408	-	-	-	100
18	408-432	-	-	--	100

\- corresponds to a species concentration (ug/l) below the detection limit of 0.5ug/l (Table 3.20).

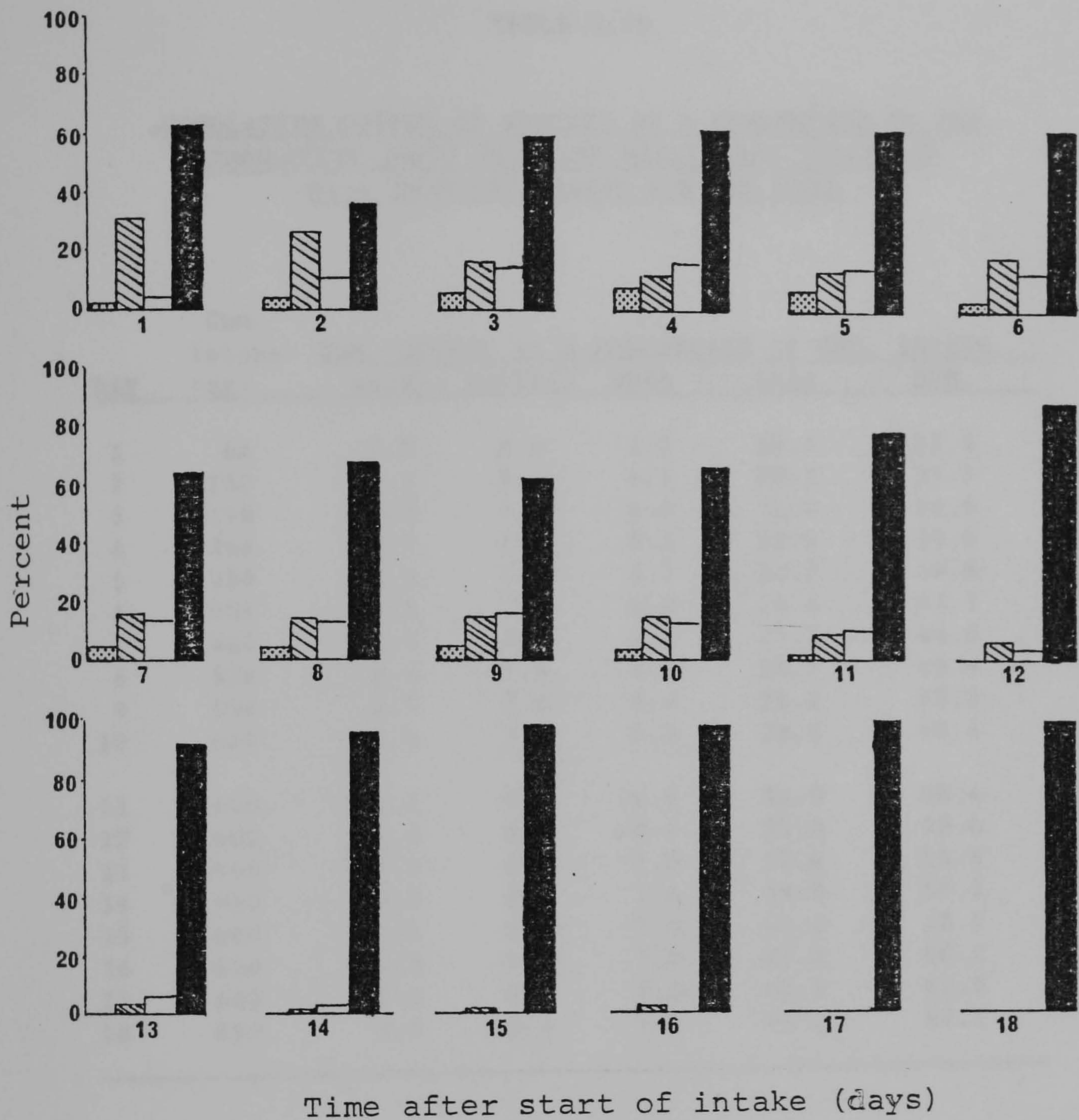


Figure 3.21 The relative proportions of each arsenic species excreted per 24hrs following the daily oral intake of 66ug inorganic As(V) for ten days by a single volunteer.

As(V) As(III) MMAA DMAA

TABLE 3.26

CUMULATIVE OUTPUT OF ARSENIC AS A PERCENTAGE OF THE CUMULATIVE INPUT FROM THE DAILY ORAL INTAKE OF 66ug INORGANIC As(V) FOR TEN DAYS

DAY	Cum. intake (ug)	CUM. OUTPUT AS A PERCENTAGE OF CUM. INTAKE				
		As(V)	As(III)	MMAA	DMAA	SUM
1	66	0.3	8.6	1.3	15.3	25.5
2	132	1.1	9.1	4.1	20.2	34.5
3	198	1.9	7.9	4.8	21.9	36.5
4	264	2.5	7.5	5.5	23.5	39.0
5	330	2.5	7.2	5.7	24.2	39.6
6	396	2.5	7.8	6.0	26.4	42.7
7	462	2.5	7.9	6.2	27.3	44.0
8	528	2.6	7.8	6.3	28.7	45.4
9	594	2.5	7.8	6.4	28.9	45.6
10	660	2.5	7.8	6.5	29.6	46.4
11	660	2.5	8.1	6.8	33.0	50.4
12	660	2.5	8.2	6.9	35.0	52.6
13	660	2.5	8.3	7.0	37.6	55.4
14	660	2.5	8.3	7.0	39.3	57.1
15	660	2.5	8.4	7.0	41.0	58.9
16	660	2.5	8.4	7.0	42.3	60.2
17	660	2.5	8.4	7.0	43.3	61.2
18	660	2.5	8.4	7.0	44.2	62.1

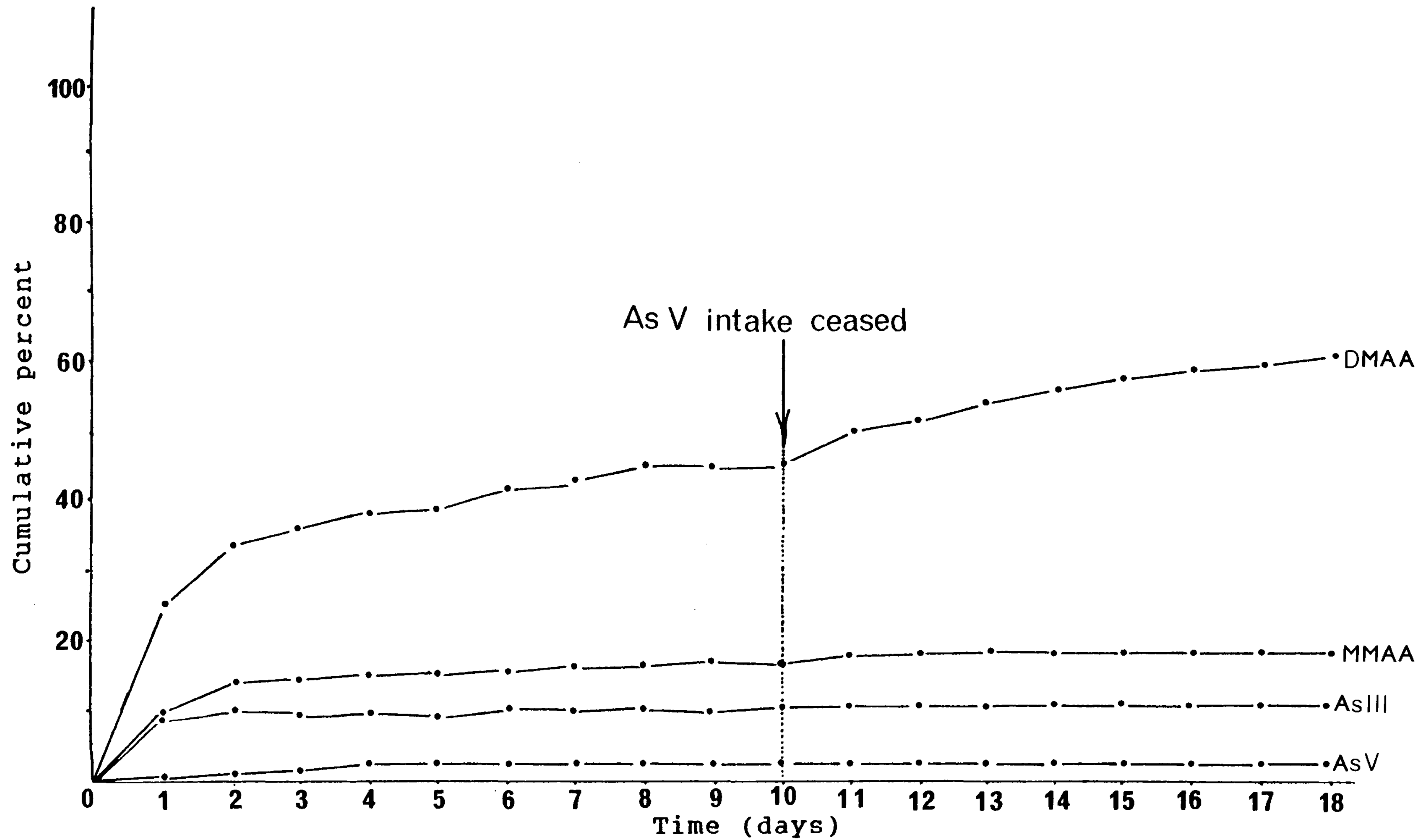


Figure 3.22 Cumulative arsenic output as a percentage of the cumulative input from the daily oral intake of 66ug inorganic As(V) for ten days by a single volunteer

3.4.5 Discussion

This experiment has shown that, on repeated exposure to inorganic arsenic, As(V), equilibrium was quite rapidly established in the urinary output of arsenic. Beginning one day after commencement of As(V) intake, the daily excreted amounts became fairly constant at levels approaching 50% of the total daily dose. Two days after the initial As(V) intake, the speciation pattern of As(V), As(III), MMAA and DMAA attained relative proportions which were maintained until cessation of intake. During the following 24 hours, this speciation pattern changed quite markedly in response to the withdrawal of As(V) exposure, in contrast to the amount excreted which was comparatively unaltered until the next day.

In view of the rapidly-established equilibrium between daily intake and output of arsenic, the slightly uneven distribution of excreted arsenic among 8-hour periods was somewhat surprising. Over the 18 days of the experiment, 39.3% of urinary arsenic appeared in the 15.30 - 23.30 samples, compared with 28.3% and 32.4% for the 07.30 - 15.30 and 23.30 - 07.30 periods, respectively. This predominance of the 15.30 - 23.30 period was independent of urine volume and arsenic speciation pattern. Likewise, as the periodic arsenic excretion pattern during the 8 days after cessation of As(V) intake (07.30 - 15.30: 29.6%; 15.30 - 23.30: 40.7%; 23.30 - 07.30: 29.8%) was

remarkably similar to that of the 10-day intake period (07.30 - 15.30: 27.9%; 15.30 - 23.30: 38.8%; 23.30 - 07.30: 33.3%), it seems unlikely that possible differences in the extent of absorption of arsenic at the different As(V) intake times, 07.30, 15.30 and 23.30, were responsible. Interestingly, in metabolic study II, the urinary arsenic excretion pattern for the same subject (A) revealed a similar pattern over 8-hour periods between 14hr and 110hr after the single As(V) intake: 08.00 - 16.00: 30.7%; 16.00 - 24.00: 36.2%; 12.00 - 08.00; 33.1%). In contrast, the pattern for volunteer (B) in metabolic study II showed little difference between periods (33.3%, 34.2%, 32.4%).

As expected, the major feature of metabolic study II, the methylation of arsenic following the administration of a single dose of pentavalent inorganic arsenic, As(V), was observed in the multi-dose experiment. It is interesting, however, to compare the excreted amounts and speciation pattern of arsenic for the first 8 hours of both experiments for (A), essentially a comparison between two single intakes of As(V), 220ug and 22ug (Table 3.27). As a percentage of the dose, the amount excreted in the low-dose experiment was similar to that excreted after the high-dose intake. The extent of methylation was also similar, the sum of the two methylated species amounting to 48.4% of the output compared with 44.7%

TABLE 3.27

COMPARISON OF URINARY ARSENIC EXCRETION OVER 8 HOURS FOLLOWING THE SEPARATE ADMINISTRATION OF 22ug AND 220ug INORGANIC As(V) TO THE SAME VOLUNTEER.

As(V) intake (ug)	AMOUNT EXCRETED (ug)					
	As(V)	As(III)	MMAA	DMAA	SUM	% of dose
22	0	0.96	0.00	0.90	1.86	8.5
220	3.48	5.97	2.85	4.82	17.13	7.8

As(V) intake (ug)	% OF EXCRETED ARSENIC			
	As(V)	As(III)	MMAA	DMAA
22	0.0	51.6	0.0	48.4
220	20.3	34.9	16.6	28.1

in the high-dose experiment. However, no MMAA was detected in the low-dose experiment whereas it constituted 16.6% of the output in the other. This finding is in line, at least qualitatively, with the suggestion (Section 3.3.5) that methylation of MMAA to DMAA may be inhibited at higher inorganic arsenic concentrations. Pentavalent inorganic arsenic, As(V), was also not detected in the low-dose experiment whereas it constituted 20.3% of the urinary arsenic output in the high-dose case (Table 3.27). This suggests a very rapid reduction of As(V) to As(III) after the 22ug As(V) intake although, in view of the postulated enhancing effect of increased urine volume on the level of As(V) excretion in the metabolic study II, it must be borne in mind that the urine output over 0 - 8 hours was only 565ml in the low-dose experiment compared with 1308ml in the high-dose study.

In the high-dose experiment 82.3% and 75.2% (68.9% and 63.9% after background correction) of the intake was excreted in the urine by volunteers (A) and (B) respectively, after 7 days. However, following the repeated oral intake of a much lower dose, over 10 days, only 62.1% was recovered in the urine over the course of the 18 day experiment. If correction for background excretion is made (5.1ug/24hrs) then the recovery after 18 days is reduced to only 48.2% of the dose. In the multi-dose metabolic experiment

however, food was ingested at the same time as the intake of the mineral water. It has been suggested that the nature of the daily diet may affect the enteric absorption of arsenic (WHO, 1981). As a milk diet has been shown to increase the elimination of arsenic in the faeces of rats (Tamura et al., 1972) it is possible that absorption of arsenic may have been reduced after the 07.30 intake of As(V), Vichy Célestins being taken along with cereal and milk. Faecal excretion in man was studied by Tam et al. (1979a) who found that only 6.1% of the radiolabelled pentavalent inorganic arsenic ingested by human volunteers was eliminated with the faeces in the 7 days after intake, and, in patients given 8.5mg As(III) solution, only 3.5% was recovered with the faeces over a 10-day period (Bettley and O'Shea, 1975, in WHO, 1981). It is possible that a small amount of arsenic could be removed in sweat although Pomroy et al. (1980) found this excretion route to be negligible in a dosed subject following a vigorous game of squash. In view of the intake/output discrepancy in this experiment, it could be postulated, contrary to the weight of published evidence (Buchet and Lauwreys, 1985), that a certain percentage of the ingested As(V) had been transformed into chemical forms of arsenic other than As(III), MMAA and DMAA, and as a consequence were not measured using the speciation techniques available. However, it was noted that

there was usually sufficiently good agreement between the sum of the four species (As(V), As(III), MMAA and DMAA) and the measured total arsenic concentration for the contribution from any other chemical forms to be considered insignificant.

It seems more likely, as discussed for the single-dose experiment (Section 3.3.5), that a significant proportion of ingested arsenic was retained in the body tissues (probably as As(III)), to be eliminated gradually over a longer period of time. The initial intake of just 22 $\pm$ 1.5ug inorganic As(V) (repeated three times a day for 10 days) results in more rapid conversion to As(III) (Table 3.27) with its greater affinity for the sulphydryl groups of enzymes and proteins and consequential longer retention capacity than As(V). Thus, it is likely that the recovery of only 48.2% of the dose, 8 days after the final intake, is due mainly to tissue retention of a significant proportion of the dose as rapidly converted As(III).

In a series of experiments in which inorganic arsenic (III) (125, 250, 500 and 1000ug as $NaAsO_2$) was administered orally once a day for five consecutive days to four volunteers, recoveries were also considerably below 100% (Buchet et al., 1981b). Over the 14 day observation period, recoveries of 54%, 73%, 74% and 64% were obtained for the 125ug, 250ug 500ug and 1000ug daily doses, respectively. The

speciation pattern of urinary arsenic excreted over the 14 days revealed that the proportion of the dose excreted as DMAA was reduced from 50 - 73% to 42% at the highest dose, suggesting significant inhibition of the conversion of MMAA to DMAA. For the three lower doses, a steady state in the urinary excretion of arsenic was reached within five days, the total amount of arsenic excreted in urine per day amounting to about 60% of the ingested dose.

This study has shown that, for a regular oral intake of 66ug (i.e. 3 x 22ug) inorganic arsenic per day, about 40% (background corrected) of the ingested dose is excreted daily in urine once equilibrium is established, assuming 100% gastrointestinal absorption. If the latter figure is reduced to 80%, then 50% of the absorbed dose is excreted per day. The inorganic arsenic intake, based on the consumption of 300ml (i.e. 3 x 100ml) Vichy Célestins per day, corresponds to the consumption of 1.32 litres of drinking water (a typical daily intake figure for the U.K. adults) of 50ug/l, the current maximum allowable arsenic concentration in water intended for human consumption (M.A.F.F., 1982; Zielhuis and Wibowo, 1984). The intake of 66ug represents about 60% of the provisional tolerable daily intake of 108ug inorganic arsenic for the 54kg subject of the experiment, based on the 2ug/kg b.w. value recommended by W.H.O. (1983). The background-

corrected mean equilibrium concentration for the sum of the arsenic species, 26ug/g creatinine, corresponding, in this case, to an output of 26.5ug inorganic arsenic per day, is considerably above the average level (5.0ug/g creatinine) for unexposed individuals (Table 4.3) but well below the peak concentrations (e.g. 81.4 and 112.0ugAs/g creatinine for volunteers A and B, respectively) observed in the 220ug single-dose experiment (Section 3.3.4, Tables 3.11, 3.12).

Previous studies of populations constantly exposed to elevated levels of inorganic arsenic in drinking water supplies have suffered from the inability to discriminate between inorganic arsenic (+ methylated metabolites) and other dietary (e.g. seafood) forms in urine (Cebrian et al., 1983; Pershagen, 1983; Southwick et al., 1983). The establishment of relationships between arsenic exposure and urinary arsenic concentration and output has also proved difficult in cases of occupational exposure where atmospheric exposure predominates and absorption may be by both respiratory and gastrointestinal routes (Smith et al., 1977; Buchet et al., 1981b; Zielhuis and Wibowo, 1984). From the linear relationship between arsenic administration and that excreted in the urine, Buchet et al. (1981b) found that for smelter workers exposed to airborne particulate arsenic trioxide, there was a reasonable

agreement between the predicted and measured urinary arsenic. The wearing of face masks (Smith et al., 1977; Buchet et al., 1981b), the relative solubilities of inhaled particulate compounds (Pershagen et al., 1982) and the extent of hand to mouth contamination (Vahter et al., 1986), however, may severely affect this relationship.

This metabolic study, along with the two previously described, has demonstrated that ingested seafood arsenic and inorganic arsenic are rapidly absorbed by the human body and largely eliminated via the kidney. For this and other reasons, urine is the preferred index of exposure in the monitoring of human exposure to inorganic arsenic. A rough guide to the sum of species concentration of inorganic arsenic and its metabolites can be provided by a direct hydride generation scan, which discriminates against the inert organoarsenicals of seafood. For accurate analysis of As(V), As(III), MMAA and DMAA, detailed analytical speciation, employing a method such as ion-exchange chromatography/HGAAS, is necessary.

In the monitoring of regular exposure (e.g. occupational, environmental, dietary), the collection of complete 24-hour urine samples, which should enable ultimate assessment of the daily output (and, possibly, intake) of inorganic arsenic, is hardly practical. Early-morning first void urine samples, where available, would enable more legitimate

comparisons between exposed and unexposed persons than random spot samples. The latter, however, are obviously more convenient to obtain but correction for creatinine content is absolutely essential.

In occupational monitoring, care should be taken in selecting the time for sampling during the working week in view of the time required to establish equilibrium between intake and output. The results of this final experiment and those of other workers show that, during the equilibrium period, some 40 - 60% of the daily inorganic arsenic intake is likely to be excreted. Once the source of exposure, or the individual, is removed, however, urinary arsenic output begins to fall about one day later. The continuation of the decline from day 12 - 18 of the multi-dose experiment yields a biological half-life, with respect to the urinary arsenic output, of 41hr, close to the 39hr (125ug) - 59hr (1000ug) found by Buchet et al. (1981b) under similar circumstances.

Information on the time of exposure may be forthcoming from the urinary arsenic speciation pattern, e.g. the extent of methylation, in particular the relative proportion of DMAA (Table 3.15, 3.16, 3.24 and Figures 3.14, 3.15, 3.21), when considered in conjunction with the magnitude of the arsenic concentration. The presence of a high percentage of inorganic arsenic implies recent exposure and the detection of the pentavalent form would suggest

probable exposure to inorganic As(V). The provision of periodic samples would clearly enable the establishment of trends, leading to a much fuller interpretation of the nature, extent and time of exposure.

3.4.5. Conclusions

1) The oral ingestion of 22ug pentavalent inorganic arsenic three times a day for ten days led, as in the single-dose metabolic study, to a rapid increase in urinary concentrations of arsenic, again composed of As(V), As(III) and the methylated metabolites MMAA and DMAA. Some seven days after arsenic intake was stopped concentrations of arsenic in urine had returned to near background levels.

2) During the first two days of regular intake of As(V), the major species excreted were As(III) and DMAA. Over the next eight days a steady speciation pattern prevailed, with mean proportions of 5.8% As(V), 15.2% As(III), 14.3% MMAA and 64.7% DMAA. After cessation of intake, DMAA quickly increased to more than 80% of the sum concentration.

3) In the 18 days covering the 10-day intake period and the remaining 8 days of the experiment, amounts corresponding to 62% (48%

after correction for likely background contributions) of the ingested arsenic were excreted in urine of which As(V) contributed 2.5%, As(III) 8.4%, MMAA 7.0% and DMAA 44.2%. The differences between oral intake and urinary output may be attributable to several factors, including dietary influences on gastrointestinal absorption of arsenic, but it seems likely that longer-term tissue retention of As(III), rapidly produced from As(V), is one of the more significant.

4) Although some fairly consistent variations in arsenic output over 8 - hour intervals during each day were observed for the subject of the study, equilibrium between the amount of arsenic excreted per day in urine and the daily intake dose was established one day after commencement of the intake and persisted for one day after cessation of the intake. The amount excreted each day after correction for possible "background contributions", averaged 40% of the daily intake.

5) The mean background-corrected equilibrium concentration of the sum of the four-hydride forming species of 26ug/g creatinine achieved during the regular daily intake of 66ug inorganic arsenic, equivalent to about 60% of the WHO (1983) provisional tolerable daily intake for the subject of this study, is

considerably in excess of the 5.0ug/g creatinine average level for unexposed individuals.

6) On the basis of this study and related investigations elsewhere, it can be reasonably deduced that 40 - 60% of the daily intake of inorganic arsenic, incurred via dietary, environmental and occupational exposure is excreted each day in urine, once equilibrium between intake and output is established. Thus, evaluation of exposure based on urinary measurements is feasible.

7) In the monitoring of people exposed to inorganic arsenic, it is clear that the use of urinary arsenic excretion is dependent on the application of appropriate analytical speciation techniques. Rapid-scan techniques for the direct measurement of the sum of the hydride-forming species can provide quick estimates but lack the necessary specificity and accuracy to yield qualitative and quantitative data of maximum value. Normalisation of urinary arsenic concentrations to creatinine content is essential for comparison purposes and data evaluation and is also a safeguard against possible anomalies introduced by sampling which is inconsistent with respect to time and type.

CHAPTER 4

URINARY EXCRETION OF ARSENIC BY THREE POPULATIONS SUBJECT TO DIFFERENT TYPES OF ARSENIC EXPOSURE

4.1 OBJECTIVES

1) To determine the concentration and speciation of urinary arsenic in the following three populations:
   a) A 'control' population unexposed to inorganic arsenic other than the small amounts present in the typical U.K. diet.
   b) An environmentally-exposed population.
   c) A number of occupationally-exposed groups.
2) To evaluate the potential exposure within each of the populations knowing the urinary arsenic excretion pattern and the likely sources of exposure.
3) To compare urinary arsenic data from the different populations and sub-groups.

4.2 CONTROL POPULATION

4.2.1. Sampling regime

To examine the typical human urinary output of arsenic, urine samples were collected from a number of adults and children from a cross section of the community who were known not to have been exposed to elevated levels of inorganic arsenic or its

derivatives MMAA and DMAA. Where possible, all volunteers were asked to collect a complete first-void sample.

Fifty samples were collected and analysed, of which 4 were 'spot' samples (i.e. only part of the sample collected at some time during the day) and 46 were first-void samples. Of the 50 samples, 40 were classified for the purposes of this study as adults (i.e. > 8 years of age) and 10 as children (i.e. ≤ 8 years of age). A division at 8 years of age was made as it was considered that any child older than this would probably not indulge in the characteristic 'hand-to-mouth' activity of younger children, with its potential for the transfer of toxic elements picked up from soils and dusts into the mouth. For all first-void samples, the pH and urine volume were measured before an aliquot (approximately 50ml) was stored for subsequent analysis. The samples were not subjected to any chemical preservation treatment but were kept under refrigeration at $4^{o}C$ or frozen at $^{-}20^{o}C$ if longer storage time was required.

4.2.2 Analytical methods

For all samples, an estimate of the sum concentration of any hydride-forming species present was obtained by direct-hydride generation as described in Section 2.4.2. All samples were then speciated by ion-exchange chromatography/HGAAS to determine the

concentrations of As(V), As(III), MMAA and DMAA (Section 2.5.3). A 10ml aliquot of each sample was analysed for the total arsenic concentration after acid digestion, along with sample duplicates, standard reference materials and reagent blanks (Section 2.4.2). Creatinine measurements were made on all the samples utilizing the Jaffe reaction as outlined in Appendix 3.2

4.2.3 Urinary arsenic excretion by a control population

Table 4.1 shows the values obtained for the total arsenic concentration (ug/l and ug/g creatinine), the direct-hydride measurement, the speciated results (ug/l), the sum of the species concentration (ug/l and ug/g creatinine), as well as the creatinine concentration, pH and volume of each sample. The results are listed in order of decreasing total arsenic concentration for male and female adults, male and female children.

Due to the influence of seafood on the total arsenic concentration in urine (Section 3.2.4), as expected for this control population, there was only a weak relationship between total urinary arsenic and the sum concentration of the hydride-forming species excreted (Spearmans rank correlation coefficient, Rs = 0.67 compared with Rs = 0.92 between the direct-hydride results and the sum of the species

TABLE 4.1

URINARY ARSENIC CONCENTRATIONS IN A CONTROL POPULATION

AGE (yrs)	VOL (ml)	pH	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat.	SUM /g creat.
SPOT SAMPLES												
ADULTS (i.e. $>$ 8 years of age)												
MALE												
23	–	–	1.93	5 – 9	56	*	*	*	6.4	6.4	29	3.3
FEMALE												
28	–	–	1.59	4 – 7	50	*	1.0	*	5.9	6.9	31	4.3
23	–	–	1.84	5 – 7	12	*	0.7	*	2.8	3.5	7	1.9
26	–	–	0.71	1 – 2	7	*	*	*	2.0	2.0	9	2.8
FIRST VOID												
ADULTS												
MALE												
30+	480	5.5	0.63	4 – 7	216	*	*	*	7.5	7.5	343	11.9
30+	55	–	1.15	2 – 3	128	*	*	*	3.7	3.7	111	3.2
30+	350	6.5	1.46	5 – 8	89	*	*	*	5.8	5.8	61	4.0
23	390	6.0	1.20	7 – 12	62	*	1.0	*	9.4	10.4	52	8.7
28	140	5.5	2.81	19 – 31	54	*	*	1.6	28.4	30.0	19	10.7
30+	407	–	0.89	2 – 3	51	*	*	*	3.0	3.0	57	3.4
30+	140	5.0	2.21	–	50	*	*	*	7.4	7.4	23	3.3
23	430	6.0	0.86	6 – 10	43	*	*	*	9.0	9.0	50	10.5
23	455	6.5	1.07	9 – 14	35	*	*	0.6	10.2	10.8	33	10.1
40+.	290	5.0	1.79	5 – 7	34	*	0.7	*	4.0	4.7	19	2.6
25	120	5.5	1.88	6 – 8	18	*	*	*	5.7	5.7	10	3.0

TABLE 4.1 cont

AGE (yrs)	VOL (ml)	pH	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat.	SUM /g creat.
30+	310	6.0	0.76	1 - 2	13	*	*	*	1.9	1.9	17	2.5
21	120	5.5	2.94	5 - 8	11	*	*	0.7	5.5	6.2	4	2.1
30+	390	5.5	1.28	4 - 7	10	*	*	*	5.7	5.7	8	4.5
24	280	5.0	1.86	6 - 9	6	*	*	*	4.3	4.3	3	2.3
26	290	5.5	0.49	2 - 3	5	*	*	*	2.0	2.0	9	4.0
30+	265	5.5	1.82	3 - 5	5	*	*	*	4.1	4.1	3	2.3
FEMALE												
30+	250	5.0	1.37	8 - 12	430	*	*	*	8.1	8.1	314	5.9
30+	155	-	0.97	6 - 9	68	*	*	*	9.0	9.0	70	9.3
23	218	5.0	1.23	6 - 8	57	*	*	*	6.8	6.8	46	5.5
30+	295	6.0	0.64	17 - 25	53	*	*	*	24.8	24.8	83	39.0
20	155	5.0	1.97	3 - 5	48	*	*	*	3.8	3.8	24	1.9
24	170	5.5	2.30	8 - 12	24	*	*	0.7	9.3	10.0	10	4.3
25	190	5.5	1.54	4 - 7	23	*	*	*	6.0	6.0	15	3.9
28	230	5.5	1.28	8 - 13	20	*	*	*	12.2	12.2	16	9.5
27	193	5.5	0.90	10 - 15	19	*	0.9	*	11.7	12.6	21	14.0
21	225	5.5	1.11	4 - 6	19	*	*	*	3.8	3.8	17	3.4
40+	498	5.0	0.25	1 - 2	16	*	*	*	1.5	1.5	64	6.0
30+	85	5.5	2.60	-	16	*	*	*	3.0	3.0	6	1.2
30+	350	5.0	0.51	1 - 2	11	*	*	*	1.4	1.4	22	2.7
24	320	6.0	1.15	6 - 8	10	*	*	*	5.7	5.7	9	5.0
30+	280	5.5	1.36	4 - 7	9	*	*	*	6.3	6.3	7	4.6
21	260	5.5	0.81	3 - 4	8	*	*	*	4.1	4.1	10	5.1
24	430	5.5	0.67	3 - 5	6	*	*	*	2.7	2.7	9	4.0
30+	400	6.5	0.53	1 - 2	6	*	*	*	2.1	2.1	10	4.0
16	320	6.0	0.64	2 - 3	3	*	*	*	1.0	1.0	5	1.6

TABLE 4.1 cont.

AGE (yrs)	VOL (ml)	pH	CREAT (g/l)	DIRECT HYDRIDE	TOTAL	As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat.	SUM /g creat.
				ARSENIC CONCENTRATION (ug/l)		SPECIES CONCENTRATION						
CHILDREN (i.e. ≤ eight years of age)												
MALE												
4	170	5.5	0.68	8 - 12	548	*	*	*	12.7	12.7	806	18.7
4	40	5.5	2.33	14 - 21	70	*	0.5	2.2	16.5	19.2	30	8.2
7	145	6.0	1.56	6 - 10	13	*	*	*	9.9	9.9	8	6.3
4	65	5.5	1.08	-	9	*	*	*	2.7	2.7	8	2.5
6	12	6.0	-	4 - 7	7	*	*	*	5.3	5.3	-	-
6	190	5.5	1.02	5 - 8	-	*	*	*	8.0	8.0	-	7.8
3	150	6.0	0.44	2 - 4	-	*	*	*	2.3	2.3	-	5.2
FEMALE												
6	210	6.0	0.93	11 - 19	1278	*	*	*	12.8	12.8	1374	13.8
8	125	6.0	0.81	6 - 10	714	*	*	*	8.1	8.1	881	10.0
6	120	6.5	1.01	15 - 22	66	*	0.5	2.2	16.9	19.6	65	19.4

* corresponds to less than the detection limit of 0.5ug/l

\- no data available

concentrations). Figure 4.1 compares the distribution of the total arsenic concentrations with the sum concentrations of the hydride-forming species (in ug/l and ug/g creatinine), the sum of the species concentration ranging from 1.0 - 30.0ug/l (1.2 - 39ug/g creatinine) while the total arsenic concentration ranges from 3 - 1278ug/l (3 - 1374ug/g creatinine). Of the control population, 78% excreted < 10ug/l arsenic as the sum of the species concentration and 80% < 10ug/g creatinine. Only one person eliminated ≥ 30ug/l (sum of species) compared with 21 people whose <u>total</u> urinary arsenic concentration exceeded 30ug/l. Only 23% of the control population had a total urinary arsenic output of < 10ug/l (30% < 10ug/g creatinine), with the majority (65%) excreting between 10 - 100ug/l (57% between 10 - 100ug/g creatinine).

Table 4.2 summarises the concentration of creatinine excreted by the control population. The mean concentration eliminated by the males was slightly higher than for the females and that of the adults slightly higher than that of the children. Application of the Student's 't' test (Appendix 4) showed however, that these differences were not significant, even at the 5% significance level. Notable effects of correction of arsenic concentration for creatinine content, however, could be seen in a number of the samples. The one sample with a sum of

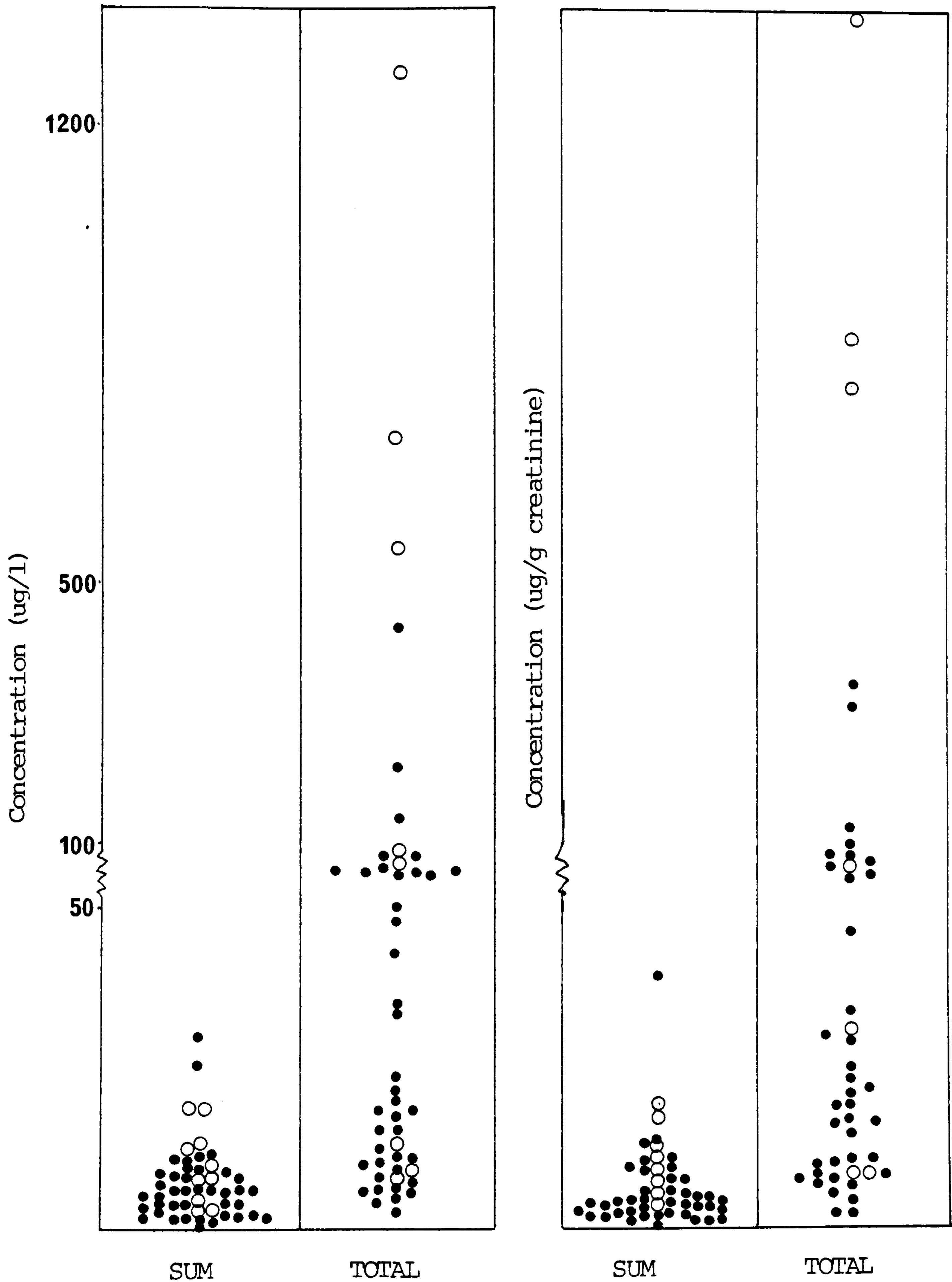


Figure 4.1 Distribution of the sum of arsenic species concentration and total arsenic concentration in a control population.
O = children ● = adults

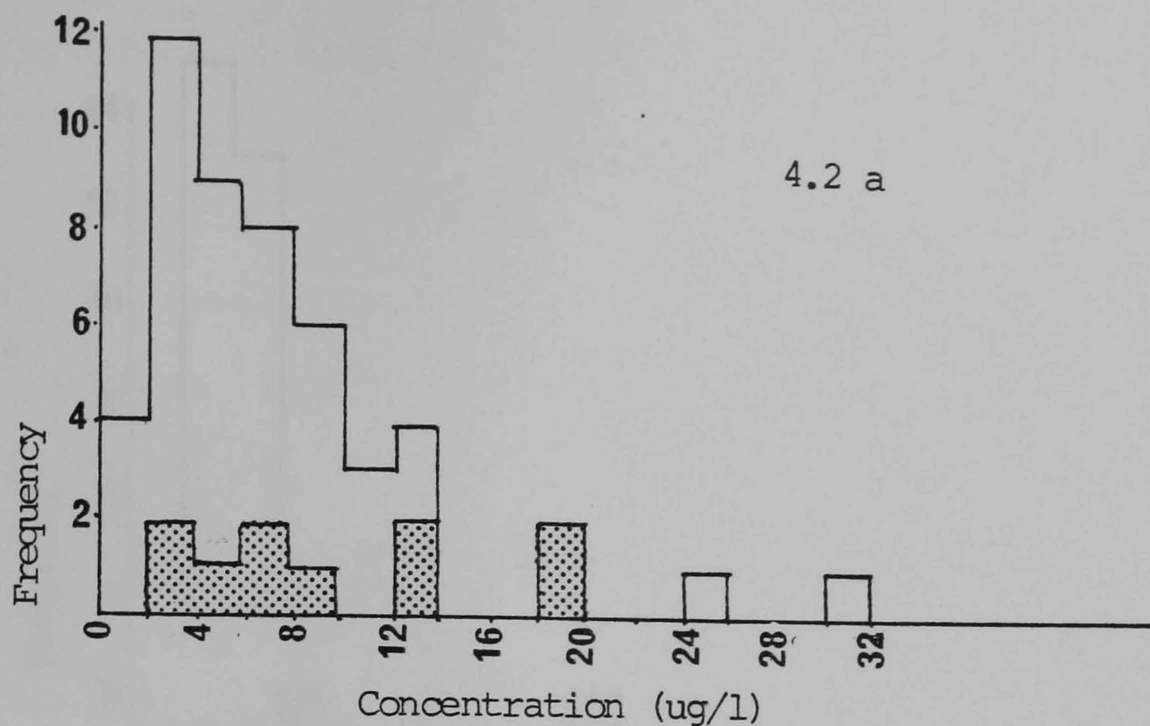
TABLE 4.2

CREATININE EXCRETION (g/l) IN
URINE SAMPLES BY A CONTROL POPULATION

	ALL	MALE	FEMALE	ADULTS	CHILDREN
n	49	24	25	40	9
Range	0.25-2.94	0.44-2.94	0.25-2.60	0.25-2.94	0.44-2.33
Arith. mean	1.26±0.60	1.37±0.68	1.14±0.58	1.30±0.65	1.09±0.55
Geom. mean	1.10	1.21	1.01	1.13	1.00
Geom. std deviation	1.70	1.66	1.70	1.71	1.61
Median	1.11	1.18	1.01	1.18	1.01

species concentration of 30.0ug/l was reduced to 10.7ug/g creatinine due to the high creatinine concentration of 2.81g/l, whereas the sample with a sum of species concentration of 24.8ug/l was increased to 39.0ug/g creatinine because of the low creatinine concentration of 0.64g/l (Table 4.1). Correction of urinary arsenic concentrations for the creatinine content is therefore essential, especially in spot samples.

