1 Introduction

BHP Copper contracted with ENVIRON International Corporation (ENVIRON) to conduct an arsenic biomonitoring study of current residents within the Northwest Study Area (NSA). In developing a plan with BHP Copper for cleanup in the NSA, the Arizona Department of Environmental Quality (ADEQ) stipulated that a two phase arsenic biomonitoring program be offered to the community as a condition to using a specific target risk goal in developing soil remediation levels for the NSA. A human health risk assessment found that arsenic levels in the study area are not expected to contribute significantly to natural background arsenic exposures from food and drinking water, nevertheless the ADEQ wishes to provide this further assurance of the minimal risk presented by the study area soils through this program. The arsenic biomonitoring program is funded by BHP Copper with oversight by the ADEQ. Participation in the program by NSA residents is voluntary.

The first sampling event occurred during the summer of 2011 (ENVIRON 2011c). The purpose of this report is to provide a data summary for ADEQ and BHP Copper of the second sampling event, which occurred in August 2012 A separate report that provides an overview of these data will also be made available to study participants.

1.1 Overview of the Arsenic Biomonitoring Study

Arsenic is naturally present in most foods and in drinking water, and is widely distributed in the environment from many natural and anthropogenic sources. Studies of background exposures to arsenic in the U.S. have found that exposures are dominated by intakes from drinking water and diet, and that intakes via incidental ingestion of soil and inhalation of air contribute a negligible amount to total exposure. Fish and seafood contain the highest amounts of total arsenic, but most of the arsenic is present as nontoxic organic forms.

Biomonitoring is the measurement of a chemical or its metabolites in body tissues and fluids. Urine, blood, bone, breast milk, exhaled air, hair, nails, fat and other tissue can be used in biomonitoring studies, depending on the chemical of interest and objectives of the study. The most reliable, least invasive, and widely used screening test to measure recent arsenic exposure is measurement of arsenic in urine (ATSDR 2007). Arsenic in urine is not a measure of health effects. However, urinary arsenic levels that are within background levels expected from diet and water are helpful in confirming that recent exposures from other sources are not significant. Accordingly, urinary arsenic testing is the focus of biomonitoring offered to the community.

Most arsenic is excreted in urine within a few days of exposure; therefore, measuring arsenic in urine captures short-term exposure to arsenic. Total arsenic measurements in urine include both the more toxic forms (e.g., inorganic forms) that are typically found in soil as well as the essentially nontoxic forms (e.g., arsenobetaine) that are found in fish and shellfish. Total urinary arsenic concentrations less than 100 micrograms arsenic per liter of urine (µg/L) are considered normal (ATSDR 2009). ATSDR (2009) notes that levels can be significantly higher (greater than 1000 µg/L) immediately following ingestion of seafood.

The National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control and Prevention (CDC) measures urinary levels of metals and selected organic chemicals for the U.S. Table 1-1 presents the NHANES results for total arsenic in urine. The most recent survey, conducted in 2009 to 2010, found that the geometric

mean total arsenic level was 9.28 µg/L (CDC 2012). Half of the people tested had a total arsenic level of 8.15 micrograms per liter (µg/L) or less and 75% of the people had levels of 18.0 µg/L or less. Fewer than 5% of the people had total arsenic levels greater than 85.6 µg/L. The higher arsenic levels likely occur in people who recently consumed a seafood meal.

In Arizona, the typical urinary arsenic levels may be higher than in the U.S. as a whole. Based on experience with other arsenic biomonitoring studies within Arizona, the Arizona Department of Health Services determined that the geometric mean for Arizona residents is 18 µg/L (ADHS 2009).

For the NSA study, total arsenic levels in urine were compared to the program reference level of 30 µg/L. Further analysis of individual samples for speciated arsenic was indicated if the total urinary arsenic[1] result for that sample exceeded this reference level. The program reference level has been used previously by the Arizona Department of Health Services in biomonitoring for arsenic exposure (ADHS 2002).

Biomonitoring for soil exposure is typically conducted during seasons in which outdoor activity involving soil contact is possible. In the warm climate of the Superior area, fewer seasonal restrictions affect exposure measurement. Accordingly, the second urine sampling event was conducted during August 2012. The second sampling event for the biomonitoring study was initiated in July of 2012 with letters mailed to residents of the NSA that provided information about the biomonitoring study and how to enroll in the study.

For the second biomonitoring field event, urine samples and exposure survey information were collected from study participants on August 14th and 15th, 2012. Sample analyses included total arsenic, creatinine, specific gravity, and, in some cases, speciated arsenic.

Individual results were provided to study participants only and

information that could be used to identify individual participants is excluded from this data summary report.

