

Evolution of chemical contaminant and toxicology studies, part 2 - case studies of Selenium and Arsenic

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ABSTRACT

As the second of a two part series discussing the evolution of the field of environmental toxicology, this paper presents two case studies: selenium and arsenic. Developments over several decades in the understanding of the behaviour of arsenic and selenium in different chemical forms in various compartments of the environment are discussed. Selenium was initially thought to be toxic, but later investigations showed it to be an essential micronutrient with a variety of biochemical functions, and, importantly, that there is a very narrow gap between the essential and the toxic body burden. Arsenic, on the other hand, has not yet had an essential role established, but enjoys an interesting and notorious history of usage. Arsenic contamination of the drinking water supplies for many millions of people has been a major catalyst for much research into understanding arsenic chemistry in aquifer systems and also arsenic metabolism and toxicity. The relationships between chemical form, bioavailability, toxicity and metabolism of these two semi-metals are being established, especially with use of sophisticated and sensitive analytical instrumentation and biochemical techniques.

Keywords: Environmental toxicology, Selenium, Arsenic

1 INTRODUCTION

This paper is the second of two papers. Part 1 (Jolley *et al.* 2003), provided an overview of the evolution of the field of environmental toxicology with particular focus on the impact of analytical technique development. This paper addresses two case studies of interest in environmental toxicology, selenium and arsenic. Arsenic is of enormous concern in drinking waters in many parts of the world especially the Indian subcontinent and South East Asia (Smedley and Kinniburgh, 2002), and high arsenic concentrations have been also been reported in shellfish and sediments from Suva Harbour, Fiji (Naidu and Morrison, 1994). Selenium is also an environmental contaminant; however, there is a complete lack of Se data published for the South Pacific region.

Arsenic and selenium are presented together in this paper for two main reasons: firstly, these are areas of current research interest in our laboratories, and secondly these semi-metals illustrate well the development of environmental toxicology. As described in this paper, various threads of developing knowledge of these elements in terms of toxic effects, geological occurrence, mineralogical and chemical behaviour, analytical determination, biochemical significance, industrial and agricultural application, slowly drew together over the years. Developments in each area came in fits and starts, sometimes depending on concomitant developments in some other area, especially that of analysis. Today the threads are significantly closer and stronger giving the environmental toxicologist knowledge from many disciplines with which to progress. Hence, in this paper, the environmental distribution, prevalence and ecological

importance of selenium and arsenic will be described in combination with a brief summary of the research directions with respect to environmental toxicity.

Trace metals are ubiquitous in the environment and are mobilised within the environment by a combination of natural and anthropogenic activities. Activities such as weathering, manufacturing processes, and industrial and human waste disposal commonly mobilize metals. Metals released into the environment may enter biological systems through dietary or passive ingestion. Within biological systems, trace metals often have dual roles; they can act as essential micronutrients; for example, playing a crucial role in metalloproteins, or they may have no biological function and be toxic in minute quantities. All trace metals are toxic at elevated concentrations; for example, copper is essential for the synthesis of haemoglobin, but in excess will result in microcytic anaemia, and cobalt is found in vitamin B₁₂, yet causes cardiomyopathy when in excess. The toxic effects of essential trace nutrients are demonstrated by the dose-response relationship for metal micronutrients (Wright and Welbourne, 2002). This relationship proceeds through deficiency, sufficiency and toxicity with increasing metal concentration (Figure 1), and the toxic effects are biological species, organism health, chemical species and dose dependent (for example, respiratory distress, immune dysfunction, cardiomyopathy).

