Title

Uranium and other heavy metals in the plant-animal-human food chain near abandoned mining sites and structures in an American Indian community in northwestern New Mexico

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Publication Date

2013

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Uranium and other heavy metals in the plant-animal-human food chain near abandoned mining sites and structures in an American Indian community in northwestern New Mexico

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy in Nursing

by

Christine B. Samuel-Nakamura

School of Nursing

2013

ABSTRACT OF THE DISSERTATION

Uranium and other heavy metals in the plant-animal-human food chain near abandoned mining sites and structures in an American Indian community in northwestern New Mexico

by

Christine B. Samuel-Nakamura

Doctor of Philosophy in Nursing

University of California, Los Angeles, 2013

Professor Wendie A. Robbins, Chair

The broad, long-term objective of this study is to identify the extent and impact of uranium (U) and other heavy metal (As, Cd, Cs, Pb, Mo, Se, Th, and V) contamination on harvested *Ovis aries* (sheep) and plants on the Diné (formerly known as Navajo) reservation. This study provides a food chain assessment of U exposure in an American Indian (AI) reservation in northwestern New Mexico. The study setting was a prime target of U mining for military purposes from 1945 to 1988. More than 1,100 unreclaimed abandoned U mines and structures remain. These abandoned U mines, structures, and tailings contaminate the land and vegetation that humans and livestock use for subsistence. The specific aims of the study are to: (1) describe the dietary behavior in Diné residents who grow and harvest their own food specifically related to ingestion of locally harvested *O. aries* and plants; (2) compare heavy metal levels in locally harvested sheep and plants in areas suspected to have high levels of environmental contamination and low environmental levels of contamination; (3) explore potential routes of heavy metal exposure for locally harvested plants and *O. aries*; and (4)

formulate a plan to disseminate study findings to the leadership and community on the Diné reservation. This is a comparison study examining contamination levels in locally harvested animals and plants across reservation areas suspected to have high levels of environmental contamination and low levels of contamination. The DiNEH (Diné Network for Environmental Health) cohort (N=1,304) served as one of the sources from which growers and harvesters were identified who provided samples for the current research. Of the DiNEH cohort respondents, those individuals who reported positively to questionnaire items about harvesting sheep (n=280) and crops (n=180) locally were eligible for the present study. A goal was to compare current results to existing DiNEH data or background levels. New participants were recruited by various snow-ball methods. Data of animal, plant, and soil U levels were determined on a scale of milligrams (mg) per kilogram (kg).Water data U levels are reported on a scale of micrograms (μg) per liter (L). Commonly harvested foodstuffs were examined in this community. Uranium and other heavy metal concentration levels are reported for harvested sheep (n=3), botanicals including herbs (n= 18), forage (n=33), crops (n=20) coupled with soil and often water utilizing Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The representative animal (*O. aries*), crops (e.g. *Zea mays*, *Cucurbita pepo*, *Phaseolus vulgaris)*. The study explored potential routes (e.g. water and soil) of U and HM exposure for locally harvested plants and *O. aries*. This research aimed to determine if locally harvested sheep and plants on the Diné Reservation are contaminated with heavy metal levels in various sheep parts, herbs, and forage. A plan has been developed to disseminated study findings and educational information to the Diné community via newsletters, community, and chapter house forums. Findings of U and other heavy metal concentration levels will be valuable for predicting U transfer to harvested food and for conducting future evaluations of the impacts of U mining on critical food chain.

