Airborne Arsenic Exposure and Excretion of Methylated Arsenic Compounds

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First void urine samples were collected from copper smelter workers exposed to inorganic arsenic and from unexposed controls. Arsenic compounds (As (III), As (V), methylarsonic acid and dimethylarsinic acid) in these samples were analyzed by selective volatilization as arsines with determination of arsenic by plasma excitation emission spectrometry. On the day preceding the urine sample collection a breathing zone measurement was made of respirable arsenic particulates for each subject. It was found that all of the subjects, including the controls excreted arsenic primarily as methylated species. Approximately 50% of the total arsenic was excreted as dimethylarsinic acid and 20% as methylarsonic acid. Slight differences in the proportion of various arsenic compounds were observed with varying levels of inorganic arsenic exposure. Amounts of arsenic species were all closely correlated with each other and with exposure. Irrespirable particulate exposures were measured on a subset of high exposure workers. Irrespirable arsenic was found to be more closely correlated with excretion of arsenic compounds than with respirable arsenic.

Introduction

There have been no previous reports of excretion of methylated arsenic compounds by industrial workers exposed to airborne arsenic dust because the methods needed to make these determinations have not been available. Data produced by Crecelius and Braman, et al., suggest that biomethylation of ingested arsenic occurs and that the primary excretion product is dimethylarsinic acid (1, 2).

The present study was undertaken to investigate the composition of urinary arsenic excreted by copper smelter workers exposed to inorganic arsenic, predominantly arsenic trioxide, and to examine the relationship between exposure and excretion.

Methods and Procedures

Copper smelter workers with three ranges of arsenic exposure were selected as potential subjects and invited to participate in the study. The three arsenic exposure groups were chosen on the basis of the arsenic exposure according to job and work area characteristics. The high exposure group was drawn from workers in the emissions control areas (baghouse, flue, cottrell, and stack) and from the reverberatory furnace area. The medium exposure workers were all from the converter area, and the low exposure group was taken for workers with no known contact with arsenic. Medium and low exposure groups were chosen such that they were matched to the high arsenic exposure group on age (within 5 years) and cigarette smoking status (present, quit ≥ 6 months previously, or never smoker).

From a total of 85 male workers invited, 82 volunteered. A control group of 43 unexposed male workers (power linemen, clerical, laboratory and copper refinery workers) from a town 200 km from the smelter were selected and also matched to the high exposure smelter workers on age and cigarette smoking status; a total of 41 workers volunteered.

First-void urine samples were collected from all subjects on two consecutive days. All urine was collected in polyethylene bottles without preservatives and divided into two portions: 200 ml was frozen, and the remainder was put in a bottle with 0.5 mg EDTA as a preservative. If the sample was scanty, all was frozen (3% of the total number of samples). The second day's 200 ml sample was fro-
zen on top of the first day to make a pooled sample for arsenic species determination. The frozen samples were analyzed by a pH and temperature selective volatilization of arsines to separate arsenic species and determination of the arsenic by plasma excitation emission spectrometry (2). All species were reported as micrograms per liter of elemental arsenic. The two preserved first-void urine samples were analyzed for total arsenic by a wet oxidation digestion of the sample by nitric, perchloric, and sulfuric acids, followed by arsine volatilization and analysis by the silver diethylthiocarbamate method (3). The two total arsenic determinations from the consecutive urine samples were averaged for each subject to provide an estimate of his total urinary arsenic excretion. All urinary arsenic values were corrected to a specific gravity of 1.018 and reported as the quantity of arsenic.