For many metal micronutrients, the borderline between a beneficial dose and one that is potentially toxic is difficult to determine. This may be because: the chemical forms of the metals that are the most bioavailable (assimilated into the biological system) are unknown; the

metabolites of these metals have not yet been identified; the action / effect of many of these chemical species (or metabolites) within biological systems is poorly understood; and the response to the chemical dose may

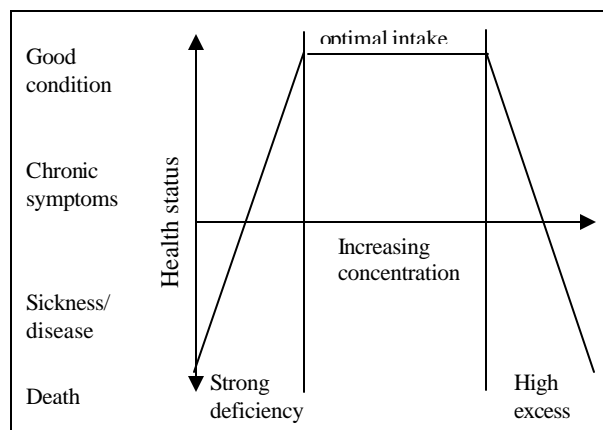


Figure 1 The general dose/response graph relating health response to ingested essential trace elements required for various metabolic functions (from Siegel, 2002).

vary between individuals, populations and species. The bioavailability, metabolic role and biological response to various metal species are the focus of many environmental toxicity studies. Two of the metals under significant current investigation are selenium (Se) and arsenic (As). Selenium is a trace nutrient whose biological role as an antioxidant is well established (Hatfield, 2001). Arsenic, however, currently has no known physiological role in humans, but has been historically used for its medicinal properties (Przygoda *et al.* 2001). Both of these elements may accumulate to high concentrations in abiotic and biological systems, but high “total metal” concentrations of As and Se correlate poorly with toxic effects. Toxicity is governed by the bioavailability of the chemical species, and the mechanisms by which the individual organisms metabolise these metal species (for example, ability or inability to metabolise and/or excrete the metals).

2 CASE STUDY 1 – SELENIUM

Selenium (Se). belongs to Group 16 period 4 of the Periodic Table, possessing chemical and physical properties that are between non-metals and metals (Haygarth, 1994). Selenium is widely distributed in the earth's crust in concentrations between 0.05 and 0.09 mg.kg⁻¹ and is the 69th most abundant element. Igneous rocks contain small amounts of selenium (often <0.2 mg.kg⁻¹); metamorphic rocks vary greatly, but Se is relatively high in carbonaceous metamorphic rocks (0.1-24 mg.kg⁻¹); sedimentary rocks are generally the highest in selenium, although these too vary widely (0.1-675 mg.kg⁻¹).

Selenium is rarely present in any environmental materials at concentrations greater than 500 mg.kg⁻¹. It is often associated with sulfur-containing minerals or as selenides of silver, copper, lead, mercury, nickel or other

metals (Alexander *et al.* 1988; Greenwood and Earnshaw, 1984).

Ground waters generally have low concentrations of Se (<0.05 µg.L⁻¹) because of the insoluble basic ferric selenite found in sediments, sedimentary rocks and shales, but higher concentrations are occasionally observed because of ground water contributions from seleniferous regions (40 µg.L⁻¹) (Cutter, 1989; Haygarth, 1994). The Se concentrations in surface waters are generally low (0.01 – 0.76 µg.L⁻¹) and influenced by pH, with lower Se in alkaline regions (Fishbein, 1983). Of the large quantities of Se reaching open water, the majority is removed from the water column by precipitation and is incorporated into marine sediment (Cooper *et al.* 1974). Marine sediment selenium concentrations lie within the range of 0.1 to 2 mg.kg⁻¹ (Cutter, 1989), and sequential chemical extraction of these sediments have revealed that the Se is mostly associated with the organic (biogenic) fraction released from the sediment following a 2 h leach with 30% hydrogen peroxide in 0.02M nitric acid at 90°C (Tessier *et al.* 1979). Marine Se is mobilised in a variety of pathways including uptake in marine organisms (assimilation and adsorption), sediment transfer to land, and volatilisation to the atmosphere through the biomethylation of inorganic Se into volatile forms, *ie.*, selenate → selenite → selenide → selenocysteine → selenomethionine → dimethylselenide gas (Terry and Zayed, 1998; Förstner, 1979). Selenium concentrations in unpolluted open ocean waters are reported at below 1 µg.L⁻¹ (Cutter, 1989).