The dissertation of Christine B. Samuel-Nakamura is approved

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Dedication

This dissertation is dedicated to my parents, loved ones, family, close friends, and many other supporters. This is a posthumous dedication to my father (Shi'zhé'é), Oscar Samuel, whom I promised that I would obtain my doctorate. He passed away my first year of college before I met my goal. My father worked tirelessly with an eighth grade education to nurture my early life and educational endeavors. My mother (Shi'máá), Betty Samuel, whom has influenced who I am and what I stand for as a Diné woman. Despite lacking a formal education, she has taught me everything including the ways of life and the importance of preserving the Diné language. She inspired within me compassion, humility, persistence, and strength. Michael Nakamura (Shi' hastiin), whose patience and love supported me and my work. My best friend, Lavina Damon (Shi'kis) who celebrated with me during the wonderful times and supported me during my difficult times. All ten of my elder brothers and sisters, for their love and support. I appreciate all the humor, inspirational words, and prayers. Connie Jo Hamilton, LAC for rejuvenating my body and mind which largely helped me to finish the last five miles of this ultramarathon. I thank Les and Judy Slade for their wonderful New Mexico hospitality.

My dissertation committee for steering my educational metamorphosis and supporting my ideas and thoughts. Your work and dedication continually motivates and inspires me.

This dissertation is also posthumously dedicated to family and friends. Jim Skeets (Shi nááli) and Desbah Samuel (Shi nááli) who stressed the importance of Diné values in my early childhood. My family, Isami Nakamura (Shi'zhé'é), Natsuye Gwen Nakamura (Shi'máá), and Robert R. Nakamura (Shi'nai). I dedicate this dissertation to those who passed away while I was actively working on my doctoral studies, Frank Benally (Shi'zhé'é ilini), Dorothy Wilson (Shi'máá yázhí), Delores Longhair (Shádí), Emmanuel Samuel (Shi' yaz), Robert Hirose (Shi'kis), Joel Okida (Shi'kis), Osamu Katano (Shi'nai), and Skip Sato (Shi'zhé'é yázhí).

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Acknowledgments

Foremost, I appreciate the participation of the Four Diné Communities (Baca/Prewitt/Haystack, Churchrock, Crownpoint, and Mariano Lake) in New Mexico who demonstrated interest in the study. I am grateful that they allowed me into their communities and lives. I value their voices, thoughts, and learned much about Diné harvesting, culture, language, and social justice issues. The study communities' struggle for environmental justice reinforced the value and need of this type of project in contaminated communities all over the world.

I value the support of The Navajo Nation Human Research Review Board (NNHRRB), the Eastern Navajo Health Board (ENHB), the Eastern Navajo Agency Council (ENAC), the UCLA Institutional Review Board (IRB), and the UCLA Office of Animal Research Oversight (OARO).

Dr. Mehdi Ali and his staff at the University of New Mexico (UNM) Analytical Chemistry Laboratory Earth and Planetary Sciences Department was invaluable to the success of my dissertation project. Dr. Ali's knowledge of sample analyses and ICP-MS instrumentation was valuable to this project. Dr. Ali made time to be available for my many questions and was eager to teach.

I would like to thank Bob Sivinski (former Botanist for New Mexico) and the UNM Herbarium for providing plant species identification of all of my samples. He provided trainings on how to collect and preserve samples needed for species identification. He was eager to share his knowledge about New Mexico plant flora and answer my inquiries.

Special thanks to Chris Shuey and the staff at Southwest Research Information Center (SRIC). Mr. Shuey was always generous with time, information, and resources.

Isaiah Mack of California Surveying and Drafting Supply Inc. was supportive by providing many hours of GIS instrument troubleshooting, teaching, assigning practice sessions, answering questions, and providing sound recommendations. Zacharias Hunt at Z World GIS™ was invaluable for his GIS mapping abilities. He provided many hours of teaching and answering questions. The UCLA School of Nursing statistical counseling was valuable in providing assistance with SPSS data analysis.

This dissertation project would have been impossible without the funding so generously provided by the National Institute of Health (NIH), National Institute of Nursing Research (NINR)/National Institute of Environmental Health Sciences (NIEHS) Grant, the Office of the Navajo Nation Scholarship & Financial Assistance (ONNSFA) Dissertation Grant, the UCLA Ethnic Studies American Indian Studies Center (AISC) Grant, the UCLA National Institute for Occupational Safety and Health (NIOSH) Grant, and various anonymous donors.