The first biomonitoring sampling event was conducted in June of 2011. NSA residents were invited to participate in either or both biomonitoring sampling events.

Results of both biomonitoring study events will be presented during a public meeting to be held in Superior, Arizona (anticipated in 2013).

[1] Where indicated, determination of reference level exceedance may be based on comparison to creatinine-corrected total urinary arsenic rather than the corresponding uncorrected total urinary arsenic result. See further discussion at section 3.2.

2 Summary of Summer 2012 Participant Recruitment and Sample Collection

This section provides a brief overview of the study participant recruitment, enrollment, and sample collection for the Summer 2012 biomonitoring sampling event. Additional details of the study objectives, scope, and planned procedures are documented in the “Northwest Study Area: Arsenic Biomonitoring Work Plan” (ENVIRON 2011a).

2.1 Recruitment and Enrollment

Recruitment of participants occurred by mailing study announcement packets to NSA residents. The announcement packets included enrollment instructions, a biomonitoring fact sheet, and a participation consent form to be returned by residents wishing to enroll in the study. A pre- addressed

stamped envelope was included with each packet to facilitate return of signed consent forms for those residents choosing to complete the enrollment process.

On July 25, 2012, 102 study announcement packets were mailed to NSA residents. Recipients interested in participating in the study were requested to return their signed consent form to ENVIRON no later than August 10, 2012. A total of ten signed consent forms were received by ENVIRON, though, as described in Section 2.4, samples were collected from only eight NSA residents who had submitted consent forms. An additional pre-prepared stamped envelope was returned to ENVIRON and received sealed, but without any contents or notes that would identify which NSA resident had returned it. Two packets were returned by the postal service as undeliverable and without a known forwarding address and one packet was returned and re-mailed to the resident with an updated address.

2.2 Sample Collection

Detailed sample collection and analysis procedures were provided to ADEQ and BHP Copper in the memorandum titled "Sample Collection, Analysis, and Quality Assurance Procedures for Northwest Study Area: Arsenic Biomonitoring Study" (ENVIRON 2011b). A summary of the procedures is provided herein.

ENVIRON provided each participant with a sample collection kit that included a pre-labeled sample collection vial, urine collection cup, re-sealable plastic bag, refrigeration pack, sample collection instructions, and a copy of the exposure survey at the beginning of the field sampling event. The kits were provided in a paper bag marked with only the participant's name. At NSA residences with more than one participant, each participant received their own sample collection kit. Participants collected their first morning void the day after receiving the sample collection kits. ENVIRON field staff retrieved the urine samples and completed exposure surveys the day of sample collection. Samples were

stored in accordance with chain-of-custody procedures.

2.3 Sample Analysis

ENVIRON (2011b) documents the urine sample analysis procedures. Briefly, Pacific Toxicology Laboratories, under contract with ENVIRON, analyzed each urine sample for total arsenic, specific gravity, and creatinine. A participant's urinary output and hydration can affect how dilute or concentrated the urine is, which can then affect interpretation of sample results. Creatinine

and specific gravity measurements are conducted to allow for correction of sample results that may be needed given a participant's urinary output and/or state of hydration.

Following analysis, the remaining sample aliquots were stored at -20° C until it was determined if total urinary arsenic levels exceeded the program reference level of 30 µg/L. Per the study work plan, further analysis for speciated arsenic was required for any samples with total urinary arsenic[2] in excess of the program reference level. Applied Speciation and Consulting, LLC analyzed these samples for arsenite (As[III]), arsenate (As[V]), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA)[3]. Figure 2-1 summarizes the process used to evaluate sample results for this study.

2.4 Field Sampling Event Summary

On August 13th, 2012, two ENVIRON staff members mobilized to Superior, Arizona to initiate the biomonitoring sampling event. Upon arrival, ENVIRON staff conducted a health and safety briefing according to the site-specific health and safety plan and

also reviewed health and safety procedures with BHP Copper staff. A designated police officer was notified of ENVIRON's presence in the area and was on-call for all three days ENVIRON was in the field.

Initially, nine participants were enrolled in the study. One participant requested that a sample collection kit be mailed to them in advance. That participant's urine sample and completed exposure survey were retrieved on the morning of Monday, August 13th from the participant's home located within the NSA. The sample collection kits for the remaining study participants were prepared in the BHP Copper field office. A total of eight sample collection kits were constructed using sample vials pre-labeled with the confidential respondent code number (RCN) designated for each participant. ENVIRON staff then delivered five of the eight sample collection kits to the study participant homes. Two kits could not be delivered due the presence of a security dog. Also, during delivery of the sample collection kits, it was determined that one of the participants had moved from the NSA to a home outside of the NSA and was ineligible to participate in the biomonitoring study. However, the current resident of that NSA home elected to participate in the study. To accommodate the newly enrolled participant, ENVIRON staff returned later in the afternoon to provide a consent form and sample collection kit using an updated RCN. This resulted in six of the eight sample collection kits being delivered; two could not be delivered due to the presence of a security dog at the participants' home. ENVIRON staff remained at the BHP Copper field office until late afternoon to accommodate residents that may have had questions or concerns related to the study.

