

Speciation and absolute bioavailability: risk assessment of arsenic-contaminated sites in a residential suburb in Canberra†

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Watson is a fully developed suburb of some 30 years in Canberra (the capital city of Australia). A plunge dip using arsenical pesticides for tick control was operated there between 1946 and 1960. Chemical investigations revealed that many soil samples obtained from the study area contained levels of arsenic exceeding the current health-based investigation levels of 100 mg kg⁻¹ set by the National Health and Medical Research Council in Australia. For the speciation study, nine composite samples of surface and sub-surface soils and a composite sample of rocks were selected. ICP-MS analysis showed that arsenic levels in these samples ranged from 32 to 1597 mg kg⁻¹. Chemical speciation of arsenic showed that the arsenite (trivalent) components were 0.32–56% in the soil and 44.8% in the rock composite samples. Using a rat model, the absolute bioavailability of these contaminated soils relative to As³⁺ or As⁵⁺ ranged from 1.02 to 9.87% and 0.26 to 2.98%, respectively. An attempt was made to develop a suitable leachate test as an index of bioavailability. However, the results indicated that there was no significant correlation between the bioavailability and leachates using neutral pH water or 1 M HCl. Our results indicate that speciation is highly significant for the interpretation of bioavailability and risk assessment data; the bioavailable fractions of arsenic in soils from Watson are small and therefore the health impact upon the environment and humans due to this element is limited.

Keywords: arsenic; metal speciation; bioavailability; contaminated sites; risk assessment

Watson is a suburb in Canberra (the capital city of Australia), approximately 6 km from the central business district. The study site is located near the north-eastern corner of Watson, where a plunge dip used arsenical pesticides for tick control between 1946 and 1960.

Chemical investigations revealed that many soil samples obtained from the study site contained elevated levels of arsenic and lead. The current National Health and Medical Research Council (NHMRC) health-based investigation levels for arsenic have been set at 100 mg kg⁻¹ in the soil. A thorough health risk assessment including biological monitoring in residents has been carried out. However, this paper concerns only the arsenic contamination of the soil related to its speciation and absolute bioavailability.

A gossan which can be encountered throughout the Australian Capital Territory (ACT) and the south-eastern region of Australia has also been identified in the Watson study area.

Gossans are geological formations formed by the weathering zones of sulfide mineralisation and are known sources of arsenic and base metals. Therefore, the major source of arsenic at Watson is likely to be naturally occurring within the rocks and soil. There is also likely to be arsenic in the soil in some sections of the study area as a result of earlier use as a sheep dip. For risk assessment purposes, the potential harmful effects of arsenic upon the environment, including the human health and ecological risk, should be assessed in the same manner, regardless whether the source of this element is natural or of anthropogenic origin.

Labile metal species are considered to be more biologically active than non-labile fractions. There are a number of tests for availability of toxic materials from single components and from mixtures,^{1,2,3} which are generally based on the assumption that greater solubility enhances bioavailability. The metal distribution value can usually be obtained using sequential extraction procedures, and several leaching schemes have been proposed and widely adopted. However, such measures seldom give anything other than qualitative guidance on the likely uptake by organisms. Quantitative data should be obtained based on actual measures of uptake of toxins, for example in animals or humans.

The bioavailability of arsenic from contaminated land has been assessed using small mammals⁴ including deer mice, meadow voles, dogs⁵ and more typically guinea pigs or rabbits.⁶ The last work refers to the term 'absolute bioavailability study', which involves orally dosing rabbits with the soil and keeping them in metabolic cages with multiple samples of 24 h urine collection over a period of days. The area under the plot of arsenic excretion against time is then compared with that for animals given an intravenous injection of the equivalent amount of sodium arsenate to derive the absolute bioavailability. Although researchers acknowledge the importance of obtaining metal speciation data at contaminated sites, and understand that the toxicology of an element is very much dependent on its chemical species, there are few reports covering both metal speciation and bioavailability data of contaminated soils in the same study.

As part of an environmental health risk assessment in Watson, we set out to measure the chemical speciation of arsenic in the contaminated soil, to determine the solubility of arsenic in aqueous solutions at pH 7.0 and 1.0 and to determine the bioavailability of As by animal experimentation. The correlation of solubility and bioavailability of As in these soils was also examined.