The Australian and New Zealand Guidelines for Fresh and Marine Water Quality give the freshwater total Se trigger value at 5 µg.L⁻¹ for 99% level of species protection (ANZECC, 2000). No trigger values have been established for sediment quality or for marine ecosystems because of the absence of relevant research. The current drinking water guideline for Se in Australia is 10 µg.L⁻¹ (NHMRC, 1996).

Australian soils typically have low Se concentrations, hence under natural conditions Australian marine organisms are not exposed to Se and tend to have low Se concentrations (Maher and Batley, 1990). Greater than 80% of Australia's population lives on the coastal plains of the East, with industry located on major estuaries (Yapp, 1986). The coastal population is exposed to major sources of aquatic Se through oil combustion, coal-fired power stations, sewage effluent (Nriagu, 1989; Maher and Batley, 1990), and consumer products such as antidandruff shampoo and antifungal creams (Johnson, 1976; Alexander *et al.* 1988; Haygarth, 1994). Most of these sources eventually feed into estuarine environments in wastewater, soil through-flow and atmospheric deposition. Consequently, coastal marine organisms are often exposed to Se concentrations not normally found in their environment.

2.1 THE BIOLOGICAL ROLES AND TOXICITY OF SELENIUM

Selenium lies directly below sulfur (S) in the Periodic Table and these two elements possess similar chemical properties which allows them to regularly interchange in

many compounds. The physiological effect of this exchange depends on the compound; in some cases there is no functional change, while in others, a unique molecule is created. In the past few decades, the exchange of these elements in biological molecules has received a lot of attention, particularly with respect to amino acids. Both S and Se exist naturally in amino acids. Se is found in selenocysteine (SeC) and selenomethionine (SeMet.) substituting the S in cysteine and methionine for Se. In biological systems there are also low molecular weight Se metabolites (e.g., hydrogen selenide, methylselenol, and seleno-diglutathione), which arise from ingested Se. The presence of Se in biological systems is essential for growth and wellbeing, but at high concentrations, it is a biological toxin.

The discovery of Se amino acids caused an expansion in Se research (biochemical, molecular and genetic), but Se has not always enjoyed its current popularity. In the 1930s and 1940s Se was believed to be carcinogenic having no beneficial biological function. Only a small number of researchers were interested in Se, attempting to unravel its biochemistry and metabolism based on its mechanisms of toxicity and excretion. In the late 1950s when Schwarz and Foltz (1957). identified Se as an essential nutrient, the focus shifted to elucidate normal Se biochemistry. Several Se deficiency disorders were identified, including white muscle disease in sheep (Muth *et al.* 1958) and mulberry heart disease in pigs. In the early 1970s, Chinese researchers identified the first major human Se deficiency disease as a childhood cardiomyopathy (Keshan disease). (Chinese Medical Association, 1979). The deficiencies were attributed to a lack of beneficial seleno-compounds.

It was not until 1973 that the first groups of functional selenoproteins were identified as glutathione peroxidase in mammals (Flohé *et al.* 1973; Rotruck *et al.* 1973). and formate dehydrogenase and glycine reductase in bacteria (Andreesen & Ljungdahl, 1973; Turner & Stadtman, 1973). This confirmed Se as an essential nutrient and indicated a role in defense against oxidative injury. It was another decade before a second mammalian selenoprotein was identified as selenoprotein P (SeP) (Motsenbocker & Tappel, 1982).

