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Use of Human Metabolic Studies and Urinary Arsenic Speciation in Assessing Arsenic Exposure

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The use of hair and nail analyses to assess human exposure to the trace metalloid arsenic (As) (Leslie and Smith 1978) is hindered by the possibility of external contamination. Even though urine represents the major excretory route, its use as an indicator of exposure is limited when no distinction is made between the non-toxic organoarsenical (arsenobetaine) excreted following the consumption of seafood and the toxic inorganic forms of As and related metabolites. The development of analytical techniques capable of separating the different chemical species of As in urine have shown that the ingestion of inorganic As (AsV or AsIII) by animals and man triggers an in vivo reduction/methylation process resulting in excretion of the less toxic species, monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) (Vahter and Marafante 1988). A necessary pre-requisite to the full interpretation of urinary As speciation data of exposed groups is to establish the uptake, bio-transformation and elimination patterns reflected in urinary As following carefully controlled experimental exposure.

MATERIALS AND METHODS

Urinary As excretion was determined following the ingestion of seafood As in a single dose and inorganic AsV in single and repeated doses. Volunteers were asked to abstain from any known As consumption prior to and during each experiment. Each collected a pre-24 hr bulk urine sample to establish background levels of urinary As. In the first study, three volunteers ingested wet prawn tissue (13.9 mgAs/Kg) corresponding to a total As consumption of 541 μg(A), 538 μg(B) and 537 μg(C). Distillation of the prawn tissue (Lunde 1973) confirmed that a stable organoarsenical was the major As species present. The consumption of a 1 L bottle of the mineral water Vichy Celestins in the second study by two volunteers constituted a single dose of 220 μg inorganic AsV (Farmer and Johnson 1985).

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In the third study, one volunteer (55 Kg) ingested 100 mL of Vichy Celestins (22 μg AsV) three times a day for 10 days corresponding to regular daily intake of 66 μg AsV, representing 60% of the provisional tolerable daily intake (W.H.O. 1983). Complete urine samples were collected in acid washed poly-propylene bottles, the total volume measured and an aliquot removed for analysis. Samples were not chemically treated but were refrigerated at 4°C or -20°C if longer storage time was required. Each sample (10 mL in duplicate) plus standard reference material (NBS, SRM 2670) and reagent blanks were analysed for total As by hydride generation-atomic absorption spectrometry (HGAAS) following digestion with a 10 mL acid mix nitric:sulphuric:perchloric in the ratio 5:1:3. Individual species, AsV, AsIII, MMAA and DMAA, were separated from untreated urine on a combined cation/anion exchange column (Grabinski 1981) and determined by HGAAS. An inter-comparison study with the Health and Safety Executive in London demonstrated close agreement for species concentrations from the same samples, thus validating the speciation method (Johnson and Farmer 1989).

RESULTS AND DISCUSSION

In accordance with previous studies (Freeman et al. 1979; Foa et al. 1984) the single oral dose of seafood As and inorganic AsV resulted in elevated urinary As concentrations within hours of intake with a subsequent decline and return to background levels several days later (Figures 1 and 2). Just 6 hr after consumption of the seafood, 25% of the dose had been eliminated at an average rate of 23.7 μg/hr, peaking within the first 2-4 hr, with almost 50% of the dose eliminated after only 20 hr. Although elevated above background excretion rates (0.18-0.4 μg/hr), urinary As elimination following the single oral intake of inorganic As was slower than in the first experiment, averaging just 1.93 μg/hr over the first 6 hr with only 5.25% of the dose eliminated. It was not until 16 hr later that 23% of the dose had been eliminated with 50% excreted 54-70 hr after intake. Even after 166 hr only 68.9% and 63.9% of the dose (background corrected) had been eliminated by the two volunteers following the ingestion of inorganic AsV, compared with 83.7%, 70.0% and 80.5% for the three volunteers following the consumption of seafood As.

A two component exponential model for the retention of As with time (Pomroy et al. 1980) showed that almost 50% of the seafood As was excreted with a short lived first component biological half life of 6.9-11.0 hr with a longer second component half life of, on average, 75.7 hr (3.15 d), in close agreement with Buchet et al. (1980) and Yamauchi and Yamamura (1984). A similar evaluation for the second experiment revealed greater arsenic retention with longer first component half lives of 24.1 and 17.7 hr and second component half lives of 7.1 and 8.6 d for A and B respectively.