Experimental

Soil treatment

Nine composite soil samples (C1–C9) of surface and sub-surface soils and one rock composite sample (C10) from sites

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containing a range of low to high levels of arsenic were randomly selected. Most samples were obtained from residential properties, except C6, which was from a community park reserve. C1–C5 were obtained in close proximity to the plunge dip site.

All soil samples had pre-treatment to ensure optimum homogeneity for purposes of chemical analysis and animal dosing, and were dried in a vacuum oven at 40 °C for at least 24 h, cooled, then ground to < 50 µm. Composite samples were made up by weighing equal portions of individual soils and mixing thoroughly in a screw-capped plastic jar. The composite samples were stored in these jars at room temperature until analysis or animal dosing.

Loose soil particles on the rocks were brushed off and washed in water. The rocks were sonicated and rinsed in water until the rinse water was clear, and given a final rinse in water purified with a Milli-Q system (Millipore, Bedford, MA, USA) before drying and grinding as above.

Arsenic speciation

The determination of the oxidation state the arsenic in the soil was based on a recently published method⁷ with slight modifications. Briefly, a 5 g sample of soil/rock (< 50 µm particle size) was extracted three times with 20 ml of 10 M hydrochloric acid and the supernatant was filtered and diluted to 100 ml. For trivalent arsenic, a 10 ml aliquot of this arsenic extract (AE) with 80 ml of 10 M hydrochloric acid was extracted with chloroform (4 × 10 ml). The arsenic in the combined chloroform extract was then back-extracted as AsCl₃ into water (3 × 15 ml) and diluted to 100 ml before ICP-MS or ETAAS analysis.

For total arsenic, a 10 ml aliquot of AE and 10 ml of 50% potassium iodide was heated in a water-bath for 30 min at 60 °C for the conversion of pentavalent to trivalent arsenic. This solution was cooled and diluted to 50 ml. An aliquot of this solution, with 80 ml of 10 M hydrochloric acid, was extracted and analysed in the same manner as for trivalent arsenic.

Animal dosing

Animal experimental protocols were approved by the UAEEC (University of Queensland Animal Experimentation Ethics Committee) and followed the NHMRC animal guidelines. Male Wistar rats, 6 weeks old and weighing 170–190 g, were obtained from the Central Animal Breeding House, University of Queensland. All animals were kept in standard polypropylene laboratory animal cages to acclimatise for 48 h before being placed in individual polycarbonate metabolic cages for 24 h urine collections prior to dosing. Soil samples suspended in 3–4 ml of water were administered orally by gavage to groups of five rats without fasting, at a dose rate of 5.0 or 0.5 mg As kg⁻¹ body mass, depending on the concentrations of arsenic in the soils. The objective was to give the animal as much soil as practically possible (1–2 g) at a given concentration in order to evaluate potential toxic effects. For positive control, groups of four rats were given the equivalent dose of arsenic by intravenous injection of 0.5 mg As kg⁻¹ body mass in the form of a solution of sodium arsenite or sodium arsenate. It is not desirable to dose rats at 5.0 mg As kg⁻¹ body mass by the intravenous route because it is a lethal dose. A negative control group of rats was given 5 ml of water by oral gavage. The dosing protocol is given in Table 1. All animals were given water and commercial rat food diet through special feeders attached to the metabolic cages. Urine samples (24 h) free from faecal contamination were collected daily for 4 d after dosing. The urine volume was recorded and the sample was divided into aliquots in plastic containers and stored at -80 °C prior to creatinine determination and elemental analysis. Rats were

anaesthetised with a mixture of carbon dioxide and oxygen according to an approved UAEEC protocol⁸ and blood samples were collected into lithium heparin tubes (Johns Professional Products, Victoria, Australia) following exsanguination 4 d after dosing for the elemental analysis. Faeces, liver and kidney were also collected for analyses. The results will not be reported here because tissue distribution data are less relevant to this study. All organs of the experimental rats appeared normal at necropsy.

Soil samples was digested in concentrated nitric acid in a CEM MDS2000 microwave-assisted digestion system (CEM, Matthews, NC, USA) based on the manufacturer's recommended procedures. The digest was diluted to contain 2% nitric acid for ICP-MS measurement. Rat urine was diluted in 2% nitric acid without digestion before analysis.

