Determination of In Vitro Bioaccessible Arsenic in Soils and Slags from Weston Solutions, Inc.

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Summary of Results for the First Set of Soils and Slags

November 1, 2002

METHODS

Sample Processing

Soils and soil/slag mixtures were sieved as received to pass a 2.0 mm screen to obtain approximately a 100 g subsample. Sieved subsamples (< 2.0 mm) were oven-dried at 70 ^{0}C for 12 h and sieved to pass a 250 μm screen. Slags were placed in a zip-lock bag and pulverized with a two pound sludge hammer to pass a 2.0 mm screen. Pulverized slags that passed a 2.0 mm sieve were oven-dried at 70 ^{0}C for 12 h and ground using a mortar and pestle to pass a 250 μm screen.

Determination of Total Metal Content

Total metal content in soils and slags were determined by acid digestion using microwave (CEM MDS 2100, Matthews, North Carolina, USA) according to U.S. EPA Method 3051. Soil (0.5 g) was digested by microwave (100% power, temperature 175 ^{0}C, 19 min total run time; 4.5 min time at temperature) in 10.0 ml of a 1:1 mixture of HNO_3:H_2O and diluted with deionized distilled water to a final volume of 25.0 ml. Triplicate analyses were conducted on all soils and slags. Digested soils and slags were syringe filtered through 0.45 μm filters and elemental analysis was conducted using a high resolution Thermo Jarrell Ash IRIS inductively coupled plasma atomic emission spectrophotometer (ICP-AES). Blanks and standard reference soil (National Institute of Standards and Testing Soil SRM 2710) were digested and analyzed for quality assurance and quality control of metals in soil.

Determination of In Vitro Bioavailable arsenic

Bioavailable As was estimated using a modified in vitro gastrointestinal (IVG) method of Rodriguez et al. (1999). The IVG method is a two-step sequential extraction; a gastric solution extraction followed by an intestinal solution extraction. Gastric solution was 0.15 M NaCl and 1% porcine pepsin (Sigma Chemical Company, St. Louis, MO, cat. no. P7000) and purged with argon for 60 min prior to use. The in vitro method was conducted using 300 ml tall glass beakers placed on a submersible stir plate in a water bath set to 37°C. Soil (1.0 g) was placed in 150 ml of gastric solution and the pH of the gastric solution was adjusted to pH 1.8 with trace metal grade HCl. Solutions were continuously stirred (simulating gastric mixing) and pH was monitored and adjusted every 5 min to maintain a pH of 1.8 during the 1-h procedure. After 1 h, 20 mL of gastric solution removed for arsenic analysis, was replaced with 20 mL of fresh gastric solution. Subsequently, the extraction solution was modified to simulate intestinal solution by adding saturated $NaHCO_3$ solution to adjust the pH to 5.5 followed by the addition of 0.53 g of porcine bile extract (Sigma Chemical Company, St. Louis, MO, cat. no. B8631) and 0.05 g of porcine pancreatin (cat. No. P1500). A small amount of anti-foam agent (decanol) was added to each reaction vessel. After 1 h, 20 mL of intestinal solution was collected for arsenic analysis. Gastric and intestinal solution samples were centrifuged for 15 min at 10,000 rpm and filtered through 0.45-µm membrane filters immediately after their collection. The samples were acidified to pH of 2 using trace metal HCl and arsenic was determined using ICP-HG. Triplicate analyses were conducted on all soils and slags in the determination of in vitro arsenic.

RESULTS

Quality Assurance/Quality Control

Blanks and standard reference soil (National Institute of Standards and Testing Soil SRM 2710) were digested and analyzed for quality assurance and quality control of metals in soil. Blanks and certified reference materials were analyzed for every six replicates of soil samples. Mean recoveries for metals in soils were excellent (i.e. > 90%) with low relative standard deviations (i.e. < 10%) for all metals. Digested blanks contained below detection limit concentrations of arsenic, Cd Cu, and Pb (Table 1). Mean recoveries of metal in standard reference soil (SRM 2710) ranged from 98 to 107% with relative standard deviations ranging from 1.17 to 1.39% (Table 1). Spike recoveries for metals in soil ranged from 95 to 100%. Agreement of replicate analyses of soils, soil/slag mixtures and slags was excellent for total metal analyses with relative standard deviations among replicates being < 10%.

Total Metal Content in Soils and Slags

Total arsenic ranged from 11.9 to 287 mg/kg with an overall mean of 72.5 mg/kg (Table 2). Mean total Cd was 16.9 mg/kg and ranged from 5.0 to 33.0 mg/kg while Cu ranged from 140 to 3541 mg/kg, averaging 1164 mg/kg for the samples. Total Pb ranged from 145 to 6238 mg/kg with an overall mean of 1021 mg/kg. The two samples that were sent to Dr. Stan Casteel for pig feeding trails contained 75.6 mg arsenic/kg (sample 17) and 71.8 mg arsenic/kg (sample 18) (Table 2).