[2] Where indicated, determination of reference level exceedance may be based on comparison to creatinine-corrected total urinary arsenic rather than the corresponding uncorrected total urinary arsenic result. See further discussion in Section 3.2. [3] Applied Speciation and Consulting, LLC routinely includes results for a fifth arsenic species, arsenobetaine, when conducting speciated arsenic analyses. Arsenobetaine is an essentially nontoxic form of arsenic that is found in fish and seafood; therefore, it is

not included in the sum of arsenic species compared to the program reference level. However, for completeness, study participants whose samples were tested for arsenic species were provided with all of their results, including arsenobetaine concentrations reported by the laboratory.

On August 14th, ENVIRON staff retrieved the participant-collected urine samples and completed exposure surveys from five of the six participants' homes; one participant elected to withdraw from the study and did not provide a sample or completed survey. The sample vials were taped to preserve the integrity of the labels and to secure the lids, and then were placed in re-sealable plastic bags. No personal identifying information was included on the samples; labels contained only the RCN and date. After sample preparation, Emily Weissinger and Meghan McKelvey delivered the remaining two sample collection kits that had been undeliverable the previous day. By close of business on August 14th, no additional NSA residents had chosen to participate in the study.

On August 15th, ENVIRON staff retrieved the remaining two participant-collected urine samples and one replicate sample, as well as the completed exposure surveys. A total of nine samples, eight investigative samples and one replicate sample, were prepared for shipment to the analytical laboratory. ENVIRON staff mailed the urine samples via FedEx with frozen gel packs and chains of custody to Pacific Toxicology Laboratories for total arsenic, creatinine, and specific gravity analyses.

2.5 Deviations from the Sample Collection, Analysis, and Quality Assurance Procedures

ENVIRON staff followed the procedures provided in the

memorandum, “Sample Collection, Analysis, and Quality Assurance Procedures for Northwest Study Area: Arsenic Biomonitoring Study” (ENVIRON 2011b), with few exceptions. The following list summarizes deviations from procedures outlined by ENVIRON (2011b):

- Applied Speciation and Consulting, LLC routinely includes results for a fifth arsenic species, arsenobetaine, when conducting speciated arsenic analyses. Arsenobetaine is an essentially nontoxic form of arsenic that is found in fish and seafood; therefore, it is not included in the sum of arsenic species compared to the program reference level. However, for completeness, study participants whose samples were tested for arsenic species were provided with all of their results, including arsenobetaine concentrations reported by the laboratory.

- One sample result exceeded the uncorrected total arsenic reference level, but not the corrected reference level. Even though the procedures for this study (outlined in Figure 2-1) state that only samples that exceed the corrected reference level will be analyzed for speciated arsenic, this sample was included in the speciated arsenic analysis as an extra precaution. No other deviations from the Sample Collection, Analysis, and Quality Assurance Procedures (ENVIRON 2011b) are noted.

3 Exposure Survey and Analytical Results

This section summarizes information reported by study participants on their exposure surveys for the Summer 2012 biomonitoring sampling event. Analytical laboratory results for

total and speciated arsenic are also presented along with associated quality control information.

3.1 Exposure Survey Results

A total of eight people residing in the NSA participated in the Summer 2012 urinary arsenic biomonitoring study and completed the exposure survey (a sample survey form is included as an appendix to ENVIRON 2011a). Based on results from the surveys, participants ranged in age from about 50 to over 80 years old[4], and six of the participants were female. All of the participants had lived at their current residence within the NSA for at least six months and all but one stayed overnight in their homes for the entire two weeks prior to sample collection. One participant reported being away from their residence for eight nights over the prior two week period. No participants reported eating seafood or locally-caught fish within the three days prior to sample collection, but three participants reported eating rice with three days of providing samples. This and other information obtained from the exposure surveys is presented in Table 3-1.

3.2 Analytical Results

Analytical results obtained during the Summer 2012 urinary arsenic biomonitoring study are summarized below.