A selenoprotein is defined as a functional protein containing Se-amino acids. It is the SeC-based selenoproteins that have been identified as the biomolecules active against oxidative injury. Currently, 21 selenoproteins have been identified in mammals and bacteria, 18 of which have biological functions attributed to them (Mostert, 2000). Selenoprotein P (SeP) is one of the most well documented selenoproteins, but its exact function is still unknown. SeP is highly conserved in bacteria, mammals and vertebrate fish (Tujebajeva *et al.* 2000) with a primary sequence (high in selenocysteine, histidines and cysteines) that suggests a function in heavy metal binding/chelation. SeP has been found to complex with Hg, Ag, Cd, Zn and Ni (Yoneda & Suzuki, 1997; Sasaku & Suzuki, 1998; Yan & Barrett 1998; Mostert *et al.* 1998), which supports earlier reports of Se-detoxifying the effects of Pd, Hg and Cd in man and marine mammals

(Kosta *et al.* 1975; Hodson *et al.* 1984; Pelletier, 1985; Osman *et al.* 1998).

Biological functions of Se compounds have been linked with mammalian health, and the dietary intake of Se has been found to protect against:

- human Se deficiency diseases, e.g., Keshan disease and myxedematous cretinism (Coppinger & Diamond, 2001);
- chemopreventive agent reduced risk of cancer or precancerous lesions., through their defense against oxidative damage (glutathione peroxidases), redox reduction (the thioredoxin reductases) and hormone regulation metabolism (iodothyronine 5'-deiodinases) (Combs & Lü, 20010);
- immune dysfunction (deficiency has demonstrated an increase in viral pathogenesis) (Beck, 2001) and aging (aging cells accumulate oxidative damage, often from UV-radiation, which among other things effects immune suppressive cytokine release) (McKenzie *et al.* 2001);
- HIV-1 progression and mortality (as HIV advances to AIDS, the prevalence of Se deficiency increases) (Baum *et al.* 2001); and,
- male infertility (Se deficiency impairs fertility in rats, mice and boars) (Flohé *et al.* 2001)

There is a close relationship between Se status and human health. Researchers have identified that selenoproteins exert the physiological effects of Se, but do not know whether the beneficial effects of dietary Se originate from the ingestion of selenoproteins or low molecular weight Se compounds. This is one of the driving forces in current Se research, particularly as Se has dual properties (essential and toxic) which are separated by a very narrow concentration range: there is only one order of magnitude between a dose of Se being essential and toxic (Rousseau *et al.* 1993).

Nutritional bioavailability, toxicity and cancer chemopreventive activity studies of Se compounds all show that the biological effects of Se depend on the chemical form administered (Quijano *et al.* 2000). This supports previous studies by Sayato *et al.* (1993), who reported that Se toxicity in mice, as LD₅₀, decreased from selenite 3.5 mg.kg⁻¹. > selenomethionine (4.3 mg.kg⁻¹) > selenate (5.5 mg.kg⁻¹) >> selenocysteine (20 mg.kg⁻¹).

The biochemical basis for selenium toxicity has been summarized by Lemly (2002). as a simple but important flaw in the process of protein synthesis due to the chemical and physical similarities between selenium and sulfur. When available in sufficient concentrations, selenium becomes erroneously substituted for sulfur and forms a triselenium linkage (Se-Se-Se) or a selenodisulfide linkage (S-Se-S), both of which prevent the formation of the essential disulfide chemical bonds (S-S) This results in dysfunctional proteins and disturbs normal cellular biochemistry.

Although many of the mechanisms and effects of selenium toxicity are known (e.g., Lemly, 2002; Spallholz and Hoffman, 2002), the actual dose of the different Se compounds that induces toxicity in various organisms is not known. Se toxicity testing, as opposed to selenium speciation analyses, in organisms other than mammals has

only recently commenced, with the first marine organism publications appearing in 2001 (e.g., Takayanagi, 2001; Hyne *et al.* 2002. Hyne *et al.* 2002) showed that juvenile amphipods (*Corophium* sp.) were five times more sensitive to dissolved Se over a 10 day exposure than the adults, and that dissolved seleno-amino acids (seleno-L-methionine and seleno- DL-cystine) were more toxic than inorganic selenate and selenite.