In Vitro Bioavailable Arsenic in Soils and Slags

IVG arsenic ranged from 4.52 to 57.9 mg/kg with an overall mean of 20.0 mg/kg (Table 3). IVG arsenic for the two samples sent to Dr. Casteel for pig feeding trails was 18.0 mg /kg (sample 17) and 17.1 mg/kg (sample 18). Percentage in vitro bioavailable arsenic averaged 35.1% and ranged from 2.93 to 79.8% (Table 3). The estimated percentage bioavailable arsenic for both of the samples sent to Dr. Casteel for pig feeding trails was 23.8% (Table 3). Agreement of replicate analyses of soils, soil/slag mixtures and slags was excellent for IVG arsenic analyses with relative standard deviations among replicates being < 10%.

Summary of Results for the First Set of Soils and Slags

December 16, 2002

METHODS

Sample Processing

Soil samples were processed similarly to the samples received in November. As requested, the second set of slag samples were crushed or unprocessed (e.g. left intact) before arsenic analysis by total (USEPA 3051) and in vitro bioavailable (Rodriguez et al., 1999) methods. Whole intact slags were placed in a zip-lock bag and physically reduced to small pieces using a two-pound sledge hammer. Because the sample preparation that was used to process and reduce the November samples to < 250 μm was very labor intensive (i.e. approximately 1.5 hours to process one sample), the second set of samples was reduced using a much faster method. These slag samples were reduced to pass a 250 μm screen using a miniature paint shaker and

corundum ball bearing grinding medium. Slag sample 7 and sample 8 were processed again and ground using the new method to allow for comparison with the second set of samples. Soil sample 13 was also reprocessed**.**

Determination of Total Metal Content in Soils and Slags

Total metal content in soils and slags were determined by acid digestion using microwave (CEM MDS 2100, Matthews, North Carolina, USA) according to U.S. EPA Method 3051. Soil (0.5 g) was digested by microwave (100% power, temperature 175 ^{0}C, 19 min total run time; 4.5 min time at temperature) in 10.0 ml of a 1:1 mixture of HNO_3:H_2O and diluted with deionized distilled water to a final volume of 25.0 ml. Triplicate analyses were conducted on all soils and slags. Digested soils and slags were syringe filtered through 0.45 μm filters and elemental analysis was conducted using a high resolution Thermo Jarrell Ash IRIS inductively coupled plasma atomic emission spectrophotometer (ICP-AES). Blanks and standard reference soil (National Institute of Standards and Testing Soil SRM 2710) were digested and analyzed for quality assurance and quality control of metals in soil.

Determination of In Vitro Bioavailable Arsenic in Soils and Slags

Bioavailable arsenic was estimated using a modified in vitro gastrointestinal (IVG) method of Rodriguez et al. (1999). The IVG method is a two-step sequential extraction; a gastric solution extraction followed by an intestinal solution extraction. Gastric solution was 0.15 M NaCl and 1% porcine pepsin (Sigma Chemical Company, St. Louis, MO, cat. no. P7000) and purged with argon for 60 min prior to use. The in

vitro method was conducted using 300 ml tall glass beakers placed on a submersible stir plate in a water bath set to 37°C. Soil (1.0 g) was placed in 150 ml of gastric solution and the pH of the gastric solution was adjusted to pH 1.8 with trace metal grade HCl. Solutions were continuously stirred (simulating gastric mixing) and pH was intermittently monitored and adjusted to maintain a pH of 1.8 during the 1-h procedure. After 1 h, 20 mL of gastric solution removed for arsenic analysis, was replaced with 20 mL of fresh gastric solution. Subsequently, the extraction solution was modified to simulate intestinal solution by adding saturated $NaHCO_3$ solution to adjust the pH to 5.5 followed by the addition of 0.53 g of porcine bile extract (Sigma Chemical Company, St. Louis, MO, cat. no. B8631) and 0.05 g of porcine pancreatin (cat. No. P1500). A small amount of anti-foam agent (decanol) was added to each reaction vessel. After 1 h, 20 mL of intestinal solution was collected for arsenic analysis. Gastric and intestinal solution samples were centrifuged for 15 min at 10,000 rpm and filtered through 0.45-µm membrane filters immediately after their collection. The samples were acidified to pH of 2 using trace metal HCl and arsenic was determined using ICP-HG. Triplicate analyses were conducted on all soils and slags in the determination of in vitro arsenic. Agreement among replicate analyses was excellent with relative standard deviations < 10%.