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Introduction

Environmental contamination contributes significantly to morbidity, lost wages, healthcare cost, early death, and disability (Rodgers, 2005). In the U.S. alone the mortality and morbidity rates related to cancer caused by environmental exposures are underappreciated and will continue to rise. In the U.S., there is significant support of greater risk of exposure to environmental toxins for ethnically diverse populations (Markstrom & Charley, 2003; Pellizzari, Perrit, & Clayton, 1999) and for people with lower socioeconomic status (Bellinger & Matthews, 1998 ; Schmidt, 1999). In the U.S. , the estimated cost of clean-up with modern conventional technology to remediate radionuclide contamination is $200 to 300 billion (Entry, Waturd, Manasse, & Vance, 1997). On a global scale, traditional societies and third world countries have been significantly affected by man-made environmental disasters (deVries, 1995). According to the literature, minority populations are disadvantaged in terms of occupational safety and environmental justice (Brugge & Goble, 2002; Grinde & Johansen, 1995).

The impact of environmental wellbeing and holism is well documented among nursing knowledge and practice (Rodgers, 2005). In fact, the nursing metaparadigm specifically includes environment in the four domains of nursing (health, client, environment, nurse). Rodgers (2005) asserts that the future focus for nursing science should encompass social, economic, and physical environments. In addition, nurses have the knowledge and opportunities to identify elements in peoples' daily lives that may affect their health and can provide prevention and protection to improve health outcomes (Chang, Robbins, Wei, Xun, Wu, Li, & Elashoff, 2006). Further yet, a goal of Healthy People 2020 is to promote the health of society's social, economic, and physical environments by providing safe water, monitoring environmentally related diseases, and decreasing risks posed by toxic substances and hazardous sites (U.S. Department of Health and Human Services, 2010).

The American Indian Community

The American Indian (AI) population residing on tribal lands or reservations are disproportionately underrepresented in terms of health care access, geographic disadvantages, and often reside in toxic environments (Dawson, 1992). The Diné people rely on the land for sustenance. For example, the land supplies a water source, forage for livestock, and areas to raise native foods and other crops. In addition, it provides areas to collect plants used for ceremonial and medicinal purposes, and a setting to practice beliefs at sacred cultural sites. The main research question is whether food harvested in the northwestern New Mexico part of the Diné reservation is contaminated with uranium and other heavy metals.

Collectively, the AI is a diverse community and comprise 1.5 percent of the U.S. population (U.S. Census Bureau, 2006). Approximately 34 percent of the population reside in AI tribal areas, 64 percent live outside tribal areas, and two percent reside in Alaskan Native Village areas (U.S. Census Bureau, 2006). Specifically, the Diné people reside on reservation land which is approximately 16 million acres and is roughly the size of West Virginia or Ireland (Brugge, Benally, & Yazzie-Lewis, 2006). The Navajo Nation spans three states: Arizona, New Mexico, and Utah and is known as the Four Corners region. In total, 276,775 individuals identify solely as Navajo and approximately 168,000 live on the Navajo Nation (Brugge et al., 2006).

The Diné Community

In the U.S., disparities among various ethnic groups are apparent and low SES among the Diné is documented in various literature (Markstrom & Charley, 2003; Taylor, 1997). Also, 37% of Diné families live below the poverty line as compared to 12.4% of the nation (U.S. Census Bureau, 2006). According to the U.S. Census Bureau (2006) the median household income for Navajo families was $23,534 as compared to the national average of $48, 201 (U.S. Census Bureau, 2007). In conjunction with low income, the Bureau of Indian Affairs (2005) reported a high unemployment rate of 52% on and near the Navajo reservation. Indian Health