Personal exposure to airborne arsenic particulate was measured for each subject on one working day, simultaneous with the urine sampling. Each of the subject’s wore a portable battery-powered air pump with a filter cassette attached to his lapel. The filters were 0.8 μm pore-size, cellulose acetate membrane type. A subset of 38 arsenic exposed workers wore a sampling unit which passed the incoming air through a 10 mm. nylon cyclone separator to collect the irrespirable (> 5 μm diameter) particulate (4). The lower portion of the cyclone was covered with a tight film of wax to prevent external contamination of the small cup which retains captured particulates. After sampling, the cyclone was carefully disassembled, and the barrel rinsed with deionized distilled water to remove any captured particulates. The wax film was stripped off the cup and the particulate in the cup removed by rinsing and ultrasonically cleaning the cup with deionized distilled water in a beaker. The filter and the solution with the irrespirable particulate were analyzed separately by digesting them with nitric, perchloric and sulfuric acids, volatilizing the arsenic as arsine and determining the quantity of arsine by the silver diethylthiocarbamate method (3). The digested particulate samples (1% nitric acid solutions) were also analyzed for lead, cadmium, zinc, and antimony by atomic absorption spectroscopy (5). Selenium in the digested urine and particulate samples was also analyzed (6).

The distribution characteristics of the exposure and urine values were tested by D’Agostino’s test for normality (7). Lognormality was evaluated by applying the test to the \( \log_{10} \) values of the measurements. Linear regressions were calculated on the log-normal data by using the \( \log_{10} \) values in the calculations. As a result, the regression equation has the form: \( Y = 10^A X^B \), where \( A \) is the intercept and \( B \) is the slope. Likewise, correlation coefficients were also on \( \log_{10} \) values where the distributions were found to be log-normal. Geometric mean and standard deviations were calculated to describe the log-normal distributions.

**Results**

As expected, there were no significant differences between the four groups with respect to age and cigarette smoking characteristics. The age of the test and control groups averaged 46 and was in the range 26–65. All four groups averaged 20 cigarettes per day and 20 years smoking with 60% present cigarette smokers, 30% past smokers, and 10% never smokers.

The arsenic and heavy metal composition of airborne particulate collected in personal exposure samples is shown in Table 1. All of the metals were found to be log-normally distributed, therefore, the geometric mean and standard deviations were used to summarize the exposure characteristics. Of the control samples, 44% contained no detectable arsenic (<1.2 μg/m³), and the maximum concentration was 12 μg/m³. The control samples also contained no detectable lead or cadmium and only small amounts of zinc and copper. The arsenic exposure groups showed the anticipated gradient in arsenic exposure. The medium arsenic exposure groups had the highest lead, zinc, copper and cadmium levels which probably reflects their work area. No detectable particulate selenium (<1.0 μg/m³) was found in any of the air samples, and SeO₂ vapor was not measured.

| Constituent | Control (n=41) | Low As exposure (n=30) | Medium As exposure (n=23) | High As exposure (n=30) |
|-------------|---------------|----------------------|--------------------------|------------------------|
| Arsenic*    | 3.6 (1.56)    | 8.3 (3.43)           | 46.1 (3.05)              | 52.7 (6.61)            |
| Lead†       | <0.8          | 3.6 (3.57)           | 50.9 (2.44)              | 10.0 (5.16)            |
| Zinc        | 5.6 (1.54)    | 14.0 (3.20)          | 59.2 (2.45)              | 14.8 (3.64)            |
| Copper      | 3.4 (2.34)    | 27.2 (4.10)          | 75.9 (6.05)              | 16.3 (4.83)            |
| Cadmium     | <0.88         | 1.20 (1.71)          | 3.41 (2.28)              | 2.50 (2.64)            |

*All constituent concentrations are expressed as μg/m³ geometric mean (standard deviation).
†Controls had 56.1% of samples less than detectable (<1.2 μg As/m³) and the low group had 20% less than detectable.
‡All control samples were less than detectable (<0.8 μg Pb/m³); 13.3% of the low group, and 8.7% of the medium group were less than detectable.
§All control samples were less than detectable (<0.88 μg Cd/m³); 83.3% of the low group, 26.1% of the medium group, and 56.7% of the high group had less than detectable.