Water and 1 M HCl solubility (leachate)

A 2 g amount of soil / rock (< 50 µm particle size) in 200 ml of neutral Milli-Q-purified water 200 ml of 1 M HCl was extracted in a conical flask by shaking (200 rpm) in an environmental chamber at 25 ± 0.5 °C for 3 d. The extract was centrifuged and filtered (0.45 µm filter membrane) prior to arsenic determination.

Results and discussion

Elemental analysis and speciation

As part of quality control/quality assurance (QC/QA) programme, QC samples were analysed after every 6–10 specimens in a run. The relative standard deviation (RSD) ($n = 20$) of the ICP-MS results using a certified reference standard solution (ICPMO 111-1; EM Science, Gibbstown, NJ, USA) was 5.8%. An in-house rock digest gave an RSD of 3.2% ($n = 6$). When the certified standard was analysed using ICP-OES, the corresponding RSD was 2.5% ($n = 4$). The analytical results for the nine soils and one rock are given in Table 2, the arsenic speciation results are given in Tables 3 and 4 and results for water and 1 M HCl leachates of As in soils are given in Table 5. All results are the means of three readings. Random samples were analysed and validated by independent operators using ICP-MS, ETAAS and hydride generation AAS.

It has been suggested that arsenate (As⁵⁺) is the predominant form of arsenic in aerated soil.⁹ When applied to soil, sodium arsenite is oxidised to arsenate and remains in this form in aerated soil, but when waterlogging and reducing conditions prevail, some reconversion back to arsenite occurs. Arsenic tends to become more tightly bound in soil with time after application, and this depends on the availability of binding sites.

Table 1 Animal experimentation dosing regime for arsenic in soil and control groups

Dosed Group	Dose rate/mg As kg ⁻¹ body mass	Route	No. of rats
<i>Experimental group—</i>			
C1 soil	0.5	Gavage	4
C2 soil	0.5	Gavage	5
C3 soil	0.5	Gavage	5
C4 soil	0.5	Gavage	5
C5 soil	0.5	Gavage	5
C6 soil	0.5	Gavage	5
C7 soil	5.0	Gavage	5
C8 soil	5.0	Gavage	5
C9 soil	5.0	Gavage	5
C10 rock	5.0	Gavage	5
<i>Negative control group</i>	0	Gavage	5
<i>Positive control group—</i>			
Sodium arsenate	0.5	Intravenous	4
Sodium arsenite	0.5	Intravenous	4

In the bound form, As is generally not readily leachable. Leachate tests reported by McDougall¹⁰ conducted on As contaminated soil obtained from a cattle tick dip site in NSW, Australia, using distilled water for the extraction revealed that for a soil containing 1000 mg kg⁻¹ of As, the concentration of the As in the water was only 0.6 mg l⁻¹. This represents a 0.06% solubility and implies that As remains tightly bound to the soil component. It appears likely that little, if any, such As would be biologically available. There are a number of tests for the availability¹⁻³ of toxic metals from contaminated soils, each using dissolution in aqueous media at various pH values as a measure of availability. However such measures are strictly qualitative with respect to the likely uptake by organisms, and

quantitative measures must rely on actual measures of uptake by animals or humans.¹¹

It has been established that inorganic arsenite is more toxic than arsenate.¹² Arsenic can exist in the organic or inorganic form, both of which are naturally occurring in the environment, or may be the product of industrial activities. An important distinction between inorganic and organic arsenic is that exposure to the latter is not generally considered to result in significant health risks.¹³ In addition, the concentrations of dimethyl arsinic acid and monomethyl arsonic acid present in the soil are usually not high enough to be considered a human health risk factor; hence they were not measured in this study. Although volatile arsine, the most toxic form of arsenic, is sometimes formed in very small amounts under suitable conditions, including microbial activity in the soil, it is considered unlikely to be a significant risk factor. In this work, only arsenite (trivalent arsenic) and total arsenic (arsenite + arsenate) were measured in the soil and rock composite samples. The arsenate portion (pentavalent arsenic) of the sample was calculated by subtracting the arsenite from the total arsenic concentration.