When the data are presented as class frequency distributions for the sum concentration of the hydride-forming species in Figures 4.2(a) (ugAs/l) and 4.3(a) (ugAs/g creatinine), there is a marked 'skew' of the data to the left of a normal distribution, with significant tailing at the higher concentration ranges, especially when the concentrations remain in ug/l (Figure 4.2(a)). Such a skewed frequency distribution pattern suggests that the data have a log/normal distribution (Langley, 1970). The data values must therefore be first converted to their natural logarithms if statistical treatment of a normal (Gaussian) distribution is to be made. Once the data are transformed in this manner, Figures 4.2(b) and 4.3(b) show that the frequency distributions do indeed conform to the accepted normal distribution pattern. Thus, when summarising the data, it is more valid to use the geometric mean and the geometric standard deviation (G.S.D) in preference



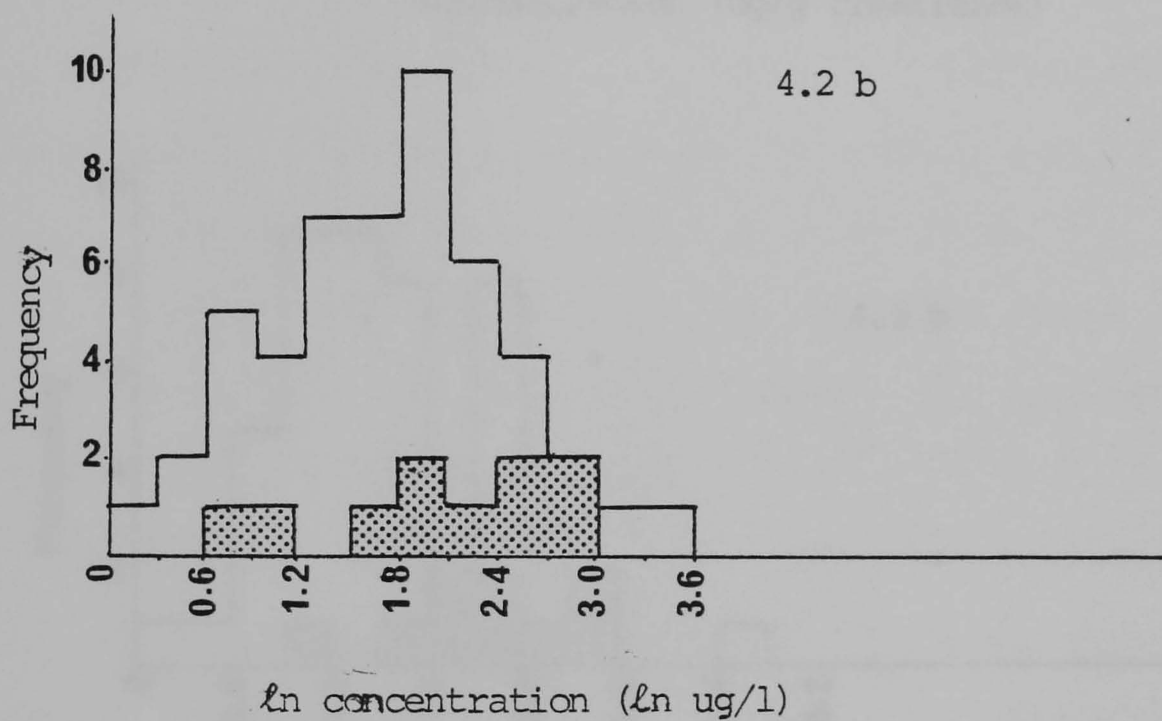
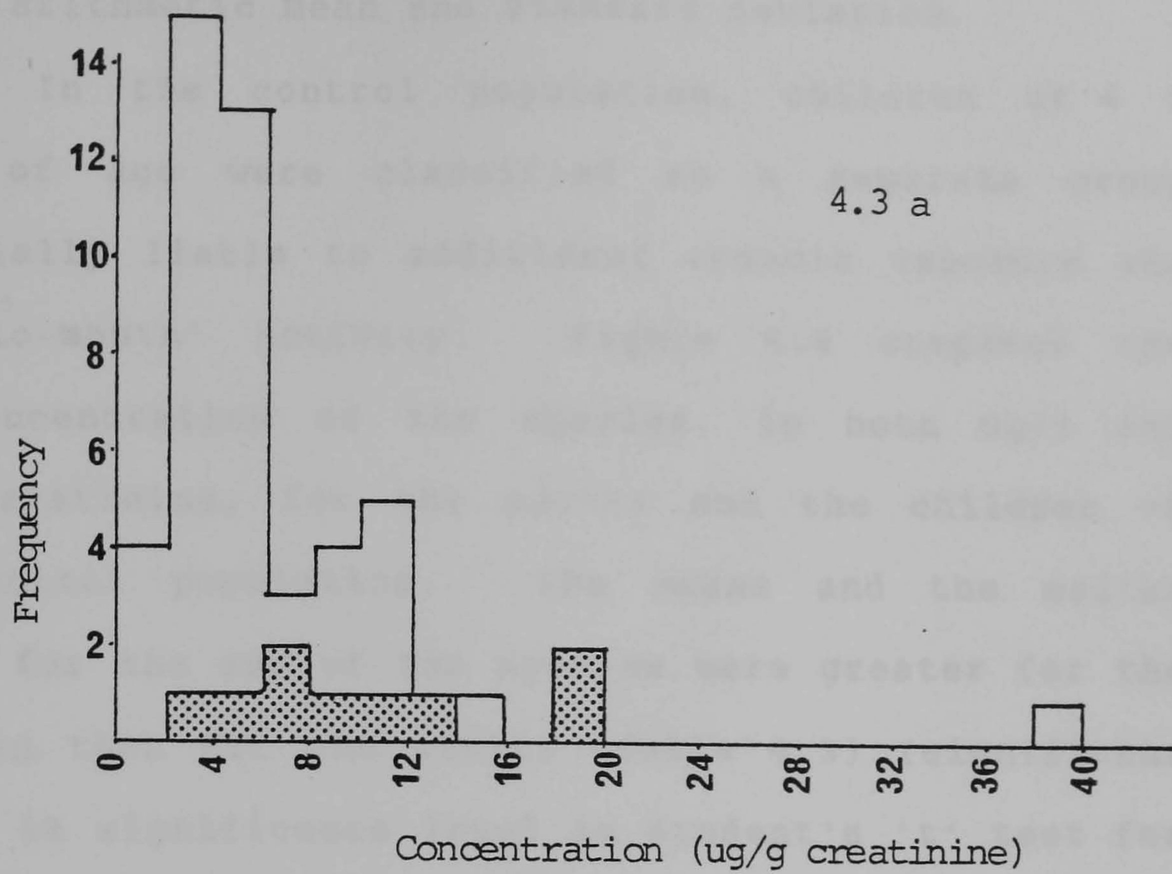


Figure 4.2 a Frequency distribution for the sum concentration (ug/l) of the arsenic hydride forming species excreted by a control population.

Figure 4.2 b Logarithmically transformed data (ℓn)

children adults



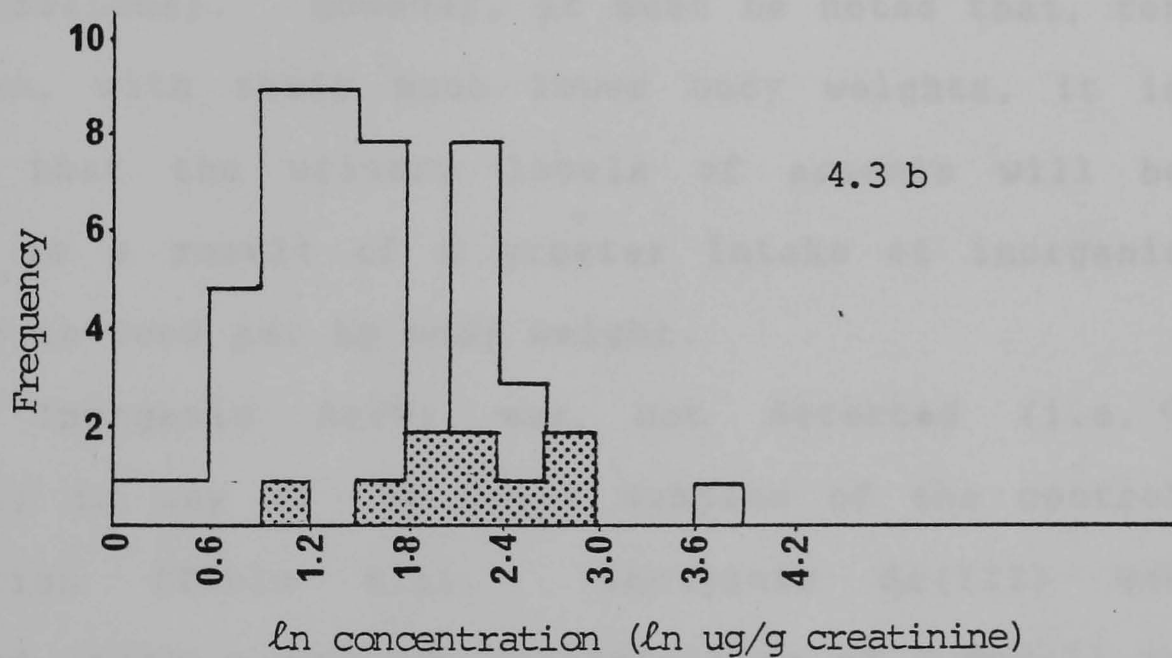


Figure 4.3 a Frequency distribution for the sum concentration (ug/g) creatinine) of the hydride forming species excreted by a control population.

Figure 4.3 b Logarithmically transformed data (ℓn).

children adults

to the arithmetic mean and standard deviation.

In the control population, children of ≤ 8 years of age were classified as a separate group potentially liable to additional arsenic exposure via 'hand-to-mouth' activity. Figure 4.4 compares the sum concentration of the species, in both ug/l and ug/g creatinine, for the adults and the children of the control population. The means and the median values for the sum of the species were greater for the children than for the adults (Table 4.3) (significant at the 5% significance level in Student's 't' test for the creatinine-corrected mean (geometric) concentrations). However, it must be noted that, for children, with their much lower body weights, it is likely that the urinary levels of arsenic will be higher as a result of a greater intake of inorganic arsenic in food per kg body weight.

Inorganic As(V) was not detected (i.e. < 0.5ug/l) in any of the urine samples of the control population (Table 4.1). Inorganic As(III) was detected (with a maximum concentration of 1.0ug/l) in only seven of the samples and MMAA (maximum concentration of 2.2ug/l) in only six. Dimethylarsinic acid was the only species detected in 39 (78%) of the 50 samples analysed and at all times exceeded 80% of the eliminated arsenic.

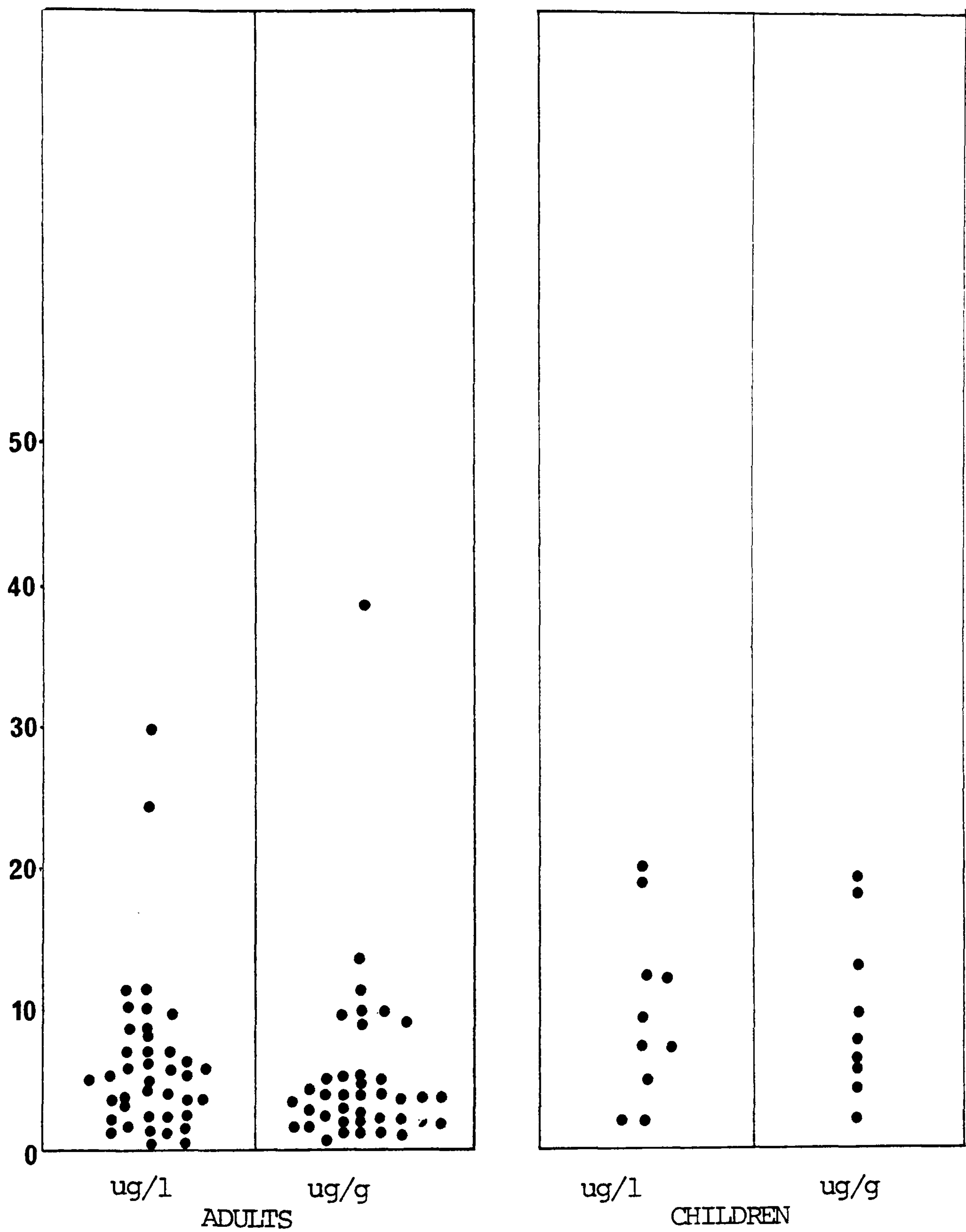


Figure 4.4 Distribution of the sum of arsenic species concentrationfor adults and children from a control population.

TABLE 4.3

SUMMARY OF THE SUM OF URINARY ARSENIC SPECIES
(As(V), As(III), MMAA, DMAA) EXCRETED BY THE CONTROL POPULATION

CATEGORY	n	Range	Arith. mean	Geom. mean	Geom. std. deviation	Med.
SUM OF SPECIES (ug/l)						
ALL	50	1.0-30.0	7.3±5.9	5.6	2.1	5.9
ADULTS	40	1.0-30.0	6.6±5.7	5.1	2.1	5.7
CHILDREN	10	2.3-19.6	10.1±6.1	8.1	2.1	9.0
SUM OF SPECIES (ug/g creatinine)						
ALL	49	1.2-39.0	6.6±6.4	5.0	2.1	4.3
ADULTS	40	1.2-39.0	5.8±6.2	4.4	2.0	4.0
CHILDREN	9	2.5-19.4	10.2±5.9	8.6	1.9	8.2

4.2.4 Exposure evaluation

The disrepancy between total urinary arsenic (geometric mean of 25.4ug/g creatinine, G.S.D. 4.2) and the sum of the hydride-forming species (geometric mean 5.0ug/g creatinine, G.S.D. 2.1) can be attributed to the contribution made by the ingested seafood organoarsenical, which, because of its inert nature and rapid elimination (Section 3.2.5), is considered to exert negligible toxic effects in the human body. In any population, the total urinary arsenic concentration will depend upon the amount of seafood in the normal diet and its time of consumption in relation to the time of urine sampling.

However, because of the known toxicity of inorganic arsenic compounds As(V) and As(III) and, to a lesser extent, the methylated derivatives MMAA and DMAA (Section 1.3.2), it is essential that detailed speciation analysis is conducted in order to evaluate the potential risk to human health from these more toxic forms. Very few studies have been carried out on the typical urinary arsenic concentrations of As(V), As(III), MMAA and DMAA in a population unexposed to elevated levels of arsenic either occupationally or environmentally, many using just the total urinary concentrations of arsenic (Buchet et al., 1980; Cant and Legendre, 1982; Takahashi et al., 1983; Beckett et al., 1986).

Although Apel and Stoeppler (1983) did not speciate urine samples, they differentiated between dietary organic arsenic and the hydride-forming species in urine of unexposed persons using direct hydride analysis. They found that the average urinary concentration of the hydride-forming species was 10ugAs/l. Valkonen and Jarvisalo (1983) reported a urinary arsenic concentration of 26ugAs/l as the sum of inorganic arsenic, MMAA and DMAA, measured collectively as 'inorganic arsenicals' after reduction with sodium borohydride, in urine from a Finnish population without occupational exposure to arsenic. More recently, Buratti et al. (1984) reported the measurement of inorganic arsenic (no As(V)/As(III) differentiation), MMAA and DMAA in the urine of 148 males without occupational exposure to arsenic. Inorganic arsenic, MMAA and DMAA each accounted for about 10% of the 17.2 $\pm$ 11.2ug/l total urinary arsenic excreted. More than 70% of the urinary arsenic was represented by other more stable forms of arsenic that could only be measured after dry ashing. The arithmetic mean for the sum of the species was 5.9 $\pm$ 2.9ug/l (range 2.0 - 21.0ug/l) compared with a slightly higher arithmetic value of 6.6 $\pm$ 5.7ug/l for the adults of the control population in this study. However, in the work described here, DMAA constituted over 80% of the urinary arsenic eliminated from the control population. Similarly, Braman and Foreback

(1973) in an early study found that 40 - 90% of the arsenic in urine of unexposed individuals was in the dimethylated form and it is now firmly established that DMAA becomes the major species excreted after dosing with inorganic arsenic (Crecelius, 1977; Tam et al., 1979a; Yamauchi and Yamamura, 1979; Buchet et al., 1981a; Section 3.3.4 and 3.4.4) and in cases of arsenic poisoning (Mahieu et al., 1981; Lovell and Farmer, 1985).

Overall, it may be concluded that for a control population, unexposed to elevated levels of inorganic arsenic, the sum concentration of the hydride-forming species excreted in urine is usually $<$ 10ug/l (corresponding to less than approximately 10ug/g creatinine). Such a species concentration of arsenic in the urine of unexposed individuals is the result of inorganic arsenic intake via inhalation and the ingestion of food and drinking water. In the metabolic study conducted in Section 3.4, it was shown that an equilibrium was established such that the daily urinary excretion of inorganic arsenic and its metabolites constituted 40 - 60% of the daily inorganic arsenic intake. From Table 4.3, the adults of the control population have a geometric mean urinary concentration of 4.4ugAs/g creatinine as the sum of the hydride-forming species. Although daily urinary excretion of creatinine can vary significantly not only among different subjects but

also in the same subject from one day to another (Duarte et al., 1980), a mean daily urinary excretion of creatinine is estimated, from values quoted in current clinical practice and in the literature, to be 1.5 ± 0.3g/day. Using this value, the daily urinary arsenic output for the adults of the control population is about 6.6ug. With an output at 40 - 60% of the intake, it follows that inorganic arsenic intake would be approximately 11.0 - 16.5ug/day. Now, if such a calculation had been based upon the uncorrected first-void urinary arsenic concentration of 5.1ug/l and an average daily urinary volume of around 1.5 litres, the average urinary output would have been 7.7ugAs/day, equivalent to an intake in the range 12.8 - 19.1ug/day, slightly higher than that for the creatinine-corrected data. For the children of the control population, a geometric mean urinary concentration of 8.6ugAs/g creatinine as the sum of the species and an estimated 24-hour creatinine output of 0.75g leads to a daily urinary output of 6.45ugAs, similar to that for the adults, which, assuming that the output is 40 - 60% of the intake as for the adults, is equivalent to an intake of 10.8 - 16.1ug As/day. Using the mean concentration of 8.1ugAs/l and a urinary volume of 750ml, the arsenic would have been eliminated at about 6.1ug/day, with an intake equivalent to 10.2 - 15.3ug/day, close to that obtained using the creatinine-corrected data.

With an average rural atmospheric concentration of 6ngAs/m^3 and an urban atmospheric concentration of 20ngAs/m^3 (Table 1.2), inhalation of arsenic for the general population is considered to make a negligible contribution to the total body content (Bennett, 1981). With the exception of seafoods (Table 3.1), most other foods have an arsenic content of $<$ 0.25mg/kg and drinking water (U.K) has a typical concentration of $<$ 10ug/l. Many of the estimates made for the total arsenic intake in the diet include a certain contribution from seafoods. Estimated intakes include 30ug/day in Canada, 90ug/day in the U.K., 40ug/day (excluding seafood) - 190ug/day in the U.S.A. and 70 - 370ug/day in Japan (Bennett, 1981). A WHO (1981) survey recorded a range of 7 - 60ug/day in Canada, France, U.K. and U.S.A., with an average of 30ug/day. M.A.F.F. (1982, 1984) estimate an average daily intake for the U.K. of 89ug, of which 75% is thought to be derived from seafood, so that approximately 22ug/day could be ingested as inorganic arsenic. The inorganic arsenic intake of 11.0 - 16.5 ugAs/day calculated from the creatinine-corrected urinary arsenic data for the adults and 10.8 - 16.1ug/day for the children is comparable with the M.A.F.F. estimate.

In the 27th report of the joint FAO/WHO expert committee on food additives WHO (1983), a provisional tolerable daily intake of 2ug/kg b.w. was set for the

intake of inorganic arsenic. Thus, for an average 70kg man this is equivalent to a daily intake of 140ug, which is close to the level of 150ug/day considered potentially responsible by The Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) (M.A.F.F, 1984) for signs of arsenicism in some individuals. The intake of 11.0 - 16.5ug/day inorganic arsenic calculated from the urinary data for the adults of the control population is well below the provisional tolerable daily intake level set by the FAO/WHO committee. Even the maximum output of 39ug/g creatinine by one individual (Table 4.1) is equivalent to an intake of only 97.5 - 146ugAs/day, still below the 150ug considered by COT (M.A.F.F., 1984) to be of concern. For a young child of 15kg weight, however, the intake of 10.8 - 16.1ug/day inorganic arsenic is closer to the provisionable tolerable daily intake of 30ug/day (at 2ug/kg b.w.) and the maximum excretion of 19.4ug/g creatinine by one child is equivalent to an intake of 24.3 - 36.4ug/day, slightly above the tolerable limit.

The potential exposure to the body from a possible maximum intake of 146ug/day to the whole body of adults can be estimated using the concept of 'exposure commitment' as proposed by Bennett (1981). Such a model assumes that the concentration of the contaminant measured in the receptor organism (in this case taken to be the whole body of man) is closely

associated with the risk of effect. Transfer factors are used to relate intake to output in each of the compartments of the model from the initial source through to the final exposure. For arsenic, the basic compartmental arrangements for such a 'pathway analysis' and a summary of the transfer relationships are given in Figure 4.5. With a possible maximum intake calculated for the adults of 146ug/day, the maximum intake per year would be 53.3mg. Assuming such an intake is via the terrestrial pathway of Bennett's model (i.e. inorganic arsenic intake from foods, other than those of aquatic origin, and from drinking water), a transfer factor of:

$$2.8.10^{-4} \frac{\text{ug yr kg-1}}{\text{ug}}$$

is used to link dietary intake to the exposure commitment in the body. It follows that the exposure commitment to the whole body would be:

$$53300\text{ug/yr} \times 2.8.10^{-4} \frac{\text{ug yr kg-1}}{\text{ug}}$$

$$= 14.9\text{ug/kg}$$

which is below the 'effect' level of 15 - 340ugAs/kg b.w. quoted by Bennett (1981).

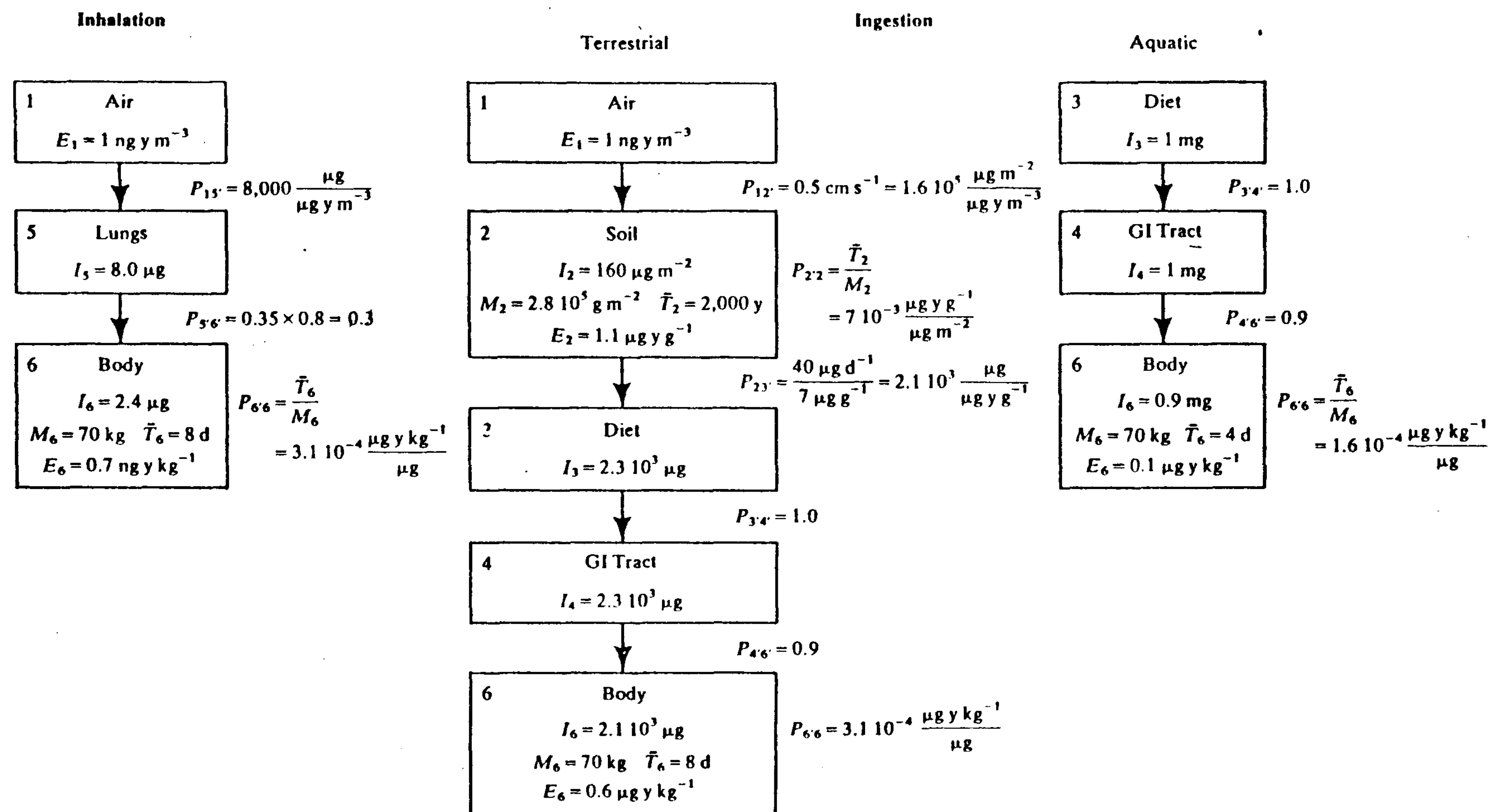


Figure 4.5 Pathway evaluations for arsenic (Bennett, 1981)

This 'effect' level is based on the association of chronic poisoning with inorganic arsenic intakes, via the consumption of drinking water, of 55 - 1200mg/yr.

With a mean intake of 11.0 - 16.5ugAs/day for the adults (equivalent to 4 - 6mg/yr) the average exposure commitment for the adults ranges from 1.1 - 1.7ug/kg. Consequently, and bearing in mind that the ingested levels are below the limits recommended by the WHO (1983) and COT (M.A.F.F., 1984), the potential health risk to the general population from a typical dietary intake of inorganic arsenic is considered to be negligible.

4.3 ENVIRONMENTALLY-EXPOSED POPULATION

4.3.1 Introduction

It was shown in Section 4.2.4 that the current background levels of arsenic in the environment are unlikely to cause a potential risk to the health of the general population. However, for those exposed to elevated levels of arsenic in the environment as a result of natural enrichment or anthropogenic contamination, the risk of exposure to this potentially toxic element is increased. For example, the drinking of well waters in geologically mineralised areas, e.g the west coast of Taiwan (Tseng, 1977; Lin et al., 1985), Antofagasta in Chile (Borgono, 1977) and Alaskan well waters (Kreiss et al., 1983), or from the inhalation of contaminated air in the vicinity of a smelter (Adamson, 1977; Milham, 1977; Pershagen et al., 1977). Long-term moderate exposure to arsenic in air, diet or drinking water can result in lesions of the skin and mucous membranes and may cause skin cancer and nervous and respiratory system damage (WHO, 1981) with possible disorders to the circulatory system (Bennett, 1981).

In the United Kingdom, the mineralised south-west peninusula of England is an area which exhibits unusually high levels of arsenic in the soils and stream sediments. Therefore, for the local population, it represents a potential source area for increased arsenic exposure. Although there are no

proven relationships between human disease and the high levels of arsenic found, Philipps et al. (1984) have observed a geographical clustering of malignant melanoma cases in the South-West in areas exhibiting elevated levels of arsenic in the stream sediments. Until now, however, no investigation of the arsenic status (e.g. urinary arsenic levels or speciation) of the population has been carried out.

4.3.2 The arsenic regional anomaly

The Wolfson Geochemical Atlas of England and Wales (1978), which is based upon stream sediment analysis (Aston et al., 1975), shows that anomalous concentrations of arsenic can be found in the south-west of England, centred upon the Penzance/Camborne and Hayle/Redruth districts as well as in the area of the Tamar Valley, west of Dartmoor (Figures 4.6, 4.7). Stream sediments with concentrations $>$ 70mg/kg are widespread in the mineralised areas, peaking to $>$ 5000mg/kg compared with $\leqslant$ 15mg/kg elsewhere (Aston et al., 1975). This distribution provides evidence of a widespread contamination of sediments and associated waters not only from natural weathering of areas underlain by mineralised copper-tin-arsenic (Cu-Sn-As) rich granites and their metamorphosed aureoles (Figure 4.7), but also exacerabated by past mining and

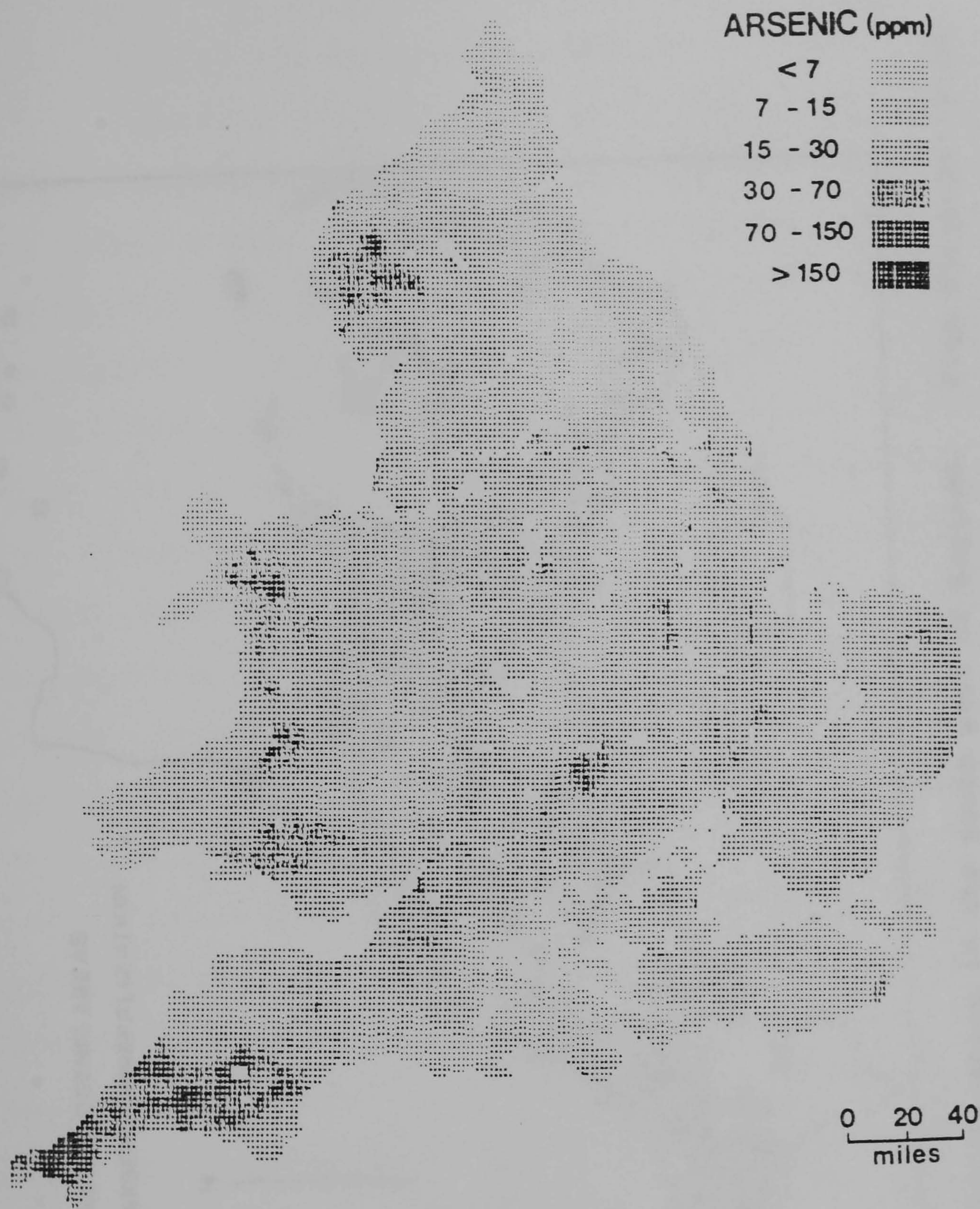


Figure 4.6 Map showing the distribution of arsenic in stream sediments in England and Wales. (Aston et al., 1975)

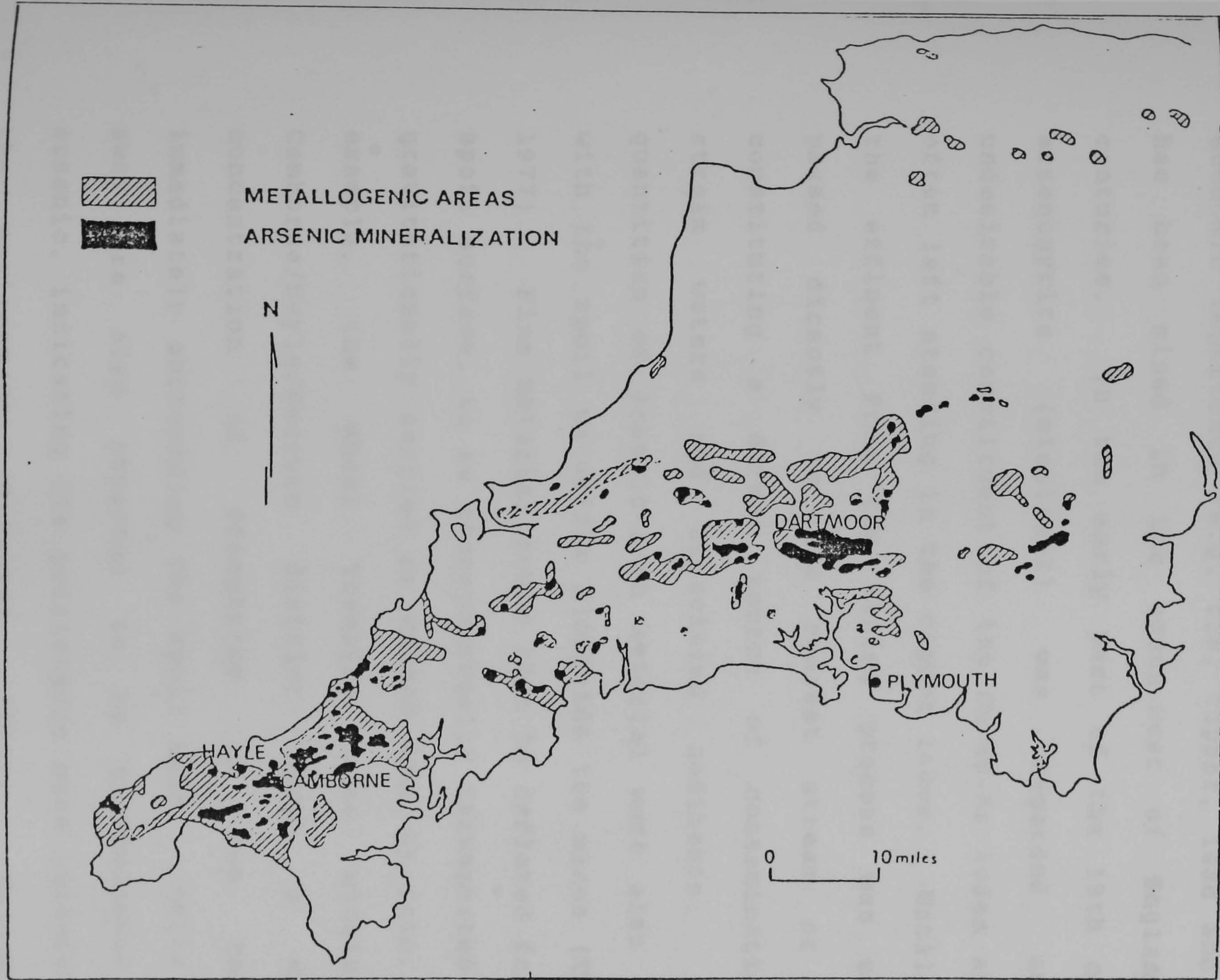


Figure 4.7 Mineralisation in the south west of England. From Abrahams (1983).

smelting of arsenical and associated metalliferous ores.

4.3.3 Mining and smelting of arsenic

Arsenic, together with other heavy metals of economic importance (e.g. tin, copper, lead and zinc), has been mined in the south-west of England for centuries. In the early part of the 19th century arsenopyrite (mispickel) was regarded as an undesirable constituent of the Cu-Sn-As lodes and was often left standing in the copper lodes. Until 1876, the effluent from the mining process was usually passed directly to the nearest stream or adit, constituting a direct source of contamination to stream waters and associated sediments. Large quantities of arsenic-rich material were also dumped with the spoil materials alongside the mines (Thomas, 1977). Fine material could then be deflated from the spoil surface, to be atmospherically transported until gravitationally settled or washed down by rain. For example, the Wheal Tremayne mine within the Camborne/Hayle/Redruth district shows a maximum concentration of 686mgAs/kg in the top-soil immediately surrounding the spoil heap. Soils 250km away are also reported to be contaminated with arsenic, indicating its persistence once released into the environment (Abrahams, 1983). During the roasting or calcination of the tin ores prior to

smelting, the arsenic would have been volatilised and oxidised primarily to arsenic trioxide constituting 80 - 90% of the 'arsenic soot' or 'crude arsenic' produced.