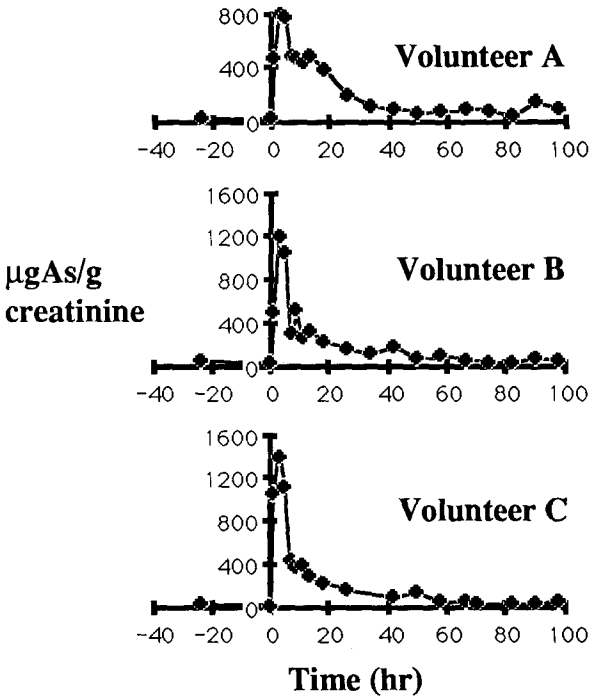

Figure 1. Total urinary arsenic concentration (μg/g creatinine) in three volunteers, A, B and C following the consumption of prawns.

Detailed chemical speciation of the urine samples demonstrated that although the uptake was high in both experiments, the differences in retention observed were probably the result of differences in As metabolism. The levels for the sum of AsV, AsIII, MMAA and DMAA excreted following consumption of the prawns were < 10 μg/L, typical of persons unexposed to these As species (Johnson and Farmer 1989) thus eliminating the possibility of in vivo biotransformation. Although arsenobetaine was not specifically identified in urine of the first metabolic study, the available evidence suggests that it was the major form of arsenic eliminated in urine. In contrast, the second study showed that AsV was rapidly reduced to AsIII and further biotransformed to the methylated metabolites MMAA and DMAA. While AsV and AsIII were the major species excreted over the first few hours following intake of AsV, DMAA rapidly became the predominant species after just 10 hr (Figures 2 and 3). Over 7 days, AsV constituted only 9-10%, AsIII 12-15%, MMAA 9-18% and DMAA 57-69% of eliminated As. The reduction in the MMAA/DMAA ratio with time is attributed to the transformation of MMAA to DMAA, the drop in MMAA production from AsIII and, in the early stages, faster direct excretion of MMAA due to differences in pKa's (MMAA 4.26 and DMAA 6.25) (Buchet et al. 1981a). Inter-individual