The area of selenium ecotoxicology is still in its infancy. Very little research has been published, and there are still many unanswered question. The current toxicity research has not addressed the complications associated with environmental toxicities, such as latency periods of certain chemical compounds, and the synergistic or antagonistic effects of other elements/compounds (e.g., the mechanism of selenium's ability to reduce methyl mercury toxicity) (Ganter *et al.* 1974). The effects of changing environmental physical and chemical conditions on Se toxicity are also unknown, and require urgent attention. Many of these issues are also evident in the next case study, arsenic ecotoxicology.

3 CASE STUDY 2 – ARSENIC

Arsenic (As) belongs to Group 15 period 4 of the Periodic Table, possessing chemical and physical properties that are between non-metals and metals. Arsenic is widely distributed in the earth's crust, with a mean crustal concentration of 3 mg.kg⁻¹ (Thornton and Farago, 1997). Arsenic occurs in over 200 minerals, as arsenate, arsenite, sulfide, arsenides, oxides and silicates (WHO, 2001). The most common arsenic containing minerals are the sulfides realgar AsS, orpiment As₂S₃, and arsenopyrite FeAsS. Arsenic also appears commonly with iron pyrites, galena, chalcopyrite. Uncontaminated soils contain 1–20 mg.kg⁻¹, influenced principally by parent rock material (Smedley and Kinniburgh, 2002; Sandberg and Allen, 1975). Marine and freshwater sediments vary more because these materials represent an accumulation of arsenic from overlying waters. Uncontaminated sediments are usually of the order of 10 mg.kg⁻¹ (Smedley and Kinniburgh, 2002).

Arsenic concentrations found in uncontaminated natural waters range from below 0.005 to 10 µg.L⁻¹, but this may rise to 100 – 5000 µg.L⁻¹ in groundwaters as a result of the geochemical environment and hydrogeology (Smedley and Kinniburgh, 2002). The Australian and New Zealand Guidelines for Fresh and Marine Water Quality give the trigger value for freshwater for As(III) for 99% level of species protection of 1 µg.L⁻¹ and for As(V) of 0.8 µg.L⁻¹ (ANZECC, 2000).

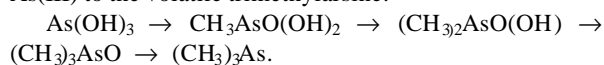
The current drinking water guideline for arsenic in Australia is 0.007 mg/L (NHMRC, 1996). Internationally, guidelines and limits are currently in a state of change (Thomas *et al.* 2001; Smedley and Kinniburgh, 2002). Following the accumulation of considerable evidence of chronic effects from As in drinking waters, many regulatory authorities are reducing the permitted level of arsenic in drinking water. The WHO guideline was provisionally reduced from 50 µg / L to 10 µg / L in 1993. While many authorities in developing countries seek to

follow WHO there are still limitations in the availability of adequate testing facilities.

The many and varied uses of arsenic have a history dating back to the time of the ancient Greeks. There is a strange dichotomy in the use of arsenicals from apparently benign where various arsenicals were used in tonics (Przygoda *et al.* 2001), to practical as pesticide application (Woolson, 1975), to malicious or homicidal. In fact, this element in various forms is recognised almost universally as toxic and the word arsenic is synonymous with poison. However, arsenic trioxide as a 1% solution with potassium carbonate and a little tincture of lavender was used as a tonic (Fowler's solution) from the early 19th century for more than 100 years. This usage has come full cycle with recent reports of remission of acute promyelocytic leukemia following administration of arsenic trioxide (Roboz *et al.* 2000). However, the toxic effects of As are well documented and commonly observed. The deleterious health effects of long-term ingestion of arsenic from contaminated drinking water are now seen in many parts of the world, e.g., China, Bangladesh, India, Chile, Argentina (Smedley and Kinniburgh, 2002). In the Ganges delta alone, this is estimated to affect 40 million people and has been described as the largest mass poisoning in history.