4.3.4 Present-day arsenic distribution

Figure 4.8 shows the present day background and anomalous distribution of arsenic in the south-west of England. Within the Tamar Valley there is considerable enhancement of metal content in both alluvial and upland topsoil, 900mg/kg arsenic reflecting the local Cu-Sn-As mineralisation (Colbourn et al., 1975). The normal range for arsenic in soils world wide is 0.1 - 40mg/kg (Bowen, 1979) and 5 - 100mg/kg for the United Kingdom (Colbourn et al., 1975; Thornton and Plant, 1980). Studies by the Applied Geochemistry Research Group, Imperial College, University of London, in the Camborne/Hayle/Redruth area in 1970 - 1973 revealed levels of 350 - 2500mg/kg arsenic, with surface contamination reflected in top soil concentrations (0 - 15cm) exceeding those of subsoil (30 - 45cm). Levels of 9.2 - 330mg/kg were subsequently found in housedusts associated with levels of 119 - 1130mg/kg in the garden soils of homes in the same area (Culbard and Johnson, 1984). Soil contamination is also reflected to a certain extent in the arsenic content of grasses and crops, although at much lower concentrations. Crops analysed in the

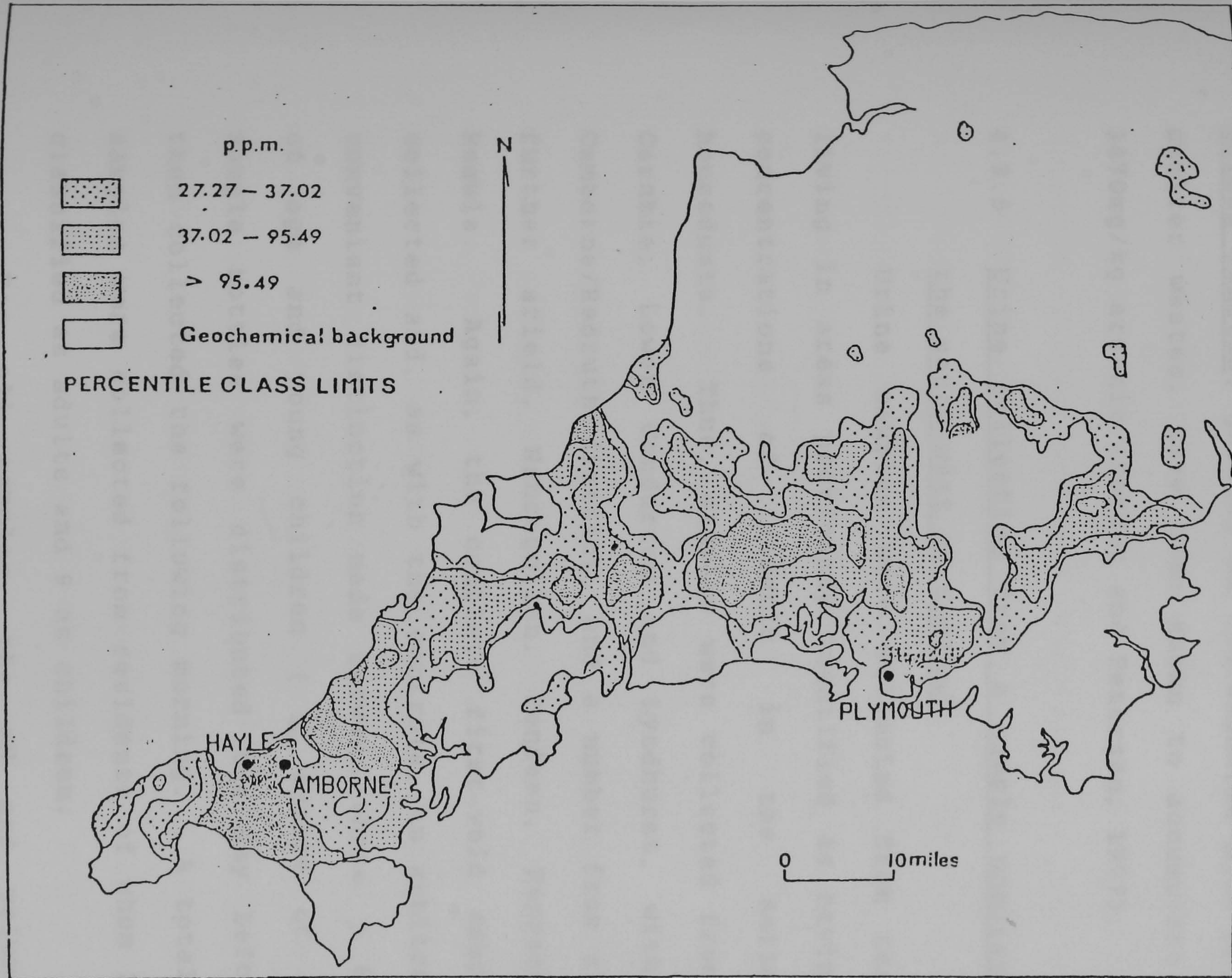


Figure 4.8 Anomolous geochemical patterns of arsenic. From Abrahams (1983).

Tamar Valley area exhibited levels of only 9.6mg/kg in pasture herbage, 0.4mg/kg in barley grain and 1.4mg/kg in lettuce (Thoresby, 1977), although common pasture types, Agrostis tenuis, Agrostis stolanifera and Holcus lanatus, growing on contaminated mine spoil and smelter wastes, have been shown to accumulate 500 - 3470mg/kg arsenic (Porter and Peterson, 1977).

4.3.5 Urine collection from a sample population in the south-west of England

Urine samples were collected from residents living in areas previously identified as having high concentrations of arsenic in the soils and housedusts. Thus, samples were collected from Brea, Carnkie, Lower Condurrow and Lyndhurst, within the Camborne/Redruth district, and a number from slightly further afield, Nancegollan, Pendeen, Penzance and Reawla. Again, the complete first-void sample was collected and, as with the controls, an arbitrary but convenient distinction made between those $>$ 8 years of age and young children ($\leqslant$ 8years of age). Sample bottles were distributed the day before and then collected the following morning. A total of 37 samples were collected from residents of whom 28 were classified as adults and 9 as children.

For all samples, the pH and volume were measured before an aliquot was stored for subsequent analysis. As before, the samples were not subject to

any chemical preservation treatment, but kept under refrigeration until required.

4.3.6 Analytical methods

As for the control population, an estimate of the sum concentration of any hydride-forming species present was obtained by direct-hydride generation (Section 2.4.2) and all samples were speciated by ion-exchange chromatography/HGAAS to determine the concentrations of As(V), As(III), MMAA and DMAA (Section 2.5.3). Total arsenic concentrations were obtained after acid digestion (Section 2.4.2) and creatinine measurements were made using the Jaffe reaction (Appendix 3.2).

4.3.7 Urinary arsenic excretion by a sample population from the south-west of England

Table 4.4 displays the arsenic data, pH, volume and creatinine concentration of each sample. The arsenic results are listed in order of decreasing total arsenic concentration (ug/l) for the adults and children from each of the individual areas. Total arsenic concentrations in ug/g creatinine are also given along with the results of direct-hydride analysis (ug/l), the speciated results (ug/l) and the sum of the species concentration (ug/l and ug/g creatinine). Again the total urinary arsenic concentration bears little relation to the sum

TABLE 4.4

URINARY ARSENIC CONCENTRATIONS IN A SAMPLE POPULATION FROM THE SOUTH WEST OF ENGLAND

AGE(y) SEX	VOL (ml)	pH	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat.	SUM /g creat.	COMMENTS
ADULTS (i.e. > 8yrs of age)													
BREA													
43 M	225	6.0	0.47	2 - 4	11	*	*	*	2.2	2.2	23	4.7	Lecturer
40 F	340	5.0	0.86	6 - 8	8	*	0.7	1.1	6.0	7.8	9	9.1	Aux. Nurse
21 F	405	5.5	0.52	2 - 4	4	*	*	*	4.2	4.2	8	8.1	Aux, Nurse
80 F	325	5.5	0.36	1 - 2	3	*	*	*	1.7	1.7	8	4.7	Pensioner
CAMBORNE													
33 M	160	5.0	2.49	21 - 30	49	*	2.6	5.6	15.5	23.7	20	9.5	Mineworker
CARNKIE													
29 F	315	6.5	0.51	4 - 6	70	*	*	1.0	1.0	2.0	137	3.9	Housewife
32 M	550	6.0	0.75	2 - 4	8	*	*	0.9	2.3	3.2	112	4.3	Self-employed
37 M	170	6.0	0.80	3 - 5	7	*	*	*	2.1	2.1	8	2.6	Engineer
32 F	145	6.0	0.31	1 - 2	2	*	*	*	2.2	2.2	7	7.1	Housewife
LOWER CONDURROW													
13 M	135	6.0	1.68	8 - 11	32	*	*	*	9.5	9.5	19	5.7	School boy
30 F	270	5.0	1.40	9 - 13	28	*	*	0.8	9.9	10.7	20	7.6	Housewife
32 F	40	6.0	1.75	6 - 9	25	*	0.6	1.8	7.1	9.5	14	5.4	Housewife
36 M	95	5.5	1.84	14 - 20	23	*	1.4	3.7	14.0	19.1	13	10.3	Seaman
43 M	250	5.0	1.93	9 - 13	21	*	0.7	1.2	9.2	11.1	11	5.8	Manager
44 F	130	5.5	1.24	2 - 4	13	*	*	*	2.2	2.2	10	1.8	Housewife
52 M	195	6.5	0.84	3 - 5	6	*	*	1.1	4.9	6.0	7	7.1	Farmer

TABLE 4.4 cont.

AGE(y) SEX	VOL (ml)	pH	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat	SUM /g creat	COMMENTS
LYNDHURST													
39 F	205	5.0	1.97	13 - 19	62	*	*	1.1	14.1	15.2	31	7.7	Housewife
41 F	485	5.0	0.85	1 - 2	15	*	*	0.9	8.6	9.5	18	11.2	Local Gvt.
NANCEGOLLAN													
49 M	320	7.0	0.85	4 - 6	21	*	*	*	3.6	3.6	25	4.2	Local Gvt.
REAWLA													
18 M	160	5.5	3.50	17 - 25	78	*	1.7	4.1	12.0	17.8	22	5.1	Motor mech.
16 M	190	6.0	2.71	16 - 23	75	*	1.0	3.2	17.2	21.4	28	7.9	Student
16 M	230	5.5	1.86	8 - 11	16	*	0.8	1.7	8.7	11.2	9	6.0	Horticulture
14 M	355	6.0	0.87	4 - 7	12	*	*	*	3.0	3.0	14	3.4	Schoolboy
37 F	505	6.5	0.66	2 - 4	9	*	*	*	3.0	3.0	13	4.5	Housewife
PENDEEN													
23 M	110	5.5	0.42	3 - 5	21	*	*	*	4.7	4.7	50	11.2	Seaman
TUCKINGMILL													
14 F	120	6.0	1.81	11 - 16	34	*	2.4	1.8	14.8	19.0	19	10.5	-
11 M	110	5.5	1.81	13 - 18	13	*	1.8	1.6	15.5	18.9	7	10.4	-
10 M	60	6.5	1.37	10 - 15	13	*	1.4	1.4	8.3	11.1	10	8.1	-

TABLE 4.4 cont.

AGE(y) SEX	VOL	pH	CREAT (g/l)	DIRECT HYDRIDE	TOTAL	ARSENIC CONCENTRATION (ug/l) SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat.	SUM /g creat.	COMMENTS
CHILDREN (i.e. ≤ 8 yrs of age)													
BREA													
3 F	115	6.0	0.82	33 - 47	48	*	1.3	5.1	33.5	39.9	59	48.7	Pre-school
5 F	130	5.5	0.90	15 - 21	24	*	0.9	0.5	17.3	18.7	27	20.8	Schoolgirl
CARNKIE													
6 M	25	5.5	0.54	5 - 7	8	*	*	0.9	4.0	4.9	14	9.1	Schoolboy
7 M	220	7.0	0.57	6 - 8	7	*	*	1.0	6.5	7.5	11	13.2	Schoolboy
6 F	235	6.0	0.40	1 - 2	3	*	*	*	2.5	2.5	8	6.3	Schoolgirl
CARNKIE/REDRUTH													
5 M	200	6.0	1.12	6 - 9	10	*	1.6	*	4.1	5.7	9	5.1	-
2 M	110	5.5	0.78	6 - 9	9	*	0.8	*	4.7	5.5	12	7.1	-
PENZANCE													
4 M	245	6.0	0.85	6 - 9	28	*	5.5	1.0	4.2	10.7	33	12.6	-
6 F	210	9.0	0.90	5 - 8	28	*	0.5	0.9	3.6	5.0	31	5.6	-

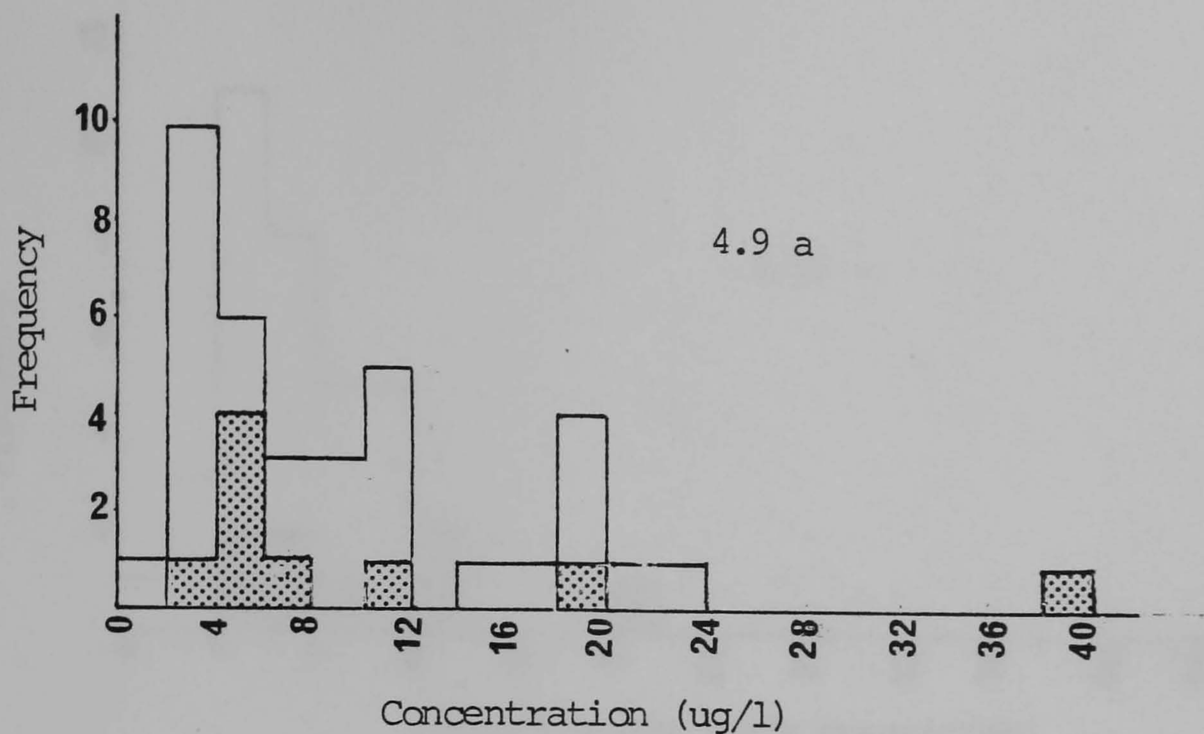
* corresponds to less than the detection limit of 0.5ug/l
\- no information available

concentration of the hydride-forming species eliminated.

Figures 4.9 and 4.10 show the frequency distribution pattern for the sum of species concentration in ug/l and ug/g creatinine respectively. As with the control population, the data are skewed towards the lower concentration ranges (Figures 4.9(a) and 4.10(a)). The data, once transformed into natural logarithms, conform to a typical normal distribution pattern (Figures 4.9(b) and 4.10(b)).

From a summary of the data for each of the districts in Table 4.5, it would appear that there is a greater urinary excretion of the hydride-forming species from the population of Brea than elsewhere. However, this is mainly due to the elevated levels excreted by the two children sampled there (Table 4.4), with sum concentrations of 48.7 and 20.8ug/g creatinine (39.9 and 18.7ug/l) respectively.

Combining the district data, Figure 4.11 reveals a greater range in concentration for the children than for the adults, but this is primarily due to the higher values for the children in Brea. It should be noted, however, that once the data are normalised for the creatinine concentration the arsenic concentration range is reduced for the adults but increased for the children (Table 4.6). Statistical analysis of the difference between the



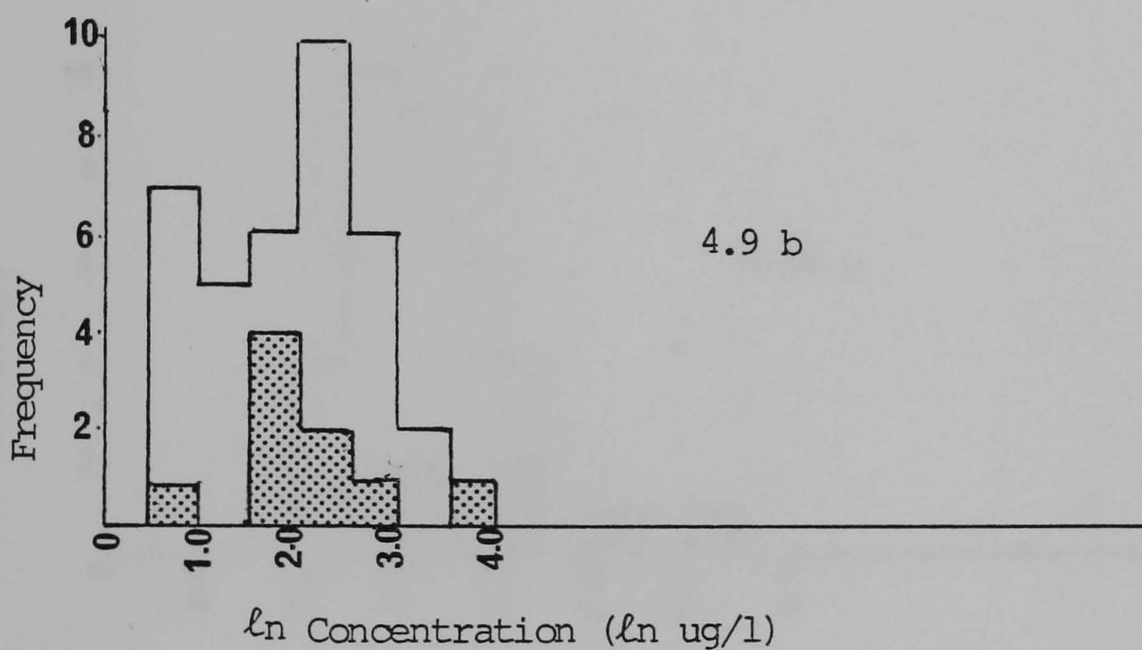
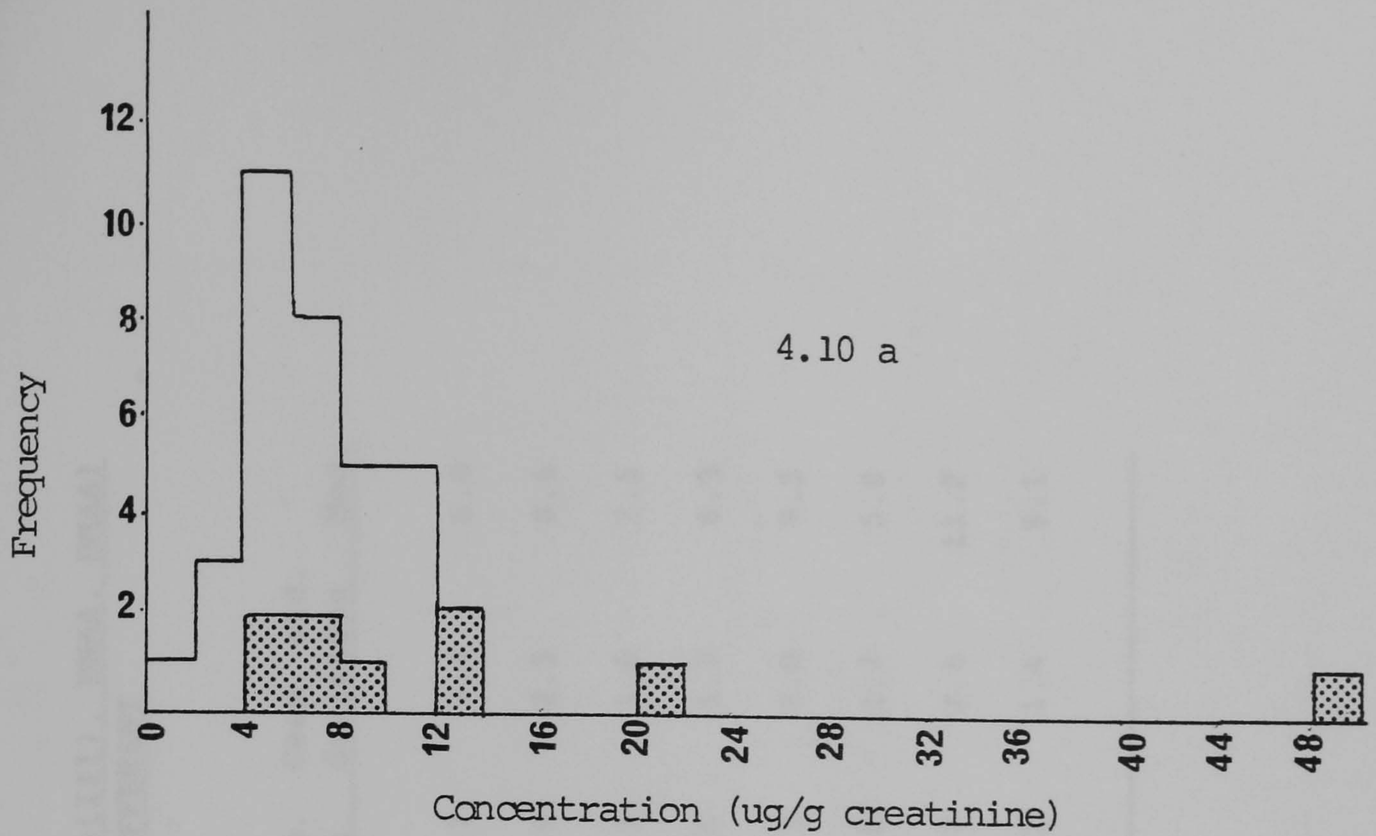


Figure 4.9 a Frequency distribution for the sum concentration (ug/l) of the arsenic hydride forming species excreted by a sample population from the south west of England.

Figure 4.9 b Logarithmically transformed data (ℓn)

children adults



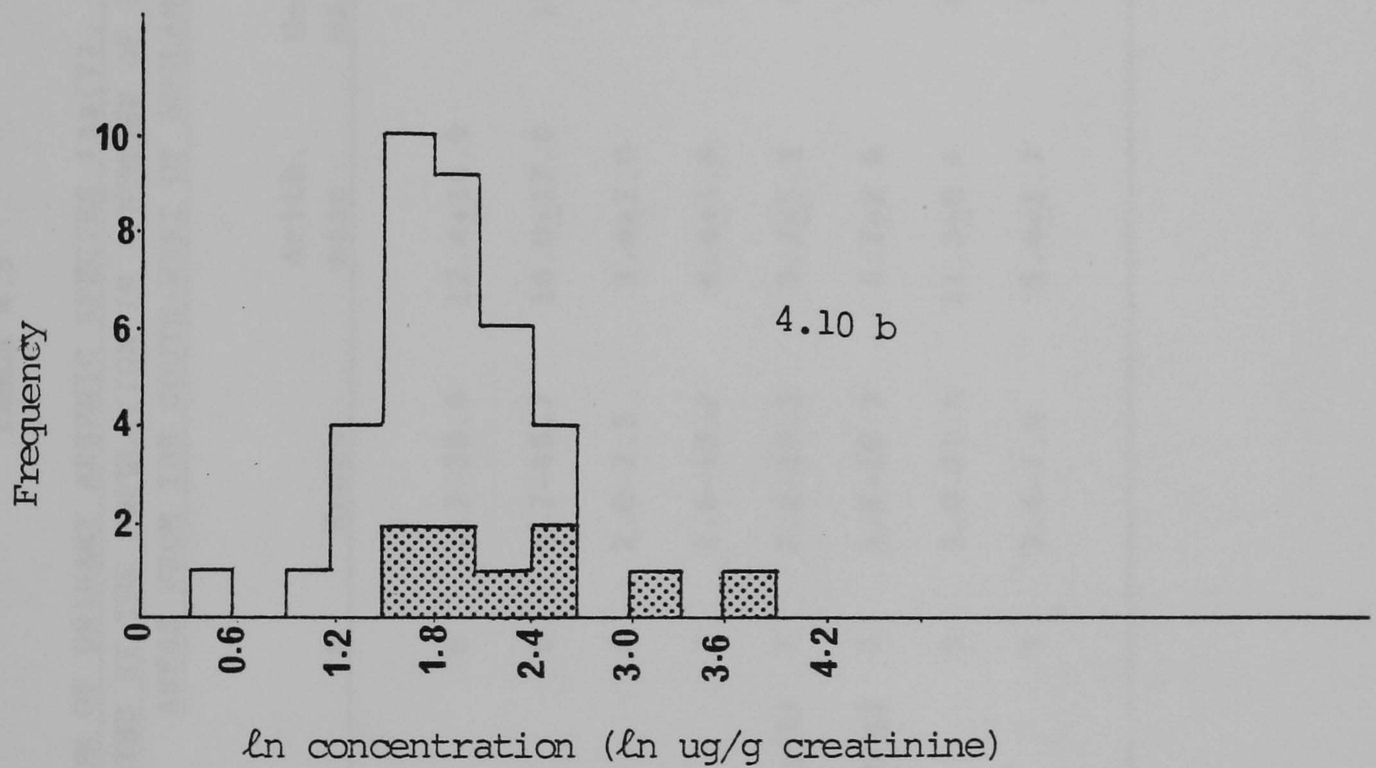


Figure 4.10 a Frequency distribution for the sum concentration (ug/g creatinine) of the arsenic hydride forming species excreted by a sample population from the south west of England.

Figure 4.10 b Logarithmically transformed data (ℓn)

children adults

TABLE 4.5

SUMMARY OF THE SUM OF URINARY ARSENIC SPECIES (As(V), As(III), MMAA, DMAA) EXCRETED BY THE POPULATION IN A NUMBER OF DIFFERENT AREAS FROM THE SOUTH-WEST OF ENGLAND

CATEGORY	n	Range	Arith. mean	Geom. mean	Geom. std. deviation	Med.
BREA (ug/l)	6	1.7-39.9	12.4±14.9	6.7	3.4	6.0
(ug/g creat.)	6	4.7-48.7	16.0±17.0	10.9	2.5	8.6
CARNKIE (ug/l)	7	2.0-7.5	3.4±2.0	3.1	1.6	2.5
(ug/g)	7	2.6-13.2	6.6±3.6	5.8	1.7	6.3
LOWER CONDURROW (ug/l)	7	2.2-19.1	9.7±5.2	8.3	2.0	9.5
(ug/g)	7	1.8-10.3	6.2±2.6	5.6	1.7	5.8
REAWLA (ug/l)	5	3.0-21.4	11.3±8.4	8.3	2.6	11.2
(ug/g)	5	3.4-7.9	5.4±1.7	5.2	1.4	5.1

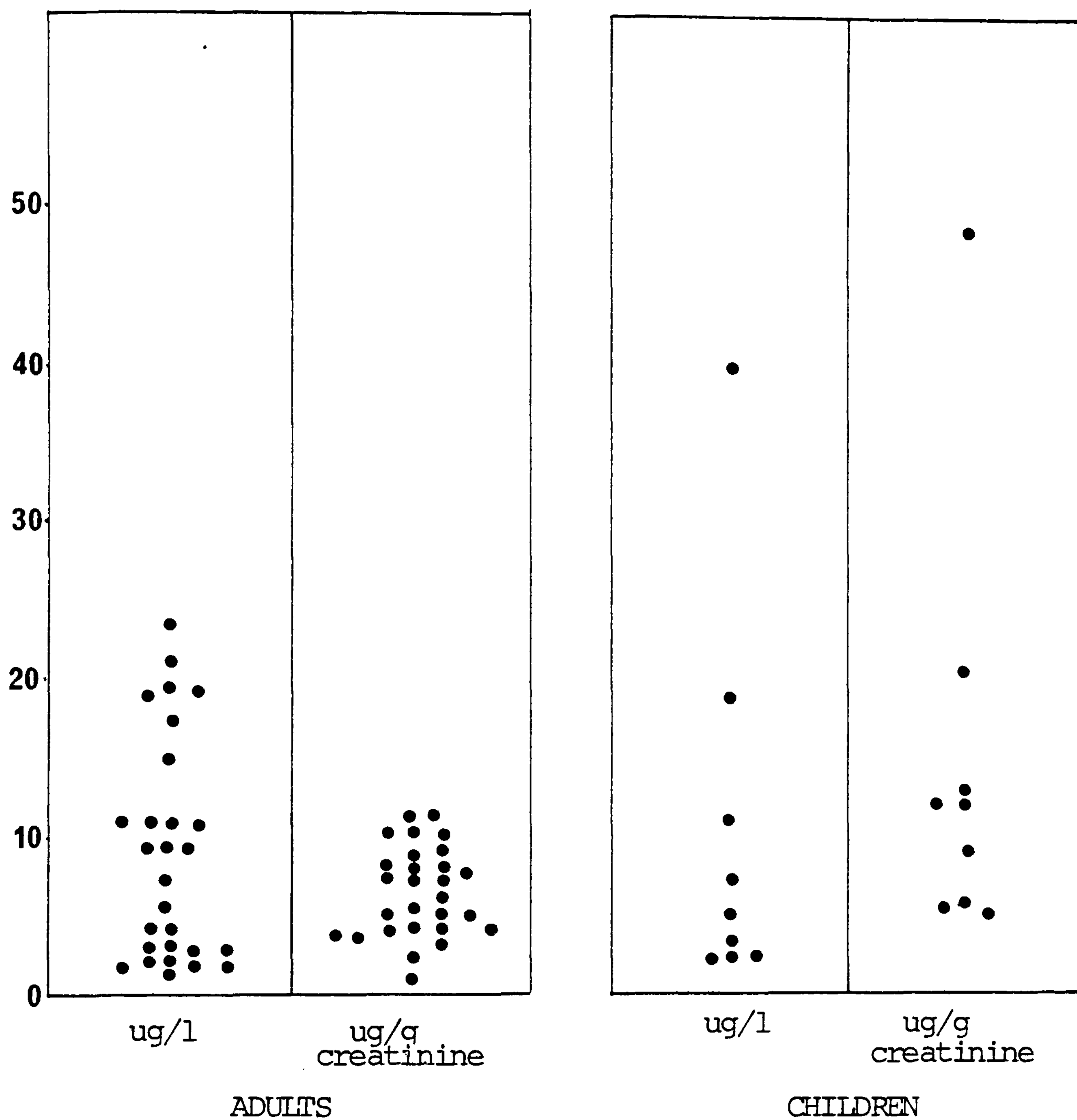


Figure 4.11 Distribution of the sum of arsenic species concentration excreted by the adults and children of a sample population from the south west of England.

TABLE 4.6

SUMMARY OF THE SUM OF URINARY ARSENIC SPECIES (As(V), As(III), MMAA, DMAA) EXCRETED BY A SAMPLE POPULATION IN THE SOUTH-WEST OF ENGLAND

CATEGORY	n	Range	Arith. mean	Geom. mean	Geom. std. deviation	Med.
SUM OF SPECIES (ug/l)						
ALL	37	1.7-39.9	9.6±8.2	6.9	2.3	7.5
ADULTS	28	1.7-23.7	9.1±6.9	6.6	2.4	8.7
CHILDREN	9	2.5-39.9	11.1±11.8	7.9	2.3	5.7
SUM OF SPECIES (ug/g creatinine)						
ALL	37	1.8-48.7	8.6±7.7	7.0	1.8	7.1
ADULTS	28	1.8-11.2	6.7±2.7	6.1	1.6	6.6
CHILDREN	9	5.1-48.7	14.3±13.8	10.8	2.1	9.1

concentrations excreted by the adults and the children using the Student's 't' test and the mean and standard deviation of the ln values, showed that the difference is significant (at the 5% significance level) for the creatinine- corrected data.

From the data collected, 62% of the sample population from the South-West excreted $<$ 10ug/l arsenic as the sum of the species, compared with 78% of the control population. As with the control population, inorganic As(V) was not detected in any of the urine samples (i.e. at concentrations below the detection limit of 0.5ug/l) while As(III) was detected (with a maximum concentration of 5.5ug/l) in 17 of the 37 samples (46%) compared with only 14% of the samples from the control group. Furthermore, MMAA could be detected (with a maximum concentration of 5.6ug/l) in 62% of samples (12% for the controls). As a result, it was in just 32% of the urine samples from the South-West, compared with 78% of the control group, that DMAA was the only species detected.

4.3.8 Comparison of the urinary arsenic excretion by the control population and by inhabitants of the south-west of England

Figure 4.12 presents a comparison between the sum concentration of the hydride-forming species (ug/l and ug/g creatinine) excreted by the control population and by those living in the south-west of England. Apart from the one elevated concentration of a young child (3 years of age) from the South West population, the data fall within the concentration range of the control population, with apparently little difference between the two sample populations. From a comparison of Tables 4.3 and 4.6, the sum concentration of the species excreted (in ug/g creatinine) by those living in the South-West was only slightly greater than by those in the control population, i.e., for the adults, a geometric mean of 4.4ugAs/g creatinine for the control population compared with 6.1ugAs/g creatinine for those from the South-West; and, for the children, a control group geometric mean of 8.6ug/g creatinine compared with 10.8ug/g creatinine for the children from the south-west of England. Further statistical analysis had to be used to determine whether these were significant. By applying the Student's 't' test to the mean and standard deviation of the ln transformed values expressed in ug/g creatinine, the urinary excretion of the hydride-forming species by the adults

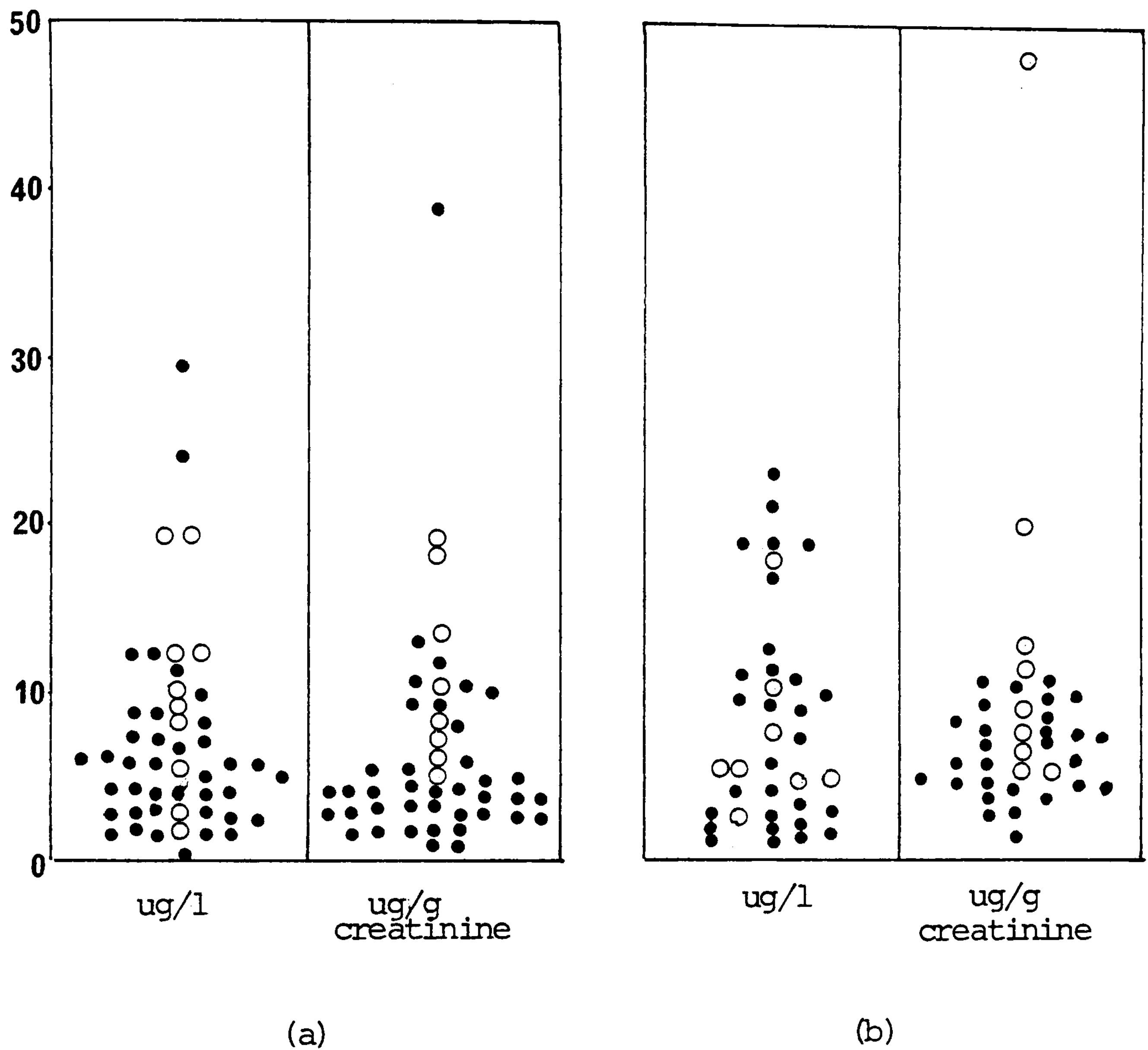


Figure 4.12 Distribution of the sum of arsenic species concentration (ug/l, ug/g creatinine) excreted by a control population (a) and by a sample population from the south west of England (b).

O children ● adults

living in the South-West was significantly greater than by the control population only at the 5% level. For the children there was no difference at the 5% significance level, but this could simply be the result of the small sample size for these two groups. It should be noted, however, that the percentage of arsenic excreted as As(III) and MMAA was greater for the adults and the children of the sample population from the south west of England than for the control population (Section 4.3.7). Although DMAA dominated urinary arsenic excretion by both populations, its relative significance for the South-West population (usually over 65% of the sum of species) was noticeably lower than for the control group (not less than 80% of the sum of species).

4.3.9 Exposure evaluation for the population of the south-west of England

From Section 4.3.8, the urinary excretion of the hydride-forming species by South-West inhabitants was not significantly greater than by the control group. With a urinary arsenic concentration of 6.1 and 4.4ug/g creatinine (geometric mean) for the adults of the South-West population and the controls, there was only an additional 1.7ugAs/g creatinine excreted, on average, by the former group. With an average creatinine output of 1.5g/day for adults (Section 4.2.4), this is equivalent to 2.55ugAs/day.

If 40% - 60% of the intake is excreted (Section 3.4.5), the additional daily intake for those living in the south-west of England is only 4.25 - 6.4ug inorganic arsenic. For the children of the South-West, a similar calculation (using an average creatinine output of 0.75g/day) reveals an additional daily intake of just 2.75 - 4.1ug inorganic arsenic. This is surprising in view of the elevated concentrations found in the surrounding garden soils, housedusts and stream sediments (Section 4.3.4).

With concentrations of 119 - 1130mg/kg found in the garden soils (Culbard and Johnson, 1984) a possible source of exposure to inorganic arsenic could be through the consumption of locally grown vegetables and salad crops. Analysis of a number of such samples taken from an allotment in Camborne (Table 4.7) revealed total arsenic concentrations well below the 1mg/kg (fresh weight) statutory limit in the U.K. Xu and Thornton (1985) also found that the total arsenic concentration (0.01 - 0.94mg/kg) in six garden crops was not high, with the exception of 3.88mg/kg for lettuce. A similar study conducted by Thoresby and Thornton (1979) concluded that the aerial parts of the plants sampled reflected to only a small extent the high concentration of the soils, e.g. a soil level of 335mg/kg corresponded to an arsenic content for barley of only 0.41mg/kg.

TABLE 4.7

TOTAL ARSENIC CONCENTRATION IN A SELECTION OF VEGETABLES FROM AN ALLOTMENT IN CAMBORNE

SAMPLE	TOTAL ARSENIC CONCENTRATION (mg/kg wet weight)
Beans	0.11
"	0.18
Brussel sprouts	0.008
"	0.007
Cabbage	0.007
"	0.009
Carrot	0.09
"	0.06
Cauliflower	0.04
"	0.03
Onion	0.03
"	0.01

However, in an earlier study, Porter and Peterson (1975) showed that the grass Agrostis Tenuis, growing in soils contaminated with arsenic from mine spoil, could accumulate a mean concentration of 670mg/kg arsenic in the leaf with up to 2000mg/kg in the seeds. Subsequent potting tests and growth experiments involving Agrostis (Porter and Peterson, 1977) showed that a tolerance to As(V) was developed in plants grown on mine waste. There therefore exists a potential for increased arsenic intake by the cattle grazing on such pasture herbage. In addition, Thornton (1984) found that it was the ingestion of contaminated soils that comprised a major exposure route for grazing animals. Such an increased intake of arsenic by cattle could present a possible route for additional exposure of the human population to arsenic.

The potential for human exposure to arsenic through the ingestion of contaminated soils and housedusts has not been fully evaluated. Most at risk are young children who have a habit of placing their hands and foreign objects in their mouths. Although, in this study, there was no statistical difference in the urinary excretion of the hydride-forming species between the children from the control population and those from the South-West, the arsenic concentrations in the urine (48.7ug/g creatinine and 20.8ug/g creatinine) of two pre-school

children in Brea, within sight of a present day working tin mine, were considerably higher than the geometric mean of 8.6ug/g creatinine for the children of the control population. With an estimated 25% of housedusts originating from the soils, the levels of arsenic encountered in a mineralised area may contribute significantly to the total body burden of very young children, who spend a considerable percentage of their time within the home environment.

Particle size analysis of housedusts in Camborne and Hayle (Johnson, 1983) revealed a tendency for arsenic enrichment in the finer fraction (< 61um). Smith (1976), in a study of particulates produced from the smelting of copper, concluded that the major fraction of arsenic was found on particles in the respirable size range of 0.25 - 10.0um as defined by Solomon and Natusch (1977) and that there was a consistent tendency for the arsenic content to increase with decreasing particle size. These findings have important implications for exposure, both environmental and occupational.