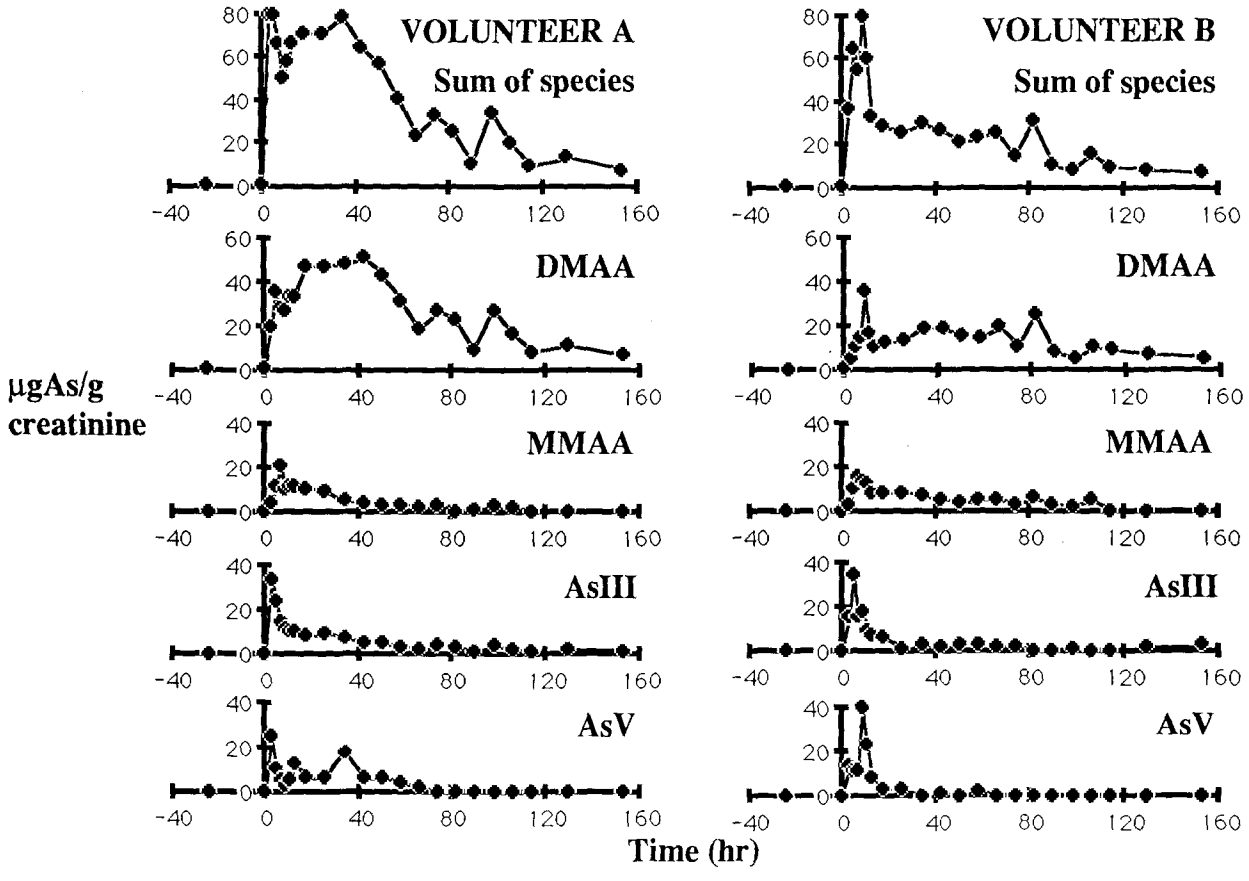

Figure 2. Urinary arsenic (µg/g creatinine) excreted by volunteers A and B following a single oral dose of 220 µg inorganic AsV.