Table 2 Total concentrations of As in nine soils (C1–C9) and one rock (C10) obtained from Watson

Soil/rock	[As]/mg kg ⁻¹	Solid/rock	[As]/mg kg ⁻¹
C1	55	C6	121
C2	32	C7	1597
C3	165	C8	867
C4	295	C9	1325
C5	67	C10	435

Table 3 Arsenic speciation results obtained for nine composite soils (C1–C9) and one rock sample (C10)

Sample	Total arsenic/ mg kg ⁻¹	Arsenite (As ³⁺) (%)
C1	55	0.33
C2	32	1.38
C3	165	1.01
C4	295	0.73
C5*	67	56
C6	121	0.32
C7	1597	0.53
C8	867	0.52
C9	1325	0.54
C10	435	44.8

* Arsenic speciation was subsequently determined in eight individual soils of the composite C5 (see Table 4).

Table 4 Arsenic speciation of eight soils in the composite C5

Soil sample	Total arsenic/ mg kg ⁻¹	As ³⁺ /mg kg ⁻¹	Arsenite (As ³⁺) (%)
SS008-A	28.9	19.9	68.7
SS030-A	20.5	18.4	89.7
SS031-A	10.9	5.1	46.7
SS040-A	98.5	16.8	17.1
SS052-A	35.1	30.1	85.7
SS301-A	155.7	6.1	3.9
SS332-A	12.8	10.7	83.8
SS421-A	40.9	10.9	26.7
			Mean 50.5 s 35.9

Table 5 Solubility (%) of As in the neutral pH water leachate and 1M HCl leachate of nine soils (C1–C9) and one rock (C10) obtained from Watson

Sample	Water leachate	1 M HCl leachate
C1	0.48	2.31
C2	<0.13	2.00
C3	<0.13	15.13
C4	0.23	22.00
C5	0.39	13.63
C6	<0.13	1.99
C7	<0.13	45.25
C8	0.24	31.88
C9	1.76	25.75
C10	<0.13	12.00

Bioavailability study

Absolute bioavailability (AB) is a definitive measurement of the bioavailability of a chemical or a drug (arsenic in this case) using an animal or human model. For the calculation of AB, urinary arsenic measured at 0, 24, 48, 72 and 96 h intervals were plotted. The area under each curve (AUC) was calculated using a graphics software package (GraphPad Prism; GraphPad Software, San Diego, CA, USA). The area under the curve for animals dosed with intravenous injection (AUC_{i.v.}) represented an arbitrary value of 100%. The AUC_{oral}, derived for animals dosed by oral gavage, was then used to calculate the AB using the following equation:

$$\%AB(As) = 100AUC_{(oral)}/AUC_{(i.v.)}$$

Total arsenic excretion curves over a 96 h period for rats dosed with soils, compared with the group of rats given a single intravenous injection of sodium arsenite at 0.5 mg As³⁺ kg⁻¹ body mass or 0.5 mg As⁵⁺ kg⁻¹ body mass are illustrated in Fig. 1. The AB values of the various soils were calculated from Fig. 1. The results are given in Table 6.

Animal models^{4-6,14} are generally used to predict the bioavailability of heavy metals. The absorption coefficient for soluble salts of arsenic has been reported to range between 0.70

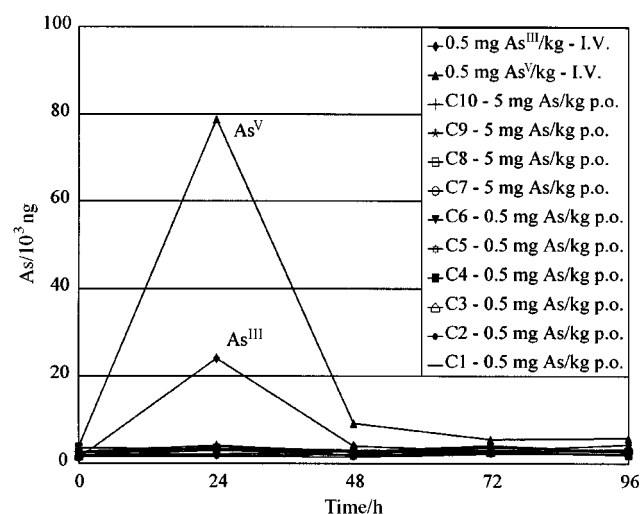


Fig. 1 Bioavailability: urinary As excretion of rats dosed by gavage with soils at a dose rate of 0.5 or 5.0 mg As kg⁻¹ compared with an i.v. injection of sodium arsenate or sodium arsenite at 0.5 mg As kg⁻¹ body mass.