Service (IHS, 2004) reports that the life expectancy of AI is 4.6 years less than all races of the U.S. population. In addition, death rates compared to other Americans from tuberculosis (TB 750% higher), alcoholism (550% higher), unintentional injuries, (150% higher), homicide (100% higher), and suicide (70% higher) (IHS, 2004) are disproportionate. IHS (2004) reported other leading causes of death and include diseases of the heart (Welty, Lee, Yeh, Cowan, Go, Fabsitz, Le, Oopik, Robbins, & Howard, 1995), diabetes (Struthers, Kaas, Hill, Hodge, DeCora, Geeishirt-Cantrell, 2003; Welty et al.,1995), malignant neoplasms (Giuliano, Mokuau, Hughes, Tortelero-Luna, Risendal, Ho, Prewitt, & McCaskill-Stevens, 2000), chronic liver disease, and cirrhosis (IHS, 2001). Of the 4.1 million that self-identify as AI, IHS provides care for approximately 1.5 million of the population (Katz, 2004). The remainder of the healthcare facilities are geographically inaccessible especially for those living in rural areas or are ineligible because they are not federally recognized tribes (Giuliano et al., 2000). Further, the funding for metropolitan AI healthcare facilities is lower than rural counterparts (Joe, 2004).

The History of the Diné Community and Uranium

In the late 1800s, uranium was discovered on Diné lands. The study area is on and adjacent to the Morrison Formation otherwise known as the Grants uranium district. The formation is geologically rich in uranium. In the U.S, New Mexico ranks second for uranium reserves and fourth in world production (McLemore, 2007). From 1951 to the early 1980s this area was actively mined (McLemore, 2007). In April 2005, the Navajo Nation President at that time, Joe Shirley, Jr., introduced into law the Diné Natural Resources Protection Act (DNRPA) that banned uranium mining and processing on the Diné reservation.

Uranium Bioeffects

Uranium is one of the heaviest metals on earth and can enter the body primarily by ingestion (contaminated water or food) or inhalation, entering the bloodstream and deposited in tissues, primarily the kidney and bone (Taylor & Taylor, 1997). Human and animal studies of

those exposed to U have shown kidney toxicity (Gilman, A.P., Villeneuve, Secours, Yagminas, Tracy, Quinn, Valli, Willes, & Moss, 1998; Haley, 1982; Tracy, Quinn, Lahey, Gilman, Mancuso, Yagminas, & Villeneuve, 1992), as well as damage to the liver, muscle, cardiovascular, and nervous system (Dang, Pullat, & Sharma, (1995; Taylor & Taylor, 1997).

Other Heavy Metals Associated with Uranium

Uranium and its constituent heavy metals were chosen to be evaluated because these HMs are a part of the decay series of U (Brugge et al., 2005). In a study evaluating the composition of tailings, Dreesen and Williams (1982) demonstrated the enrichment of a suite of Uranium geochemical analogue elements which included Arsenic (As), Molybdenum (Mo), Selenium (Se), and Vanadium (V). Frequently heavy metals generally occurred with sulfide minerals such as (Cobalt) Co, Copper (Cu), Nickel (Ni), and Lead (Pb). A number of trace elements are frequently enriched in epigenetic sandstone uranium ore deposits, the predominant ore type presently being mined in the United States. According to several reports (Devoto, 1978; Brookins et al, 1977), the deposits have As, Mo, Se, and V enrichment along with U. Further, heavy metals specific to the Colorado Plateau include U, V, Mo, Se, As, Cu, Co, Pb, Ni, Sulfer (S), Radium (^{226}Ra, Dreesen & Williams, 1982; Dreesen & Marple, 1979; Squyres, 1970; Cannon, 1964). Colorado Plateau ores are known to contain elevated concentrations of Pb as a result of the presence of pyrite or as base sulfide minerals (Dreesen & Williams, 1982). Lead enrichment also results from being the final products of the U^{238} and U^{235} decay series.