Lepow et al. (1974) studying the lead intake by young children, postulated that a child might have 10mg soil on its hands each day. With a mean concentration of 424mg/kg in the Camborne/Hayle soils it is possible that a child could be subjected to an arsenic intake of 4.2ug/day (close to the extra intake of 2.75 - 4.1ug/day calculated from the urinary data)

compared with an intake of 0.07ug/day from the soils in the rest of the U.K. (assuming an average concentration of 7mg/kg (Table 1.2)). This would be in addition to that acquired from the consumption of locally grown salad crops and local drinking water supplies. However, with reduced reliance on food grown in the immediate locality together with increased population movement, the risk of additional arsenic intake arising from the consumption of local produce is greatly reduced (Thomas, 1977). In a study of the potential uptake of lead by young pre-school children from the sucking of dirty fingers and contaminated objects, Duggan and Williams (1977) estimated that as much as 100mg of dust could be ingested each day. This would lead to a theoretical additional intake of 42ug/day from the soils of the South-West (or 113ug/day using the maximum soil arsenic concentration of 1130mg/kg), in line with the maximum range of 60.8 - 91.3ug/day intake of inorganic arsenic calculated from the urinary data for the three year old child in Brea. With a maximum concentration of 330mgAs/kg found in the housedusts from the same area (Johnson, 1983), the maximum additional intake would be 33ug/day in the home compared with a maximum intake of only 5ug/day for a control group elsewhere in the U.K..

Concern over increased exposure to the toxic inorganic forms of arsenic for the population of the

south-west of England has, in the past, prompted a number of studies on the levels of arsenic in the stream sediments (Aston et al., 1975; Philipps et al., 1984) soils and dusts (Colbourn et al., 1975; Thomas, 1977; Culbard and Johnson, 1984) and in locally grown crops (Porter and Peterson, 1975; 1977; Thoresby and Thornton, 1979; Xu and Thornton, 1985). Due to the elevated levels of arsenic found in these studies, there exist numerous possible pathways for the transfer of this toxic trace element to the local population. In this study, the observed levels and chemical forms of arsenic in urine collected from a sample population have enabled the first direct quantitative assessment of the arsenic status and exposure of humans in the area to be made. Although the average levels for the sum of the hydride-forming species for the adults and children of the South-West population are a little higher than the corresponding values for a U.K. control population, the slight differences are of no real significance. Furthermore, the levels found are much lower than elsewhere in the world where observable health effects have been linked with significant environmental sources of arsenic. For example, a mean urinary arsenic concentration of 63.4 $\pm$ 29.7ug/l has been associated with a number of cases of blackfoot disease in an area of Taiwan supplied by artesian well waters with a mean arsenic concentration of 600ug/l

(Lin et al., 1985; Tseng, 1977), and a mean urinary level of 300 ± 180ugAs/l with cutaneous signs of chronic arsenic poisoning in North Mexican inhabitants subject to exposure via the consumption of drinking water with an average arsenic concentration of 410ug/l (Cebrian et al., 1983, 1985). It is worth noting, however, that the inorganic arsenic species and MMAA were detected with greater frequency in urine from the South West inhabitants than from the control population. This phenomenon and, more importantly, the elevated arsenic levels excreted by the two children under 5 years of age in Brea, with their lower acceptable tolerable daily intake, when their lower body weight is taken into consideration warrant further investigation.

4.4 OCCUPATIONALLY-EXPOSED GROUPS

4.4.1 Introduction

Although there has been a decline in the extraction and use of arsenic since 1925, it is still used in a number of industries in the United Kingdom e.g. in the glass industry and in the manufacture and use of wood preservatives, pesticides and herbicides (Table 1.1). People working in such industries may be subject to greatly increased exposure to inorganic arsenic. Beckett et al. (1986) reported on an association of chronic motor and sensory polyneuropathy after prolonged low level exposure to inorganic arsenic while a number of epidemiological studies have shown increased incidence of skin and lung cancer in exposed individuals (Perry et al., 1948; Milham and Strong, 1974; Ott et al., 1974; Pershagen et al., 1977; Pinto et al., 1977).

As the most common route of occupational exposure is through inhalation, exposure levels are often estimated from data on the concentrations of arsenic in the atmosphere of the working environment. As pointed out by Vahter et al. (1986), a major disadvantage with air measurements is that they provide only a rough estimate of the inhaled amount, even if measured in the breathing zone. The actual dose may vary to a great extent between individuals due to variations in the burden of work, which will influence the rate of respiration, and to

exposure via other routes, e.g. ingestion or skin absorption (Beckett et al., 1986). The exposure is most likely to be to inorganic arsenic trioxide in the glass industry (Foa et al., 1984) and in the smelting of copper (Cant and Legendre, 1982; Pinto et al., 1976), to inorganic pentavalent arsenic in the manufacture and use of wood preservatives (Gollop and Glass, 1979; Takahashi et al., 1983) and to MMAA and DMAA in the manufacture and use of pesticides and herbicides (WHO, 1981; Pershagen, 1983). The metabolic studies of Sections 3.3 and 3.4 have shown that, as the main route of excretion for these chemical forms is via the kidneys, the concentration of arsenic excreted in the urine is a useful 'biological indicator' (Alesso and Bertelli, 1983; Berlin, 1983) of occupational exposure.

In a study of smelter workers conducted by Pinto et al. (1976), however, only a weak correlation ('r' of 0.53) was found between airborne arsenic and the total concentration of arsenic in the urine. This resulted from the use of total arsenic concentrations which are known to be greatly influenced by the dietary intake of organic arsenic compounds, mainly arsenobetaine, in seafood (Section 3.2.4). In contrast, Smith et al. (1977) showed that urinary concentrations of As(III), MMAA and DMAA were much better correlated with airborne arsenic.

Thus, in the monitoring of workers exposed to arsenic it is clearly very important to determine the concentrations of the more toxic forms of arsenic, inorganic arsenic and metabolites MMAA and DMAA, excreted in the urine, as distinct from arsenic of a dietary origin.

For the purposes of this study, urine samples were collected for arsenic speciation analysis from workers liable to exposure to the toxic forms of arsenic, at different levels, in a number of different industries.

4.4.2 Sampling and analysis

Samples were collected from a selected number of individuals working within the following occupational groups :

A: Semi-conductor industry: arsenic is used as arsine gas in the 'doping' of the semi-conductor chips to enhance the conduction of the silicon or germanium crystal (Skoog and West, 1980). Possible exposure is through the cleaning operations of the plumbers and by the work of those involved in electrical maintenance. The operators can be exposed to arsenic trioxide through the handling of the wafers, although this is kept to a minimum through the use of tongs and automated systems.

B: Electronics and electrical engineering (a university department); again, arsenic is used in the manufacture of semiconductors (gallium arsenide) with potential exposure to arsenic trioxide when cleaning out equipment.

C: Glass manufacturing industry: inorganic arsenic trioxide is used as a decolourising agent in the manufacture of specialist glass, e.g. in lead-based lenses. Possible exposure to airborne arsenic trioxide can occur during the weighing out of the constituents for each batch of glass and during the mixing of the chemicals. With a semi-automated system employed at the mixing stage, handling is kept to a minimum.

D: Wood preservative firm: pentavalent arsenic is used in combination with chromium and copper for the prevention of wood decay. Exposure is to airborne inorganic pentavalent arsenic during the mixing of the chemicals and via possible skin contact through the handling of the dried wood after treatment and removal from the solution. As in the previous industries, the exposure is particularly enhanced during the cleaning of equipment, in this case the tanks used to contain the wood preservative.

E: <u>Chemical firm</u>: exposure to both inorganic pentavalent and trivalent arsenic in the manufacture of arsenic containing compounds. For the plant operators, there is the possibility of exposure to i) arsenic trioxide as a result of dust escape during the discharge of material into the reactor system and ii) arsenic pentoxide and sodium arsenate due to possible dust escape during the filling of drums with the final product. In addition, there is a greater potential for exposure to these inorganic arsenicals during maintenance work than during normal production work. Urine samples were sent from those individuals thought to be subject to increased exposure.

F: <u>Health and Safety Executive (HSE)</u>: selected samples were provided by the HSE from workers in a number of different occupations with different levels of exposure to the inorganic forms of arsenic:

F1: <u>Glass industry</u>: exposure as in group C

F2 <u>Chemical firm</u> : arsenic used in the production of arsenic acid.

F3 <u>Scrap-metal firm</u>:

F4 <u>Production of electronic components</u>:

Where possible, first-void samples were collected, preferably towards the end of the working week to allow the pattern of arsenic excretion to be established (Section 3.4.4). For those working with arsenic, the first sample of the day, taken at home, is less prone to external contamination. 'Spot' samples were collected when first-void samples were not available, and, for some groups, a distinction was made between pre-shift and post-shift samples. As before, the samples were not chemically treated but stored under refrigeration. The pH and volume (first-void samples) were recorded.

As with the controls and the environmentally-exposed group, all urine samples were analysed by direct-hydride generation (Section 2.4.2) and speciated by ion-exchange chromatography/HGAAS (Section 2.5.3) while total arsenic concentrations were determined after acid digestion (Section 2.4.2). Creatinine measurements were made following the Jaffe reaction (Appendix 3.2).

4.4.3 Urinary arsenic excretion by occupationally-exposed groups.

Tables 4.8 to 4.13 display the urinary arsenic results, listed in order of decreasing total arsenic concentration (ug/l) for each of the urine samples collected from groups A - E, while for group F, the sample order was kept as received from the Health and

TABLE 4.8

URINARY ARSENIC CONCENTRATIONS EXCRETED BY WORKERS IN THE SEMICONDUCTOR INDUSTRY (GROUP A)

VOL (ml)	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat	SUM /g creat	COMMENTS
FIRST VOID SAMPLES											
148	0.77	5 - 6	140	*	*	*	4.1	4.1	182	5.3	Personnel no exposure
46	0.41	8 - 10	46	*	*	*	9.1	9.1	113	22.2	Personnel no exposure
278	1.12	4 - 6	24	*	*	*	4.1	4.1	22	3.7	Medical
388	0.80	4 - 7	21	*	*	*	6.1	6.1	26	7.6	Control
650	0.31	2 - 4	15	*	*	*	1.6	1.6	49	5.2	Post-shift - operator
205	-	5 - 9	13	*	*	1.0	6.4	7.4	-	-	Pre-shift waste management
660	0.24	1 - 2	10	*	*	*	1.1	1.1	42	4.6	Pre-shift - operator
295	0.77	4 - 5	10	*	*	*	3.6	3.6	13	4.6	Pre-shift - operator
438	0.29	3 - 4	8	*	*	*	2.0	2.0	26	6.9	Personnel no exposure
287	1.83	14 - 21	-	*	*	*	19.5	19.5	-	10.7	-
260	1.97	6 - 9	-	*	*	*	8.4	8.4	-	4.3	-
SPOT SAMPLES											
-	1.52	14 - 26	29	4.2	3.1	2.2	20.0	29.5	19	19.4	Waste management
-	1.01	1 - 3	19	*	*	*	3.0	3.0	19	3.0	Plant maintenance
-	1.20	2 - 4	18	*	*	*	2.0	2.0	15	1.7	Plant maintenance

* corresponds to less than the detection limit of 0.5ug/l

\- no data available

TABLE 4.9

URINARY ARSENIC CONCENTRATIONS EXCRETED BY WORKERS IN ELECTRONICS AND ELECTRICAL ENGINEERING (GROUP B)

CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION (ug/l) As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat	SUM /g creat	COMMENTS
SPOT SAMPLES										
1.72	14 - 22	204	*	*	*	19.7	19.7	119	11.5	Fish 9hrs prev.
1.27	3 - 4	150	2.6	*	0.9	3.0	6.5	118	5.1	–
2.31	25 - 38	66	*	7.0	5.6	30.3	42.9	29	18.6	exposed (B1)
1.13	14 - 22	53	*	3.8	*	14.0	17.8	47	15.8	exposed (B2)
1.19	13 - 17	17	0.9	1.5	2.0	10.6	15.0	14	12.6	–
1.21	8 - 12	17	*	*	*	5.6	5.6	14	4.6	unexposed
2.16	9 - 16	12	2.9	1.1	2.8	11.0	17.8	6	8.2	exposed (B3)

* corresponds to less than the detection limit of 0.5ug/l

– no data available

TABLE 4.10

URINARY ARSENIC CONCENTRATIONS EXCRETED BY WORKERS IN THE GLASS INDUSTRY (GROUP C)

		ARSENIC CONCENTRATION (ug/l)							
CREAT (g/l)	DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat	SUM /g creat
SPOT SAMPLES									
1.62	14 - 25	465	*	2.7	2.3	23.0	28.0	287	17.3
-	7 - 31	322	0.7	*	2.1	51.0	53.8	-	-
-	9 - 15	159	*	1.3	*	11.0	12.3	-	-
-	6 - 11	125	*	1.8	1.6	14.0	17.4	-	-
-	3 - 5	49	*	*	*	2.9	2.9	-	-
-	10 - 17	46	*	0.8	4.8	19.0	24.6	-	-
1.38	7 - 14	41	*	0.9	1.2	10.0	12.1	30	8.8
2.69	9 - 16	33	*	2.6	5.3	9.3	17.2	12	6.4
1.20	6 - 12	15	*	0.9	4.7	4.8	10.4	13	8.7
1.03	6 - 12	14	*	0.8	1.2	2.7	4.7	14	4.6
1.12	7 - 13	13	*	1.5	2.2	5.2	8.9	12	7.9
1.48	10 - 18	12	*	*	2.5	4.6	7.1	9	4.8

* corresponds to less than the detection limit of 0.5ug/l

\- no data available

TABLE 4.10 cont.

SAMPLE NO.	VOL (ml)	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat	SUM /g creat
PRE-SHIFT FIRST VOID SAMPLES											
1	590	0.43	6 - 10	6	*	1.4	*	7.2	8.6	14	20.0
2	238	0.64	6 - 9	7	2.4	2.7	*	3.8	8.9	11	13.9
3	620	0.51	12 - 18	16	*	0.6	0.7	13.8	15.1	31	29.6
4	198	2.38	6 - 10	9	*	*	*	4.8	4.8	4	2.0
5	289	1.15	10 - 15	24	*	1.9	2.8	7.1	11.8	21	10.3
6	160	0.29	8 - 12	8	*	3.5	1.8	5.5	10.8	26	37.2
7	185	1.41	18 - 28	70	*	3.1	4.2	12.7	20.0	50	14.2
8	95	1.80	12 - 18	32	*	1.2	2.2	10.2	13.6	18	7.6
9	147	2.76	25 - 37	72	*	4.0	6.6	21.8	32.4	26	11.7
POST-SHIFT FIRST VOID SAMPLES											
1	225	0.48	6 - 10	10	*	1.7	1.3	6.4	9.4	21	19.6
2	1890	0.60	5 - 8	34	*	1.6	1.0	3.0	5.6	57	9.3
3	318	1.18	9 - 15	94	*	0.8	1.2	10.0	12.0	80	10.2
4	180	1.27	7 - 11	8	*	1.8	*	7.3	9.1	6	7.2
5	330	0.36	7 - 11	13	*	2.6	2.0	3.6	8.2	36	22.8
6	620	1.39	6 - 10	15	*	1.2	1.3	3.0	5.5	11	4.0
7	155	0.41	6 - 10	14	*	1.6	1.6	5.0	8.2	34	20.0
8	125	1.12	6 - 9	6	*	1.3	1.0	3.0	5.3	5	4.7
9	140	1.67	16 - 26	70	*	3.0	4.2	15.0	22.2	42	13.3

* corresponds to less than the detection limit of 0.5ug/l

TABLE 4.11

URINARY ARSENIC CONCENTRATIONS EXCRETED BY WORKERS IN THE WOOD PRESERVATIVE FIRM (GROUP D)

VOL (ml)	pH	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat	SUM /g creat	COMMENTS
FIRST VOID SAMPLES												
160	5.5	1.23	44 - 63	135	3.5	8.6	7.7	38.0	57.8	110	47.0	24hrs after exposure
260	6.5	0.45	51 - 73	111	3.0	9.4	8.2	36.3	56.9	247	126.4	6hrs after exposure
420	6.0	0.77	20 - 29	38	2.7	4.8	5.4	10.8	23.7	49	30.8	Yard worker
370	5.5	0.81	8 - 13	15	*	0.6	*	9.4	10.0	19	12.3	Cabinet maker
488	6.5	0.16	17 - 24	14	*	2.4	3.4	12.2	18.0	88	112.5	Plant operator

* corresponds to less than the detection limit of 0.5ug/l

TABLE 4.12

ARSENIC CONCENTRATIONS IN SPOT URINE SAMPLES FROM WORKERS AT THE CHEMICAL FIRM (GROUP E)

pH	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat.	SUM /g creat.
5.5	1.05	10 - 14	2500	1.5	1.5	1.9	10.3	15.2	2381	14.5
7.0	1.35	860 - 1380	1450	100.4	252.6	209.5	728.6	1291.1	1074	956.4
5.5	1.70	760 - 1200	1170	314.5	181.5	96.8	255.7	848.5	688	499.1
6.5	1.41	640 - 1020	1150	43.8	176.3	156.8	589.8	966.7	816	685.6
7.0	1.02	460 - 740	900	50.0	83.0	79.9	407.1	620.0	882	607.8
6.5	1.69	540 - 860	840	36.4	140.5	148.1	324.6	649.6	497	384.4
5.5	1.98	410 - 650	840	21.9	58.3	90.6	321.6	492.4	424	248.7
5.5	1.57	440 - 720	750	38.4	91.1	129.2	345.4	604.1	478	384.8
5.0	1.65	460 - 740	720	17.5	57.6	138.6	467.0	680.7	436	412.5
5.0	2.02	520 - 760	716	61.5	88.1	137.2	342.1	628.9	354	311.3
7.0	0.67	280 - 390	675	37.3	62.4	127.0	199.2	425.9	1007	635.7
6.5	2.83	410 - 580	610	20.9	65.1	113.8	330.4	530.2	216	187.3
5.0	1.30	480 - 700	600	20.2	38.9	93.3	307.3	459.7	462	353.6
-	2.38	300 - 540	562	7.2	53.7	68.3	337.2	466.4	236	196.0
-	0.80	5 - 9	441	*	*	*	5.0	5.0	551	6.3
5.5	1.81	200 - 290	380	8.2	41.1	63.9	126.1	239.3	210	132.2
5.0	1.24	200 - 290	310	15.2	21.9	46.6	225.6	309.3	250	249.4
-	1.41	162 - 288	309	17.2	25.7	35.0	149.0	226.9	219	160.9
7.5	1.43	138 - 198	300	1.5	13.1	28.3	138.0	180.9	210	126.5
8.0	0.40	170 - 250	262	29.7	6.3	41.1	163.0	240.1	655	600.3
5.0	1.07	160 - 230	220	15.4	26.4	44.6	95.0	181.4	206	169.5
6.5	0.87	100 - 150	166	1.8	7.6	17.8	137.4	164.6	191	189.2
6.0	0.79	90 - 150	118	1.9	12.4	18.8	73.9	107.0	149	135.4
-	1.46	60 - 100	110	0.9	5.8	7.2	53.8	67.7	75	46.4
5.5	1.32	60 - 100	91	*	6.8	12.2	40.7	59.7	69	45.2
6.0	0.54	28 - 39	59	*	1.5	6.5	32.9	40.9	109	75.7
5.5	0.66	5 - 8	35	*	*	*	4.9	4.9	53	7.4

* corresponds to less than the detection limit of 0.5ug/l

\- no data available

TABLE 4.13

URINARY ARSENIC CONCENTRATIONS IN SAMPLES FROM THE HEALTH AND SAFETY EXECUTIVE (GROUP F)

SAMPLE NO.	pH	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat	SUM /g creat
SPOT SAMPLES											
F1 Glassworkers											
1	7.0	1.30	68 - 110	143	8.0	8.1	21.0	82.9	120.0	110	92.3
2	5.5	1.02	60 - 96	129	1.5	8.8	18.9	68.5	97.7	126	95.8
3	5.5	1.45	80 - 132	167	4.4	9.6	20.0	83.6	117.6	115	81.1
4	6.0	1.06	56 - 92	110	2.4	8.9	12.3	63.6	87.2	104	82.3
5	5.5	0.79	64 - 100	113	2.9	8.9	14.9	59.5	86.2	143	109.1
6	5.5	3.26	174 - 276	320	3.9	25.7	51.4	201.9	282.9	98	86.8
7	6.0	2.10	112 - 180	178	2.5	21.1	31.1	114.2	168.9	85	80.4
8	5.5	2.26	125 - 200	207	4.8	25.4	36.4	138.6	205.2	92	90.7
9	5.5	1.80	96 - 150	145	3.3	18.5	26.5	89.9	138.2	81	76.8
10	5.5	1.69	108 - 174	180	6.5	28.3	29.2	97.9	161.9	107	95.8
14	5.5	0.92	45 - 74	98	*	8.0	13.7	39.9	61.6	107	67.0
15	5.5	1.73	186 - 306	350	3.7	41.8	68.0	206.0	319.5	202	184.7
16	7.5	0.75	30 - 48	-	*	1.0	4.9	24.0	29.9	-	39.9
17	7.0	0.50	27 - 44	44	*	2.3	8.9	27.4	38.6	88	77.2
18	5.5	0.49	168 - 276	270	6.0	26.8	71.5	149.0	253.3	551	516.9
19	9.5	0.61	18 - 29	56	*	*	4.6	18.5	23.1	92	37.9
20	8.5	1.22	-	-	*	*	1.7	29.2	30.9	-	25.3
21	5.5	0.79	17 - 28	92	*	0.8	1.0	22.1	23.9	116	30.3
D	5.5	1.81	102 - 144	210	*	10.0	24.7	96.0	130.7	116	72.2
E	6.5	1.17	34 - 48	63	4.6	8.5	9.3	25.6	48.0	54	41.0
F	5.5	1.21	78 - 108	146	2.1	13.4	16.6	72.5	104.6	121	86.4
G	5.5	1.24	60 - 80	78	4.0	17.9	14.5	29.7	66.1	63	53.3

TABLE 4.13 cont.

SAMPLE NO.	pH	CREAT (g/l)	ARSENIC CONCENTRATION (ug/l) DIRECT HYDRIDE	TOTAL	SPECIES CONCENTRATION As(V)	As(III)	MMAA	DMAA	SUM	TOTAL /g creat.	SUM /g creat.
Glassworkers cont.											
H	5.5	1.39	84 - 120	129	2.2	9.3	19.4	78.2	109.1	93	78.5
I	5.0	1.92	80 - 112	128	1.7	15.2	26.1	51.0	94.0	67	49.0
N	5.5	1.38	120 - 180	347	1.9	21.3	19.8	111.2	154.2	251	111.7
O	5.5	0.92	90 - 126	119	1.8	19.7	18.8	70.1	110.4	129	120.0
P	5.0	-	130 - 190	202	3.4	28.2	16.3	105.8	153.7	-	-
Q	6.0	1.19	160 - 200	248	7.0	36.8	35.1	122.7	201.6	208	169.4
F2 Chemical firm											
A	5.5	1.30	25 - 36	54	3.0	3.6	2.7	21.9	31.2	42	24.0
B	5.0	0.86	22 - 31	34	2.5	2.2	3.6	19.5	27.8	40	32.3
C	6.0	0.88	81 - 114	146	15.6	6.6	16.9	66.3	105.4	166	119.7
F3 Scrap-metal firm											
11	5.5	1.14	12 - 20	65	*	*	*	19.6	19.6	57	17.2
12	5.5	1.18	5 - 9	45	*	*	*	9.0	9.0	38	7.6
13	6.0	1.82	5 - 10	22	*	*	*	4.6	4.6	12	2.5
F4 production of electrical components											
J	6.0	1.13	6 - 10	9	*	*	*	5.8	5.8	8	5.1
K	7.5	2.71	12 - 17	39	*	1.1	*	10.3	11.4	14	4.2
L	5.5	1.35	7 - 10	25	*	1.2	1.3	6.5	9.0	19	6.7
M	7.0	2.15	5 - 8	11	*	*	*	5.3	5.3	5	2.5

* corresponds to less than the detection limit of 0.5ug/l
\- no data available

Safety Executive. The volume of the first void sample is also listed along with the urinary creatinine concentration (g/l) for all samples. The pH was measured in only a selected number of the samples. Tables 4.8 to 4.13 also list the results of direct-hydride analysis (ug/l), speciation of As(V), As(III), MMAA and DMAA (ug/l) and the sum of the species concentration (ug/l and ug/g creatinine). Total arsenic concentrations in ug/g creatinine are also given. Table 4.14 provides a summary of the sum concentration of the hydride-forming species excreted by each of the groups.

As before (Tables 4.1, 4.4), the total arsenic concentration was often very much greater than the sum concentration of the hydride-forming species (Tables 4.8 - 4.13).

The sum concentrations of the hydride-forming species eliminated by workers from the semiconductor industry (Group A, Table 4.8) were low, with DMAA constituting over 80% of the sum of the species excreted, as in the control population. In only one of the samples analysed, with a sum of species concentration of 19.4ug/g creatinine, were the inorganic species excreted in any significant amounts, i.e. 4.2ug/l As(V) (14.2% of the sum) and 3.1ug/l As(III) (10.5% of the sum). The individual concerned was involved with the waste management and perhaps did not adhere to the stringent working conditions

TABLE 4.14

SUMMARY OF THE SUM OF THE URINARY ARSENIC SPECIES (As(V), As(III), MMAA, DMAA) EXCRETED BY EACH OF THE OCCUPATIONALLY EXPOSED GROUPS

GROUP	Units	n	Range	Arith. mean	Geom. mean	Geom. std. deviation	Med.
A	ug/l	14	1.1-29.5	7.3±8.0	4.7	2.6	4.1
	ug/g creatinine	13	1.7-22.2	7.6±6.3	5.9	2.0	5.2
B	ug/l	7	5.6-42.9	17.9±12.4	14.7	2.0	17.8
	ug/g creatinine	7	4.6-18.6	10.9±5.3	9.7	1.7	11.5
C	ALL ug/l	30	2.9-53.8	13.6±10.4	11.1	1.9	10.6
	ALL ug/g creat.	26	2.0-37.2	12.4±8.4	10.0	2.0	9.8
	Spot ug/l	12	2.9-53.8	16.6±13.9	12.5	2.2	12.2
	First void ug/l	16	4.8-32.4	12.5±7.1	11.1	1.6	10.1
	Preshift ug/l	9	2.0-37.2	16.3±11.0	12.6	2.3	13.9
	Postshift ug/l	9	4.0-22.8	12.3±7.0	10.5	1.9	10.2
D	ug/l	5	10.0-57.8	33.3±22.5	26.9	2.1	23.7
	minus control	4	18.0-57.8	39.1±21.2	34.4	1.8	40.3
	ug/g creatinine	5	12.3-126.4	65.8±50.7	47.9	2.6	47.0
	minus control	4	30.8-126.4	79.2±47.3	67.4	2.0	79.8

TABLE 4.14 cont.

SUMMARY OF THE SUM OF THE URINARY ARSENIC SPECIES (As(V), As(III), MMAA, DMAA) EXCRETED BY EACH OF THE OCCUPATIONALLY EXPOSED GROUPS

GROUP	n	Range	Arith. mean	Geom. mean	Geom. std. deviation	Med.
E						
ALL ug/l	27	4.9-1291.1	389.2±326.6	207.8	4.5	309.3
ALL ug/g creatinine	27	6.3-956.4	289.7±243.3	169.1	3.8	196.0
All minus 'control values'						
ug/l	24	40.9-1291.1	436.8±315.1	316.4	2.5	442.8
ug/g creatinine	24	45.2-956.4	324.7±235.4	244.8	2.3	249.1
F						
ALL ug/l	38	4.6-319.5	96.0±80.9	58.3	3.3	90.6
ALL ug/g creatinine	37	2.5-516.9	77.6±86.7	45.5	3.4	76.8
F1 Glassworkers						
ug/l	28	23.1-319.5	122.1±77.3	97.7	2.1	109.8
ug/g creatinine	27	25.3-516.9	98.1±91.5	79.4	1.8	81.1
F2 Chemical Workers						
ug/l	3	27.8-105.4	54.8±43.9	45.0	2.1	31.2
ug/g creatinine	3	24.0-119.7	58.7±53.0	45.3	2.4	32.3
F3 Scrap metal						
ug/l	3	4.6-19.6	11.1±7.7	9.3	2.1	9.0
ug/g creatinine	3	2.5-17.2	9.1±7.5	6.9	2.6	7.6
F4 Electronics						
ug/l	4	5.3-11.4	7.9±2.9	7.5	1.4	7.4
ug/g creatinine	4	2.5-6.7	4.6±1.8	4.4	1.5	4.7

maintained throughout the rest of the plant. Statistical analysis, using the Student's 't' test and the mean and standard deviation of the ln values, (ln of the geometric mean in ug/g creatinine) showed that there was no statistically significant difference (even at the 5% level) between group A and the adults of the control population with respect to the urinary excretion of inorganic arsenic and its metabolites.

Speciation of seven urine samples from workers in occupational group B (Electronics and Electrical Engineering, Table 4.9) showed that, while the sum of species concentrations were low, As(V), As(III) and MMAA were detected at slightly higher concentrations than in the control group (Table 4.1), especially for one particular individual (samples B1 to B3). Direct-hydride analysis of additional samples from this group, however, (Appendix 5) showed that the concentrations of the hydride-forming species were generally within the range excreted by the control population.

For occupational group C (glass industry, Table 4.10) statistical analysis showed there was no significant difference between the urinary excretion of arsenic between the spot and first-void samples collected nor between the pre-shift or post-shift samples. When sample pairs for the pre-shift and post-shift exposure were considered, the anticipated increase in urinary arsenic excretion in the

post-shift samples was not observed to any significant extent. As(V) was detected in only two of the samples, while As(III) and MMAA were detected in most of the samples. DMAA accounted for 69.5 ± 16.5% of the sum of species excreted. An output of 10.0ugAs/g creatinine (geometric mean) as the sum concentration of the hydride-forming species for all samples was significantly greater, at the 1% significance level, than the 4.4ugAs/g creatinine excreted by the adults of the control population, showing that this group was subjected to increased exposure to the inorganic forms of arsenic.

For those working with the wood preservatives (Group D), urinary levels of the hydride-forming species were notably higher than in the previous three groups (Table 4.11). The sum concentration of 126.4ug/g creatinine in one individual six hours after exposure (cleaning of the preservative containing tanks) and 112.5ug/g creatinine in another were approximately 27 times the average control value of 4.4ug/g creatinine. The sum concentration of 12.3ug/g creatinine (10ug/l) for the cabinet maker of this group was considered to be within the 'normal' range. Of the remaining four samples, there was a significant excretion of As(V) even 24 hours after the exposure, constituting an average 5.7 ± 4.7% of the sum of species, with As(III) and MMAA eliminated at 16.3 ± 3.0% and 17.4 ± 4.4% respectively, while 60.7 ±

10.2% was excreted as DMAA.

The urine samples from occupational group E (the chemical firm, Table 4.12) exhibited the highest urinary concentrations of inorganic arsenic and its metabolites MMAA and DMAA for all the groups analysed, with a maximum concentration of 956.4ug/g creatinine (1291.1ug/l) as the sum of the hydride-forming species for one individual. For three of the samples analysed, the urinary arsenic concentrations could be classified as 'normal'. If these three were omitted from the exposed group then the geometric mean was increased from 169.1ugAs/g creatinine to 244.8ugAs/g creatinine (Table 4.14). As(V) was detected at very high concentrations in many of the samples, reaching a maximum of 314.5ug/l, reflecting the exposure to the pentavalent inorganic form of arsenic (Section 4.4.2). As(III) concentrations were also high, with a maximum level of 252.6ug/l in one of the samples. Despite the high concentrations of the inorganic species, most of the excreted arsenic was in the methylated forms, MMAA at 18.2 $\pm$ 4.9% and DMAA at 63.6 $\pm$ 12.1% with As(V) and As(III) excreted at an average 6.1 $\pm$ 7.4% and 12.1 $\pm$ 5.2% (n = 24), respectively, of the sum. However, it is noted that when there is such excessive exposure to the inorganic forms of arsenic, the concentration of DMAA excreted rarely exceeds 70% of the sum of the species. The high levels of the species excreted, especially of the

inorganic forms of arsenic by this group, is evidence of increased exposure and high absorption of arsenic by those working in this particular occupation.

Table 4.13 shows that it was essentially the glassworkers (F1) and chemical workers (F2) of the last group who excreted the elevated concentrations of the toxic forms of arsenic, while those working in the scrap-metal industry (F3) and in the production of electrical components (F4) were considered to excrete arsenic within the 'normal' range. For the chemical workers in group F2, inorganic As(V) and As(III) were detected in all the samples, with As(V) constituting 14.8% of the sum of species in sample C while only 62.9% was eliminated as DMAA. The urine samples from group F1 exhibited a greater range in arsenic concentrations than those of the glassworkers from group C (Table 4.14), with a geometric mean of 79.4ugAs/g creatinine and 10.0ugAs/g creatinine as the sum of the species for groups F1 and C respectively. As(V), with a mean concentration of 2.8 $\pm$ 2.3ug/l, was detected in many of the samples from group F1 unlike in group C, indicating that there must have been some form of exposure to the pentavalent inorganic form of arsenic. The concentrations of As(III), MMAA and DMAA were higher in F1, with As(III) ranging from 0.8 - 41.8ug/l (compared with 0.6 - 4.0ug/l for group C) and MMAA reaching a maximum of 68ug/l. However, DMAA constituted a similar 69.0 $\pm$ 10.0% of the sum of

species compared with the 69.5 ± 16.5% for group C.

In group F1, samples 1 - 5 were from the same subject handling arsenic trioxide in the glass industry over a five day period and samples 6 - 10 were from the same individual over another period of five days. Average urinary arsenic levels for this individual were elevated at 88.9 ± 9.8ug/g creatinine as the sum of the species compared with 4.4ug/g creatinine for the adults of the control population (Table 4.3). His exposure to the trivalent form of arsenic, as with most of the glass industry workers from group F1, was reflected in the consistent detection of elevated concentrations of As(III), with only small amounts of As(V) detected. It is possible that excretion of As(V) in urine could be the result of oxidation of the airborne As(III) from the chemical used (Yamamura and Yamauchi, 1980) followed by inhalation, absorption and excretion of small amounts of the pentavalent form. The percentages of As(V), As(III), MMAA and DMAA eliminated (3.0/10.9/17.7/68.5%) for this worker were similar to those from the other occupationally exposed groups, namely groups C, D, and E, although for those exposed to As(V), a slightly higher percentage of this species was eliminated in the urine, e.g. for the wood preservative workers (group D) 5.7/16.3/17.4/60.7% and for the chemical workers (group E) 5.8/11.1/16.7/66.4%.

For the eight subjects from the glass manufacturing firm (samples 14 - 21), As(V) was detected in only the two samples of very high sum of species concentrations (i.e. 184.7 and 516.9ug/g creatinine, sample numbers 15 and 18, Table 4.13) constituting 1.2 and 2.3% of the sum. It was noted that for this group of samples there was a greater variation in the concentration and the percentage of each of the species excreted, indicating that, within the same firm, the exposure may vary considerably between individuals.

Sample D, F and H and E, G and I are pre-shift, post-shift and end of working week urine samples from two employees involved in the production of glass containers. For both, there was an increase in the creatinine-corrected levels of arsenic excreted in the post-shift sample and in the end of the week sample when compared with the pre-shift sample and with the control group (Table 4.13). The ingestion of fish two days prior to sampling by individual N, is reflected in the high total arsenic concentration of 347ug/l relative to the sum of species concentration of 154.2ug/l. Samples O, P and Q are from the same person, with sample Q, taken after a five-day working week, exhibiting a greater urinary concentration of the sum of arsenic species (201.6ug/l) than either the pre-shift (153.7ug/l) or back-shift samples (110.4ug/l).

Figure 4.13 shows the range in the sum concentration of the hydride-forming species excreted (ug/g creatinine) by each of the 6 groups and the control group. It can be seen that the concentration range of groups A and B was very similar to that of the control group, while for groups C, D, E and F the range was considerably greater. The concentrations for the sum of the hydride-forming species excreted by workers from the chemical firm (group E) exhibited the greatest range, values for the other occupational groups decreasing in the order E (chemical firm) > F1 (glass industry) > D, F2 (wood preservatives, chemical workers) > C (glass industry) > A, B, F3, F4 (semiconductor industry, electronics department, scrap metal workers, electrical component production).

4.4.4 Exposure evaluation

The speciation of arsenic in urine samples collected from those working with the trace metalloid in a number of different occupations showed that the extent of the exposure varies considerably between industries. For most of the workers in the semiconductor industry and in the university electronics and electrical engineering department, the urinary excretion of As(V), As(III), MMAA and DMAA was within the typical range for the control population, and they could therefore be considered as unexposed. The urinary excretion of arsenic by the workers of

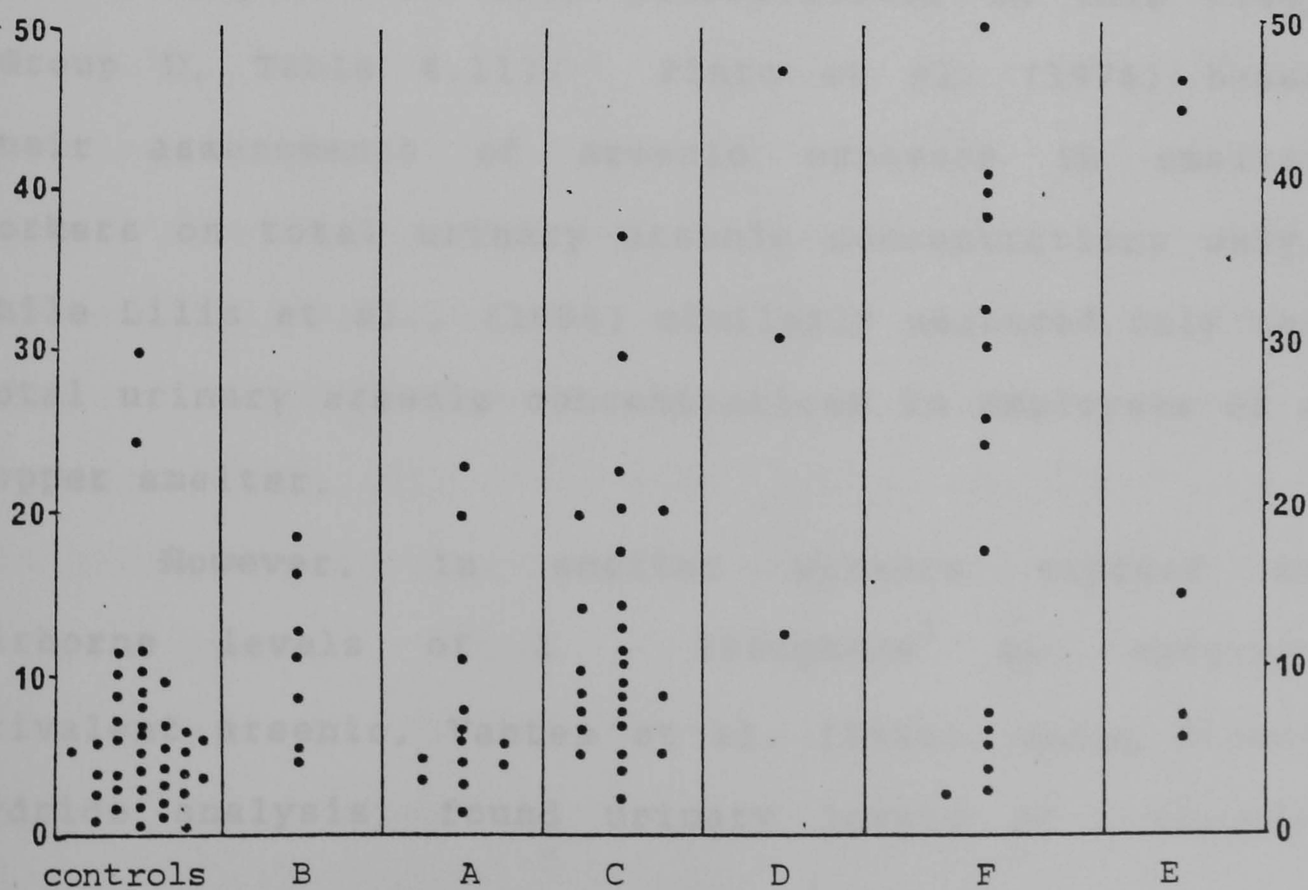


Figure 4.13 Distribution of the sum of arsenic species (ug/g creatinine) excreted by the controls (Adults) and the occupationally exposed groups.

group C (the glass industry) was only slightly elevated, especially when compared with the highly significant enhancements in the arsenic concentrations of urine from those employed at the wood preservative firm (group D), the chemical firm (group E) and groups F1, the glassworkers, and F2, the chemical workers, who submitted samples to the Health and Safety Executive Laboratories.

The literature contains very few reports of the speciation of arsenic in urine from occupationally exposed workers. In timber treatment operators, Gollop and Glass (1979) measured a mean total urinary arsenic concentration of 222ug/l compared with a mean of 27ug/l for a control group, while for workers exposed to arsenic-based wood preservatives, Takahashi et al. (1983) found a mean total urinary arsenic concentration of 103ugAs/l, both greater than for the workers exposed to wood preservatives in this study (Group D, Table 4.11). Pinto et al. (1976) based their assessments of arsenic exposure in smelter workers on total urinary arsenic concentrations only, while Lilis et al., (1984) similarly measured only the total urinary arsenic concentrations in employees of a copper smelter.