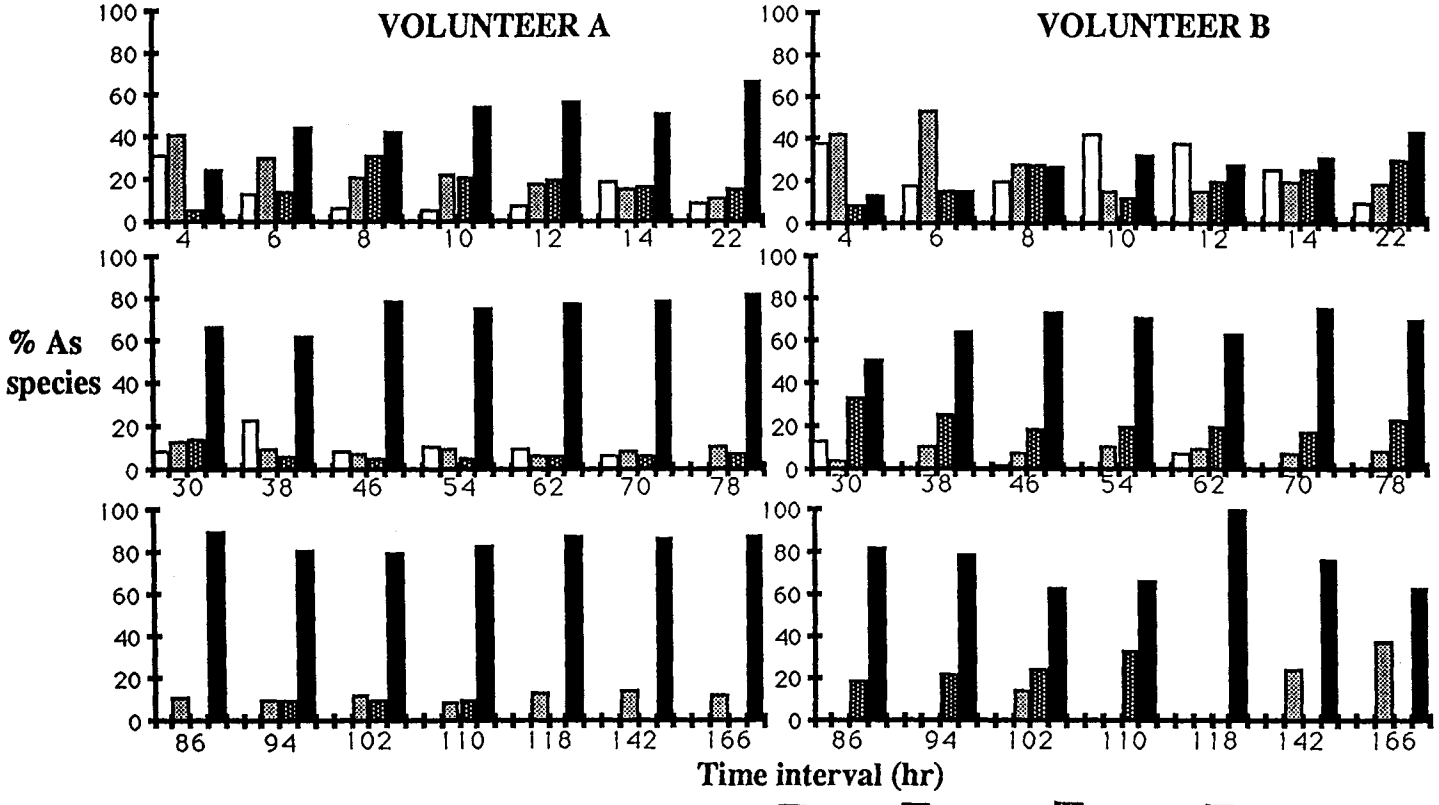

Figure 3. Relative proportions of each arsenic species (AsV □, AsIII ▨, MMAA ▩, DMAA ■) excreted in urine by volunteers A and B following a single oral dose of 220 µg inorganic AsV. Samples were collected over 2 hr intervals from 0-14 hr, 8 hr intervals from 14-118 hr and 24 hr intervals from 118-166 hr.

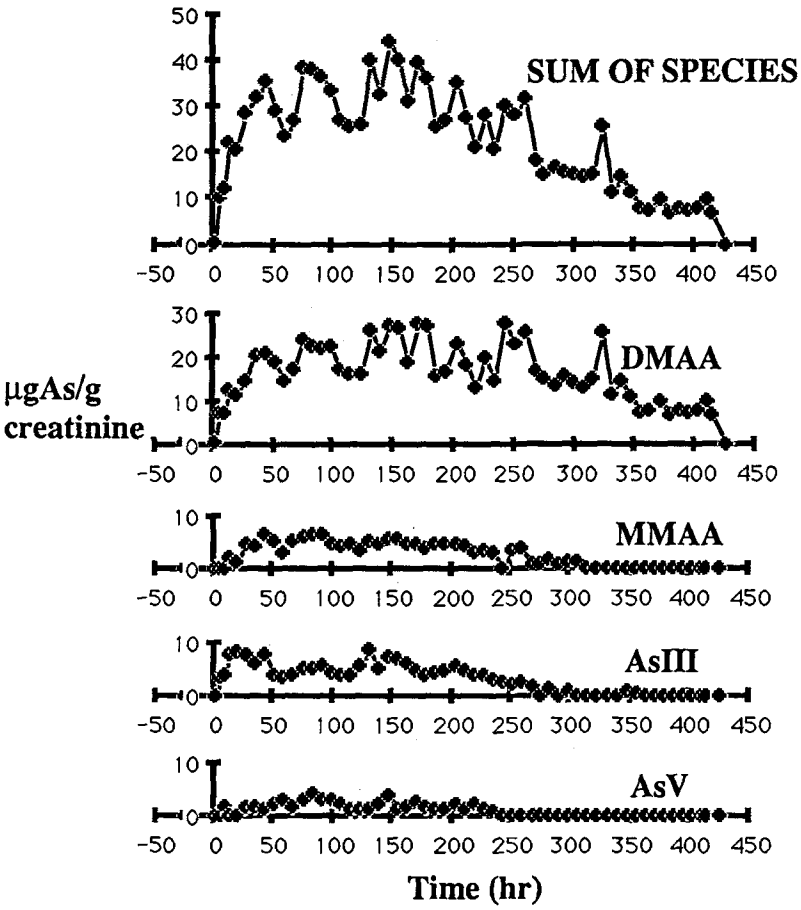

Figure 4. Urinary arsenic excretion (μg/g creatinine) following oral intake of 22 μg inorganic AsV three times a day for ten days by a single volunteer.

differences in speciation pattern, especially in the MMAA/DMAA ratio over the first 22 hr, could be at least partially due to differences in the extent of liver uptake of inorganic As by A and B, AsIII being known to exert an inhibitory effect on the conversion of MMAA to DMAA (Buchet and Lauwerys 1985).