Diné and the History of *Ovis aries*

Wild game meat was the primary meat source for the Diné before the introduction of domesticated subsistence animals (Darby, Adams, Pollard, Dalton, McKinley, 1956). *Ovis aries* (*O. aries*) or sheep is a European livestock that was introduced to the Americas in 1494 by Spaniards. In 1598 sheep were introduced to the U.S. Southwest. Following the Pueblo Revolt of 1680, the Diné and Pueblo people acquired their own sheep flocks (Sponenberg & Taylor,

2009). Currently, there are various breeds of sheep that are utilized by the Diné for food, textile making, and as an economic resource. The Navajo-Churro is the longest standing breed of sheep on the reservation and has been utilized for meat and textile weaving in the southwest for over 400 years (Blunn, 1943; Sponenberg & Taylor, 2009). The average inbreeding level for the Navajo-Churro was found to be 3.8% in 2004 (Sponenberg & Taylor, 2009). In 2000, of the Navajo-Churro, Maiwashe and Blackburn (2004) found the average inbreeding level to be 1.2% and 1.0% in flocks (N=22) from New Mexico, Arizona, Utah, Colorado, Wyoming, and Texas. Various other sheep breeds exist in Diné herds today; a full identification of genetic breeds reservation-wide is lacking. Goats often comprise part of the Diné herd. Sheep are considered to be more economically valuable for the raw materials they produce which are used for trade items (wool, clothing, blankets, etc.) in addition to providing subsistence meat (Bailey & Bailey, 1986).

Over time, sheep as a meat staple of the Diné diet has been consistent as evidenced in the literature. A historical review of the Diné diet by Kopp (1986) identified mutton as one of the primary foods eaten others include coffee, wheat flour, and potatoes in the 1930s. Carpenter and Steggerda (1939, p. 303) also reported that "if available, the Navajo would make meat, chiefly mutton, 60 to 80% of his diet." Due to government mandated stock reductions in the 1930s, sheep intake among the population changed. During that era, in one region of the reservation Darby et al. (1956) reported that well-to-do (larger herders) families ate mutton at three meals of the day, two meals for moderately well-to-do families, and only potatoes and tortillas for indigent families (smaller herders). During the 1960s, Kopp (1986) reports that 71% of the population reported harvesting some of their own food which included sheep (46%), corn (41%), squash (21%), cattle (11%), beans (six percent), and potatoes (five percent). In the late 1960s, a study in the Greasewood area of the reservation, by Rogers and Reisinger (1969) collected 24-hour recall diet data and demonstrated that mutton and corn remain the diet staples.

The sheep is a highly valued resource or cultural animal and is a known source of socio-cultural identity for the Diné (Sponenberg & Taylor, 2009). The sheep provides food sustenance and a psychological sense of security (Witherspoon, 1973). In fact, a common Diné adage is "sheep is life." Early Diné economy was solely based on a subsistence economy contrary to the contemporary wage economy (Kopp, 1986). The sheep is commonly viewed as deterrent of hunger and poverty. The original Diné subsistence economy was based on herding, farming, hunting, and gathering (Bailey & Bailey, 1986). In addition, there exists a central conceptual relationship between sheep and motherhood. Sheep similar to motherhood provided reproduction and sustenance of life (Witherspoon, 1973). Roles of responsibility and leadership were bestowed on those members of the tribe that cared well for their flock (Witherspoon, 1973). In fact, those community members who possessed the largest flocks wielded the most power and influence in their residence group.