However, in smelter workers exposed to airborne levels of 1 - 194ugAs/m^3 as inorganic trivalent arsenic, Vahter et al. (1986), using direct hydride analysis, found urinary levels of inorganic

arsenic and metabolites to range from 16 to 328ugAs/g creatinine, with a geometric mean of 79ugAs/g creatinine. Wojeck et al. (1982), measured a sum of species concentration of up to 263 $\pm$ 101ugAs/l and 120 $\pm$ 24ugAs/l in urine of the 'suppliers' and the 'applicators' involved in the spraying of grapefruit with lead arsenate. Of the arsenic eliminated, the proportions of the species (inorganic arsenic: MMAA:DMAA) were 10:23:67 and 18:20:62 for these two groups respectively. The chemical species of arsenic in the hair, blood and urine of workers exposed to the dust of arsenic trioxide in the production of arsenic acid were studied by Yamamura and Yamauchi (1980). Airborne levels found in two separate areas of the plant were 202ug/m^3 (Plant A) and 334ug/m^3 (Plant B), with 80% as trivalent and 20% as pentavalent arsenic, confirming the use of arsenic trioxide as one of the raw materials. The after-work concentrations of As(V), As(III), MMAA and DMAA (sum of species concentration of 158.8 $\pm$ 57.7ug/l) in urine were higher than in the before-work samples (sum of species concentration of 134.9 $\pm$ 46ug/l). As in the urine samples from employees in the chemical firm (group E) of this study, DMAA excretion rarely exceeded 70% of the sum of species, in marked contrast to the control group sampled here in which DMAA always constituted $>$ 80% of the arsenic excreted. In a study of glassworkers occupationally exposed to

arsenic trioxide, Foa et al. (1984) found airborne levels of 4.5 - 619ug/m^3 for the glass-mix makers, associated with an average urinary sum concentration of inorganic arsenic and methylated metabolites of 81.2ug/l, similar to the 97.7ug/l found in this study for the group of glassworkers F1. Of the sum of species excreted, Foa et al. (1984) reported a mean inorganic arsenic concentration of 17.5ug/l, representing 21.6% of the sum (no differentiation made between As(V) and As(III)) with MMAA constituting 16.9% of the arsenic excreted and DMAA 61.6%. These authors also noted that, for those involved with mixing the glass, there was frequent opportunity for exposure to arsenic through skin contact, which may lead to the absorption of some trivalent inorganic arsenic.

With the elevated concentrations of urinary arsenic indicating enhanced exposure to the toxic inorganic forms of arsenic in occupational groups D, E and F1 and F2, there exists the potential for chronic arsenic poisoning from the continued absorption of the dust of arsenical compounds whether by inhalation, ingestion or through the skin. Such poisoning may manifest itself in a variety of ways, but the symptoms are chiefly related to the skin, mucous membranes, gastrointestinal and nervous systems (HSE, 1976; NAS, 1977; WHO, 1981). Arsenical dust in contact with

exposed or moist areas of skin acts as an irritant causing inflammation and eczematous dermatitis and may also cause conjuctivitis and nasal irritation. In chemical workers making arsenical compounds and in workers in the batch mixing departments of glassworks where arsenic trioxide is used, dusts of arsenic trioxide and arsenite have caused ulceration or perforation of the nasal septum (HSE, 1976). In very chronic cases, the skin may become pigmented with warty growths (hyperkeratosis) which may become malignant years later. High levels of arsenic are often found in the skin because of its richness in keratin, containing sulphydryl groups to which inorganic trivalent arsenic may bind.

A number of studies in man have linked the appearance of lung cancer to exposure to inorganic arsenic compounds. Ott et al. (1974) examined the causes of death in 173 workers exposed to airborne levels of 180 - 19,000ug/m^3 of inorganic arsenic, including trivalent arsenic, in the production of insecticides. Respiratory malignancies accounted for 16.2% of the deaths in the exposed group compared with 5.7% in the rest of the workers. In contrast, Nelson et al. (1973) found that orchard workers with the Dow Chemical company who used lead arsenate had no evidence of increased mortality from cancer, heart disease or vascular lesions. With atmospheric concentrations ranging from 78 - 1034ug/m^3 in a

factory producing sheep dip powder from sodium arsenite (Perry et al., 1948), a significant 31.8% of the deaths were attributable to cancer of the respiratory organs. Pershagen et al., (1977) found increased mortality from lung cancer among workers at a Swedish copper smelter where arsenic-rich ores were processed. More recently, Mabuchi et al., (1979) studied the mortality among workers at a U.S. pesticide plant. The 23 deaths from lung cancer constituted a significant excess, corresponding to a Standard Mortality Ratio (SMR) of 168 compared with a normal SMR of 100. The link between arsenic exposure and lung cancer was further supported by an increase in arsenical keratoses among lung cancer cases when compared with those dying of other causes.

A few of the studies have attempted to quantify possible correlations between the concentrations of urinary arsenic, the levels of airborne arsenic and the risk of contracting lung cancer (Pinto et al., 1976, 1977; WHO, 1981; Vahter et al., 1986). Pinto et al., (1976) found a correlation between urinary arsenic levels and airborne arsenic concentrations and subsequently concluded (Pinto et al., 1977) that a group of workers with more than 25 years exposure to airborne arsenic at concentrations associated with urinary arsenic levels of 50 - 200ug/l, i.e. up to around 40ug/m^3, showed a SMR of 277.8. In a study of airborne arsenic and urinary

arsenic excretion among smelter workers, Vahter et al. (1986) found that urinary concentrations of 50 - 200ugAs/l were equivalent to airborne levels of 1.7 - 53ugAs/m^3. Using their empirically established linear relationship (y=45+2.9x, where y=concentration of inorganic arsenic and metabolites ugAs/l and x=airborne arsenic concentration ugAs/m^3), the average urinary arsenic concentration of 207.8ug/l for the chemical workers (occupational group E) would be equivalent to an average airborne level of 56ug/m^3, above the level concluded by Pinto et al. (1977) to be associated with increased mortality from lung cancer amongst workers. The concentration range of 4.9 - 1291.lugAs/l for this group E would be equivalent to airborne levels of 0 - 430ugAs/m^3, above the United States Threshold Limit Value (TLV) of 200ugAs/m^3 for arsenic in the workroom air (quoted by Zielhuis, 1984). While only six of the urinary arsenic levels in group E correspond to airborne arsenic concentrations exceeding 200ug/m^3, 20 of the 27 analysed exceed the 40ug/m^3 level associated with increased mortality from lung cancer.

Zielhuis and Wibowo (1984) observed, however, that evaluations of the risk of contracting lung cancer using atmospheric/urinary arsenic correlations and extrapolations should be viewed with caution. These usually take little account of the different chemical forms of arsenic in the atmosphere, with

their differing solubilities and retention in the lungs, or of the additional hand-to-mouth transfer (e.g. via smoking) of arsenic that may occur in the working environment. In their study of workers in a smelter environment, Vahter et al. (1986) noted that for four of the workers, the daily urinary excretion of arsenic was over 100% of the inhaled amount, implying that they were indeed exposed to arsenic other than via the inhalation pathway. It should also be noted that the Threshold Limit Value (TLV) of 200ugAs/m^3 quoted by Zeilhuis (1984) is based upon total arsenic concentrations with no differentiation between the different chemical forms. The Health and Safety Executive (1976) recognised the need to set different TLV values for the different chemical forms of arsenic of differing toxicities. While a TLV value of 500ug/m^3 was set for arsenic and arsenic compounds, a limit of 250ug/m^3 was proposed for the handling and use of arsenic trioxide, and an even more stringent limit of 50ug/m^3 set for the <u>production</u> of arsenic trioxide.

Vahter (1983) noted that the respiratory deposition and absorption of arsenic depends on the size of the inhaled arsenic particles as well as on the chemical form of the arsenic. Larger particles are deposited in the upper respiratory tract where they are removed by ciliary action and transported to the gastrointestinal tract. In workers exposed to

inorganic arsenic in a copper smelter, Smith et al. (1977) found a stronger relationship between irrespirable particles (> 5um) and urinary arsenic excretion because it was these particles that were more effectively captured and transported to the gastrointestinal (GI) tract for absorption. Of the arsenic compounds deposited in the lungs, animal studies have indicated that water-soluble compounds such as sodium arsenate and DMAA are rapidly absorbed (Stevens et al., 1977). The long retention of certain arsenic compounds in the lungs is indicated in a report by Wester et al. (1981). Following the inhalation of arsenic, smelter workers, some of whom were retired, had a sevenfold increase of arsenic in the lungs as well as a threefold increase in the liver compared with unexposed controls.

Sections 3.3.4 and 3.4.4 showed that probably more than 80% of inorganic arsenic, in solution, was absorbed from the GI tract. It is expected that, following the transport of inorganic arsenic on dust particles to the GI tract, absorption will depend, at least partially, on the solubility of the particles in the gastric juices. Although arsenic trioxide is only slightly soluble in water, it must be remembered that the pH of the stomach is very acidic and therefore more conducive to the dissolution of the dust.

For the samples received from occupational group E (the chemical firm), the average concentration for the sum of species excreted, 244.8ug/g creatinine, is equivalent to an output of 367.2ug/day (based on an excretion of 1.5g creatinine). If a large proportion of the arsenic on inhaled dust particles is absorbed through the GI tract, then this would be equivalent to an intake (using the 40 - 60% relationship established in Section 3.4.5) of 612 - 918ug inorganic arsenic/day. Employing the model proposed by Bennett (1981) and a working year of 240 days, this corresponds to an intake of 146.9 - 220.3mg/yr and an exposure commitment of 41 - 62ug/kg. In contrast, for occupational group A, with an average urinary arsenic excretion of 5.9ug/g creatinine, an exposure commitment of only 1 - 1.5ug/kg is obtained. It has to be noted, of course, that some of the arsenic excreted in urine by occupationally-exposed workers may have been absorbed via the gastrointestinal tract and some via the much less efficient lung retention and lung to blood transfer mechanisms. It follows that back calculation of inorganic arsenic intake using the output/intake equilibrium ratio of 0.4 - 0.6 established for efficiently absorbed ($\geqslant$ 80%) ingested arsenic, may, underestimate the actual combined intake via the inhalation and ingestion. Nonetheless, it is interesting to note that Vahter et al. (1986), after exclusion of a few anomalous results, found that the

average daily urinary excretion of arsenic by smelter workers was 42% of the inhaled amount derived from direct measurements of airborne inorganic arsenic.

The WHO (1983) recommended a provisional maximum tolerable daily intake of ingested inorganic arsenic of 2ug/kg b.w.. Thus, for a 70kg man, this is equal to a provisional tolerable daily intake of 140ug inorganic arsenic. This corresponds to an output of 56 - 84ug arsenic, based upon an output of 40 - 60% of the intake, Assuming that 1.5g creatinine is excreted daily, this gives a urinary arsenic concentration of 37 - 56ug/g creatinine. Similarly, the daily intake of 150ug inorganic arsenic (equivalent to drinking 1.5 litres of water with an average inorganic arsenic concentration of 0.1mg/l), considered by COT (M.A.F.F., 1984) to give rise to 'presumptive toxicity', corresponds to a urinary arsenic concentration of 40 - 60ug/g creatinine. From Tables 4.11 - 4.13, 22 of the 27 urine samples analysed in group E, 20 of the 27 in group F1, 2 of the 5 group D and 1 of the 3 in F2, exceeded the upper levels of 56 and 60ug/g creatinine. COT also reported that a daily intake of 1500ug inorganic arsenic (equivalent to drinking 1.5 litres of water with an inorganic arsenic concentration of 1mg/l) could produce signs of 'overt chronic arsenicism' in some individuals. This corresponds to a daily output of 600 - 900ug arsenic (based upon 40 - 60% of the

intake) and gives a urinary arsenic concentration of 400 - 600ug/g creatinine, assuming excretion of 1.5g creatinine/day. In occupational group E, five of the samples exceeded a concentration of 600ugAs/g creatinine, with seven of this group and one of group F1 higher than 400ug/g creatinine. The maximum urinary arsenic concentration for group E was 956.4ug/g creatinine for one individual, which is equivalent to a daily output of 1434.6ug and an intake of 2391 - 3587ug inorganic arsenic per day, far in excess of the recommended limits.

The analytical speciation of arsenic in first-void and spot urine samples from workers occupationally exposed to inorganic arsenic enables those individuals at risk from increased exposure to be identified. Using the levels of inorganic arsenic and metabolites excreted and the established empirical relationship between the output and the intake, it is possible to calculate the likely intake of inorganic arsenic by the different occupational groups and individuals. Such levels can then be placed within the context of recommended limits and levels implicated as having a deleterious effect on health. Due to the rapid urinary excretion of absorbed inorganic arsenic and its metabolites MMAA and DMAA (Sections 3.3.4, 3.4.4), it is recommended that analysis of the urine from the same individual over an extended period of time is carried out, or at

least for spot sample analysis to be carried out on a regular routine basis so that any changes in exposure can be detected and evaluated.

4.5 CONCLUSIONS

1) A study of control, environmentally-exposed and occupationally-exposed populations demonstrated that urinary arsenic is a good indicator of human exposure to arsenic from the diet, the general environment and the workplace. As the total urinary arsenic concentration is influenced by the dietary intake of the stable organic form of arsenic in seafood, analytical arsenic speciation to detect inorganic arsenic (As(V), As(III)) and its metabolites (MMAA, DMAA) is essential. Correction of urinary arsenic concentrations for urinary creatinine content is recommended to facilitate comparative assessment of the data.

2) For the general population, urinary arsenic concentrations of the sum of the hydride-forming species (As(V), As(III), MMAA, DMAA) are usually less than 10ug/l, with over 80% in the DMAA form. On the basis of the average sum concentrations of 4.4ug/g creatinine for adults and 8.6ug/g for the children, daily inorganic arsenic intakes in

the U.K. are calculated to be 11 - 16.5ug for adults and 10.8 - 16.1ug for children, comparable to M.A.F.F. estimates derived from dietary studies and well below the FAO/WHO provisional maximum tolerable daily intake of 2ug/kg b.w.. Health risks to the general population are considered to be negligible.

3) In the first direct study of the arsenic status of individuals residing in the mineralised area of Cornwall in the south-west of England, where the arsenic content of soil and dust is highly elevated, average sum of species concentrations of urinary arsenic of 6.1ug/g creatinine for adults and 10.8ug/g creatinine for children only slightly exceeded control values. The influence of environmental arsenic, however, was reflected in the more frequent occurrence of As(III) and MMAA at detectable levels (but at concentrations not exceeding 6ug/l) in urine samples from the Cornwall population. Calculated additions to daily inorganic arsenic intake, as a result of this environmental exposure, amount to only 4.25 - 6.4ug for adults and 2.75 - 4.1ug for children, increments unlikely to contribute significantly to the incidence of diseases (e.g. skin cancer) which have been linked with

high levels of environmental arsenic elsewhere in the world. Nevertheless, high urinary arsenic concentrations (48.7 and 20.8ug/g creatinine) for two pre-school children from Brea suggest that more detailed investigation of the arsenic status of children in the area would be justified.

4) A wide range of urinary arsenic (As(V)+As(III)+MMAA+DMAA) concentrations, up to a maximum of 956ug/g creatinine, was found for workers exposed to inorganic arsenic in industries involved in the manufacture, applications or use of inorganic arsenic compounds. Average concentrations for the major groups studied were 244.8ug/gcreatinine for chemical workers preparing inorganic arsenicals, 79.4ug/gcreatinine for glassworkers, 67.4ug/gcreatinine for wood preservative applicators, 10.0ug/gcreatinine for a group of glassworkers from another firm and 9.7 and 5.9ug/gcreatinine for separate groups of operators engaged in the manufacture of electronic (semi-conductor) components. Urinary speciation patterns for the four most exposed groups were similar, average group proportions falling within the range 1 - 6% for As(V), 11 - 17% for As(III), 15 - 18% for MMAA and 60 - 70% for DMAA. Pentavalent

inorganic arsenic was relatively more prominant for employees of the chemical and timber treatment firms. Despite uncertainty over the relative contributions of the inhalation/lung and inhalation/ingestion/ gastrointestinal tract pathways to the absorption of inorganic arsenic, the highly elevated urinary arsenic concentrations ($>$ 60ug/g creatinine) found for the chemical workers, in particular, and some of the glassworkers and timber treatment personnel reflect uptake corresponding to daily intakes in excess of the FAO/WHO provisional maximum tolerable intake of 2ug ingested inorganic arsenic per kg body weight and the 150ug considered by COT to give rise to presumptive toxicity. Indeed, for a few chemical workers, calculated intake of inorganic arsenic actually exceeds 1500ug per day, a level COT associates with overt chronic arsenicism. For the chemical workers, and, to a lesser extent, the glassworkers (F1), some of the workplace atmospheric arsenic concentrations, derived from recently published relationships between atmospheric and urinary arsenic for smelter workers appear to exceed recommended and statutory limits as well as levels implicated elsewhere with an

increased incidence of lung cancer. It is recommended, therefore, that further research into occupational exposure to inorganic arsenic in these industries should incorporate, in addition to the analytical speciation of urinary arsenic, direct measurement of atmospheric arsenic and close scrutiny of all potential exposure routes and intakes/uptake pathways arising from the workplace conditions and practices peculiar to each particular firm or industry.

CHAPTER 5

ARSENIC IN THE SEDIMENTS OF FRESHWATER LOCH LOMOND, THE DUBH LOCHAN AND THE NEIGHBOURING SEALOCHS OF THE CLYDE SEA AREA

5.1 INTRODUCTION

The freshwater bodies of Loch Lomond, the Dubh Lochan and neighbouring sealochs of the Clyde Sea Area are situated on the west coast of Scotland, approximately 30 - 80km from Glasgow (Figure 5.1). In the late eighteenth century and early nineteenth century, Glasgow became the centre of a large industrial complex which caused considerable pollution of the surrounding atmosphere, rivers, estuary and sealochs (Farmer, 1983). With the present day population at around 2.5 million (1.7 million in the Clydeside conurbation) there is a significant influx of domestic sewage and industrial discharges into the Clyde estuary and the sealochs via the River Clyde and the River Leven. However, because of their geographical location, Loch Lomond and the neighbouring Dubh Lochan have not been subject to the same pollutant inputs. They are both isolated from the sealochs and are surrounded by much uninhabited mountainous terrain in the north and lightly populated farmland in the south. The small scale local iron

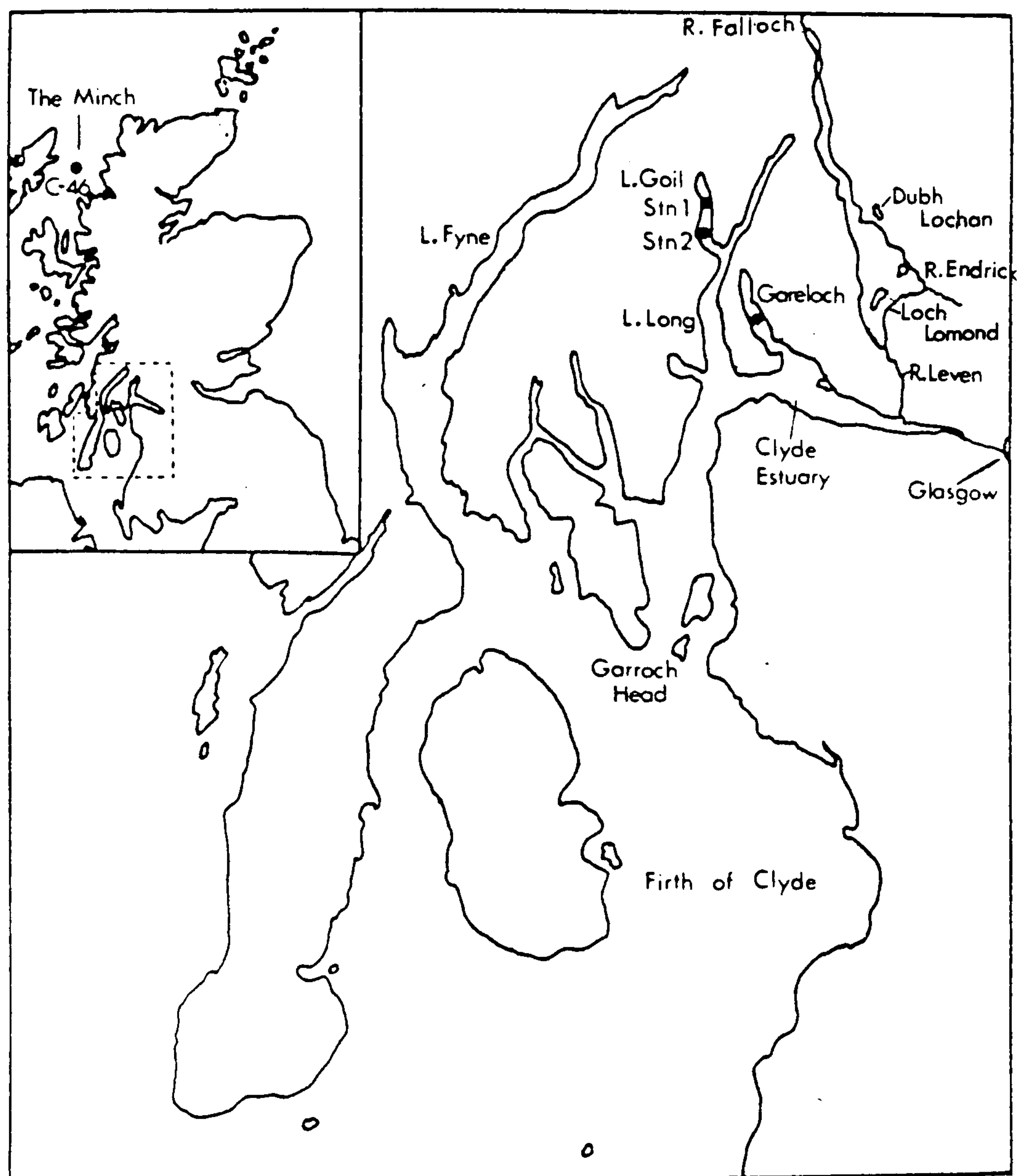


Figure 5.1 Location of freshwater Loch Lomond, the Dubh Lochan and the neighbouring sealochs of the Clyde Sea Area and the Minch off the west coast of Scotland. (modified from Farmer, 1983)

smelters near Rowardennan and tanneries of the seventeeth century situated in the vicinity of Loch Lomond are no longer in existence. In the mineralised area of Tyndrum to the north, the mining of lead and zinc ores, which may have introduced dissolved metals in Loch Lomond via the River Falloch in the past, has long since ceased.

5.2 GEOLOGICAL AND ENVIRONMENTAL SETTINGS OF LOCH LOMOND, DUBH LOCHAN AND THE SEALOCHS OF THE CLYDE SEA AREA

The geology of the west coast of Scotland is dominated by a major fault line, 'The Highland Boundary Fault', which separates Scotland into the Highlands, characterised by metamorphosed Dalradian mica schists to the north and, the Lowlands, dominated by softer sedimentary rocks of the Carboniferous and Devonian to the south (Slack, 1957). As a result, the narrow glacial valley of Loch Lomond, running north/south across this fault line, is characteristically divided into a northerly steep-sided upper basin set in the Trossach mountain range and a southerly more shallow and wider lower basin, surrounded by undulating farmland. The Dubh Lochan, situated on the northern side of the fault line, is underlain by metamorphosed slates, grits and schists (Anderson, 1947) and is surrounded by mountains on three sides. While Loch Lomond and the

Dubh Lochan now exist as isolated freshwater lochs, Loch Goil to the west remains as a steep-sided fjordic sealoch running north into the highlands. In contrast, the Minch, 275km to the north-west of the Clyde Estuary, is a deep sea channel between the north-west coast of Scotland and the Outer Hebrides (Figure 5.1).

5.2.1 Loch Lomond

Loch Lomond is the largest freshwater body in Great Britain with a surface area of $71km^2$, a length of 36.4km and a maximum width of 8km. The northern upper loch is steep sided with a maximum depth of 200m, in marked contrast to the broad southern, lower loch where the maximum depth is only 31m. The main inflow is from the north via the River Falloch which, because it drains the nutrient-deficient peaty moorland of the surrounding catchment area, contributes to the acidity and oligotrophic nature of the upper loch. In contrast, the southern basin is considerably more biologically productive, with the River Endrick draining the productive arable farmland on beds of boulder clay and glacial and marine deposits (Lovell, 1985) although the concentrations of the major nutrients are still low for most of the year (e.g. phosphate 0.003 - 0.012mg/l, silicate 0.37 - 1.15mg/l) (Chapman, 1965). These two basins are separated by a central basin of intermediate

characteristics. The main outflow from Loch Lomond is via the River Leven in the southern basin.

Due to a rapid overturn of its shallow water, a stable thermocline in the southern basin is developed only from June to August. In contrast, the more stable nature of the deep north basin allows a thermocline to remain from May to January. However, despite this and the fact that the deep waters remain cold all year round, the dissolved oxygen levels remain high (i.e. greater than 72% saturation) throughout the loch (Maulood and Boney, 1980).

While the sediments of the north are black micaceous oozes overlain by thin, dark-brown unconsolidated material, those of the south are brown, with a thin near-surface rust-red layer. The organic matter content is higher in the north, with its significant allochthonous input, than in the south, where the more autochthonous planktonic debris prevails (Slack, 1954; Lovell, 1985). Previous work conducted on Loch Lomond sediments has demonstrated considerable enhancements of manganese and iron in the uppermost few centimetres of the sediments, manganese levels reaching 9.1% in the 1 - 2cm section at the deepest sites, relative to background levels of 0.15 - 0.5%. Similarly, arsenic levels were found to be elevated in the near-surface sediments with concentration peaks coinciding with the maximum iron

levels, while the manganese peaks were displaced approximately 1cm above the arsenic/iron peak (Figure 1.4) (Farmer and Cross, 1979, Lovell, 1985). The sedimentary concentration profile for arsenic was also found to be similar to that of the diagenetically mobile phosphorus, a chemical analogue of arsenic (Lovell, 1985; Farmer and Lovell, 1986). The development of such profiles for iron and manganese has been attributed to post-depositional diagenetic remobilisation processes, with upward migration of the soluble divalent cations from reduced zones via the porewaters, culminating in subsequent reoxidation in the aerobic surface layers, yielding the observed enrichment profiles (Section 1.4.2). From the sedimentary data previously gathered, it has been deduced that a similar mobilisation process governs the vertical distribution of arsenic in Loch Lomond sediments. This has been partly confirmed by an upward declining trend in total arsenic porewater concentrations followed by a sudden depletion at the same depth as a peak in the sedimentary arsenic concentration in the near surface sediments, reflecting oxidation and removal to the solid phase via precipitation and/or adsorption (Lovell, 1985).

5.2.2 Dubh Lochan

The Dubh Lochan is situated approximately 10km north of the Highland Boundary Fault on the east shore of Loch Lomond adjacent to the central basin. With a length of 550m and a width of 128m, it is considerably smaller than the main loch, with a surface area of only $7.06 \times 10^4 m^2$ & a maximum depth of only 11.1m, (average depth of 4.8m). As with Loch Lomond, the Dubh Lochan is considered to be nutrient-poor (e.g. phosphorus 0.001 - 0.02mg/l, nitrate 0.02 - 0.1mg/l and sulphate 0.1 - 0.3mg/l). With a small catchment area of forested mountainous land and much vegetation surrounding the water's edge, the sediments have a high organic content with a significant quantity of undegraded vegetation settling on the surface of the sediment.

Thermal stratification commences early (April) and breaks up around late September/early October when the surface water temperature declines to less than 11^oC (Klarer, 1978). There is occasional ice formation on the surface in the winter. Unlike the main loch, the Dubh Lochan can be divided into two separate areas according to dissolved oxygen concentrations. The greater demand for oxygen in the more biologically active summer months can often cause oxygen levels below the metalimnion to decline to less than 5% saturation and sometimes to completely anoxic conditions in the deeper waters in some parts of the

lochan. Under such conditions, Klarer (1978) found that there was an increase in iron and manganese concentrations in the hypolimnion, possibly as a result of gradual release from the sediments. Iron concentrations were found to increase from 1ug/l in surface waters to 625ug/l at a depth of 9m and, similarly, manganese concentrations increased from 192 to 450ug/l at 9m. In view of known temporal changes in redox potential at the sediment/water interface at one location, while the rest of the lochan remains oxic throughout the year, there exists the opportunity to examine how changes at the sediment/water interface may modify the sedimentary profiles of arsenic, iron and manganese.

5.2.3 Sealochs

The sealoch, Loch Goil (Figure 5.1), is 8km long, with a characteristic glacial steep sided and flat-bottomed profile (average width only 1.2km) and a shallow protective sill at the entrance. The major freshwater inputs are the River Goil and the Lettermay Burn. Loch Goil, however, also receives a heavy input of suspended particulates as a result of dredging and dumping operations in the Clyde estuary. The sediments of the deeper waters are dominated by silty clays, in which the discovery of ferromanganese concretions and nodules suggested the presence of diagenetic mobilisation processes (Calvert and Price,

1970). This was later confirmed by Farmer (1983) who found up to 3% manganese in the top few centimetres of sediment cores compared with background concentrations of 0.5 - 1.0% at a depth of 5 - 15 cm. Unlike the narrow manganese sediment peak, the vertical extent of the lead, zinc, chromium and copper enrichment profiles and the comparatively insoluble nature of their sulphides under reducing conditions, strongly suggests that the enhanced levels of these trace metals are due primarily to contamination, presumably originating from the heavily populated and industrialised region centred on Glasgow.

In contrast, the Minch, situated in the remote Atlantic sea channel between the Outer Hebrides island group and the mainland of north-west Scotland, exhibits only a small enhancement in the levels of the pollutant trace metals lead and zinc. While copper could not be detected and the chromium concentration remained constant, manganese was only slightly elevated with surface sediment concentrations of 0.018% compared with a mean of 0.013 $\pm$ 0.007% below 1cm (Farmer, 1983).

5.3 SAMPLING STRATEGY

5.3.1 Loch Lomond

To fully understand the processes controlling the mobilisation and distribution of arsenic in the sediments of Loch Lomond and to determine whether

biomethylation is an important process, it is necessary that the different chemical forms of arsenic in the associated porewaters are determined via analytical speciation. In addition, measurement of the nutrient levels, e.g. sulphate, could be used as an indicator of the change in redox potential with depth. Thus, one good quality core from the southern basin of Loch Lomond was taken, from which the porewaters were abstracted at 1cm intervals for arsenic speciation and sulphate determination (Section 5.4.2). The concentrations of arsenic, iron and manganese were also determined in the sediments from which the porewaters had been abstracted, enabling the sediment and porewater results to be directly related. To evaluate what were the arsenic concentrations of the settling material in the loch, water samples, plankton and seston samples were also collected from the southern basin for arsenic analysis (Sections 5.4.3, 5.4.4).

5.3.2 Dubh Lochan

As there is no previous information on the concentrations of arsenic, iron or manganese in the sediments of the Dubh Lochan and, with the possibility of comparing two sites of differing stratification patterns (i.e. one remaining oxic all year, the other exhibiting seasonally anoxic conditions), it was decided that sediment cores be taken from both sites

at regular intervals, during the period of likely change from anoxic to oxic conditions in the hypolomnion at the deeper site. As with Loch Lomond, sediment sections were analysed for concentrations of arsenic, iron and manganese and the porewaters abstracted for arsenic speciation and sulphate determination. At the same time as the sediment cores were taken, the temperature and oxygen saturation profiles of the overlying water column were measured. Water samples, plankton and seston samples were also collected at intervals.

5.3.3 Sealochs

The sealoch sediments collected in 1976 - 1978 had previously been analysed for the concentrations of a number of trace metals, including iron and manganese. It was decided that the same sediment sections be redigested and reanalysed for the arsenic, iron and manganese concentrations, to establish whether arsenic enrichment profiles similar to those in Loch Lomond sediments also exist in the neighbouring coastal marine environment or whether such features are unique to that particular freshwater system.

5.4 SAMPLE COLLECTION

5.4.1 Sediment cores

Sediment cores were taken from Loch Lomond and the Dubh Lochan using a Jenkin surface-mud sampler (Figure 5.2). The sampler has been specifically designed to take the sediment core with minimal disturbance of the sediment at the important sediment/water interface. As previous sedimentary data from Loch Lomond have shown arsenic, iron and manganese to be concentrated in the near-surface sediment sections, it is vital that this part of the core remain intact. The sampler is lowered to the loch bed where it settles into the sediment. The sample is collected in a 4.8cm diameter perspex tube (A) upon closure of the two spring loaded rubber sealed lids (B). A 15 - 20cm depth sediment core is thus taken, together with the water immediately overlying it, in a relatively undisturbed state. If disturbance of the sample is suspected, (e.g. cloudiness in the overlying water) the sample must be discarded.

A single sediment core was taken from the southern basin of Loch Lomond on 09/12/85 just north east of Inchmurrin (Figure 5.3).

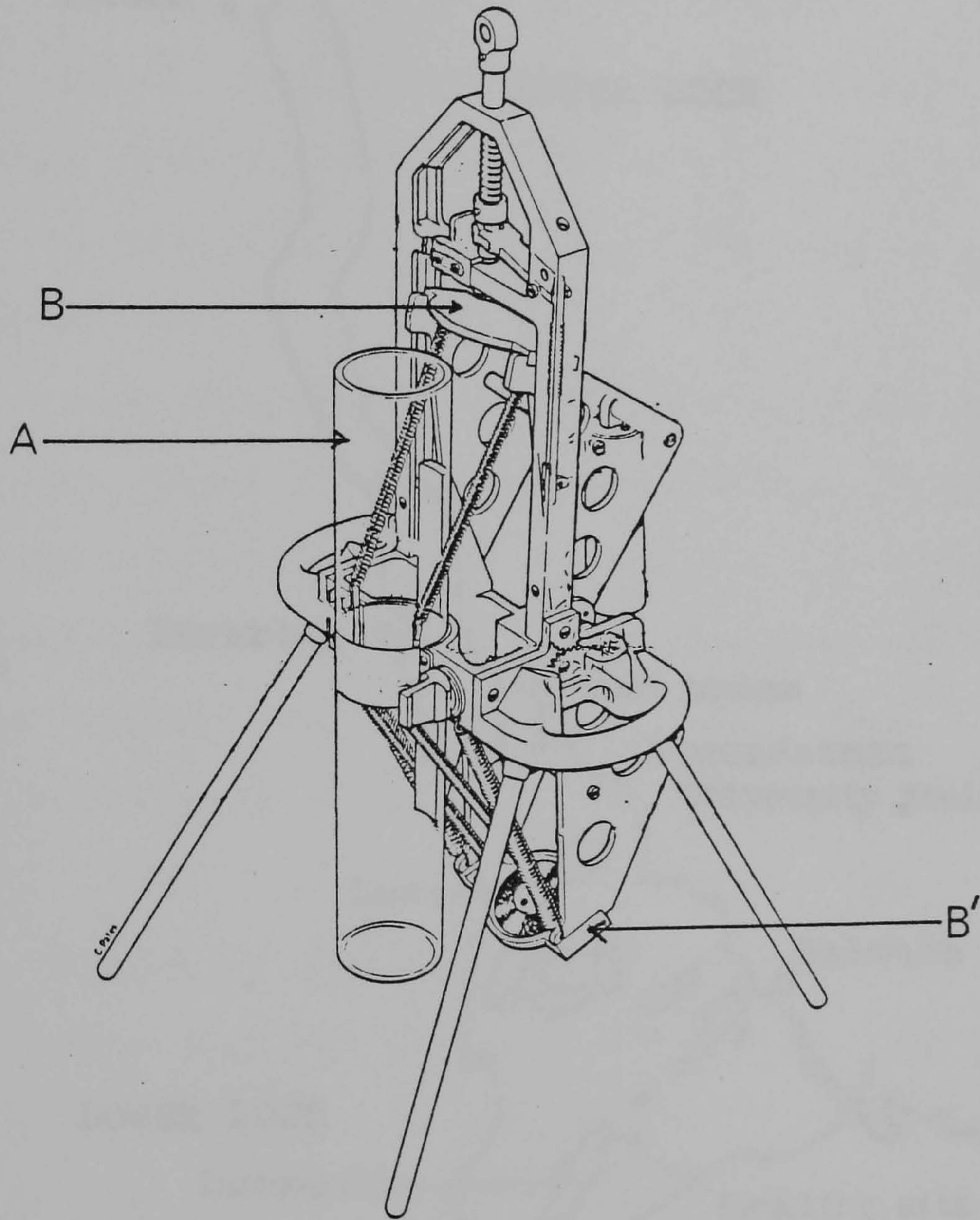


Figure 5.2 The Jenkin surface-mud sampler (Modified from Ohnstad and Jones, 1982)

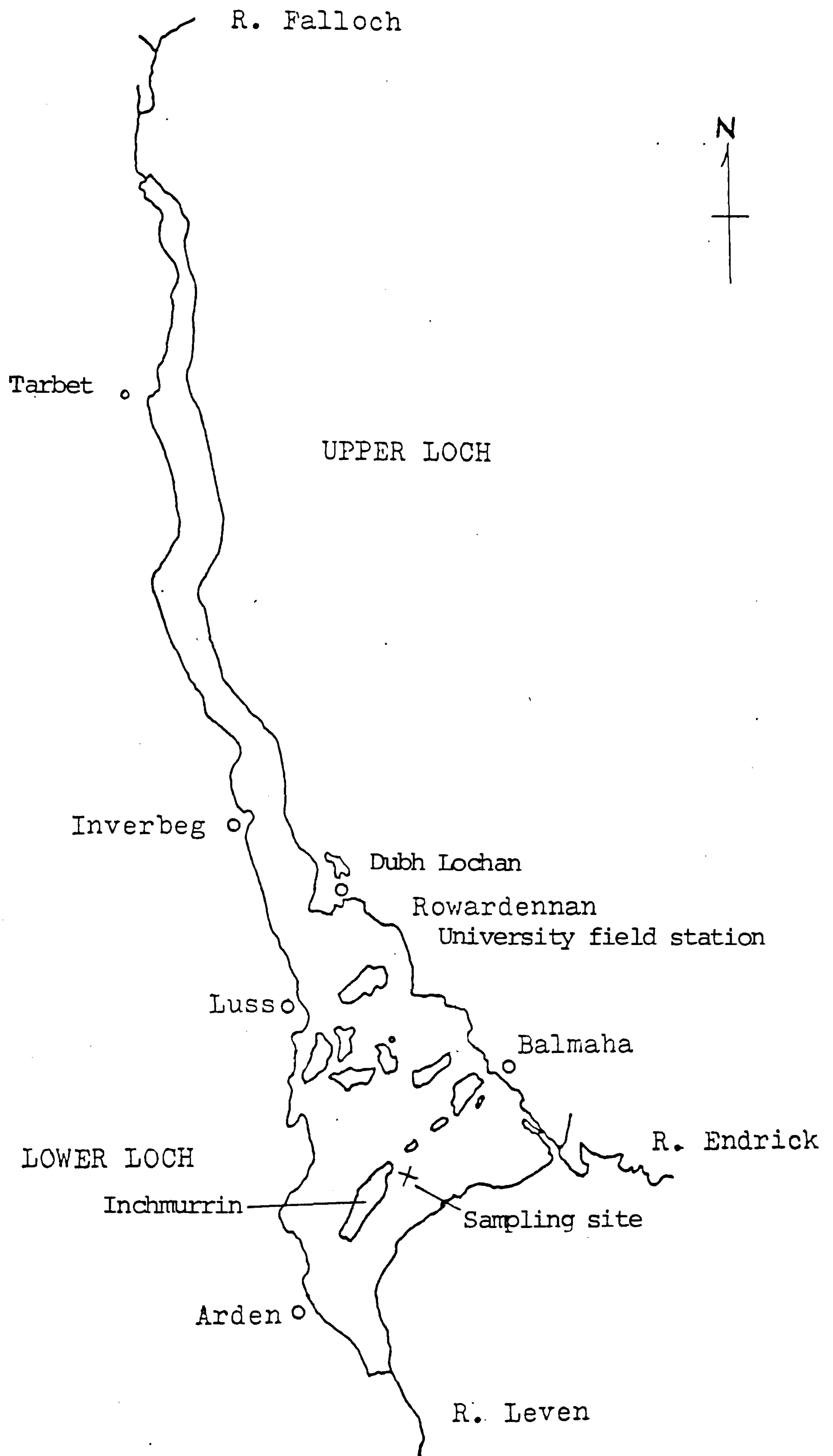


Figure 5.3 Location of sampling site on Loch Lomond. (modified from Lovell, 1985)

Core samples were taken from two sites in the Dubh Lochan: (Figure 5.4)

Station A with a maximium water depth of 11m, at which the sediment-water interface frequently becomes anoxic for 1 - 2 months during the summer.

Station B with a maximum water depth of 5m and which remains oxic throughout the year.

Cores were taken at approximately two-week intervals from 22/08/85 to 02/10/85 (Table 5.1) in an effort to sample during the possible anoxic period at the deeper site.

Once collected, the cores were returned to the University Field Station at Rowardennan (Figure 5.3) where the 'bottom water' was immediately syphoned off to prevent mixing with the sediment surface and seepage through the core. At all times, care was taken to maintain the core in an upright position.