In the third metabolic experiment, urinary As concentrations also increased from a background 3.5 μg/g creatinine to 10.1 ug/g creatinine in 4-8 hr following the intake of just 22 μg AsV. The subsequent intake of 22 μg AsV at regular 8 hr intervals for 10 d was reflected in a fairly constant daily urinary output of 26.7-38.2 μg As (av. 31.6 μg) from day 2 to day 11 (Figure 4).

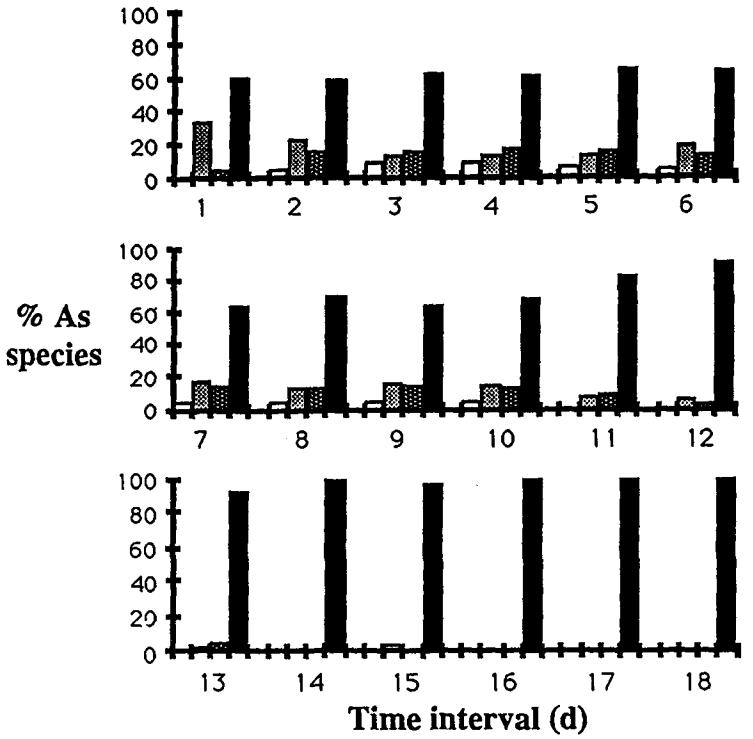

Figure 5. Relative proportions of each arsenic species (AsV □, AsIII ▩, MMAA ▩, DMAA ■) excreted per 24 hr following the daily oral intake of 66 μg AsV for 10 d by a single volunteer.

Assuming 80-100% gastrointestinal absorption and correcting for background excretion (5 μg/d), this corresponds to an average 40-50% of the daily dose, similar to the 60% found by Buchet et al. (1981b). As in the second metabolic study, excretion of arsenic in urine reflected the reduction/methylation mechanisms following the ingestion of AsV. However, unlike the single dose study, repeated exposure to AsV for 10 d established a constant speciation pattern two days after the initial intake, with AsV/AsIII/MMAA/DMAA at 5.8/15.2/14.3/64.7%. One day after removal of the exposure, the speciation pattern changed markedly with DMAA exceeding 80% of the sum of species (Figure 5). In addition, only 48.2% (background corrected) of the dose was recovered over 18 d of this third study compared with 68.9% and 63.9% in 7 d following the intake of 220 μg in a single dose. With insignificant amounts of As recovered in faeces (3.5% and 6.1% of the dose, Tam et al. 1979) and in sweat (Pomroy et al. 1980), it is likely that a significant proportion of the ingested As was retained in the body, probably as rapidly formed AsIII, with its affinity for the sulphydryl groups

of proteins and enzymes, to be eliminated gradually over a longer period of time (Buchet et al. 1981b).

The urinary excretion of As within hours of intake provides a powerful medium for detecting exposure. The ability to differentiate between As excreted as a result of seafood consumption (non-toxic) and that excreted due to exposure to the toxic inorganic species is of utmost importance and essential when assessing potential risk from occupational and/or environmental exposure. The chemical speciation pattern observed can be compared with those obtained from the metabolic studies enabling the time, type and extent of exposure to be determined and hence aid decisions regarding future action.

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