O. Aries Anatomy, Digestive Physiology, and Mineral Utilization

Ovis aries or sheep are ruminants. Ruminants by definition are cud-chewing, even-toed ungulates; they possess four stomach compartments (reticulum, rumen, omasum, and abomasum). According to the National Research Council (NRC, 2007), *O.aries* are grass-roughage eaters which means they possess a highly developed fermentation system that enables them to digest cellulosic fractions of plant cell walls that characterize the high fiber content of graminoid diets. Grass-roughage eaters have short lips and broad mouths which maximizes intake of grasses and sedges at low biomass and the long torus of these species is associated with a cornified tip of the tongue which aids in tearing grass and sedges of high biomass (Hofmann, 1989). The lining of the small intestine is the primary site of absorption of nutrients for distribution throughout the body (NRC, 2007). Most of the protein digestion is completed before the contents enter the large intestine. It is for this reason that samples will be collected from the small intestine for this study.

Some minerals are required for various important functions in the sheep body. Mineral requirements for the sheep depend on soil and pH, fertilization practices, climatic conditions related to growth rate, plant maturity, and adequacy of feed resources. Minerals that are needed in gram quantities per pay are classified as macroelements (NRC, 2007). Minerals that are found in low concentrations in the animal body and are needed in small quantities are called microelements (NRC, 2007). For this study, importance will focus more on microelements. Essential microelements needed by sheep are Mo and Se. Toxicity can also occur in excess intakes of As, Cadmium (Cd), Pb, Mo, Se, and V. The upper limit of Arsenic toxicity in water is 0.2 ppm in contaminated water (Pugh, 2002). Cadmium toxicity levels are reached at 10mg/kg diet of dry matter (NRC, 2005) and 0.01to 0.05 ppm in contaminated water (Pugh, 2002). Lead is poorly absorbed in sheep at three to 10 percent (Fick, Ammerman, Miller, Simpson, & Loggins, 1976). The maximum tolerance of Pb in sheep is 100mg/kg diet of dry matter (NRC, 2005) or 0.05 to 1ppm in contaminated water. Molybdenum absorption occurs from the small intestine. The dietary requirement for Mo is 0.1 mg/kg diet of dry matter (NRC, 1985 and 1975) up to 0.5 mg/kg in a diet for sheep (Pugh, 2002). Molybdenum toxicity is reported to be 5 μg/g or 5,000 mg/kg (Dreesen & Williams, 1982). The Se requirement for sheep is 0.10 to 0.20 ppm or 0.10 to 0.20 μg/g (Pugh, 2002). A single toxic dose for *O. aries* has been reported to be 2.2 mg/kg orally or chronic ingestion of 0.25 mg/kg of body weight (Pugh, 2002; Garry, Chew, Rings, Tarr, & Hoffsis, 1990). The upper limits of potential Se toxicity for sheep in water is 0.05 ppm (0.05 μg/g, Pugh, 2002). Sheep absorb little vanadium: 1.6% (Paterson, Hansard, Ammerman, Henry, Zech, & Fisher, 1986). The recommended standard for the upper limit of toxicity for V in water is 0.1 ppm (0.1 μg/g).

The Use of *O. Aries* in the Contemporary Diné Diet

In contemporary time, mutton remains a staple of the Diné diet (Ballew, White, Strauss, Benson, Mendlein, & Mokdad, (1997; Kopp, 1986). For the current study, the most commonly

eaten locally raised meat in the community is domesticated *O. aries* or sheep. In preliminary data from the DiNEH cohort, 76.5% exclusively raised and consumed sheep on their ranch, 2.4% raised cattle exclusively, and the remaining 2.1% were all other categories of meat combined. Other categories of meat are traditionally considered taboo and include fowl (Kopp, 1986; Darby et al., 1956), eggs (Kopp, 1986), fish (Fessler & Navarette, 2003; Kopp, 1986), bear (Kopp, 1986), and coyote (Kopp, 1986).