5.4.2 Porewater abstraction

Once the bottom water had been syphoned off, sediment porewaters were collected using the method described by Lovell (1985), as adapted from that of Davison et al. (1982). A 30ml Plastipak syringe fitted with a specially designed 'wide bore' stainless steel needle, was inserted through the 4mm diameter holes drilled at 1cm intervals through the perspex tubing (sealed with tape before sampling). The

TABLE 5.1

DATES OF SEDIMENT SAMPLING AND LENGTHS OF THE CORES COLLECTED

TIME	CORE LENGTH (cm)	
	STATION A	STATION B
DUBH LOCHAN		
1) 22/08/85	15	12
2) 17/09/85	17	14
3) 02/10/85	17	20
LOCH LOMOND		
09/12/85	15	

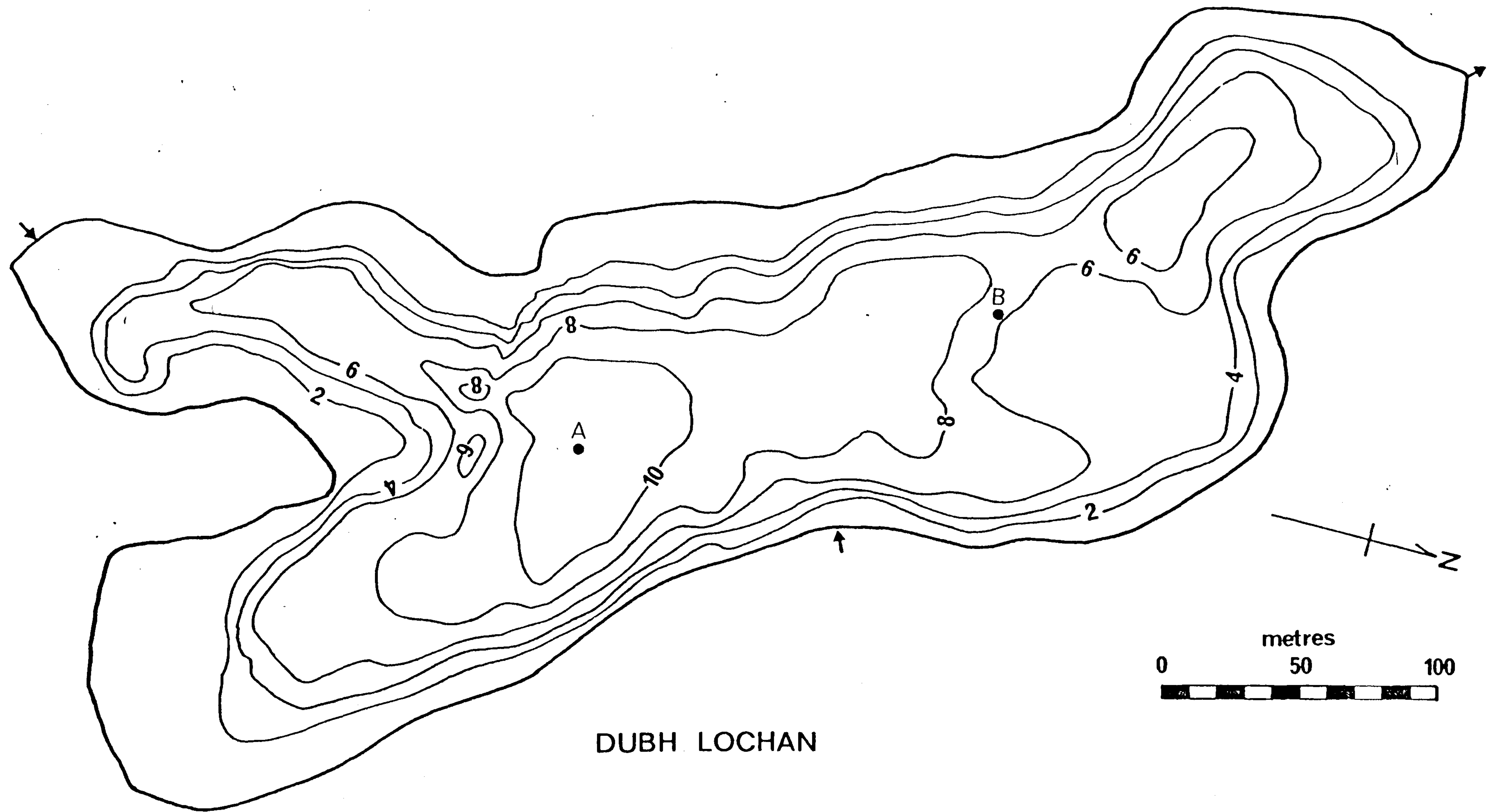


Figure 5.4 Location of sampling sites A and B in the Dubh Lochan (Klarer, 1978)

sediments and their associated porewaters were then sucked into the syringe and transferred into an enclosed 450nm Millipore (MHW1 037 AO) air filter. The porewaters were sucked through the filter into a second syringe and then injected into nitrogen-filled self sealing vials and stored at $4^{o}C$. Porewaters were abstracted in this manner from the top to the base of the core to prevent seepage from the upper sections into the lower layers. It is noted that in an effort to maintain the reducing conditions of the sediments at depth, all processing of the material had to be carried out as quickly as possible using air filters purged with argon, nitrogen-filled vials for sample storage and separate sterile syringes for each of the samples. Using this method, between 2 and 4ml of the porewater could be collected.

Following porewater abstraction, the cores were sectioned at 1cm intervals and later dried at $30^{o}C$, ground and homogenised for subsequent arsenic, iron and manganese determination.

The sediment collected on the filter paper was kept and similarly dried and ground for analysis.

5.4.3 Loch water

On a number of occasions, loch water was collected, at depths of 1m, 3m, 5m and, where possible, 10m and 30m from both Loch Lomond and the Dubh Lochan using a Van-Dorn water sampler. For each

6-litre sample collected, a 60ml aliquot was taken and stored at $4^{o}C$ in an acid-washed polypropylene container.

5.4.4 Biological material

Plankton were collected frequently throughout the period of sediment coring, by trawling a 0.1mm mesh net very slowly to sample at approximately 1m depth below the water surface (i.e. in the photic zone).

A number of weed samples (Labillia, myriophalum and Patamageton natans), rooted in the sediment surface of the southern basin were also collected. These were thoroughly washed in deionised water and separated into roots, shoots, and leaves.

Organic and inorganic particulate material settling through the water column was collected as seston, in 2 x one litre plastic measuring cylinders, (two anchored 1m above the sediment surface and two 1m below the water surface) from both Loch Lomond and the Dubh Lochan. At intervals of approximately one month, the cylinders were retrieved, the contents collected in acid-washed one-litre containers and the seston subsequently filtered out.

To determine whether arsenic is incorporated into the food chain, bottom-feeding Powan were also collected by those working at the Field Station. The fish were dissected into muscle, stomach and liver

tissue.

All the above biological material collected was frozen, freeze-dried and then ground and homogenised before being processed for subsequent analysis.

5.5 SAMPLE ANALYSIS

5.5.1 Sediment

The total arsenic, iron and manganese contents of the samples were determined in the filtered and sectioned sediment from Loch Lomond and the Dubh Lochan. In addition, arsenic, iron and manganese were determined in the samples previously collected from the sealochs.

All samples were digested by dry ashing with magnesium nitrate and then taken up in concentrated HCl as outlined in Section 2.4.3. For total arsenic analysis, aliquots of the digest solutions were pre-reduced with acidified potassium iodide (Section 2.4.1.2) and analysed by hydride generation/atomic absorption spectrometry (HGAAS) (Section 2.2.3). Iron and manganese concentrations were determined using conventional flame AAS on the solutions resulting from sample digestion.

5.5.2 Porewater and Loch water

For the porewaters abstracted from Loch Lomond sediments, arsenic speciation was carried out on a 2ml sample aliquot by ion exchange chromatography (Section 2.5.3) before HGAAS determination of arsenic in each of the 3ml eluant fractions collected.

For the Dubh Lochan porewaters, As(V)/As(III) speciation was carried out using pH control (Section 2.5.2)

Total arsenic concentration of the loch water was obtained by direct hydride analysis (Section 2.2.3) using a maximum 50ml sample aliquot.

5.5.3 Biological material

Total arsenic concentrations were determined on the freeze-dried plankton, fish tissue and seston by HGAAS following acid digestion with nitric:sulphuric:perchloric acids (Section 2.4.1)

Subsamples of the plankton material were speciated for inorganic/organic arsenic using distillation with concentrated HCl (Section 2.5.1).

5.6 LOCH LOMOND

5.6.1 Results

Table 5.2 lists the total arsenic concentration (mg/kg) with depth in the 1cm sections of the Loch Lomond sediment core and the arsenic speciation data (ug/l) for the associated porewaters

TABLE 5.2

SEDIMENT ARSENIC, IRON AND MANGANESE AND ASSOCIATED POREWATER ARSENIC AND SULPHATE CONCENTRATIONS IN THE SOUTHERN BASIN OF LOCH LOMOND (09/12/85)

	SEDIMENT			POREWATER					
Section (cm)	As (mg/kg)	Fe percent	Mn percent	As(V) (ug/l)	As(III) (ug/l)	As(V) relative amounts (%)	As(III) relative amounts (%)	As(III)/As(V) ratio	SO_4^{2-} (mg/l)
0-1	38	3.79	0.59	0.8	*	100	0	0	9.1
1-2	74	4.30	1.46	1.5	5.1	23	77	3.4	5.6
2-3	283	6.33	1.40	3.6	22.2	14	86	6.2	3.3
3-4	179	5.45	0.41	14.0	53.8	21	79	3.8	3.8
4-5	103	4.76	0.31	17.4	70.6	20	80	4.1	5.3
5-6	55	3.89	0.21	19.2	81.5	19	81	4.2	3.6
6-7	46	3.52	0.20	11.2	64.9	15	85	5.8	5.6
7-8	55	4.02	0.24	19.4	83.2	19	81	4.3	5.7
8-9	48	4.12	0.24	13.6	75.9	15	85	5.6	4.4
9-10	42	3.42	0.19	19.4	77.9	20	80	4.0	2.0
10-11	41	3.72	0.20	12.4	91.0	12	88	7.3	-
11-12	50	3.87	0.23	-	-	-	-	-	-
12-13	36	4.00	0.18	9.1	65.0	12	88	7.1	3.7
13-14	41	4.18	0.17	4.1	35.0	11	89	8.5	3.9
14-15	34	4.55	0.17	*	30.6	0	100	0	-

* less than the detection limit of 1ug/l

\- no data collected

at each centimetre interval. The table also includes results for the iron and manganese concentrations (as percent) for each sediment section and the sulphate concentration (mg/l) of the porewaters. Results for the arsenic, iron and manganese sediment concentrations are shown in Figure 5.5, while Figure 5.6 shows the sediment arsenic profile alongside the porewater arsenic speciation data and sulphate concentrations.

5.6.2 Discussion

The profiles in Figure 5.5 clearly show sub-surface maxima in the concentrations of arsenic, iron and manganese in Loch Lomond sediments overlying more uniform concentrations at depth, enhancements in line with those found by Farmer and Cross (1979) and Lovell (1985). The sub-surface nature of the arsenic peak, the low concentration of arsenic in the loch water ($\leqslant$ 0.0001mg/l) and in the plankton (2.5mg/kg) and the arsenic contents for seston of 8.4 $\pm$ 1.6mg/kg (1m below the water surface) and 28.6 $\pm$ 11.2mg/kg (1m above the sediment surface), strongly suggests that the near surface arsenic peaks cannot merely be the result of input from the overlying water column. Manganese reached a maximum concentration of 1.46% in the 1 - 2cm section in contrast to a background concentration of 0.21% while iron and arsenic concentrations peaked slightly lower down the core,

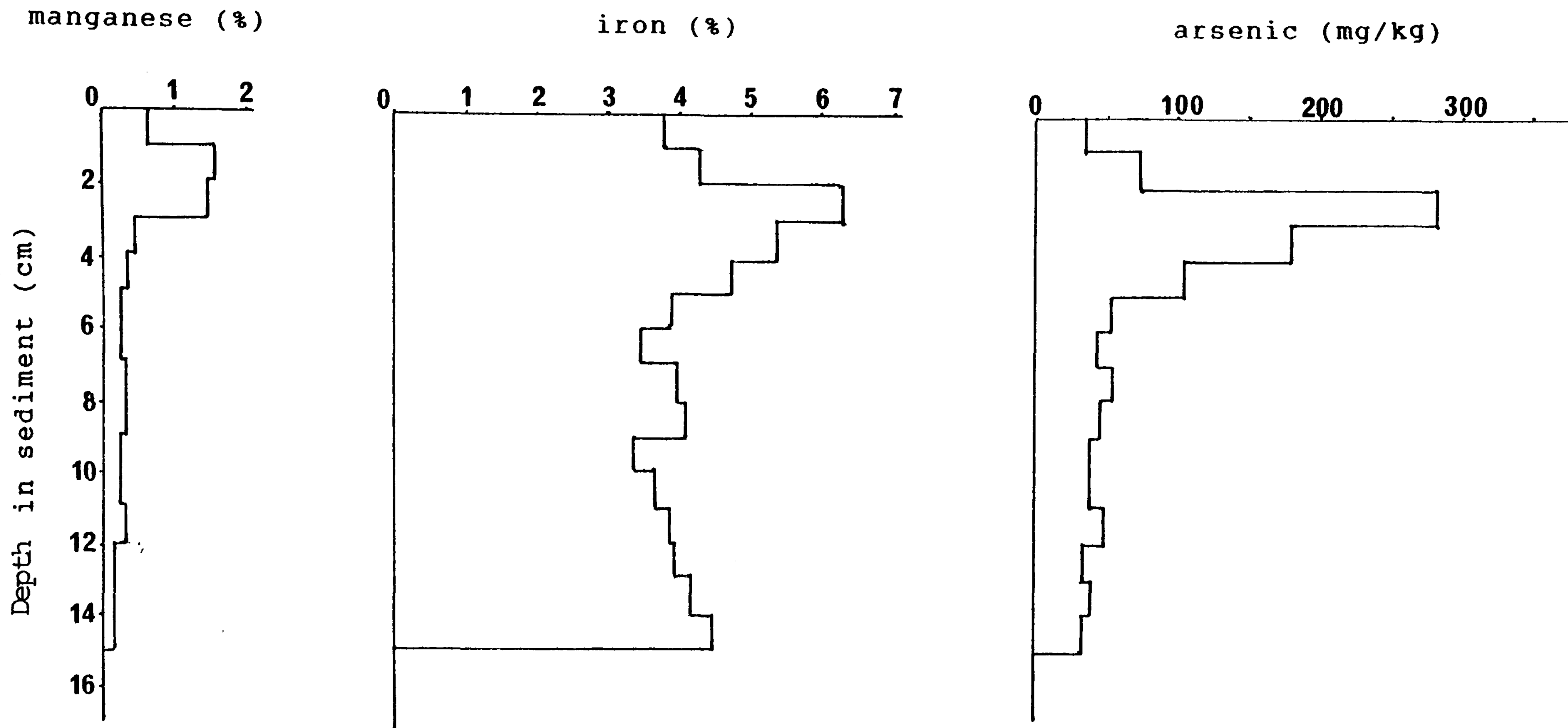


Figure 5.5 Profiles of manganese, iron (percent) and total arsenic concentration (mg/kg) in the sediment core taken from the south basin of Loch Lomond.

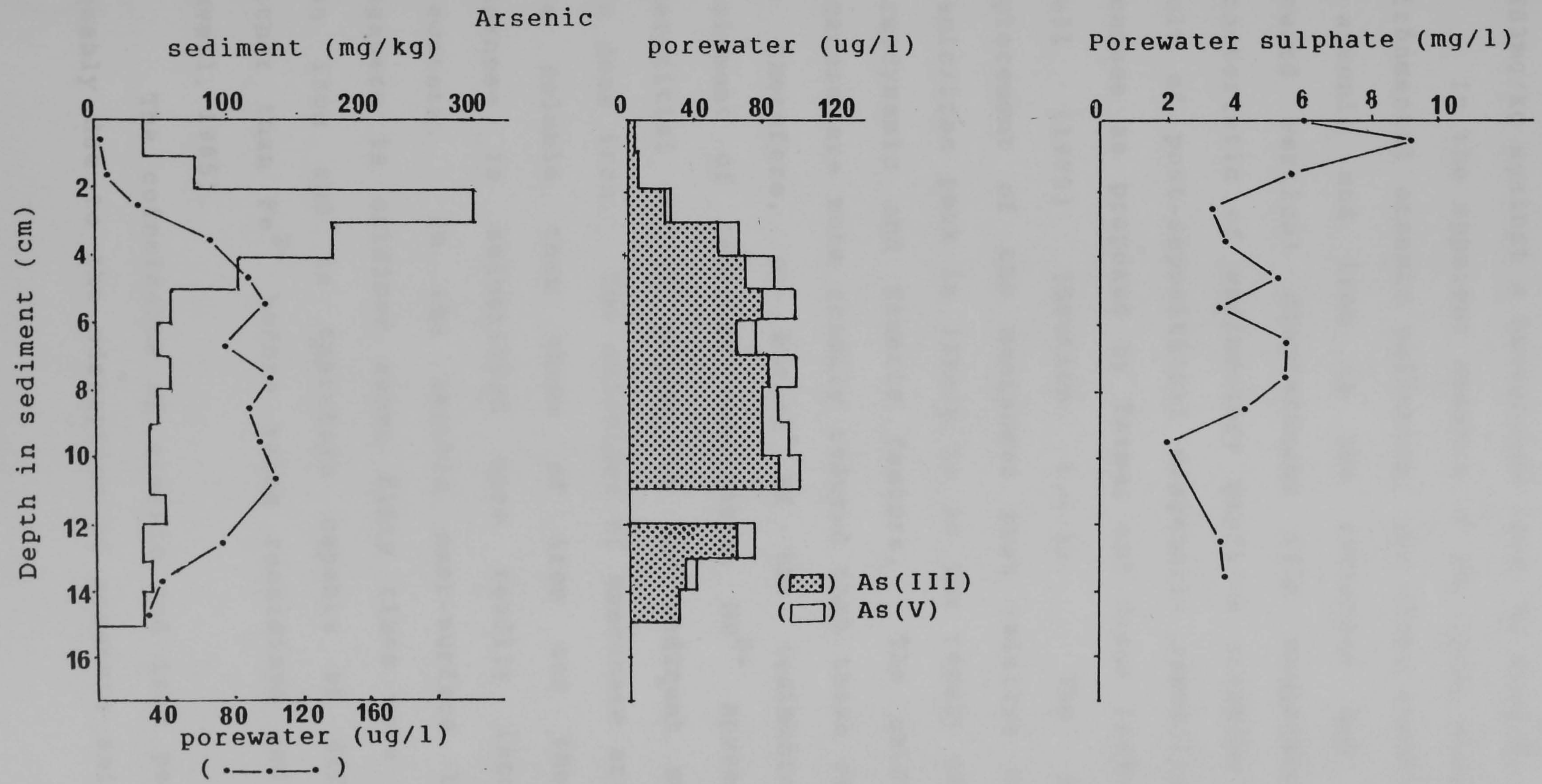


Figure 5.6 Sediment/porewater total arsenic profile, porewater speciation data and sulphate concentrations for the Loch Lomond sediment core.

coinciding at 2 - 3cm with an iron maximum of 6.33% over a background level of 3.93% and an arsenic peak of 283mg/kg against a background level of 45mg/kg.

In the apparent absence of any local source of environmental arsenic pollution, the close association of arsenic and iron in the sediments and their downward vertical displacement from manganese are characteristic of sedimentary profiles produced as a result of post-depositional diagenetic remobilisation processes as proposed by Farmer and Cross (1979) and Lovell (1985) (Section 5.2.1). The upward displacement of the manganese peak relative to the arsenic/iron peak is likely to be the result of both thermodynamic and kinetic factors. The oxides of manganese are more readily reduced than those of iron and therefore, on burial of the sediments and attainment of reducing conditions, Mn^{2+} appears in interstitial waters nearer to the sediment surface than does iron. The sulphides of manganese are also more soluble than those of iron and therefore manganese is solubilised more readily into the porewaters. In the aerobic near-surface layers, manganese is oxidised about fifty times more slowly than iron and is therefore capable of diffusing further than Fe^{2+} before being reoxidised and fixed (Lovell, 1985).

The coincidence of arsenic and iron peaks is probably due to the adsorption of arsenate anions on

amorphous ferric oxides and hydroxides in the oxygenated environment of the uppermost sediment layers and is thought to be an important mechanism for arsenic fixation and retention (Kanomari, 1965; Ferguson and Gavis, 1972: Crecelius, 1975: Price, 1976). In the sediments of Lake Washington, Crecelius (1975) found that the mean maximum (0 - 1 cm) arsenic concentration of 95mg/kg coincided with a mean elevated iron concentration of 4.8% and similarly, Aggett and O'Brien (1985) found that arsenic peaks of 6000mg/kg occurred in the same surficial sections as elevated iron levels of 13% in the sediments of Lake Ohakuri, New Zealand, one of eight hydroelectric lakes supplied by the Waikato River from a geothermally active area. Lovell (1985) found that arsenic/iron peaks coincided in 5 of the 6 cores from the southern basin of Loch Lomond, a phenomenon which was further substantiated by selective chemical extraction procedures. With the burial of the surface sediments, the prevailing pH and redox potential of the sediments at depth gives rise to the reduction of solid phase FeIII to soluble FeII. The solubilisation of oxides and hydroxides of iron would therefore release the adsorbed arsenate, which would then perhaps be reduced to arsenite and migrate upwards through the porewaters, to be reoxidised/ readsorbed/reprecipitated with the iron in the aerobic environment of the near surface sediments. Thus, the

sedimentary data collected here are in accord with those found in the previous studies on Loch Lomond and, therefore, are consistent with the theory advanced in explanation (Lovell, 1985; Farmer and Lovell, 1986).

Direct evidence for arsenic reduction/ solubilisation/mobilisation in the sediment core is provided by the porewater data (Table 5.2, Figure 5.6). Supporting evidence for the decrease in the redox potential (necessary for the solubilisation of arsenic, iron and manganese from the sediments into the surrounding porewaters) is shown by the slight decrease in the porewater sulphate concentrations with depth. Below a depth of a few centimetres, the depletion of the dissolved oxygen levels for the microbiological degradation of the organic matter, leads to the production of anoxic or sub-oxic conditions. Other inorganic oxidising agents (namely the electron acceptors, NO_3^-, MnO_2, $Fe(OH)_3$ and SO_4^{2-}) are then used to aid the oxidation of organic matter in the thermodynamically-favoured sequence, nitrate $\longrightarrow$ manganese-oxides $\longrightarrow$ iron-hydroxides $\longrightarrow$ sulphate $\longrightarrow$ disproportionation of organic matter with the production of methane (Berner, 1980, 1981). Although the concentrations of iron and manganese were not measured in the porewaters of Loch Lomond, previous studies have shown that at depth in sediments, the environment is sufficiently reducing

for iron and manganese (presumably as Fe^{2+} and Mn^{2+}) to enter into solution with oxidation of the upwardly diffusing divalent cations to insoluble Fe^{3+} and Mn^{4+} within the oxidised surface layer (Lovell, 1985; Carignan and Nriagu, 1985; Takamatsu et al., 1985; Peterson and Carpenter, 1986). In the porewaters of Loch Lomond, Lovell (1985) found that Fe^{2+} and Mn^{2+} concentrations peaked at depth over 10 - 15cm and declined with decreasing depth towards the sediment surface as solid phase iron and manganese concentrations increased, reflecting the change in the redox conditions to an increasingly oxidising environment and precipitation as amorphous oxides and hydroxides in the top few centimetres of the sediments, consistent with the concept of diagenetic control. In this study, there was a significant peak in the porewater arsenic concentrations over 5 - 11cm, with a maximum concentration of 103.4ug/l at 10 - 11cm, a thousand times the concentration in the overlying water column, as a direct result of the reduction and solubilisation of iron and hence the release and reduction of at least part of the arsenic under the reducing conditions at depth, with diffusion away to regions of lower concentration in both upward and downward directions. The abrupt decrease in the porewater arsenic concentration from 3 - 4 to 2 - 3cm depth, at the same depth as an increase in the sediment arsenic concentration, is therefore, an

indication of the increase in the redox potential in the aerobic surface sediments and oxidation/ precipitation/adsorption/fixation of soluble arsenic in the near surface sediments probably with the oxides and hydroxides of iron.

Speciation of the porewaters by ion-exchange chromatography showed that only inorganic As(V) and As(III) were present above the detection limit of lug/l. Despite the fact that both Ferguson and Gavis (1972) and Wood (1974) predicted that the formation of the methylated arsenic species is important in sedimentary systems, neither MMAA or DMAA could be detected in the porewaters of Loch Lomond. Andreae (1979) also reported the absence of the methylated species in ocean sediments, nor were they found in the sediments of Lake Okahuri by Aggett and O'Brien (1985) who concluded that the mobility of arsenic in the sediments cannot be dependent on the formation of these species.

Except for the surface 0 - 1cm section, where inorganic As(V) constituted 100% of the porewater arsenic, As(III) was the dominant porewater species throughout the rest of the core, especially in the reduction zone from 5 - 11cm, averageing 85 ± 6.1% of the total throughout and reaching 100% at a depth of 14 - 15cm (Table 5.2). In the porewaters of Lake Ohakuri, Aggett and O'Brien (1985) found that As(III) was normally the major arsenic species and its

concentration was often $>$ 90% of the total arsenic concentration. Peterson and Carpenter (1986) similarly reported that in all the porewaters of the Puget Sound and Lake Washington, As(III) predominated over As(V), with an As(III)/As(V) ratio (excluding the surface sediments) ranging between 1.5 - 4.0. From Table 5.2 it can be seen that the As(III)/As(V) porewater ratio for Loch Lomond increases from 3.4 in the 1 - 2cm section to 8.5 in the 13 - 14cm section which, at least qualitatively, is indicative of the increase in reducing conditions with depth in the sediments.

Figure 5.7 represents diagrammatically the thermodynamic stability fields for arsenic over a range of pH and redox potential (represented by pE where pE = -log of the electron concentration) (Cherry et al., 1979). At a measured pH of 5.5 - 6.0 for the porewaters throughout the core, Figure 5.7 shows that because sulphate was still detected at depth in the porewaters, the conditions are not sufficiently reducing for arsenic to be in a sulphidic environment (Figure 5.7b). Thus, the inorganic As(III) and As(V) species present are likely to be undissociated arsenous acid $H_3AsO_3^0$ (As(III)) and dissociated arsenic acid $H_2AsO_4^-$ (As(V)) (Figure 5.7a).

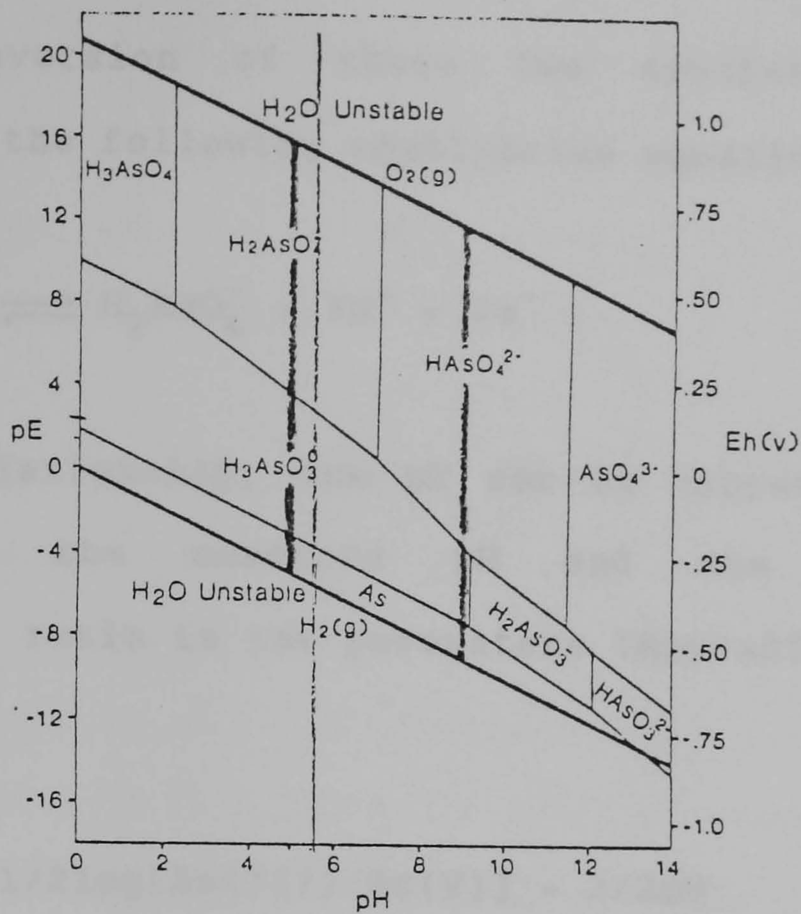


Figure 5.7(a) pE-pH diagram for the As-H_2O system at 25°C. Total dissolved arsenic species is set at $10^{-6.176}$ mol/l(50ug/l). The area within the vertical bars represents the common pE-pH domains for natural waters. (from Cherry et al., 1979)

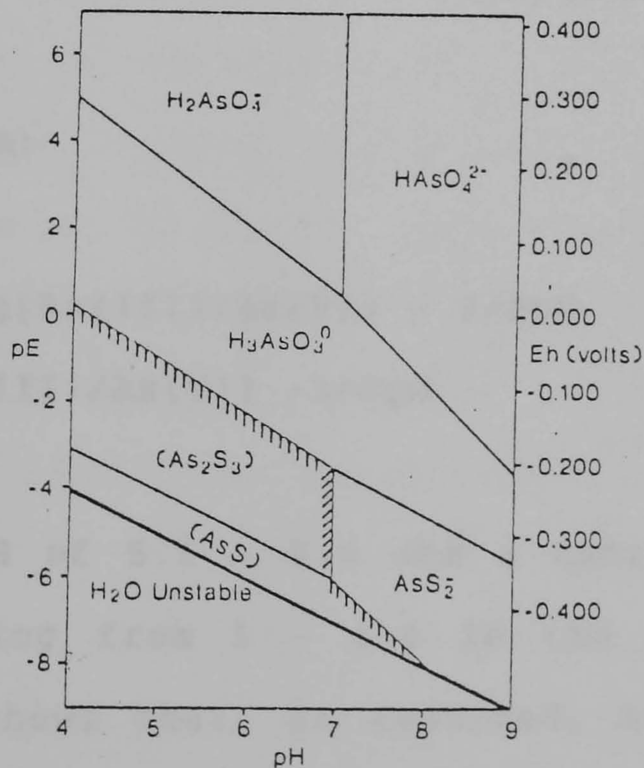
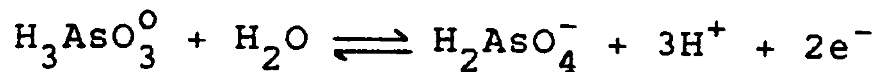


Figure 5.7(b) pE-pH diagram for the As-S-H_2O system at 25°C. Total dissolved arsenic species is set at $10^{-7.176}$ mol/l(5ug/l) and S species is set at 10^{-3}mol/l(32mg/l). The area within the hatched lines denotes that the solid phases are predominant (total dissolved As species are present at less $10^{-7.176}$mol/l(5ug/l). (from Cherry et al., 1979)

The interconversion of these two species can be described by the following equilibrium equation:



$$H_3AsO_3^0 + H_2O \rightleftharpoons H_2AsO_4^- + 3H^+ + 2e^-$$

From this relationship, the pE can be expressed as a function of the measured pH and the measured As(III)/As(V) ratio in the porewaters (Appendix 6).

Thus:

$$pE = 1/2pK - 1/2\log[As(III)/As(V)] - 3/2pH$$

The pK for this system can be calculated from the values for Gibbs free energy of the species involved and the relationship:

$$\Delta G = -RT\ln K \qquad \text{(Appendix 7)}$$

Thus by substitution:

$$pE = 1/2(22) - 1/2\log[As(III)/As(V)] - 3/2pH$$

$$pE = 11 - 1/2\log[As(III)/As(V)] - 3/2pH$$

With a porewater pH of 5.5 - 6.0 and a corresponding calculated pE ranging from 1 - 2.4 in the reduction zone, Figure 5.7a shows that, as expected, As(III) as $H_3AsO_3^0$ must be the dominant form of arsenic in the porewaters of Loch Lomond. This is also in the theoretical pE range (at a pH of 5.5 - 6.0) required

for the reduction of Fe^{3+} to Fe^{2+} (Hemm, 1977). Thus, conditions are sufficiently reducing for the solubilisation of iron and release of associated arsenic from the solid phase into the surrounding porewaters.

In the oxidation zone, calculation of pE based upon the As(III)/As(V) ratios reveals very little change in the value of pE. At first this seems surprising because for the oxidation and precipitation of the oxides and hydroxides of iron and manganese and the oxidation and adsorption of arsenic to occur. there must be a considerable increase in the redox potential (assuming the pH remains unaltered) in the porewaters. Indeed, Lovell (1985) found that there was a rapid decrease in the levels of iron and manganese in the porewaters of Loch Lomond in the oxidation zone and in the particular study described here, total porewater arsenic concentrations decreased suddenly from 67.8ug/l in the 3 - 4cm section to 25.8 and 6.6ug/l in the 2 - 3 and 1- 2cm sections respectively (Table 5.2). However, as noted by Farmer and Lovell (1986), the characteristic porewater arsenic profile may not only be governed by the pH and the redox potential of the system, but by the differences in the adsorption characteristics of the As(III) and As(V) species, which may well produce deviations from thermodynamically-predicted equilibrium (the absence of the methylated species

rules out the possibility that arsenic mobility is partly governed by biological activity). In a series of laboratory-controlled experiments, Pierce and Moore (1982) showed that the oxidised form, arsenate, has a greater affinity for the oxides and hydroxides of iron than the reduced form, arsenite. Thus, accompanying the oxidation of As(III) to As(V) in the aerobic surface sediments, there is likely to be increased adsorption of arsenic, as As(V), on iron compounds. Indeed, in the general trend of decreasing As(III)/As(V) ratios with decreasing depth (Table 5.2), there is an abrupt and significant increase in the ratio of 6.2 at a depth of 2 - 3cm, which, as noted before, is exactly the same depth as the maximum arsenic concentration in the sediments. This appears to be the direct result of As(III) oxidation to As(V) with the preferential adsorption of the latter onto the oxides and hydroxides of iron in the near surface sediments. In addition, Peterson and Carpenter (1986) noted that, when Fe^{2+} and Mn^{2+} are oxidised, the oxidation of As(III) (e.g. to $H_2AsO_4^-$) does not produce a species with low solubility (as in the case of $Fe(OH)_3$ and MnO_2). Therefore, in terms of the solid sediment profile, arsenic, theoretically, should not be as sensitive as those iron and manganese compounds to pE changes across a redox boundary. The fact that the arsenic and iron peaks do coincide in the sediments of Loch

Lomond suggests adsorption or coprecipitation of arsenic with the oxides and hydroxides of iron as a major controlling factor in the oxidation zone. By measuring a close correlation between the rates of dissolution of the arsenic anions and that of iron with EDTA in lake sediments of Lake Ohakuri, and from the constancy of the iron:arsenic ratios during dissolution, Aggett and Roberts (1986) concluded that arsenic may be incorporated into the sediments by coprecipitation onto iron oxides and hydroxides at the time of their formation rather than solely by adsorption onto previously formed surfaces. This explanation of the solid sediment and porewater arsenic data also shows why it is not possible to calculate the prevailing pE from the ratios of As(III)/As(V) in the porewaters of the oxidation zone because of the different adsorption characteristics of the two species.

5.6.3 Conclusions

1) The concentrations of arsenic (283mg/kg), iron (6.33%) and manganese (1.46%) were found to be significantly elevated in the near surface sedimentary layers of Loch Lomond, in contrast to background levels of 45mg/kg, 3.93% and 0.21% for arsenic, iron and manganese respectively. The lack of a recent source of arsenic contamination, the coincidence of the arsenic/iron peaks, the upwards displacement

of the manganese peak and the sub-surface nature of the peaks are in accord with the postulated diagenetic remobilisation theory for the sedimentary distribution of arsenic.

2) The observed peak in the porewater arsenic concentrations in the reduction zone (ranging from 76.1 - 103.4ug/l) is consistent with the solubilisation of iron compounds under reducing conditions at depth, with the release of arsenic followed by upward migration, oxidation and readsorption in the near surface oxic layers at the same depth as a concomitant increase in solid phase arsenic.

3) The speciation of arsenic in the porewaters revealed an As(III)/As(V) ratio of $>$ 4.0 in the reducing zone, indicating that As(III) was the dominant species. At a pH of 5.5 - 6.0, the species present are likely to be undissociated arsenious acid $H_3AsO_3^o$ and dissociated arsenic acid $H_2AsO_4^-$. Calculation of the redox potential reveals a theoretical pE of 1 - 2.4 in the reduction zone, consistent with that necessary for the dissolution of iron oxyhydroxides and the release of solid phase arsenic and reduction at depth. However, in the surface oxidation zone where iron precipitates out and porewater arsenic levels decline rapidly, As(III)

species still predominate because of the preferential adsorption of the oxidised species, As(V), with the oxides and hydroxides of iron. Thus, in this zone, theoretical calculation of pE cannot be based on the ratios of As(III)/As(V) in the porewaters.

4) Thus, within Loch Lomond, sedimentary arsenic profiles are dominated by a self-contained arsenic enrichment cycle. The oxygenated bottom waters are unlikely to release arsenic into the overlying water column unless anoxic conditions are produced through eutrophication or if acidification of the overlying waters disturbs the As/Fe stability fields and complexes. These phenomena may well be of consequence elsewhere and merit further investigation.

5.7 DUBH LOCHAN

5.7.1 Results

The temperature and dissolved oxygen levels measured at 1m intervals for both stations are listed in Table 5.3 with the oxygen profiles shown in Figure 5.8. Tables 5.4 to 5.9 list the concentrations of arsenic, iron and manganese in the sediments and the arsenic and sulphate concentrations of the associated porewaters for cores taken from both sites. This data is represented in Figures 5.9 to 5.11, while Figure 5.12 shows the relative amounts of As(V) and As(III) constituting the total porewater arsenic concentrations.

5.7.2 Discussion

Tables 5.4 to 5.9 and Figures 5.9 to 5.11 show that at both sites in the Dubh Lochan, the overall arsenic, iron and manganese concentrations of the sediments at both sites are considerably lower than in the sediments of Loch Lomond. For example, the maximum arsenic level recorded was 64mg/kg in the 0 - 1cm section at station B (Table 5.7) in contrast to the arsenic maximum of 283mg/kg for Loch Lomond. Neither the sedimentary arsenic profiles, nor those of iron and manganese show the pronounced enrichment profiles characteristic of Loch Lomond sediments. These phenomena may be the result of higher sedimentation rates in the Dubh Lochan having a

TABLE 5.3

TEMPERATURE AND DISSOLVED OXYGEN LEVEL IN THE WATER COLUMN OF THE DUBH LOCHAN

	STATION A		STATION B	
DEPTH (m)	TEMP. C	OXYGEN (%)	TEMP. C	OXYGEN (%)
27/08/85				
Surface	14.5	80	14.5	86
1	14.0	82	14.5	87
2	14.0	84	14.0	85
3	13.5	82	14.0	84
4	13.5	81	13.5	80
5	13.5	80	-	-
6	13.0	78	-	-
7	13.0	76	-	-
8	12.0	10	-	-
9	11.0	2	-	-
10	10.0	1	-	-
11	9.5	0	-	-
20/09/85				
Surface	13.0	88	13.0	92
1	13.0	88	13.0	92
2	13.0	88	13.0	92
3	12.5	86	12.5	90
4	12.5	86	12.5	88
5	12.5	84	12.5	88
6	12.5	84	-	-
7	12.5	84	-	-
8	12.5	84	-	-
9	12.5	84	-	-
10	11.5	10	-	-
02/10/85				
Surface	12.0	84	11.0	84
1	12.0	84	11.0	80
2	11.5	84	11.0	80
3	11.5	82	10.5	80
4	11.5	82	10.5	78
5	11.0	82	10.5	78
6	11.0	80	-	-
7	11.0	80	-	-
8	10.5	78	-	-
9	10.5	78	-	-
10	10.5	60	-	-
11	10.5	60	-	-

\- No data collected

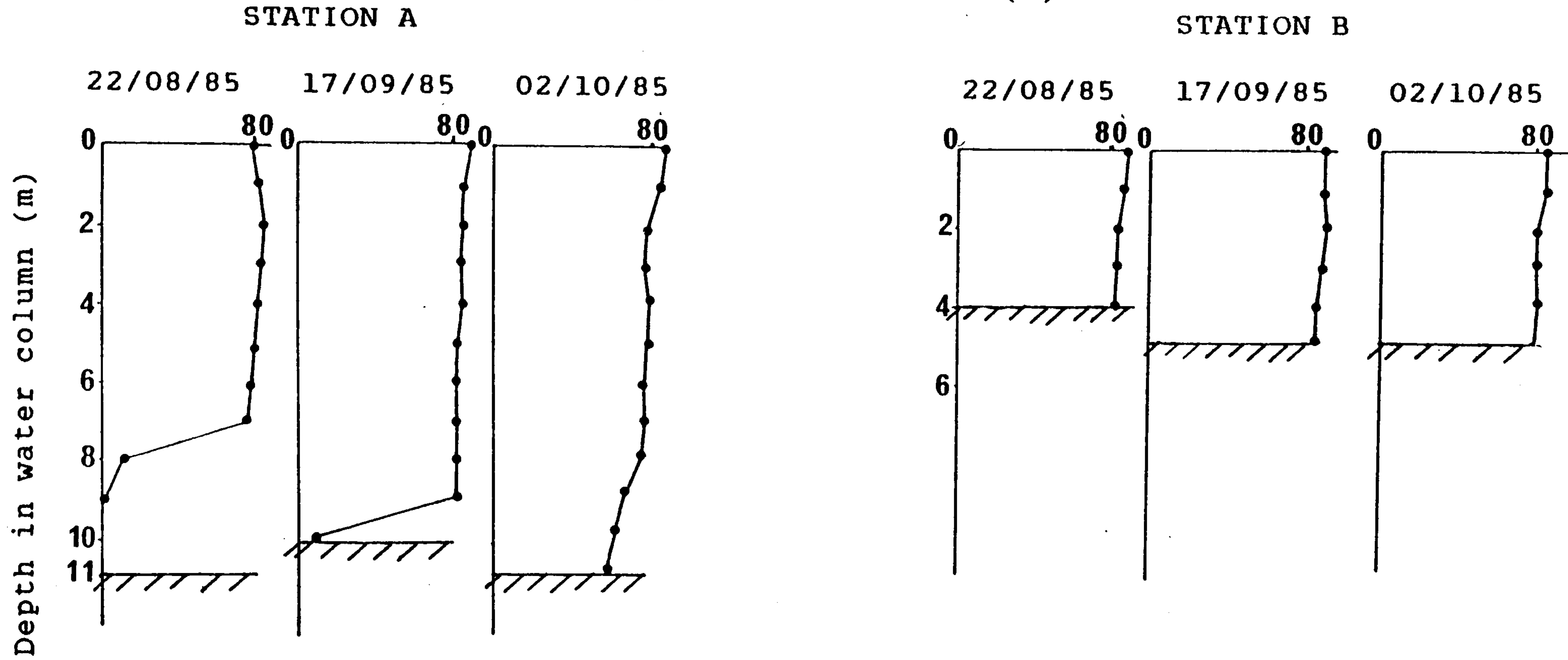


Figure 5.8 Dissolved oxygen profiles at stations A and B in the the Dubh Lochan.