and 0.98.¹⁵ This absorption factor has often been assumed and conservatively taken as 100% biologically available when the worst case scenario is assumed in risk assessment. It has been established that the rat is different from other mammals in that it accumulates arsenic in the blood.^{16,17} In our experience, rats accumulate arsenic in the blood up to 30–60 times more than guinea pigs when given the same amount of sodium arsenate and sodium arsenite, respectively. Therefore, rats could be used as sensitive indicators for body absorption of arsenicals.¹¹ The rat model was chosen for the biological availability study for the reason.¹⁸ A perfect animal model for studies on arsenic toxicity and metabolism has not yet been identified.¹⁹ In this study, the specific aim was to evaluate the bioavailability of arsenic contaminated soil in relation to arsenate and arsenite, and rat can therefore be used for this purpose.

The relevant arsenite and arsenate bioavailability data (AB) were found to have no significant correlation with the water leachate or the 1 M HCl leachate solutions of various soils. The correlation coefficients (r^2) were calculated to be 0.0395 and 0.0341 when compared with water leachate and 0.3026 and 0.3081 when compared with the acid leachate relative to the AB values for arsenite and arsenate, respectively. These results agreed with a previously reported¹⁸ low correlation ($r^2 = 0.54$) between acid leachates and bioavailabilities of As from an anthropogenic origin. Our results suggest that one cannot use merely the leachate data for the prediction of bioavailability of As from these soils. The effect of elevated temperature (for example, at 37 °C, body temperature) on the leachate concentration has not been investigated. However, it is believed that elevated temperature would not have improved the correlation significantly since the extraction regime was a continuous and thorough leaching process over 3 days.

Eight out of ten soils tested in this study contained about 99% of arsenic as arsenate (As^{5+}), the exceptions being C5 and C10. For practical purposes, one could calculate the AB using the As^{5+} curve alone (Fig. 1), and the AB for these samples were found to range from 0.55 to 2.98%, as shown in Table 6. The AB's of C10 were 0.26 and 1.02% relative to arsenate and arsenite, respectively, and those of C5 were 2.98 and 9.58%, respectively. The actual AB for C10 was calculated to be 0.64% (average of 0.26 and 1.02%) and 6.28% (average of 2.98 and 9.58%) for C5, since the As^{3+} concentration was about 50% for both samples (Table 5). The bioavailability of As relative to arsenate in the soil from disused mining areas has been reported as 11%²⁰ and 24%¹⁴ in soil impacted by smelter activities; arsenic speciation was not determined in these studies. These bioavailability results were likely to be under-estimated if there were significant concentrations of arsenite in the soil samples. Our results illustrate the need for and importance of speciation in order to obtain reliable bioavailability data.

It is concluded that traditional risk assessment protocols assuming 100% bioavailability will be over-conservative, which in turn may result in unnecessary and expensive

remediation. The speciation of arsenic is highly relevant in providing meaningful risk assessment data. Our results also substantiate the need for site specific risk assessment. Since the bioavailability of arsenic was relatively low in soils tested in Watson, one could suggest that there would be very limited health impact on the environment and humans in the study area under the current residential usage of the land. This is consistent with the biological monitoring results obtained from 31 residents, whose urinary arsenic levels were below the NHMRC guideline value of 150 $\mu\text{g g}^{-1}$ creatinine. Notwithstanding that the risk for the community in the suburb of Watson is limited, the biological monitoring programme should be continued at government level to safeguard the health status of people who are living at this 'contaminated site'.

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Table 6 Absolute bioavailability (AB) relative to 0.5 mg kg⁻¹ i.v. of As^{5+} or As^{3+}

Sample	Dose mg kg ⁻¹	AB (cf. As^{5+}) (%)	AB (cf. As^{3+}) (%)
C1	0.5	2.31	8.50
C2	0.5	1.27	4.31
C3	0.5	2.68	9.87
C4	0.5	1.44	5.56
C5	0.5	2.98	9.58
C6	0.5	2.46	7.25
C7	5.0	0.55	1.86
C8	5.0	0.59	1.18
C9	5.0	0.67	1.96
C10	5.0	0.26	1.02