O. aries are herded on a regular basis for grazing and therefore are called range sheep (Subcommittee on Sheep Nutrition, 1985). Opposite that, sheep that are raised in a non-free range environment are referred to as pen-raised sheep. On the Dine reservation range sheep are predominant. During grazing, a herder or sheep dog(s) (or both) watch or monitor the flock (Black & Green, 1985). Some herds graze great distances in a day. Most herding is done on foot; occasionally herding is done on horseback. The majority of the home-raised sheep diet consists of local grasses, bushes and/or plants. The animals drink water from livestock wells (unregulated), regulated public water or seasonal water sources (streams or ponds). The animals are sheltered in corrals to deter weather and predatory animals. In a Diné reservation-wide study, Black and Green (1985) frequently found that *hogans* (homes) and corrals are within 100 meters or less of each other. During the winter the sheeps' local forage diet can be supplemented with fodder (e.g. alfalfa hay, grain. etc.). Tainted or unclean meat is considered unsafe for human consumption. Similar to *kosher* and *halal* meat, the animal should be lively at the time of harvesting, untainted with medications, and free of illness and acute injury. For the Diné, most parts of harvested meat are consumed except the contents of the digestive tract, the bladder, the gallbladder, bones, and wool hair (Kopp, 1986). Some literature indicate that sheep bones may be boiled; the "red marrow (Ruttenbur, Kreiss, Douglas, Buhl, & Millard, 1984)" and bone soup can be consumed (Millard, Lapham, & Hahn, 1986) but the bone itself is not ingested (Kopp, 1986). Sheep wool is commonly used to weave textiles in the Diné population. Lambs and wool

are an economic resource and therefore can be sold on the market (Blunn, 1945). Foods harvested from animals are considered gifts to the *five-fingered* (humans) and permission and thanks are offered to the harvested food (Griffin-Pierce, 2000). Prayers, songs, and gifts are offered for plants as well. In exchange for such gifts the harvester is obligated to take the life of the animal swiftly, consume and use all parts of the animal, and share the food gift with other people.

The History of Diné Agriculture

Agriculture may have been adopted by the Diné from the Pueblo people (Dunmire & Tierney, 1997; Kopp, 1986) subsequent to the Spanish introduction of *O. aries* (Teufel, 1996). The four sacred plants of the Diné are corn, squash, bean, and tobacco (Griffin-Pierce, 2000). Crops are an important food source and were also reported to have medicinal value (Hill, 1938). In a landmark study by Carpenter and Steggerda (1939) sixty six samples of daily Navajo diet were collected and analyzed for their energy value. In the same study, sheep foodstuffs, corn, beans, and squash were the primary food sources. The highest energy values were found in the meats, and certain berries, and seeds. In the mid-1950s, Darby et al. (1956) identified the chief crops grown for home consumption to be corn (the most abundant), squash, melon, pumpkins, bean, and potatoes on the Diné reservation. Rogers and Reisinger (1969; Kopp,1986) reported that 71% of Lower Greasewood reservation residents raised their own food which included: corn (41%), squash (21%), beans (6%), and potatoes (5%). The frost-free growing season ranges from 148 to 220 days (Dick-Peddie, 1993).

DiNEH Study and Agriculture

Preliminary DiNEH data on the population recruited for this dissertation research demonstrated that 49% of the community (N=180) consume the crops they raise. The five most commonly harvested crops in order of frequency are: squash, corn, melon, chili, and beans. Other less frequently grown crops are potatoes, onions, tomatoes, and peaches. Similar to the

study in the mid-50s by Carpenter and Steggerda (1939), a more contemporary study (Teufel, 1996) still reported the same dominantly harvested crops (corn, beans, and squash). In unpublished DiNEH data, only a small subset of respondents reported irrigating their crops with various sources and including most from public water systems, and natural rain fall with fewer using hauled water from various sources, private wells, spring/pond/dam, and livestock wells. A few respondents reported utilizing various combinations of irrigation sources (n=3).

Diné Ethnobotany

Dunmire and Tierny (1997) identified that there are over three thousand species of plants that grow in the wild in New Mexico. Of those, two-thirds of the local American Indians utilize plants for various applications. The plants have three main functions or uses. First, most of those plants are used for medicinal purposes (phytotherapy). The use of plants for a food source is the second most common use. Lastly, plants are utilized for developing dyes and paints.