Arsenic excretion by the test groups is shown in Table 2. The majority of arsenic was excreted as
dimethylarsinic acid, and the proportion of arsenic species was independent of exposure level: 9% As (III), 6% As (V), 20% methylarsonic acid, and 65% dimethylarsonic acid. All arsenic species but As (V) showed log-normal distributions, and As (V) was very close to this type of distribution. As a result, the geometric means and standard deviations were used to present this data. Total arsenic excretion and all arsenic species followed the anticipated arsenic exposure gradient. Total urinary arsenic and all arsenic species, except As (V), showed a sixfold increase from the control to the high exposure group. The As (V) showed a threefold increase over the control levels. Total arsenic excretion showed no significant change from the controls to the low exposure group, but all of the arsenic species showed an increase.

Table 2. Concentration of arsenic species in urine samples from test and control groups.

| Urinary species | As (III) control (n=41) | Medium As exposure (n=30) | High As exposure (n=30) |
|-----------------|-------------------------|--------------------------|------------------------|
| As (III)        | 1.3 (1.58)              | 2.2 (2.19)               | 4.8 (2.08)             |
| As (V)          | 1.3 (1.59)              | 1.6 (2.32)               | 2.4 (2.86)             |
| Methylarsonic acid | 3.4 (1.63)          | 4.9 (2.13)               | 9.7 (1.90)             |
| Dimethylarsinic Acid | 11.5 (1.47)     | 17.0 (1.96)              | 32.7 (1.71)            |
| Total urinary arsenic | 21.2 (2.04)    | 24.7 (2.01)              | 51.8 (1.61)            |

*All concentrations are expressed as µg/l of elemental arsenic, geometric mean (standard deviation).

Urinary selenium excretion was found to be similar in all of the test groups and in the controls, 55 µg/l. No significant relationship was found between urinary selenium and any of the urinary arsenic measurement or any of the airborne metal values.

The pairwise correlation coefficients between the concentrations of arsenic species and total arsenic are given in Table 3. All of these measurements were highly correlated with each other. However, As (V) was not as strongly correlated with the other species, as they were with each other.

Since the data in Table 2 suggested that the arsenic species may be more closely related to exposure than total urinary arsenic, regressions were calculated for total airborne arsenic versus total urinary arsenic excretion and for total airborne arsenic exposure versus each of the arsenic species excretion. The regressions were all highly significant, p<0.001. However, the total airborne exposure only explained 27% (r=0.52) of the variability in total urinary arsenic excretion, whereas the regressions for As (III), methylarsonic, and dimethylar-

![Figure 1](image.png)

**Table 3. Correlation matrix for urinary arsenic species and total arsenic.**

|               | As (III) | As (V) |
|---------------|----------|--------|
| Methylarsinic acid | 1.000    | 0.810  |
| Dimethylarsinic acid | 0.908    | 0.673  |
| Total urinary arsenic | 0.764    | 0.604  |

*All correlation coefficients are significant at the p<0.001 level and all contain 124 observations.

This regression was also highly significant, p<0.001, with r=0.68. This regression accounted for 46% of the variability which was a substantial improvement in the degree of fit over that for total urinary arsenic. It should be noted that this relationship included approximately half of the controls, who had detectable airborne arsenic exposure. The figure is a log-log graph, because the distribution of both variables was log-normal. Note the regression equation is a geometric progression.

For a set of 38 arsenic-exposed workers, both respirable and irrespirable particulates were measured. Table 4 shows the distribution of arsenic with respect to particle size for each of the exposure groups. For most of these workers more than half of the airborne arsenic was present in irrespirable particulates, but in some work areas, e.g., the medium exposure group, the majority was respirable. Correlation coefficients were calculated for urinary arsenic excretion with the quantity of arsenic in various
**Discussion**

The findings strongly suggest that inhaled inorganic arsenic [primarily As (III) As$_2$O$_3$] was metabolized to methylated species. This was supported by three major findings. First, the proportion of arsenic species was relatively constant and independent of exposure. Second, the urinary concentrations of all arsenic species were closely related to airborne arsenic. Finally, urinary concentrations of As (III), methylarsonic acid, and dimethylarsinic acid were all substantially better correlated with airborne arsenic than was total urinary arsenic. The later was important because total urinary arsenic includes the small quantities of dietary arsenic, such as the stable organic compounds found in seafood.