Industrial use of various arsenic compounds developed from the late 17th century and includes use as insecticides, soil sterilants and defoliants. In the 1960s the use of inorganic arsenicals gave way to the use of methylated compounds as herbicides (Hiltbold, 1975). Arsenic compounds have also been used as feed additives for poultry and pigs, and as wood preservatives, desiccants and fungicides (Calvert, 1975; Woolson, 1975). Agricultural use is declining, currently arsenic finds use in metallurgical applications and in electronic materials.

Investigations into the toxic behaviour of arsenic species began over one hundred years ago. In the late 19th century, Gosio demonstrated the formation of volatile compounds of arsenic from arsenic trioxide. The identity of the gaseous compound remained unknown until Challenger and coworkers made investigations into the biological methylation of arsenic in the 1930s by exposing *Scopulariopsis brevicaulis* to inorganic and various alkylated arsenic compounds. The identity of the volatile compound as trimethylarsine was established by melting point determination of an arsine derivative. Challenger proposed a sequential methylation pathway from inorganic As(III) to the volatile trimethylarsine:



By using ethylarsonic acid as an arsenic source and isolating ethyldimethylarsine as the only product with no mixed alkylated products, Challenger demonstrated that the methylation occurred sequentially, with no loss of alkyl groups already present (Cox, 1975).

Investigations into the form and toxicity of arsenic were carried out as long ago as the 1930s when Coulson *et al.* (in Woolson, 1975). fed rats with shrimps containing As in an undetermined form. A second population of rats was fed As in the form of As₂O₃ at the same dietary concentration. Coulson reported the shrimp fed rats

excreted the 98% of the ingested arsenic in 4 days, whereas the As_2O_3 fed rats excreted only 21% of the As taken in, and As liver concentrations were 20 times higher in the As_2O_3 fed rats than in the shrimp fed rats. At that stage the forms of the arsenic in the shrimp and in the excreted urine were unknown.

Over the next few decades till the late 1960s various reports of arsenic contamination or accumulation were made, including in groundwaters associated with arsenical pyrites, in some marine organisms, aquatic plants, algae and in some freshwater invertebrates. At this stage most results were reported in terms of total arsenic found. Attempts at various methods of speciation were made in order to determine the species present, ascertain toxicity of the various forms, and determine pathways of arsenic metabolism (Woolson, 1975).

Separations of different arsenic compounds were developed based on a variety of chemical and physical properties (Talmi and Feldman, 1975). Boiling point separation of cold trapped, NaBH_4 generated arsines was used to determine arsenate (As(V), arsenite (As(III)), monomethylarsonic acid (MMAs(V) and dimethylarsinic acid (DMAs(V) in various materials (Braman and Foreback, 1973). Various methods using synthesis of volatile arsenic derivatives suitable for GC were also developed (Talmi and Bostick, 1975). Further development of chromatographic methods based on ion exchange for separation of As(V), MMAs(V) and DMAs(V) were reported (Yamamoto, 1975). With the advent of differential pulse polarography (voltammetry) hydride generation AAS and graphite furnace AAS, greater sensitivity was also achieved (Iverson *et al.* 1979). The analytical techniques now available allowed some progress in the elucidation of arsenic behaviour. However, there remained a scarcity of information and the need for the determination of active molecular forms of arsenic in every environmental compartment (Brinkmann *et al.* 1977).

In aerated fresh and marine waters, arsenic is present mainly in the form of inorganic arsenate As(V), as the ions HAsO_4^{2-} and H_2AsO_4^- . In zones of lower redox / anoxia or high productivity inorganic arsenite As(III), present as the nonionic H_3AsO_3 , can constitute a major portion of the total arsenic. Redox potential is controlled by the major elements, O, C, N, S, and minor components respond slowly, especially as many of the reactions involved are heterogeneous. In interstitial waters of lake sediments, arsenic was found to be >90% As(III) (Aggett and Kriegman, 1988). Organic arsenic compounds have been reported in marine and estuarine waters, but are usually a minor component of the total arsenic present (Smedley and Kinniburgh, 2002).