TABLE 5.4

SEDIMENT ARSENIC, IRON AND MANGANESE AND ASSOCIATED POREWATER ARSENIC AND SULPHATE CONCENTRATIONS AT STATION A IN THE DUBH LOCHAN (22/08/85)

	SEDIMENT			POREWATER				
Section (cm)	As (mg/kg)	Fe percent	Mn percent	Total (ug/l)	As(III) (ug/l)	As(V) relative amounts (%)	As(III) relative amounts (%)	SO_4^{2-} (mg/l)
0-1	40	2.42	0.037	12.0	8.4	30	70	5.0
1-2	37	2.90	0.045	36.0	34.6	4	96	4.5
2-3	37	2.63	0.040	26.8	23.6	12	88	4.8
3-4	46	2.71	0.043	59.2	62.0	0	100	-
4-5	36	2.63	0.041	36.4	30.0	18	82	3.9
5-6	38	2.60	0.043	21.2	26.8	0	100	-
6-7	37	2.50	0.042	28.8	25.2	12	88	4.3
7-8	39	2.68	0.047	-	-	-	-	-
8-9	-	-	-	-	-	-	-	-
9-10	41	2.42	0.048	22.4	16.8	25	75	2.2
10-11	55	3.06	0.055	56.8	43.4	24	76	-
11-12	-	-	-	-	-	-	-	-
12-13	49	3.17	0.047	37.6	28.4	24	76	2.6
13-14	46	3.21	0.058	52.4	76.0	0	100	2.8
14-15	35	2.87	0.050	30.8	30.0	3	97	3.4

\- no data collected

TABLE 5.5

SEDIMENT ARSENIC, IRON AND MANGANESE AND ASSOCIATED POREWATER ARSENIC AND SULPHATE CONCENTRATIONS AT STATION B IN THE DUBH LOCHAN (22/08/85)

	SEDIMENT			POREWATER				
Section (cm)	As (mg/kg)	Fe percent	Mn percent	Total (ug/l)	As(III) (ug/l)	As(V) relative amounts (%)	As(III) relative amounts (%)	SO_4^{2-} (mg/l)
0-1	39	4.75	0.39	3.6	*	100	0	6.7
1-2	33	3.10	0.20	6.0	*	100	0	7.7
2-3	30	2.97	0.13	8.4	*	100	0	12.3
3-4	24	2.29	0.071	9.0	*	100	0	9.2
4-5	27	2.51	0.086	8.2	*	100	0	10.8
5-6	26	2.40	0.056	14.0	13.2	6	94	4.0
6-7	20	2.24	0.053	29.2	28.0	4	96	4.7
7-8	17	2.26	0.053	46.8	55.6	0	100	-
8-9	17	2.13	0.053	34.0	56.0	0	100	1.8
9-10	13	2.09	0.052	37.2	33.3	10	90	-
10-11	13	1.92	0.047	39.2	52.4	0	100	2.1
11-12	14	1.85	0.050	36.0	67.6	0	100	-

* less than the detection limit of 0.5ug/l

\- no data available

TABLE 5.6

SEDIMENT ARSENIC, IRON AND MANGANESE AND ASSOCIATED POREWATER ARSENIC AND SULPHATE CONCENTRATIONS AT STATION A IN THE DUBH LOCHAN (17/09/85)

	SEDIMENT			POREWATER				
Section	As	Fe	Mn	Total	As(III)	As(V)	As(III)	SO_4^{2-}
(cm)	(mg/kg)	percent		(ug/l)		relative amounts (%)		(mg/l)
0-1	37	2.49	0.033	9.0	5.0	72	28	3.6
1-2	49	2.68	0.037	19.0	22.0	42	58	4.3
2-3	37	3.03	0.042	25.0	39.2	22	78	4.2
3-4	37	2.66	0.039	22.0	32.6	26	74	-
4-5	33	3.16	0.039	23.5	32.6	31	69	2.3
5-6	42	2.96	0.042	47.0	70.0	26	74	3.0
6-7	38	2.66	0.042	66.0	139.2	0	100	-
7-8	39	2.60	0.043	42.0	85.0	0	100	-
8-9	-	-	-	-	-	-	-	-
9-10	47	2.67	0.051	44.0	99.0	0	100	2.5
10-11	-	-	-	-	-	-	-	-
11-12	44	4.06	0.068	49.0	95.6	2	98	1.9
12-13	46	4.41	0.074	32.0	60.0	6	94	1.7
13-14	38	4.05	0.068	44.0	94.0	0	100	1.2
14-15	-	-	-	-	-	-	-	-
15-16	30	3.82	0.065	30.0	58.4	3	97	2.6
16-17	28	4.44	0.070	14.0	29.0	0	100	2.1

- no data collected

TABLE 5.7

SEDIMENT ARSENIC, IRON AND MANGANESE AND ASSOCIATED POREWATER ARSENIC AND SULPHATE CONCENTRATIONS AT STATION B IN THE DUBH LOCHAN (17/09/85)

	SEDIMENT			POREWATER				
Section	As	Fe	Mn	Total	As(III)	As(V)	As(III)	SO_4^{2-}
(cm)	(mg/kg)	percent		(ug/l)		relative amounts (%)		(mg/l)
0-1	64	6.17	0.087	3.0	*	100	0	4.9
1-2	43	5.65	0.072	15.0	*	100	0	4.5
2-3	37	4.69	0.064	20.0	*	100	0	2.8
3-4	30	3.72	0.046	-	-	-	-	3.2
4-5	27	4.18	0.054	21.6	12.0	44	56	3.4
5-6	24	3.95	0.054	22.0	15.4	30	70	2.1
6-7	27	4.46	0.052	17.0	13.2	22	78	2.1
7-8	31	4.59	0.055	25.0	29.0	0	100	2.1
8-9	36	3.37	0.047	44.0	36.4	17	83	3.0
9-10	35	3.42	0.048	48.0	31.0	35	65	3.2
10-11	35	3.57	0.051	44.0	22.4	49	51	2.0
11-12	26	3.18	0.046	35.0	22.4	36	64	2.6
12-13	24	3.08	0.043	33.0	22.4	32	68	3.0
13-14	18	2.71	0.039	19.0	10.0	53	47	2.0

* less than the detection limit of 1ug/l

\- no data available

TABLE 5.8

SEDIMENT ARSENIC, IRON AND MANGANESE AND ASSOCIATED POREWATER ARSENIC AND SULPHATE CONCENTRATIONS AT STATION A IN THE DUBH LOCHAN (02/10/85)

Section	SEDIMENT			POREWATER				
Section	As	Fe	Mn	Total	As(III)	As(V)	As(III)	SO_4^{2-}
(cm)	(mg/kg)	percent		(ug/l)		relative amounts (%)		(mg/l)
0-1	39	3.00	0.038	8.0	3.0	63	37	4.8
1-2	61	4.48	0.061	8.0	3.0	63	37	5.3
2-3	47	3.39	0.045	15.2	10.0	34	66	5.0
3-4	36	2.93	0.040	27.6	13.6	51	49	2.8
4-5	37	2.87	0.037	48.0	31.2	35	65	3.5
5-6	43	2.94	0.040	40.0	18.0	55	45	2.8
6-7	36	3.34	0.045	50.8	19.2	62	38	2.3
7-8	38	3.31	0.049	31.6	12.0	62	38	3.5
8-9	39	3.18	0.048	18.0	6.0	67	33	2.3
9-10	44	3.56	0.052	21.6	4.0	81	19	4.0
10-11	-	-	-	-	-	-	-	-
11-12	56	3.96	0.058	20.0	4.0	80	20	2.8
12-13	60	3.48	0.062	23.6	4.4	81	19	3.0
13-14	60	3.58	0.060	32.8	11.8	64	36	3.3
14-15	57	3.44	0.062	40.0	12.0	70	30	-
15-16	-	-	-	-	-	-	-	-
16-17	37	3.08	0.059	36.0	14.8	59	41	3.3

\- no data collected

TABLE 5.9

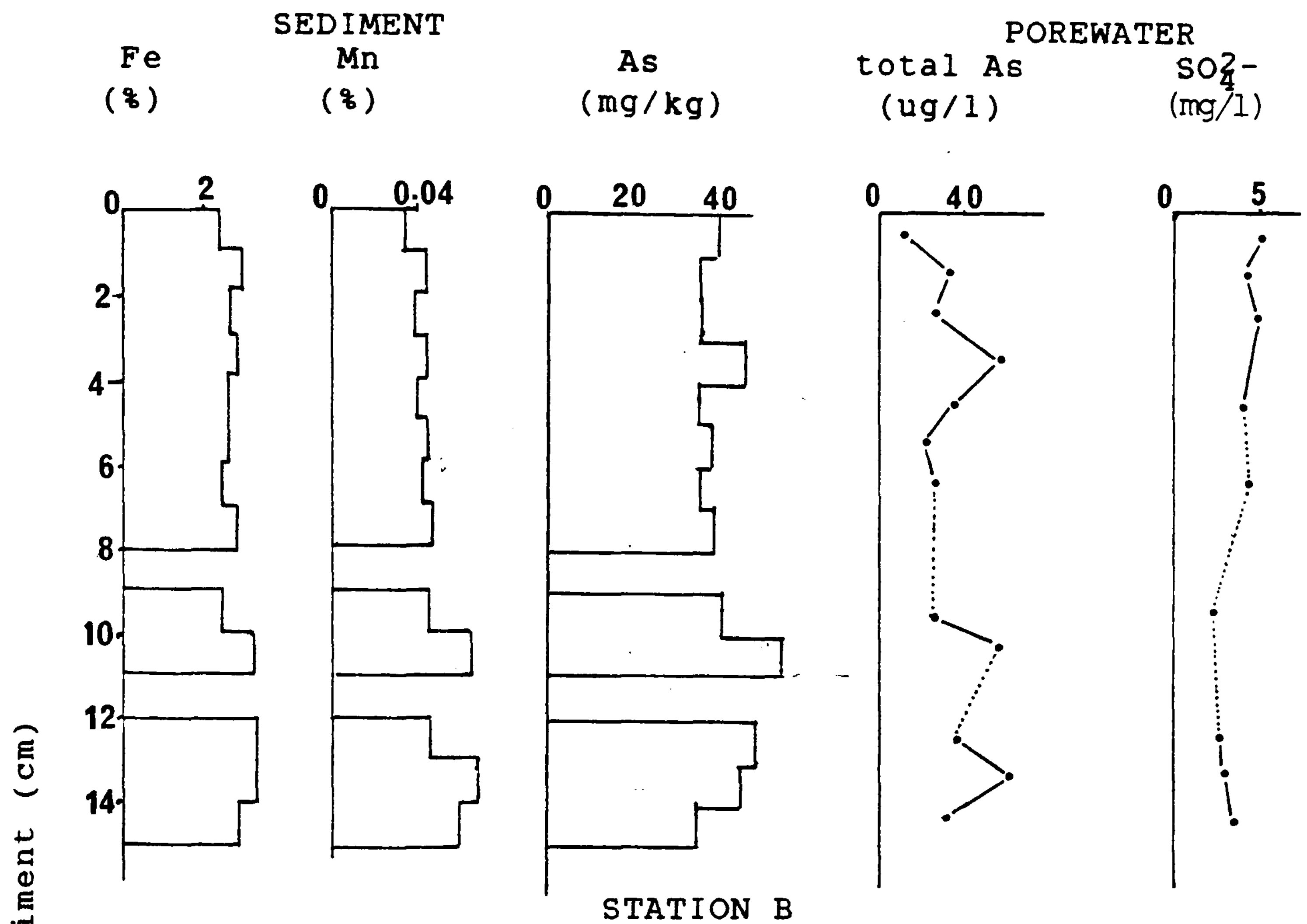
SEDIMENT ARSENIC, IRON AND MANGANESE AND ASSOCIATED POREWATER ARSENIC AND SULPHATE CONCENTRATIONS AT STATION B IN THE DUBH LOCHAN (02/10/85)

Section (cm)	SEDIMENT As (mg/kg)	Fe percent	Mn percent	POREWATER Total (ug/l)	As(III) (ug/l)	As(V) relative amounts (%)	As(III) relative amounts (%)	SO_4^{2-} (mg/l)
0-1	46	4.33	0.260	2.6	*	100	0	5.2
1-2	37	4.97	0.271	2.6	*	100	0	5.2
2-3	37	3.61	0.137	6.0	*	100	0	3.0
3-4	36	3.21	0.080	21.2	*	100	0	2.2
4-5	34	3.48	0.072	34.0	19.2	44	56	2.0
5-6	34	3.18	0.069	48.0	26.4	45	55	2.4
6-7	29	2.96	0.059	42.8	28.0	35	65	2.6
7-8	28	2.66	0.056	38.0	20.4	46	54	2.7
8-9	25	2.74	0.055	29.2	22.0	25	75	2.4
9-10	24	2.74	0.052	30.0	22.0	27	73	2.5
10-11	19	2.50	0.051	24.4	17.6	28	72	2.9
11-12	18	2.56	0.049	22.4	8.8	61	39	2.9
12-13	17	2.44	0.049	33.2	16.0	52	48	3.1
13-14	16	2.52	0.047	49.2	32.4	34	66	3.2
14-15	16	2.67	0.047	50.8	32.4	36	64	3.0
15-16	16	3.46	0.046	40.0	25.2	37	63	3.5
16-17	14	2.28	0.043	41.6	44.0	0	100	3.7
17-18	14	2.17	0.043	22.8	16.0	26	74	3.4
18-19	13	1.94	0.037	14.0	8.8	37	63	3.4
19-20	15	2.29	0.044	31.6	20.4	35	65	3.4

* less than the detection limit of 0.5ug/l

\- no data available

STATION A



STATION B

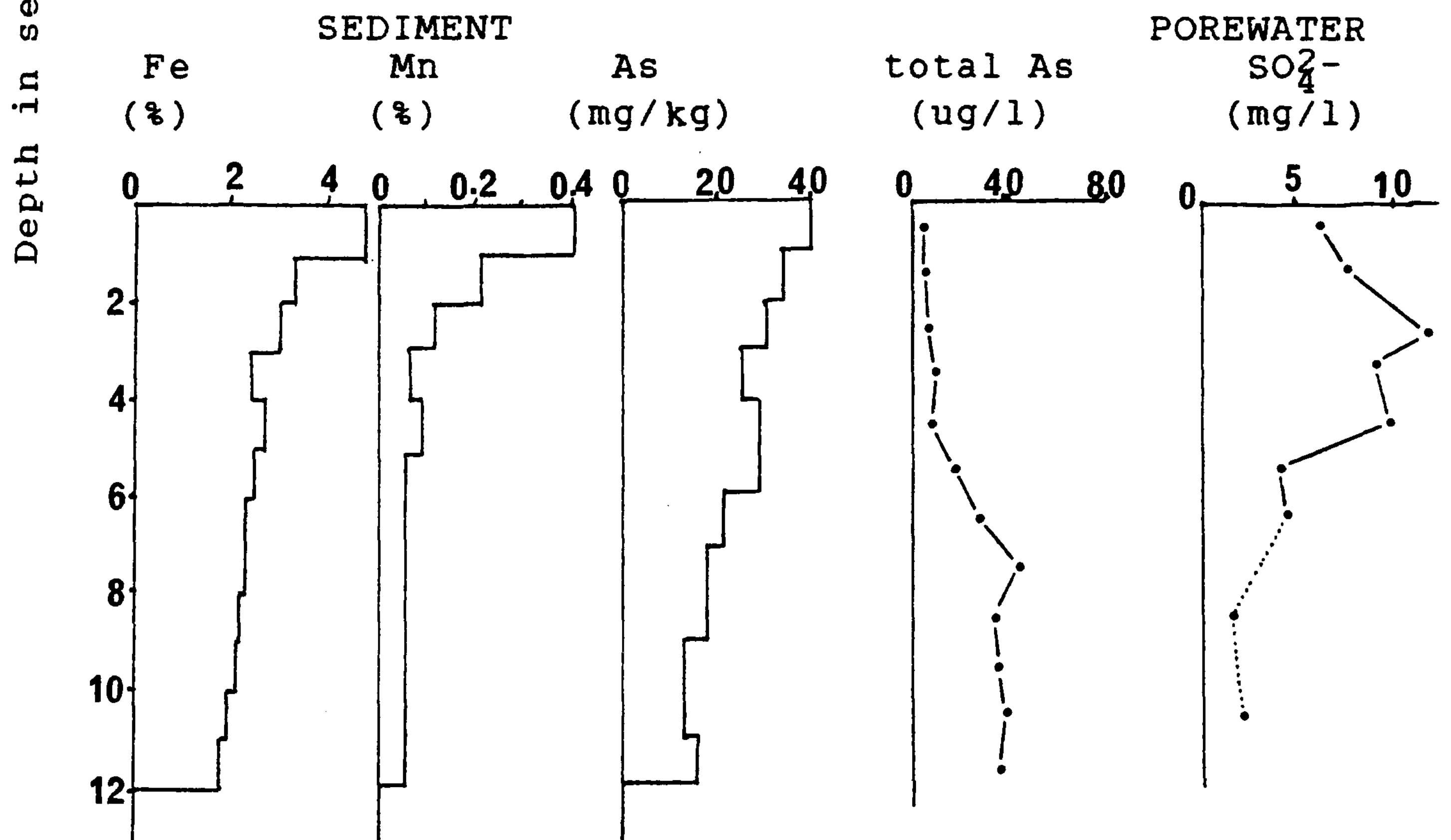
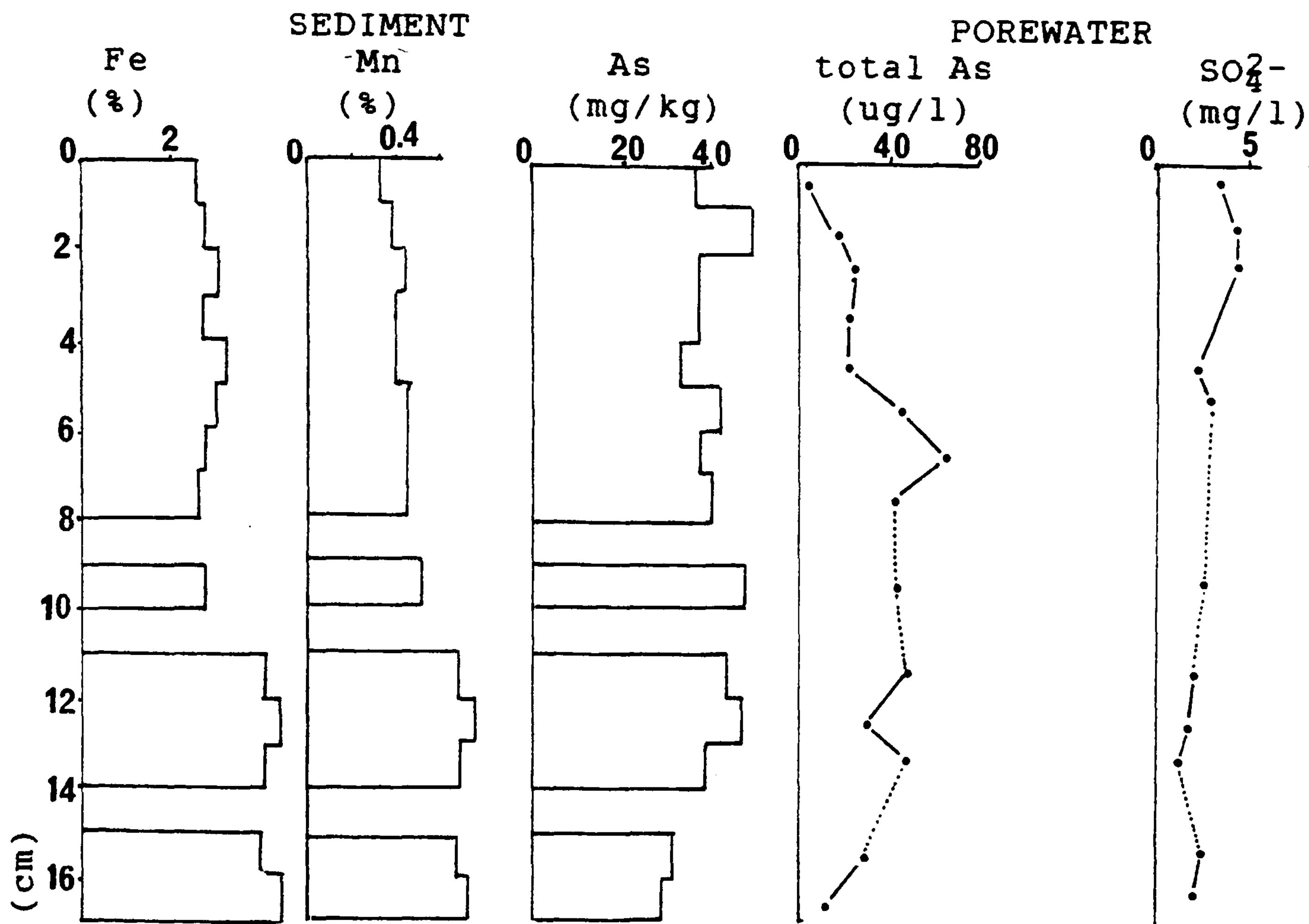


Figure 5.9 Sediment arsenic, iron and manganese and associated porewater arsenic and sulphate concentrations at stations A and B in the Dubh Lochan (22/08/85).



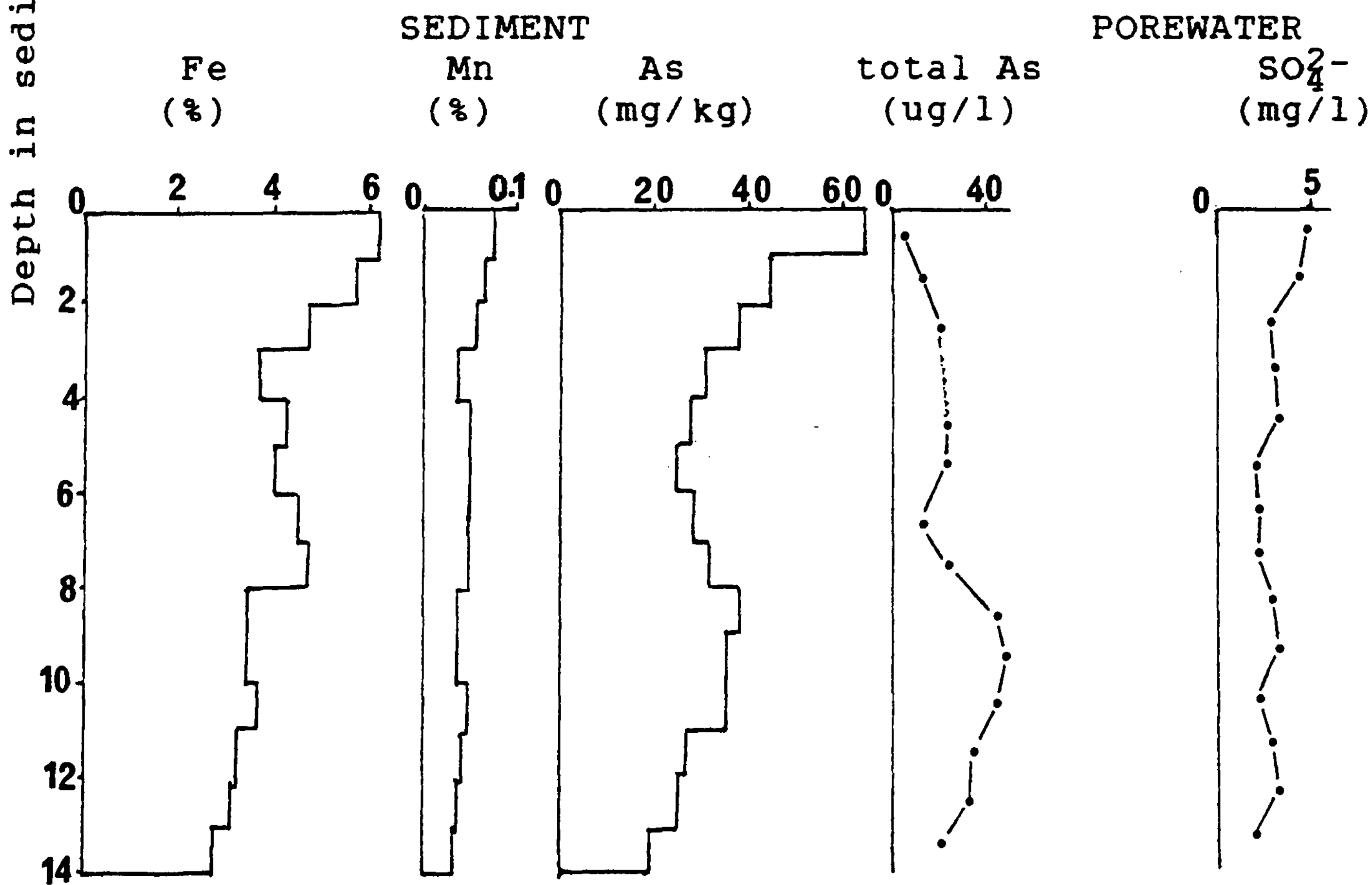


Figure 5.10 Sediment arsenic, iron and manganese and associated porewater arsenic and sulphate concentrations at stations A and B in the Dubh Lochan (17/09/85).

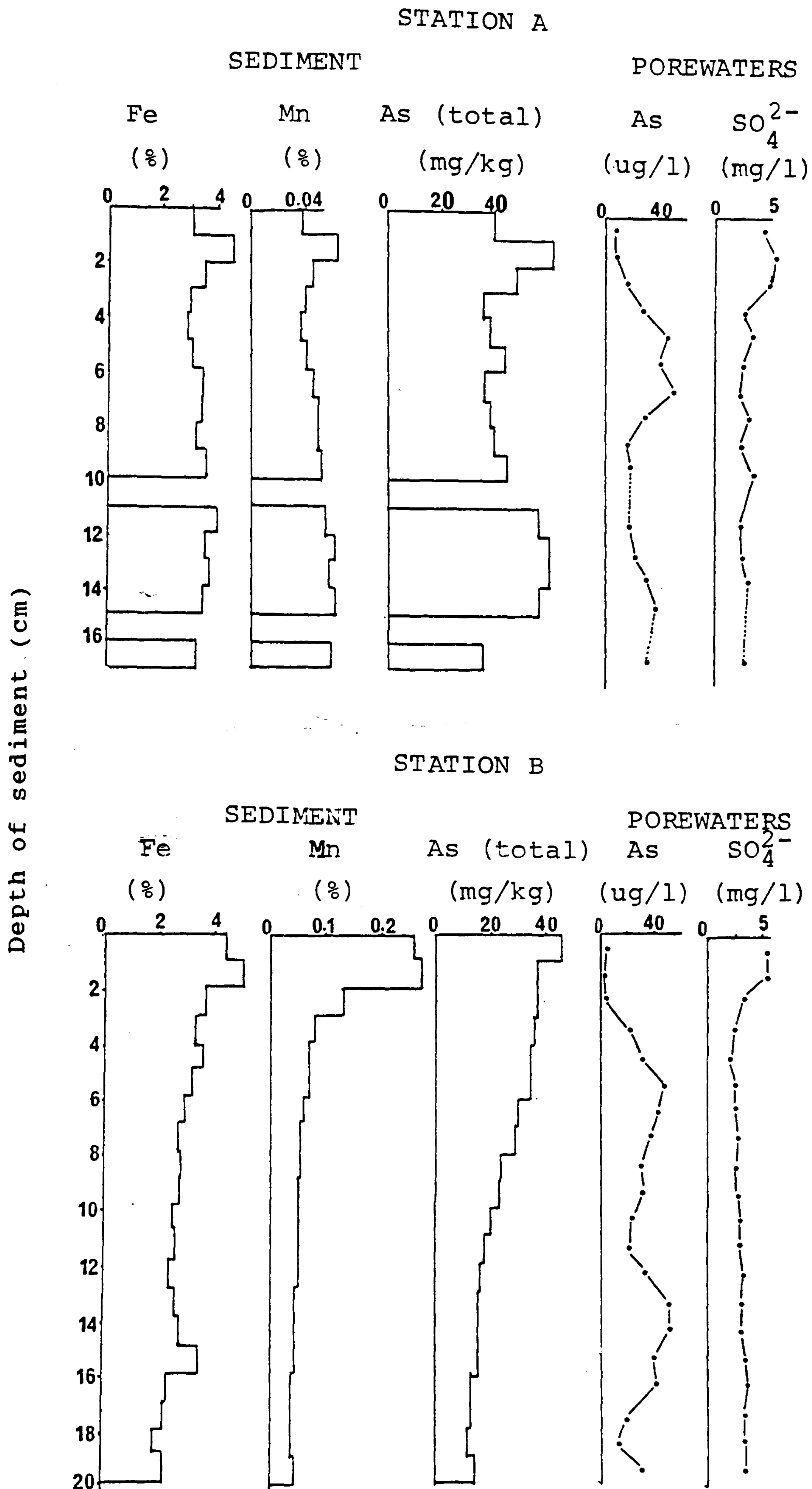


Figure 5.11 Sediment arsenic, iron and manganese and associated porewater arsenic and sulphate concentrations at stations A and B in the Dubh Lochan (02/10/85).

'diluting' effect on the concentrations present and it is highly probable that mixing of the surficial sediments via bioturbation is more significant in the more biologically active Dubh Lochan. In addition, the greater organic content throughout the Dubh Lochan sediments suggests that the resultant redoxcline may be less pronounced, leading to thicker and less well defined zones of enhancement (Farmer and Lovell, 1986).

From measurements of the temperature and dissolved oxygen levels in the water column above the sediments at each of the sites, (Table 5.3, Figure 5.8) it was observed that the bottom waters remained oxygenated at all times at station B, while, at station A, the overlying waters became thermally stratified during August, with the oxygen level at 0% at the sediment/water interface. From then on, the decline in water temperatures at the water surface caused a breakdown of the stratification, with bottom waters becoming increasingly saturated with respect to the dissolved oxygen content.

With bottom waters remaining at over 78% saturation with respect to the dissolved oxygen concentration, Figures 5.9 to 5.11 show that at station B, in general, there is a slight enhancement in the concentrations of arsenic, iron and manganese in the surface sediments, although with a maximum peak of only 64mg/kg (background of 22 ±7mg/kg) compared with 283mg/kg for Loch Lomond, the enhancement is of

only a modest nature. For all three cores taken from this site, it is noted that the peak concentrations for arsenic, iron and manganese occur in the uppermost section and are not sub-surface as in Loch Lomond. As with the Loch Lomond core, there was a decline in the porewater sulphate concentrations with depth. Although the concentration gradient was less marked than that of the Loch Lomond porewaters, the decreasing concentrations are still indicative of the more reducing conditions at depth in the sediments. All three cores taken from site B show a steady decline in the porewater total arsenic concentrations with decreasing depth. Porewater arsenic levels peaked below a depth of 6cm (around 46 - 50ug/l) presumably by the same reduction and solubilisation mechanism of iron at depth with the release of solid phase arsenic into the surrounding porewaters. Similarly, there appears to be a decreasing porewater arsenic concentration gradient towards the surface sediments and hence upward migration of arsenic via the porewaters from the reduction zone into the oxidation zone. However, unlike Loch Lomond, where there is the greatest decrease in the porewater arsenic concentrations in the uppermost sections of the core, there is no dramatic increase in the solid phase arsenic concentrations. Figures 5.9 to 5.11 show that the enrichment profile (although slight) is more gradual, with the peaks occurring in the 0 - 1cm

surface sections. Indeed, arsenic speciation of the porewaters showed that, while As(III) was the predominant species at depth (as in Loch Lomond), in the uppermost sections of the core where it might have been expected that As(V) would adsorb onto the near surface sediments, As(V) constituted 100% of the total arsenic concentration of the porewaters and did not decline until the 0 - 1cm sections where iron was seen to precipitate out. However, the overall pattern is consistent with diagenetic remobilisation of arsenic at depth and subsequent oxidation and readsorption with the oxides and hydroxides of iron in the oxidation zone.

In contrast, the arsenic, iron and manganese profiles for station A exhibit no significant enrichments in the near surface sediments (Figures 5.9 to 5.11). While for the first core taken from this site, the arsenic, iron and manganese sediment concentrations were virtually uniform throughout the core at 41 $\pm$ 6mg/kg, 2.75 $\pm$ 0.26% and 0.046 $\pm$ 0.006% respectivley, there was a slight peak in the arsenic level of 49mg/kg at 1 - 2cm in the second core and, for the third core, there was a definite indication of peak formation for arsenic (61mg/kg), iron (4.48%) and manganese (0.06%) in the 1 - 2cm section. As with the second and third cores taken from site B, all three cores at A exhibited a very slight decrease in the porewater sulphate concentrations with depth,

indicating that there was a slight decrease in the redox potential with depth. Porewater arsenic concentrations showed that it was only when the solid phase arsenic peaks began to appear (Figures 5.10 and 5.11) that the porewater arsenic profiles decreased significantly in the uppermost sections of the cores. Table 5.3 and Figure 5.8 show that when the first core was taken, the oxygen content of the bottom waters were at 0% saturation i.e. anoxic and unlike station B, As(III) was the dominant form of arsenic in the sediment porewaters (Figure 5.12) constituting 87 $\pm$ 12% of the total compared with 13 $\pm$ 11% as As(V). With the metalimnion at 7 - 8cm (i.e. where there is a rapid change in temperature over a short depth) there is very little dissolved oxygen throughout the bottom 3m of water. With the development of anoxic conditions throughout the sediment, as far as the sediment/water interface, the reduced forms of arsenic, iron and manganese will not precipitate out, but remain in solution. In this reduced form, it is highly probable that they will simply diffuse from the sediments into the overlying water column, similar to the well established transfer of phosphorus from sediments to the overlying water under low oxygen conditions (Mortimer, 1971; Wetzel, 1975). Indeed, Klarer (1978) noted an increase in the conductivity of the hypoliminion (bottom waters below the metalimnion) in the Dubh Lochan during thermal stratification of

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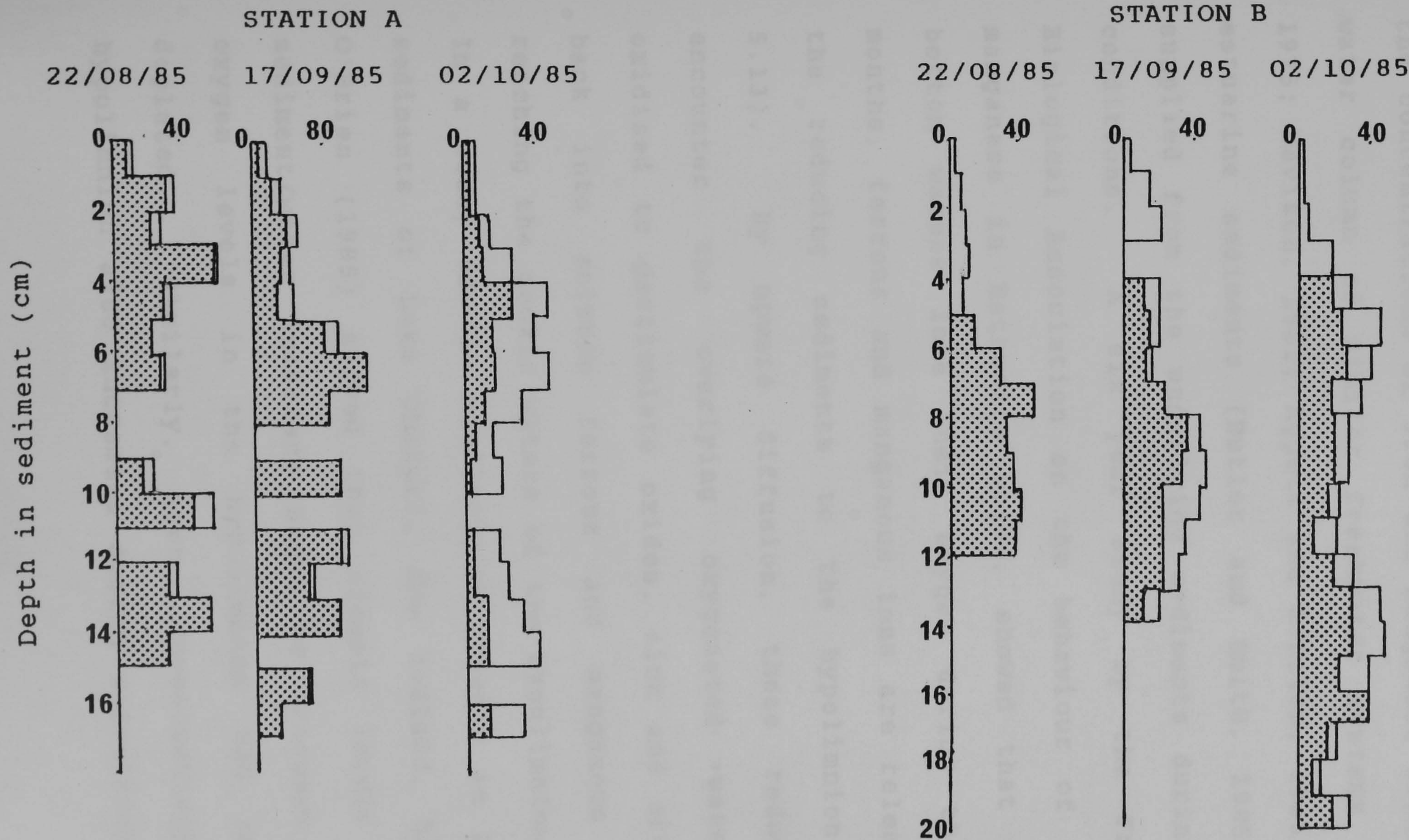


Figure 5.12 The relative proportions of As(V)(□) and As(III) (▒) in the porewaters of sediment cores taken from stations A and B in the Dubh Lochan.

the water column, which was believed to be due to the release of Fe^{2+} and Mn^{2+} from the sediments in a similar manner to that reported by Mortimer (1971) for the sediments of the Great Lakes.