Dimethylarsinic acid appears to be a better biological monitor of airborne exposure than total urinary arsenic. It was more sensitive to small changes in exposure at low airborne levels than was total urinary arsenic excretion and dimethylarsinic acid was better correlated overall with exposure. Further, it does not appear to be sensitive to interference from arsenic in seafood. The standard silver diethylthiocarbamate method may be adaptable for routine measurement of dimethylarsinic acid in urine, if the arsenic generation conditions are performed under the proper pH and with sodium borohydride as the reducing agent.

It should be noted that the relationship between total airborne arsenic and arsenic excretion was probably modified by the smelter worker’s use of chemical cartridge respirators to filter their breathing air. As a result, it is likely that the external arsenic concentrations over estimate the quantity of arsenic inhaled, especially at the higher concentrations.

Arsenic (V) showed a significant relationship with exposure but did not show as much increase with exposure as was seen for the other arsenic species. No analyses were performed to determine As (V) in the particulate but it was presumed that most was present as As (III) based on measurements of stack emissions. Some As (V) may also have been injected with the diet. Another postulated source of As (V) may have been oxidation of a portion of the As (III) within the body. The data suggest that approximately 5% of the total arsenic excreted by the exposed workers may have been converted to As (V), if it is assumed that all of the As (V) excreted by the controls was from dietary sources. Further research will be needed to clarify this question.

Selenium and arsenic are biologically antagonistic (8), and it has been postulated by Frost that excessive arsenic intake depletes body stores of selenium and susceptibility to cancer is increased because the protective effect of selenium is lost (9, 10). Urinary selenium was virtually identical for the controls and all three exposure groups, approximately 55 μg/l. Since there was no reason to suspect that the arsenic exposed subjects had altered their intake of selenium to compensate for an arsenic effect, it appears that arsenic exposure at the levels monitored in this population had no detectable effect on selenium excretion. Therefore, if arsenic exposure does alter the selenium balance it must occur at higher levels of arsenic exposure than were observed in this test population.

A significant difference in the strength of the relationship between arsenic excretion and arsenic ex-
posure was found according to particle size. Although both respirable and irrespirable arsenic were strongly correlated with arsenic excretion, the relationship between irrespirable arsenic and excretion was much stronger. This was attributed to two factors. First, the irrespirable particles are more efficiently captured by the respiratory system than are the respirable: about 100% capture versus about 60%, respectively. Even though a large portion of the irrespirable particles are transported into the gastrointestinal system, there is no reason to believe that As$_2$O$_3$ is less readily absorbed there than in the respiratory system. Second, the majority of workers tested had more than half of their total arsenic exposure via irrespirable particles. As a result, the dose of arsenic received was substantially larger for irrespirable than respirable dust; and, therefore, it is reasonable that irrespirable arsenic be better correlated with excretion than the respirable. The implication of this finding is irrespirable dust exposure maybe an important source of exposure for toxic agents with no significant difference between gastrointestinal absorption and respiratory absorption.

In conclusion, it was found that inorganic arsenic can apparently be biomethylated, principally to dimethylarsinic acid. The question is raised as to what would take place for workers exposed to arsenic in the form of dimethylarsinic acid, calcodylic acid, as in the case of forestry workers. This question can only be answered by examining persons exposed to calcodylic acid and determining the urinary composition of arsenic species.

The authors gratefully acknowledge the support of the Smelter Environmental Research Association and the Anaconda Company whose support made this work possible and those individuals who contributed their time and thoughts: George McArthur, Scott Walker, Larry Kresan, Charlie O'Donald, Ken Nelson and Mike Varner.

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