The binding of arsenic to various sediment phases, especially forms of iron oxyhydroxides, was extensively investigated through the 1980s, both with in lab surface adsorption studies (Pierce and Moore, 1982) and by analysis of sediments (de Vitre *et al.* 1991). Vertical profiling of total arsenic in sediment cores was found to demonstrate surface enrichment and suggested redox sensitive mobilization of the deposited arsenic following diagenetic solubilization of iron oxyhydroxides (Aggett

and O'Brien, 1985). There are numerous reports of the release of arsenic (and phosphate) from below the 'oxic' zone of sediments (Smedley and Kinniburgh, 2002). In anoxic conditions where sulfate reduction has occurred, arsenic is found to be associated with iron sulfides (Moore *et al.* 1988). Surface analytical investigations of the binding of arsenic to specific phases have been reported (Fendorf *et al.* 1997), but generally the concentration of arsenic in sediments is too low for application of these techniques. Further characterization of the binding of arsenic in sediments has been made via a variety of selective extraction schemes, where the nature of the binding is operationally defined (Manning and Martens, 1997; Keon *et al.* 2001). By the 1990s many different organic compounds of arsenic had been shown to occur in various biological organisms, however the forms of arsenic most often found in water or sediment remained thus far the four: As (V), As (III), monomethylarsenate and dimethylarsinate (Manning and Martens, 1997).

Groundwater environments with elevated levels of arsenic have been much investigated especially in response to chronic arsenicism encountered in many parts of the world due to potable use of these waters. Smedley and Kinniburgh (2002), in their extensive review of sources, behaviour and distribution of arsenic in natural waters have discussed common factors related to aquifer chemistry and hydrogeology associated with elevated levels of arsenic. Two distinct 'trigger' situations are described in which arsenic is released from the solid phase into groundwaters; a high pH (>8.5) condition in arid/semi-arid areas, leading to desorption of arsenic, and a second strongly reducing condition at near neutral pH, where arsenic is desorbed from mineral oxyhydroxides and released from iron and manganese oxyhydroxides on their reductive dissolution. Smedley and Kinniburgh call for theoretical modelling of these complex dissolution and surface reactions, and laboratory studies to establish the sequence of chemical events and the kinetics and stoichiometry of release. Concomitant with understanding the release mechanisms, is the need to understand how the hydrogeology of the aquifer affects the movement of arsenic. There is high variability in arsenic groundwater concentrations found in high arsenic areas. The occurrence of high concentrations of arsenic in groundwater appears to depend on the combination of a number of critical factors: the appropriate release chemistry (the trigger conditions described above), the age of the aquifer material (areas of young sedimentary material may not yet have been flushed of arsenic), and the nature of the groundwater flow (in areas of low flow dissolved arsenic accumulation in the aquifer is possible). Thus, on a local scale, small variations in relief and in drainage and mixing patterns can lead to the development of volumes of water with a range of different arsenic concentrations, demonstrating that predicting possible arsenic contamination of the groundwater of a particular area is difficult.

Over the past three decades many investigations into metabolism of arsenic have been carried out. Arsenic is listed as a carcinogen, and worldwide observations yield strong epidemiological evidence. Long-term exposure has

been associated with increased prevalence of many forms of cancers (Thomas *et al.* 2001). Much work has been based on epidemiological studies related to the consumption of contaminated drinking water and the associated health effects (Tseng *et al.* 1968, Saha *et al.* 1999). These cases involve ingestion of arsenic in the form of inorganic As(V) and As(III). These inorganic forms undergo methylation within the body and the methylated forms are excreted in the urine. Thus methylation has been considered a detoxification process. Methylation pathways have been described, based on the analysis of metabolic products in urine (Vahter *et al.* 2000; Le *et al.* 2000) and the isolation of specific enzymes (Lin *et al.* 2002). The pathway essentially involves reduction of As(V) to As(III), followed by a cycle of oxidative methylation and glutathione mediated reduction to yield first MMAs(V), then MMAs(III), and finally DMAs(V). However, it appears that some species of primates lack specific methyltransferase enzymes and other pathways of detoxification probably exist which may involve a form of protein binding (Wildfang *et al.* 2001).