3.2.1 Total Arsenic

Table 3-2 presents the total urinary arsenic data collected in this biomonitoring study. Of the eight samples collected, arsenic was detected in six samples above the detection limit of 10 µg/L. Detected concentrations of total arsenic, uncorrected, ranged from 11.9 µg/L to 41.8 µg/L. Two of the eight sample results exceeded the uncorrected program reference level. The average total urinary arsenic concentration was 17.4 µg/L (uncorrected), assuming that all nondetect results are equal to one half of the detection limit, or 5 µg/L.

The validity of spot (untimed) urine sample measurements is indicated by review of creatinine and specific gravity results for each sample, both of which allow for corrections for varying hydration states of study subjects. Creatinine is a natural waste product of the body found in urine, which may be used to correct for variable water excretion rates (i.e., dilution) at the time of spot urine specimen collection (Barr et al. 2005). Creatinine correction assumes, on average, an individual excretes one gram of creatinine per liter of urine based on total daily mean urinary output volumes and total daily mean creatinine excretion. This is a theoretical value which may be above or below measured urinary creatinine in a given sample. Creatinine concentrations are primarily affected by the individual's hydration state, but creatinine excretion is also influenced to a lesser degree by other factors[5], including gender, age, and lean body mass. Specific gravity

[4] This range applies to six of the eight participants, because two participants did not report their age. [5] The World Health Organization (WHO 1996) guideline range for urinary creatinine is 0.3 g/L to 3 g/L. Creatinine concentrations within this range are routinely used to adjust targeted analyte concentrations

represents the ratio of the density of a urine specimen to the density of water and will also vary depending on the individual's hydration state at the time of sample collection. As with creatinine, specific gravity measurements may also vary with age, gender, and health status.[6]

Because creatinine and specific gravity measurements are predominantly influenced by the volume of urinary output at the time of sample collection, they are used to adjust a sample for dilution effects that can influence interpretation of results for arsenic (Barr et al. 2005; Pactox 2012). For example,

considering individuals of similar age, gender, and health status, a person who is dehydrated will have a more concentrated urine sample with higher specific gravity and higher levels of salts, creatinine, and arsenic than someone who is sufficiently hydrated. Conversely, someone who is very well hydrated will have a more dilute urine sample with lower specific gravity and lower levels of salts, creatinine, and arsenic. In the first case, the arsenic level in the urine may be artificially high unless adjusted for normal urine output. In the second case, the arsenic level in the urine may be artificially low without adjustment.

In this study, arsenic levels were adjusted for creatinine to correct for variable urinary output that would affect interpretation of arsenic concentrations in the samples. As discussed above, creatinine correction can also account for some of the variability in renal function and lean body mass among individuals (CDC 2009). As is typically the case, creatinine levels in this study were lower in older participants than in middle age participants. Accordingly, some of the variability in creatinine-corrected urinary arsenic results may have been due to differences in creatinine output between older and younger participants as well as other factors.

Creatinine correction was conducted according to the following equation (Pactox 2012):

$$Creatinine-Corrected\ Total\ Urinary\ Arsenic\ \left(\mu \frac{g}{g}\right) = \frac{Total\ Urinary\ Arsenic\ \left(\frac{g}{L}\right)}{Creatinine}$$

An assumed average daily urinary creatinine output of 1 g/L at normal hydration levels (Hee 1993) allows for direct comparison of creatinine-corrected results reported as “µg/g” to uncorrected results and to a creatinine-corrected program reference level of 30 µg/g.

μ $\frac{g}{L}$

(e.g., arsenic) in spot urine samples for variability in the individual's urinary output at the time of sample collection. However, this range is not absolute. An analysis of the NHANES dataset by Barr et al. (2005) found that the WHO range may not encompass heterogeneous populations. For example, Barr et al. report 15 percent of female NHANES participants over the age of 70 had creatinine levels less than 0.3 g/L. As urinary creatinine concentrations have been shown to correlate with muscle mass, with higher urinary creatinine typically found in men than in women (Barr et al. 2005). With increasing age and coincident decreases in muscle mass, urinary creatinine concentrations decrease in both men and women (Barr et al. 2005).

[6] The range of normal specific gravity in urine varies by laboratory, but range of 1.002 to 1.035 is generally considered acceptable for individuals with normal kidney function (Cadogan et al. 2011). Specific gravity measurements for NSA study participants ranged from 1.012 to 1.020 (Table 3-2) indicating further support for the validity of the NSA participants' urinary sample results.