RESULTS

Quality Assurance/Quality Control

Blanks and certified reference materials were analyzed for every six replicates of soil samples. Digested blanks contained below detection limit concentrations of arsenic, Cd Cu, and Pb (Table 4). Mean recoveries for metals in soils were excellent

(i.e. > 90%) with low relative standard deviations (i.e. < 10%) for all metals. Mean recoveries of metal in standard reference soil (SRM 2710) ranged from 96 to 102% with relative standard deviations ranging from 3.36 to 5.00% (Table 4). Spike recoveries for metals in soil ranged from 95 to 100%. Agreement of replicate analyses of soils, fine slags (i.e. these did not require crushing) and crushed slags was excellent for total metal analyses with relative standard deviations among replicates being < 10%. However, it was impossible to weigh out the same sized particles in the analyses of the whole intact samples which resulted in extremely high relative standard deviations (i.e., RSD > 30%) for these samples.

Total Metal Content

Samples 20 and 21 had extremely high total values. Mean total metals, in parentheses, for sample 20 were asenic (26663 mg/kg), Cd (7885 mg/kg), Cu (198100 mg/kg), and Pb (61715 mg/kg) (Table 5). Sample 21 averaged 26967 mg arsenic/kg, 7740 mg Cd/kg, 194325 mg Cu/kg and 61118 mg Pb/kg. The means for total metals of the other samples were greatly influenced by these two highly contaminated samples. Therefore, median values will be used in the discussion of other samples. Total arsenic ranged from 10.7 to 26967 mg/kg with an overall median of 62.3 mg/kg (Table 5). Median total Cd was 9.96 mg/kg and ranged from 1.66 to 7885 mg/kg while Cu ranged from 342 to 198100 mg/kg with a median value of 1953 mg/kg. Total Pb ranged from 40.1 to 61715 mg/kg with an overall median of 708 mg/kg (Table 5).

In Vitro Bioavailable arsenic

Samples 20 and 21 had extremely high IVG arsenic values . Mean IVG arsenic for sample 20 was 9786 mg/kg while mean IVG for sample 21 was was 9905 mg/kg (Table 6). The mean for IVG arsenic of the other samples was greatly influenced by these two highly contaminated samples. Therefore, the median value will be used in the discussion of IVG arsenic in the other samples. IVG arsenic ranged from 1.10 to 9905 mg/kg with a median of 18.6 mg/kg (Table 6). Percentage in vitro bioavailable arsenic averaged 31.4% and ranged from 4.10 to 82.3% (Table 6). Agreement among replicate in vitro bioavailable arsenic analyses was excellent for the soil samples, fine slags, and crushed slags with relative standard deviations < 10%. However, it was impossible to weigh out the same sized particles in the IVG analyses of the whole samples which resulted in extremely high relative standard deviations (i.e. some RSD > 30%) for these samples.

Crushes Slag vs. Intact Slag Samples

Comparison of crush slag and whole slag results in Table 6 show crushing the sample had a great effect. Crushing increased total arsenic, IVG arsenic and hence calculated bioavailable arsenic (IVG/Total) of the slag samples. This is consistent with the ability of the extraction methods to dissolve arsenic in the sample. Crushed samples have much more surface area and are more readily dissolved by acid solutions used for total arsenic (USEPA 3051) and for IVG bioavailable arsenic. Increased dissolution of arsenic in crushed samples increases the amount of slag arsenic dissolved and measured as in vitro bioaccessible.

Crushed samples (< 250 µm) are used to estimated hand-to-mouth activity associated with incidental ingestion of contaminated soil by children. Results in this study showed there is considerably less risk associated with ingestion of intact slag (e.g. uncrushed).

Comparison of Results from November 1 with December 16, 2002

There were differences between results reported on Nov 1 with those reported on December 16. Results from two slags (samples 6 and 8) and one soil (sample 13) were compared. Results are summarized below.

Sample 13

This soil had the highest IVG bioavailable arsenic of all samples reported on Nov 1. Re-inspection of the data showed that one of the three ICP readings was very high. The high reading was likely due to carryover error from an arsenic standard. The inaccurate "bad" ICP replicate reading was removed to obtain the new values in Table 7 of 35.8% bioavailable arsenic. The 35.8% value is in agreement with both analyses reported on Dec 16. The reprocessed sample was the same sample as Nov 1, but ground using a ball. A new sample, sent from Weston, gave the same IVG arsenic.

Samples 6 and 8

IVG results for both slags processed by crushing with a hammer (Nov 1) were as accurate as using a ball mill for grinding –which produced a much more homogeneous mix. Therefore, results obtained for these slag on Dec 16 should be used instead of Nov 1 results. Other Nov 1 results were soil materials, not all slag, and these samples were easily homogenized and results are accurate.

Summary

A summary of determinations for IVG bioaccessible (i.e. bioavailable) arsenic for all soils and slags are summarized in Table 8. In general, most IVG bioavailability is <50% for most materials. Bioavailable As is very low (<10%) in uncrushed slag. The low bioavailability of the slags and soils should be considered when determining risk-posed by arsenic from ingestion of these materials.