The toxicity of arsenic relates to the behaviour of the various arsenicals disrupting normal metabolic processes. The toxicity of As (III) appears to relate in part to its reactivity with protein sulfhydryl groups, blocking the activity of enzymes by doing so. The action of As(V) relates to the interference of this anion in the role of phosphate in metabolic processes. The mode of toxic action of organoarsenicals is still under investigation and part of the difficulty lies in selection of suitable animal models to use for toxicity studies (Wang *et al.* 2002). Another difficulty lies in the complex array of arsenic metabolites formed in the body and in sorting out which of these actually induces a toxic action. Recent studies have raised the issue of whether the methylation pathway is in fact a detoxification pathway. Some of the various intermediates formed, which have been determined in urine, notably MMAs(III), are now reported to be of greater toxicity (cytotoxic and genotoxic, than the precursor inorganic As(III) (Petrick *et al.* 2000).

The recent use of mass spectral methods with electrospray ionization (ESI-MS) allows the characterization and identification of organoarsenicals. For example these methods have been used to identify various arsenosugars and methylated arsenosugars extracted from brown algae (Gong *et al.* 2002). These powerful techniques will be of much use in characterizing the form of arsenic in many biological media, especially arseno – protein moieties. This information will contribute much to determination of the various pathways involved in the metabolism of arsenic, especially in terms of understanding the mode of toxicity of the various arsenicals and how some appear to have beneficial effects. Biogeochemical cycling of arsenic will also be better characterized.

For continuing toxicological studies, two analytical issues are of note. There remain organoarsenic compounds separated by chromatography and detected as containing arsenic, but as yet unidentified (Francesconi *et al.* 2002). With the use of techniques such as ESI – MS the structure of these compounds can now be established. A second

and related issue also tests the analytical skills. Although it is now possible to analyse materials for several different compounds of arsenic, studies need to be undertaken to ensure that the process of extraction and analysis does not result in incomplete recovery of, or chemical changes to, the arsenic compounds present.

With respect to the continuing consumption of arsenic in drinking water, there are specific development needs that must be acknowledged. Analytical methods suitable for rapid on-site determinations are needed for assessment of drinking water supplies in rural communities in developing countries. Such field test kits are under development (Kroll, 2001; Kinniburgh and Kosmus, 2002). Most important for the supply of uncontaminated water to the millions of people affected, is the development of simple easily constructed treatment systems for removal of arsenic from drinking water (Khan *et al.* 2000).

4 CONCLUDING REMARKS

For most metals the relationship between chemical form and biological availability is poorly understood, which is clearly demonstrated by these case studies for selenium and arsenic. Once assimilated, physiological effects of these metal species is further complicated by the synergistic and antagonistic effects of other biomolecules and ions, metabolic variations (for example, age and gender), and physico-chemical conditions (such as pH temperature, redox condition). The information that is available for metal species is focused on mammalian systems, primarily human toxicology. There is a significant lack of published information on the environmental toxicity of various metal species, particularly for the marine environment. The lack of literature may be due to analytical instrumentation limitations, as addressed in Part 1 (Jolley *et al.* 2003). For example the spectral interferences caused by high chloride concentrations when using inductively coupled plasma mass spectrometer (ICP-MS) quantification. Many scientists may also be awaiting the publication of more literature before commencing research in this discipline. Irrespective of the reason, there is an urgent need for further research on the toxic effects of metals in the environment.

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