ENVIRON

μ $\frac{g}{L}$ $Specific\ Gravity\ Corrected\ Total\ Urinary\ Arsenic$

(Miller et al. 2004):

$$= Total\ Urinary\ Arsenic\ \mu g \times \frac{Average\ Specific\ Gravity - 1\ L}{Sample\ Specific\ Gravity - 1}$$

Arsenic Biomonitoring Results – Summer 2012

Based on creatinine correction, detected sample results ranged

from 21.1 µg/g to 51.3 µg/g (Table 3-2, Figure 3-2). Three samples exceeded the creatinine-corrected program reference level.

Specific gravity corrections were also conducted to provide an additional point of comparison for total urinary arsenic results. For specific gravity corrections, the following equation is used

The specific gravity correction uses the average value for the population to normalize the arsenic concentrations based on the average dilution for the sample population. The average specific gravity for the eight samples in this study is 1.017. Detected specific gravity corrected total urinary arsenic ranged from 13.8 µg/L to 38.3 µg/L (Table 3-2, Figure 3-3) with one result above the program reference level. Specific gravity adjustments rely on input of a population- specific average specific gravity, thus the small number of participants and wide range of ages for participants in this study may introduce greater uncertainty in the reliability of specific gravity adjusted results versus creatinine-adjusted results for this study.

3.2.2 Speciated Arsenic

Three of eight samples analyzed for total arsenic in urine exceeded the creatinine-corrected program reference level of 30 µg/g and were analyzed for speciated arsenic. Speciation analysis was also conducted on one additional sample where the total urine arsenic exceeded the reference level prior to creatinine correction, but was below the reference level once corrected for creatinine. Speciated arsenic results are presented in Table 3-3 and Figure 3-4.

The toxicity of arsenic generally corresponds to its form or species. Inorganic arsenic species are the most toxic forms of arsenic and high concentrations in drinking water have been linked to increases in lung, bladder, and skin cancer in some regions of the world. Organic arsenic species are much less toxic than inorganic arsenic species and some organic forms (e.g.,

arsenobetaine) are thought to be essentially nontoxic. Inorganic forms of arsenic (i.e., As(III) and As(V)) and its metabolites (i.e., MMA and DMA)7, were summed for each participant with speciated results, and this sum was compared with the program reference level of 30 µg/L (or µg/g creatinine). For all four participants, the sum of As(III), As(V), MMA, and DMA was less than the program reference level. Creatinine-corrected results for summed species ranged from 1.16 to 13.0 µg/g (Table 3-3 and Figure 3-4).

[7] Some inorganic arsenic is converted in the human body to MMA and DMA. 3027339D 8

3.3 Quality Assurance and Quality Control

All samples were collected and stored according to procedures outlined by ENVIRON (2011b).

A single replicate sample was collected. Both the investigative and replicate sample were below the detection limit of 10 µg/L. Although the fact that both were non-detect provides some information about the agreement between the samples, it was not possible to calculate relative percent difference (RPD)[8] between them. However, in the Summer 2011 sampling event, the RPD between the investigative (16.9 µg/L) and replicate (17.1 µg/L) samples was 1.2%, falling well below the target RPD of 20%. RPD is calculated using the following equation:

$$RPD(\%) = 100 \times \frac{Result\ 1 - Result\ 2}{Average(Result\ 1, Result\ 2)}$$

[8] RPD is a measure of the consistency of the analytical method and provides information on the reliability of the analytical results.

4 Discussion and Conclusions

A limited number of NSA residents elected to participate in the second biomonitoring sampling event. Participants chose to volunteer for the study (i.e., they were not pre-selected based on any characteristic other than residence within the NSA) and may or may not be representative of NSA residents as a whole in terms of health status, activity patterns, diet, and other factors that may influence urinary arsenic concentrations.

For these eight individuals, total urinary arsenic was reported above the detection limit in six samples (75 percent detection rate). Of these six total arsenic detections, three exceeded the creatinine-corrected study reference level and were therefore analyzed for speciated arsenic. A fourth sample exceeded the uncorrected, but not the corrected, reference level. Speciated arsenic analysis was conducted for this sample as a check on the process outlined in Figure 2- 1. For all four samples with speciated arsenic analysis, the sum of inorganic arsenic compounds was less than the study reference level, indicating that environmental exposure to arsenic in soil was not significant.

Urinary arsenic measurements capture recent arsenic exposures and may not be indicative of exposures occurring over a longer period of time. However, the range of total and speciated urinary arsenic concentrations reported are well within levels considered normal by ATSDR and consistent with average concentrations determined for Arizona residents based on ADHS involvement in other biomonitoring programs.

These findings are consistent with the site-specific human health risk assessment that found that arsenic levels in the study area are not expected to contribute significantly to natural background

arsenic exposures from food and drinking water and provide further assurance of the minimal risk presented by the study area soils in the vicinity of NSA residents.