A number of previous studies have attributed the concentrations of iron and manganese found in the water column of similar freshwater systems (Wetzel, 1975; Davison, 1981; Aggett and O'Brien, 1985) and in estuarine sediments (Butler and Smith, 1985) to be supplied from the underlying sediments during anoxic conditions. A six year study by the Freshwater Biological Association on the behaviour of iron and manganese in Esthwaite water, showed that when the bottom waters lose their oxygen during the summer months, ferrous and manganous ions are released from the reducing sediments to the hypolimnion (Figure 5.13). By upward diffusion, these reduced ions encounter the overlying oxygenated waters, are oxidised to particulate oxides, sink and are reduced back into soluble ferrous and manganous ions on reaching the anoxic waters of the hypolimnion again.
In a study of the mobility of arsenic in lacustrine sediments of Lake Ohakuri, New Zealand, Aggett and O'Brien (1985) showed that arsenic levels near the sediment/water interface began to increase once the oxygen levels in the hypolimnion had been fully depleted. Similarly, iron concentrations in the hypolimnion also increased during stratification with

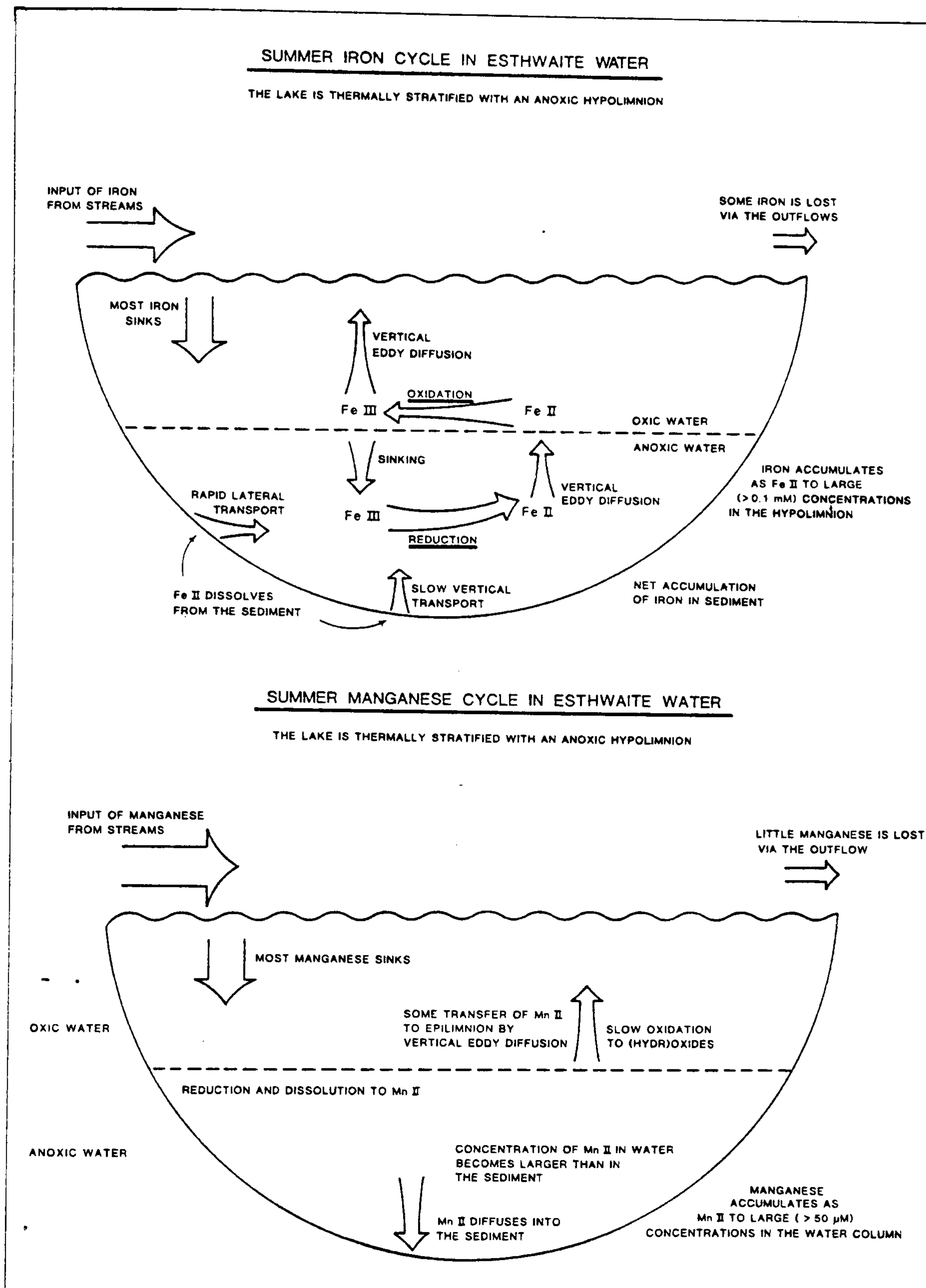


Figure 5.13 Summer iron/manganese cycle in Esthwaite water (N.E.R.C. Newsletter, June 1985)

the overall pattern being very similar to that of arsenic. The iron and arsenic concentrations vs depth profiles were indicative of diffusion from the sediment surfaces rather than dissolution of particles by reduction of suspended material which would, according to Davison (1981), produce more uniform concentrations of the elements with depth. Table 5.3 and Figure 5.8 show that when the second core was taken, the bottom waters were no longer anoxic, but were at 10% saturation with respect to the dissolved oxygen concentration. Although As(III) predominated at depth (99 $\pm$ 2%, Figure 5.12), As(V) now constituted a significant 37 $\pm$ 19% in the upper 6cm. With the oxygenation of the bottom waters the top few sections of the core should be sufficiently aerobic for arsenic, iron and manganese to be oxidised and fixed in the near surface sediments by precipitation/ adsorption mechanisms, with depletion of the porewater arsenic levels and initiation of a more marked concentration gradient and upward diffusion of dissolved arsenic from depth as observed for station B (Figures 5.10, 5.11). With bottom waters at 60% oxygen saturation (Figure 5.8) when the third core was taken, As(V) was detected in the porewaters throughout the core to a depth of 17cm (Figure 5.12) consitituting an average 62 $\pm$ 14% of the total porewater arsenic. As pointed out by Aggett and O'Brien (1985) much of the upward diffusing iron and

arsenic will only be temporarily held in the oxidised upper zone, as increased biological activity (in the newly aerated sediments) will lead to some release of the ions into the overlying loch water (as well as encourage the penetration of dissolved oxygen at depth) and destroy the diagenetically produced enrichment profiles. This, in addition to that lost on the return of anoxic conditions during the summer, means that there is insufficient time for the enrichment profiles characteristic of Loch Lomond and, to a certain extent also, station B in the Dubh Lochan, to develop at this seasonally anoxic site.

5.7.3 Conclusions

1) Sedimentary enrichment profiles of arsenic (64mg/kg), iron (6.17%) and manganese (0.39%) are less clearly defined in the Dubh Lochan than in the main loch, Loch Lomond. Although concentrations and enhancements are much lower, however, the Dubh Lochan does allow a comparison between an oxic and a seasonally anoxic site.

2) At the shallower site, B, the oxygenated conditions of the bottom waters did appear to result in a pattern of arsenic, iron and manganese enrichment in the near-surface sediments, associated with a decline in the porewater arsenic concentrations in the

aerobic surface environment. Speciation of the porewater arsenic showed that, for the uppermost 4 - 5cm of the core, all of the porewater arsenic was oxidised, while, at depth, As(III) was predominant, thus providing evidence for the solubilisation/reduction at depth, followed by oxidation and precipitation/adsorption in the upper layers, in line with the diagenetic remobilisation hypothesis. The gradual nature of the profiles could be due to the higher organic content and less well defined redoxcline, as well as biological mixing in the top few centimetres, thereby preventing the development of well defined enrichment layers.

3) The initial lack of any structure to the arsenic, iron and manganese sediment profiles at station A is thought to be due to the diffusion of these elements from the porewaters to the overlying water column when the bottom waters became anoxic during a period of thermal stratification over the summer months. With the onset of the turnover period and the reoxygenation of the bottom waters, there was a suggestion that arsenic, iron and manganese might be fixed in the near surface sediments. This was confirmed by the porewater data, which, during

anoxia, did not decline significantly in the near surface layers, but which, during reoxygenation of the bottom waters, did show the development of a more pronounced concentration gradient in the upper oxygenated sedimentary layers. Speciation of the sediment porewaters from station A confirmed the change from reducing conditions at depth where the porewater arsenic was largely in the reduced form, As(III), to the oxygenated conditions near the surface where As(V) became the predominant species.

5.8 SEALOCHS

5.8.1 Results

Tables 5.10 to 5.13 list the sedimentary arsenic, iron and manganese concentrations for Loch Goil and the Minch. As it was suspected that any near surface iron peaks might be masked by high background concentrations and increased organic carbon content near the surface, the iron concentrations were normalised to aluminium using the aluminium concentrations obtained for the appropriate sections in each of the cores by Farmer (1983). Figures 5.14 to 5.16 show the profiles obtained from the Craib surface cores (C) and an 'average' for the elements at depth as determined from the gravity cores (G).

TABLE 5.10

ARSENIC, IRON (IRON/ALUMINIUM RATIO) AND MANGANESE CONCENTRATIONS IN LOCH GOIL SEDIMENTS USING A CRAIB CORER (LGC4) AND GRAVITY CORER (LGG7)

	SECTION (cm)	As (mg/kg)	Fe (%)	Fe/Al	Mn (%)
LGC4 (St.2)	0-1	32	4.24	0.57	0.703
	1-2	33	4.20	0.54	0.617
	2-3	36	4.49	0.54	0.646
	3-4	39	4.73	0.58	0.486
	4-5	40	4.74	-	0.300
	5-6	34	4.70	-	0.198
	6-7	30	4.55	0.50	0.189
	7-8	22	4.77	-	0.149
	8-9	21	4.50	-	0.137
	9-10	19	4.82	0.55	0.169
	10-11	21	4.49	-	0.156
	11-12	22	4.76	-	0.133
	12-13	25	4.77	0.53	0.125
	13-14	29	5.19	-	0.146
	14-15	26	4.83	-	0.094
	15-16	25	4.69	0.49	0.084
	16-17	18	4.73	-	0.080
LGG7	17-18	26	4.21	-	0.084
	24-28	21	2.89	-	0.052
	34-38	17	3.21	-	0.050

- no aluminium data available

TABLE 5.11

ARSENIC, IRON (IRON/ALUMINIUM RATIO) AND MANGANESE CONCENTRATIONS IN LOCH GOIL SEDIMENTS USING A CRAIB CORER (LGC9) AND GRAVITY CORER (LGG5)

	SECTION (cm)	As (mg/kg)	Fe (%)	Fe/Al	Mn (%)
LGC9 (St.1)	0-1	64	5.29	0.77	2.704
	1-2	77	5.42	0.78	2.839
	2-3	100	5.66	0.78	2.354
	3-4	100	6.36	0.82	0.783
	4-5	112	5.91	-	0.558
	5-6	90	6.23	-	0.554
	6-7	87	5.38	-	0.581
	7-8	68	5.65	-	0.678
	8-9	48	4.91	-	0.860
	9-10	44	5.16	0.65	0.910
	10-11	45	5.22	-	1.000
	11-12	43	4.91	-	0.994
	13-14	46	5.16	-	2.104
	14-15	47	5.25	-	1.436
	15-16	49	5.14	0.64	1.940
LGG5	15-16	24	5.30	-	0.316
	19-20	27	5.89	-	0.249
	26-27	22	5.12	-	0.277
	31-32	19	4.93	-	0.406
	35-36	27	5.02	-	0.566

\- no aluminium data available

TABLE 5.12

ARSENIC, IRON (IRON/ALUMINIUM RATIO) AND MANGANESE CONCENTRATIONS IN LOCH GOIL CORE LGBJe

	SECTION (cm)	As (mg/kg)	Fe (%)	Fe/Al	Mn (%)
LGBJE	0-1	72	4.62	0.71	2.533
	1-2	116	4.84	0.67	2.589
	2-3	109	5.36	0.74	0.748
	3-4	72	5.54	0.77	1.168
	4-5	47	5.11	0.63	0.881
	5-6	42	4.69	0.59	1.001
	6-7	60	4.60	0.62	2.092
	7-8	55	5.40	0.65	1.864
	8-9	48	4.97	0.61	1.374
	9-10	58	5.59	0.66	1.342
	10-11	55	5.40	0.63	1.832
	11-12	56	5.23	0.62	1.168
	12-13	47	5.38	0.66	0.984
	13-14	41	4.77	0.55	1.032
	14-15	40	5.10	0.57	0.768
	15-16	36	5.14	0.60	0.634

TABLE 5.13

ARSENIC, IRON AND MANGANESE
CONCENTRATIONS IN THE MINCH CORE KNORR C-46

	SECTION (cm)	As (mg/kg)	Fe (%)	Mn (%)
Knorr				
C-46	0-1	4.7	1.35	0.028
	1-2	4.1	1.33	0.023
	2-3	3.6	1.20	0.022
	3-4	3.8	1.33	0.023
	4-6	3.3	1.24	0.022
	6-8	3.4	1.35	0.023
	8-10	3.2	1.71	0.024
	10-12	3.9	1.51	0.022
	12-14	3.7	1.23	0.022
	14-16	3.9	1.32	0.022
	16-18	3.6	1.17	0.012
	18-20	5.7	1.42	0.023
	20-24	4.6	1.31	0.022
	24-28	5.1	1.37	0.022
	28-32	5.0	1.31	0.022
	32-36	5.6	1.51	0.024

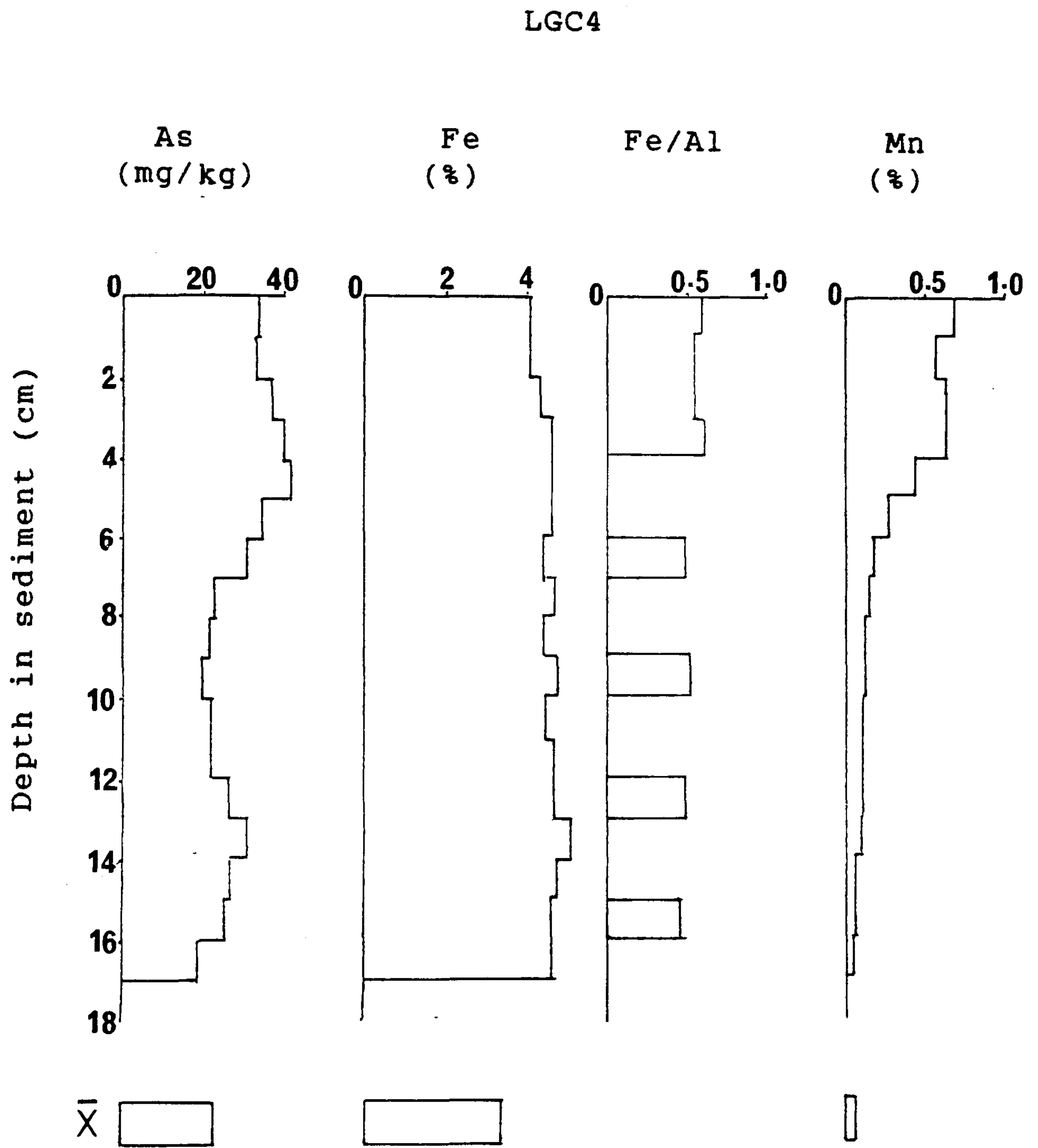


Figure 5.14 Concentration/depth sediment profiles of arsenic, iron, iron/aluminium ratio and manganese in sediment core LGC4 with corresponding 'average' levels (X̄) at depth in LGG7.

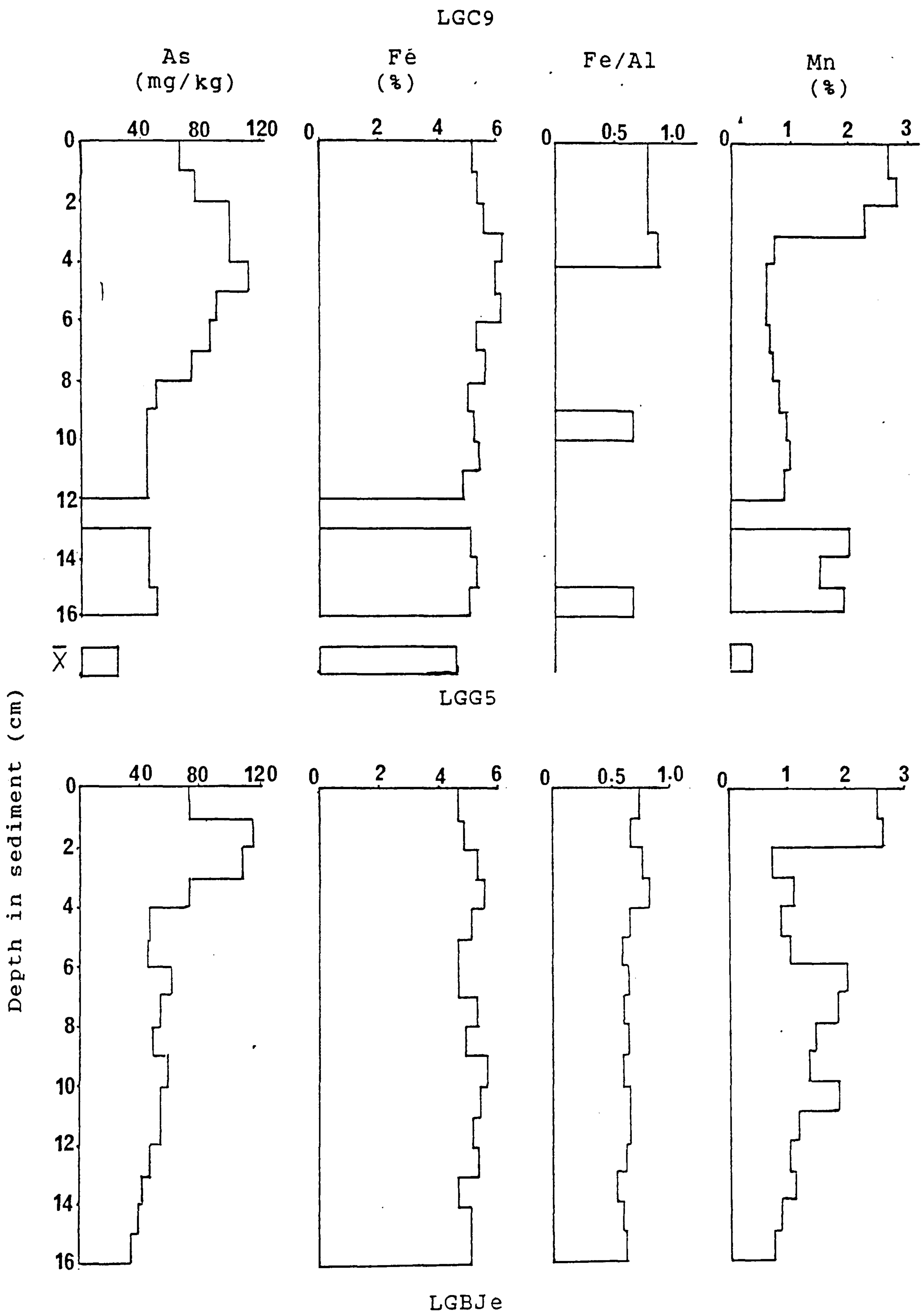


Figure 5.15 Concentration/depth sediment profiles of arsenic, iron, iron/aluminium ratio and manganese in Loch Goil sediment cores LGC9 and LGBJe with corresponding 'average' levels ($\bar{x}$) at depth in LGG5

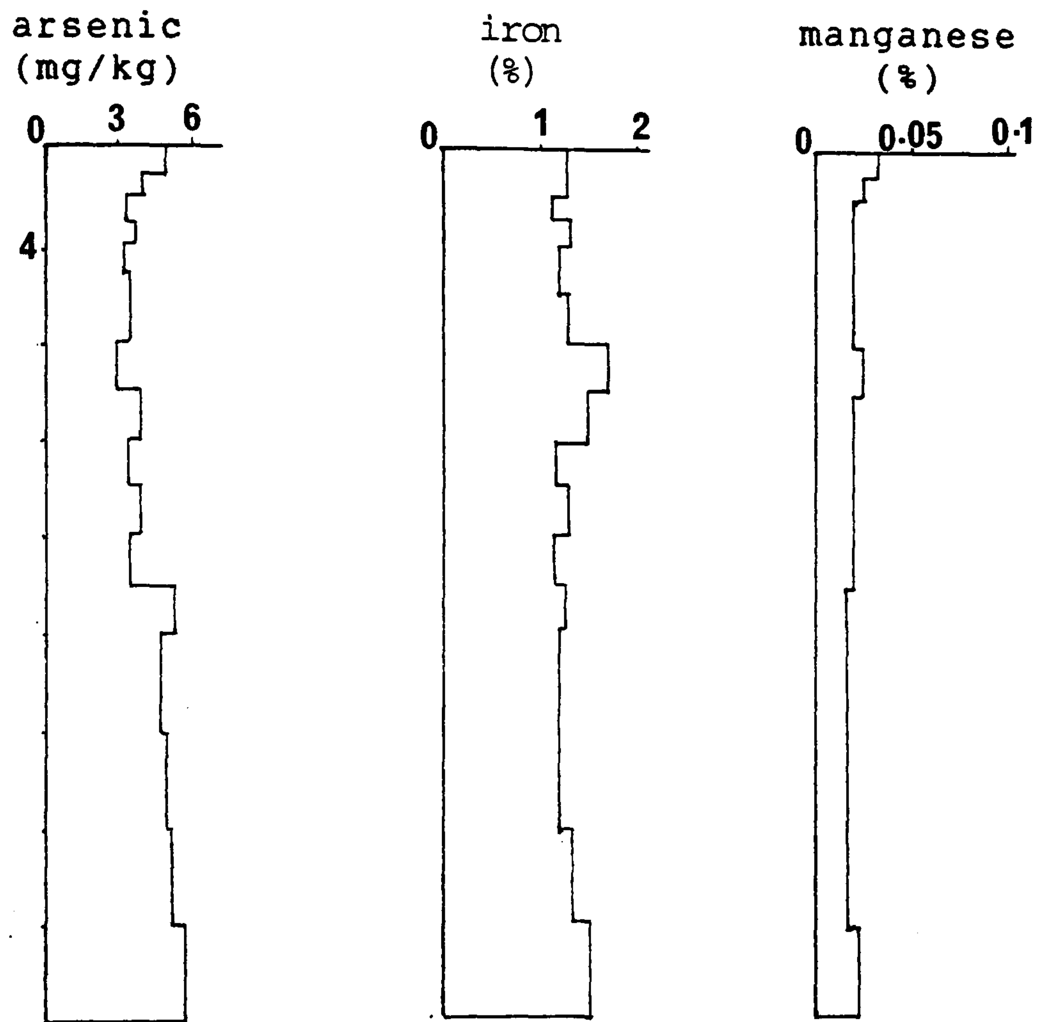


Figure 5.16 concentration/depth sediment profile for arsenic, iron and manganese in the Minch sediment core Knorr - C46.

LGC9 and LGG5 represent Craib surface cores and small diameter (6cm) gravity cores respectively from station 1 in Loch Goil (LG), while LGC4 and LGG7 another core pair from station 2 (Figure 5.1). The Loch Goil core LGBJe was subsampled from a box core collected from the same location as LGC9. The core from the Minch, Knorr C-46, was taken using a large diameter (21.3cm) gravity core (Farmer, 1983).

5.8.2 Discussion

From the sediment cores taken from the two sites in Loch Goil using the Craib corer, Figures 5.14 and 5.15 show that there are indeed sub-surface enhancements in the levels of arsenic (40 and 112mg/kg) and manganese (0.703 and 2.839%) in LGC4 and LGC9 respectively in comparison to background arsenic levels of 21 and 24mg/kg and background manganese levels of 0.062 and 0.363% for LGG7 and LGG5 respectively (Tables 5.10, 5.11).

Once the iron concentrations are normalised to the aluminium content, Figures 5.14 and 5.15 show that there is also a slight elevation for iron in the near surface sediments in cores LGC9 and LG BJe. It is noted that, in agreement with the sedimentary profiles from Loch Lomond, the arsenic/iron peaks are closely correlated, with the manganese peaks placed slightly higher in the sediment profile. However, unlike the sediment/concentration profiles for Loch Lomond, the

peaks are spread over a greater depth, e.g. in LGC9, the arsenic peak is from 2 - 8cm compared with 2 - 4cm in the Loch Lomond core and in LGC4 the manganese peak is between 1 - 5cm compared with 1 - 3cm in Loch Lomond. As in the Dubh Lochan, such differences in the characteristic profiles for the sealochs may be due to differences in (a) sedimentation rates, higher sedimentation rates having a diluting effect on the levels present, (b) the degree of compaction, with greater compaction resulting in narrower enrichment zones, (c) the extent of bioturbation and mixing of the profiles produced, and (d) differences in the redox gradient and pH of the porewaters.

The sediment core taken at the same site as LGC9, LGBJe (Table 5.12 Figure 5.15), shows a similar surface peak for arsenic, iron and manganese with maximum concentrations of 116mg/kg As and 2.59% Mn respectively. However, for the arsenic profile in particular, it is noted that the concentration maximum is found in the 1 - 2 cm section (compared with 4 - 5cm in LGC9) and may be due to the loss of the first few centimetres of the sediment core as result of using the box corer (Farmer, 1983). Similarly, the manganese peak was within the first 2cm of this core compared with at least 3cm in LGC9, again suggesting some loss of the uppermost sediment sections.

Both cores, LGC9 and LGBJe exhibited a secondary manganese peak of approximately 2% at a

depth of 14 - 16cm (Figure 5.15) similar to those observed by Farmer (1983) at the same depth. At depth, the reduced Mn^{2+} ions in solution may well precipitate out in the sediments as Mn carbonates, as suggested by the finding of mixed Mn-Ca carbonates in nearby Loch Fyne sediments (Figure 5.1) (Calvert and Price, 1970).

In contrast to the sediment cores from Loch Goil (with background arsenic concentrations of 20mg/kg and 24mg/kg for LGG7 and LGG5 respectively) arsenic levels for Knorr C-46 from the Minch were very much lower with an average concentration of 4.2 $\pm$ 0.8mg/kg and a maximum of only 5.7mg/kg in the upper most section (Table 5.13, Figure 5.16). Manganese concentrations, similarly were much lower at an average of 0.0223% (223mg/kg) with a very slight near surface enhancement of 0.0279% over a background of 0.0200% below 10cm. Although the levels of iron were lower than in the Loch Goil core, they were of similar magnitude with an average of 1.35 $\pm$ 0.14%. The lower concentrations in the Minch are thought to be due to the coarser grained sandier nature of the sediments, reflected in the lower aluminium levels and higher silicon levels (Farmer, 1983), and hence a different geological source material from Loch Goil or Loch Lomond.

Despite the peak arsenic zones in LGC4, LGC9 and LGBJe being more elongated than those observed in

the Loch Lomond core, their pronounced subsurface nature and comparatively limited vertical extent differentiates them from the profiles for the pollutant metals lead, zinc, copper and chromium in the same cores (Farmer, 1983). The elevated profiles for these elements characteristically originated from a depth greater than 15cm and continued to the surface 0 - 1cm section, reflecting continued anthropogenic metal contamination via the overlying water. The similar background concentrations of arsenic and manganese in Loch Goil (As 22mg/kg, Mn 0.363%) and Loch Lomond (As 45mg/kg, Mn 0.213%) reflect their common source area in the form of the Dalradian mica schists of the surrounding catchment area. As with Loch Lomond, the runoff from the surrounding rural catchment area is unlikely to make a significant direct unmodified contribution to the elevated arsenic and manganese concentrations found in the near surface sediments of Loch Goil. Thus, although past direct input of arsenic from discharges into the River Clyde and Clyde Estuary cannot be ruled out, the common nature of the enrichment profiles for arsenic, iron and manganese found in the sediments of the sealochs suggests that arsenic is predominantly controlled by a similar diagenetic remobilisation mechanism as found for freshwater Loch Lomond.

Some workers however, have suggested that surface sediment enrichment could be the result of

'scavenging' of arsenic from the overlying water column. By chemical extraction of the sediments from the North Atlantic ocean, Neal et al. (1979) showed arsenic to be associated with iron in the same proportion as found in ferromanganese nodules, and suggested that the arsenic was 'scavenged' from the seawater by iron phases. A strong correlation between arsenic and iron in deep sea ferromanganese nodules was also found by Calvert and Price, (1970, 1977) and similarly attributed to adsorption from seawater by hydrous ferric oxides or associated with the formation of ferric phosphate. Peterson and Carpenter (1983, 1986) reported a strong correlation between solid phase arsenic and manganese concentration maxima in surficial sediments of the Washington coast and the Saanich Inlet in Vancouver, which they postulated was due either to 1) intense scavenging of the overlying water by manganese or iron rich phases or 2) to solid phase arsenic dissolution at depth followed by upward diffusion and coprecipitation with manganese or iron oxides at or near the sediment/water interface. The finding of porewater arsenic concentration maxima, 10 - 60 times greater than in the overlying water, at depth and below the solid phase arsenic/manganese maxima, suggested the release of arsenic from the sediments to the porewaters in response to a decrease in the redox potential, with a rapid drop off indicative of solid

phase incorporation in the near surface sections. While differences in the arsenic content of upper sediments from LGC4 and LGC9/LGBJe could perhaps be explained in terms of scavenging from the overlying water, (especially as LGC9/LGBJe were taken from a deep water site possibly more conducive to nodule formation (Calvert and Price, 1970)), the similarity of trends in arsenic/iron and manganese sedimentary profiles between the sealochs and Loch Lomond, where it has been shown via porewater analysis that diagenetic remobilisation is a major factor, strongly suggests that the latter phenomenon is of considerable significance in the sealoch sediments. Detailed direct porewater analysis would clearly aid resolution of the various removal, uptake, redistribution and remobilisation mechanisms potentially involved.

5.8.3 Conclusions

1) Near-surface enrichments in the concentrations of arsenic (115mg/kg), iron (6.36%) and manganese (2.839%) were found in the sediments of the sealoch, Loch Goil.

2) The close association, and vertical displacement from the manganese peak, of the arsenic/iron concentration peaks, their sub-surface nature and limited vertical penetration (unlike the concentration/depth

profiles for the pollutant metals) are similar to those found in Loch Lomond. This suggests that the arsenic profiles are strongly influenced by the same post-depositional diagenetic remobilisation processes, although alternative, and possibly coexisting phenomena associated with ferromanganese nodule formation at the sediment/water interface cannot be ruled out in the absence of detailed porewater information.

APPENDICES AND REFERENCES

APPENDIX 1.1

INORGANIC ARSENIC CONCENTRATION IN 3 ml FRACTIONS FROM THE DISTILLATION OF NBS ORCHARD LEAVES SRM 1571

FIRST FIFTY ml ADDED

Vial No.	Fraction (ml)	Vol.of 3ml used (ul)	ng measured	ug As in 3ml	cumulative ug
1	3	50	23.2	1.40	1.40
2	6	50	6.4	0.38	1.78
3	9	100	5.8	0.17	1.95
4	12	250	10.2	0.12	2.07
5	15	250	6.4	0.08	2.15
6	18	500	9.6	0.06	2.21
7	21	500	5.6	0.03	2.24
8	24	1000	7.8	0.02	2.26
9	27	1000	7.0	0.02	2.28
10	30	1000	5.8	0.02	2.30
11	33	1000	5.4	0.02	2.32
		SECOND FIFTY ml ADDED			
12	36	1ml	5.1	0.02	2.34
13	39	1	4.9	0.01	2.35
14	42	1	4.6	0.01	2.36
15	45	1	4.3	0.01	2.37
16	48	1	4.0	0.01	2.38
17	51	1	3.4	0.01	2.39
18	54	2	3.0	0.005	2.395
19	57	3	2.5	0.003	2.398
20	60	3	N.D	N.D	2.398
21	70	10	N.D	N.D	2.398
Washings			N.D		2.398

N.D. = less than 1ng

APPENDIX 1.2

INORGANIC ARSENIC CONCENTRATION IN 3ml FRACTIONS AFTER THE DISTILLATION OF PRAWN TISSUE

FIRST FIFTY ml ADDED

Vial No.	Fraction (ml)	Vol. of 3ml used (ml)	ng measured	ng As. in 3 ml	cumulative ng
1	3	3.0	5.0	5.0	5.0
2	6	1.0	1.5	4.5	9.5
3	9	3.0	3.3	3.3	12.8
4	12	3.0	3.3	3.3	16.1
5	15	3.0	3.5	3.5	19.6
6	18	3.0	3.7	3.7	23.3
7	21	3.0	3.8	3.8	27.1
8	24	3.0	4.5	4.5	31.6
9	27	3.0	5.0	5.0	36.6
10	30	3.0	4.8	4.8	42.4
11	33	3.0	8.8	8.8	51.2
SECOND FIFTY ml ADDED					
12	36	3.0	7.8	7.8	59.0
13	39	3.0	3.3	3.3	62.3
14	42	3.0	3.0	3.0	65.3
15	45	3.0	3.0	3.0	68.3
16	48	3.0	2.0	2.0	70.3
17	51	3.0	1.5	1.5	71.8
18	54	3.0	1.3	1.3	73.1
19	57	3.0	1.0	1.0	74.1
20	72	15.0	N.D	N.D	74.1
21	81	9.0	N.D	N.D	74.1
Washings				2.5	76.6

N.D. = less than 1ng

APPENDIX 2

THE INORGANIC ARSENIC CONTENT OF A SELECTED NUMBER OF BOTTLED MINERAL WATERS

MINERAL WATER**	COUNTRY	As CONC. (ug/l)
Vichy Célestins	France	220 ± 15
Vichy St. Yore	France	26
Volvic	France	18
Badoit*	France	3.3
Highland Spring	U.K.	3.1
San Pellegrino	Italy	1.4
Prewetts*	W. Germany	1.1
Brecon Mountain Spring	U.K.	0.7
Dorset Spring	U.K.	0.35
Malvern	U.K.	0.3
Champney's Pure Chiltern*	U.K.	0.3
Sainsbury's Cwm Dale	U.K.	0.3
Ashbourne	U.K.	0.25
Buxton Spring Water	U.K.	0.25
Evian	France	0.25
Perrier*	France	0.25
Benderloch	U.K.	0.2
Contrex	France	0.2
Mont-Clair	France	0.2
Spa-Marie Henriette*	Belgium	0.15
Isabelle	France	0.1
St. Leger	France	0.1
Spa-Reine Spring	Belgium	0.05

* Labelled as carbonated or sparkling

** One bottle of each mineral water was sampled, except for Vichy Célestins (3 bottles).

APPENDIX 3.1

THE USE OF URINARY CREATININE CONCENTRATION TO CORRECT FOR THE EFFECT OF SAMPLE VOLUME

Creatinine concentrations have been used by other workers when examining the urinary output of other elements. Creatinine is a waste product derived from creatine and is excreted by the kidneys. Creatine itself is synthesised in the liver and pancreas from three amino acids, arginine, glycine and methionine, and is diffused into the muscles where it becomes phosphorylated. It therefore acts as a reservoir of high energy readily convertible to ATP in the muscles and other tissues. Creatinine is removed from the plasma by glomerular filtration and then excreted in the urine without being reabsorbed by the tubules - resulting in a relatively high clearance rate, (Duarte, 1980). The validity of using creatinine correction is based on the assumption that in a normal subject under steady state conditions, creatinine is released from the muscle stores at a constant rate throughout the day.

APPENDIX 3.2

DETERMINATION OF URINARY CREATININE CONCENTRATIONS USING THE JAFFE REACTION

Analysis of the creatinine concentration (mmol) in urine samples was carried out at the Royal Infirmary, Glasgow using a technique based upon the the precipitation of creatinine with picric acid, first noted by Jaffe in 1886. In alkaline solution, creatinine forms a yellow-orange precipitate with picric acid. The concentration of the coloured precipitate (measured spectrophotometrically at a wavelength of 510nm) formed over a certain reaction time is a measure of the creatinine concentration. Concentrations in mmol were then converted into g/l to be used in the correction of the urinary arsenic concentrations.

APPENDIX 4

STUDENT'S 't' TEST

The Student's 't' test is used to determine whether a statistically significant difference exists between two sample means. The test requires that the two sets of data approximate a normal Gaussian distribution, that the sample size (n) and the variance (S^2) are similar for the two groups. Using the formula below, the null hypothesis that no significant difference exists between the means is tested, using a two-tailed test with a significance level of 5% (i.e. the probability that the null hypothesis is correct is no greater than five chances in 100).

$$t = \frac{X_1 - X_2}{\sqrt{\frac{(S_1)^2}{n_1 - 1} + \frac{(S_2)^2}{n_2 - 1}}}$$

where

t = Student's 't'

X_1 and X_2 = mean values for each sample population

S_1 and S_2 = standard deviation for each sample population

n_1 and n_2 = number of observations in each sample population

$$(n_1-1) + (n_2-1) = \text{degrees of freedom}$$

Thus, if the calculated value for Student's 't' using the above is greater than the critical value of 't' obtained from a set of tables using the appropriate degrees of freedom and significance level, the null hypothesis is rejected and the alternative hypothesis accepted i.e. that there is a significant difference between the two sample means.

APPENDIX 5

ANALYSIS OF ADDITIONAL URINE SAMPLES FROM THE UNIVERSITY ELECTRONICS AND ELECTRICAL ENGINEERING DEPARTMENT

	ARSENIC CONCENTRATION		
	DIRECT HYDRIDE	TOTAL	
SAMPLE No.	(ug/l)	(ug/l)	ug/g creat.
1	6 - 10	206	*
2	7 - 11	74	*
3	5 - 6	56	*
4	8 - 12	44	*
5	5 - 7	41	*
6	9 - 14	38	*
7	4 - 7	37	*
8	5 - 7	30	*
9	3 - 5	23	*
10	1 - 2	23	*
11	3 - 5	21	*
12	3 - 5	21	*
13	1 - 2	13	*
14	2 - 5	12	8
15	2 - 4	12	8
16	5 - 8	11	*
17	2 - 5	11	14
18	1 - 3	10	13
19	1 - 3	9	8
20	1 - 3	8	6
21	2 - 5	7	6
22	3 - 4	3	*
23	1 - 2	2	*

* no creatinine data available

APPENDIX 6

DERIVATION OF pE FROM THERMODYNAMIC EQUILIBRIUM EQUATIONS

For the equilibrium between arsenite and arsenate

$$H_3AsO_3^0 + H_2O \rightleftharpoons H_2AsO_4^- + 3H^+ + 2e^-$$

$$K = \frac{[H_2AsO_4^-][H^+]^3[e^-]^2}{[H_3AsO_3][H_2O]}$$

$$\log K + \log[H_3AsO_3] + \log[H_2O] = \log[H_2AsO_4^-] + 3\log[H^+] + 2\log[e^-]$$

$$2\log[e^-] = \log K + \log\left[\frac{[H_3AsO_3]}{[H_2AsO_4^-]}\right] - 3\log[H^+] + \log[H_2O]$$

$$\log[e^-] = 1/2\log K + 1/2\log\left[\frac{[H_3AsO_3]}{[H_2AsO_4^-]}\right] - 3/2\log[H^+] + 1/2\log[H_2O]$$

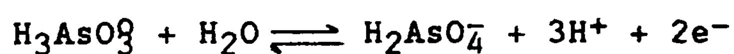
$$-\log[e^-] = -1/2\log K - 1/2\log\left[\frac{[H_3AsO_3]}{[H_2AsO_4^-]}\right] + 3/2\log[H^+] + 0$$

$$pE = 1/2pK - 1/2\log\left[\frac{[H3AsO3]}{[H_2AsO_4^-]}\right] - 3/2pH$$

APPENDIX 7

DERIVATION OF pK FROM GIBBS FREE ENERGY VALUES FOR ARSENITE/ARSENATE EQUILIBRIUM REACTIONS

For the equilibrium between arsenite and arsenate:



$$H_3AsO_3^0 + H_2O \rightleftharpoons H_2AsO_4^- + 3H^+ + 2e^-$$

ΔG_f° -154.4 -56.69 -181 Kcal mol^{-1}

$\Delta G = +30.09$

using: $\Delta G = -RT\ln K$

$$30090 = -2.303 \times 1.986 \times 298 \log K$$

$$-\log K = \frac{30090}{2.303 \times 1.986 \times 298}$$

$$pK = 22.07$$

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