The Ute Mountain people once used about 300 different plants for phytotherapy (Dunmire & Tierny, 1997). In one region of the Diné reservation, Wyman and Harris (1951) listed 450 plants utilized by the Diné for medicinal purposes and various other applications. Plants and their applications will vary depending on regional climate, availability, and accessibility. Dunmire and Tierney (1997) categorized the uses of plants as: food (edible plants, beverages), medicine, construction (structure construction) and/or fuel (cooking and fuel wood), ceremonial objects (prayer sticks, masks) and implements (agricultural tools, cradles, hunting tools, etc.), basketry and cordage, textiles (matting), and dyes (including paints, soaps, etc.). Most of the medicinal applications are for human use, but in certain instances animals may be fed herbs or plants before slaughter for ceremonial purposes (Sponenberg & Taylor, 2009).

Darby and colleagues identified several "wild foods" in a dietary survey in a study that was published in 1956. They specifically named "*C'îl dehî*" or "wild mountain tea" as a plant

used by the community. A bundle of dried tea measuring one inch diameter by five inches in length was placed in boiled half gallon of water for 20 minutes to concoct a hot beverage. The tea genus and species were not identified in the publication.

Medicinal plants may become contaminated during growth and processing. Some plants selectively accumulate some toxic elements which can be affected by geochemical makeup of the soil. Elements can be transported via rainfall, fertilizers, atmospheric dust, or adsorbed via leaf blades (Basgel & Erdemoglu, 2006).

This community relies on harvesting activities for sustenance and the community's concerns about the contamination of their food chain (deLemos et al., 2009) have propelled this current investigation. The overall goal of this study to explore whether the local food chain is indeed contaminated with heavy metals as a result of uranium mining. This dissertation is organized as four chapters which will be submitted in part or as individual manuscripts for publication.

The first manuscript or Chapter One presents the specific aims of the dissertation, the implications for nursing research, and identifies the gaps in the literature.

Chapter Two is a data-based manuscript that reviews the historical research progression of U research; an emphasis on studies of dietary risk factors associated to harvested animals and plants in U mining areas will be presented.

The third manuscript or Chapter Three presents the Host Agent Environment Triad (HAET) model and the Haddon model utilized to develop the dissertation model.

The fourth paper or Chapter Four presents the methodology of the dissertation and includes the design, procedures, and proposed data analyses.

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Chapter One

Aims, Background, and Significance

This chapter includes the dissertation aims, a historical review of mining in the northwestern New Mexico area, a description of uranium (U) and its properties, a review of U biokinetics with associated health affects, and the gaps in the literature.

The primary aim of the study is to describe dietary behavior of the Diné residents specifically related to ingestion of locally harvested *Ovis aries* and plants. The DiNEH cohort data demonstrates there are sufficient numbers of the community participating in subsistence harvesting activities. Agricultural, harvesting, and consumption patterns of present-day Diné people warrant further evaluation and description.

The second aim of the study will compare U and other associated heavy metal levels in locally harvested sheep and plants from areas known to have high levels of environmental U contamination to those from areas known to have low levels of environmental U contamination. A comparative study has the potential to test and demonstrate differences among the exposure group with a parallel non-mining community in another area of the Diné reservation. The third aim is to explore potential routes of U and heavy metal exposures from locally harvested plants and *O. aries*. Understanding the nature and extent of the exposure need further evaluation. For example, the migration of U into harvested food via tailings or waste piles or contaminated water sources needs further exploration. Animal husbandry activities need further exploration. Of the DiNEH study cohort (deLemos, 2009), 40% self-reported living *near* (within 2 miles) a U mine, 25% demonstrated herding livestock near U mines, mills or waste dumps, and four percent sheltered livestock in abandoned mines.

The fourth aim is disseminate study findings to the leadership and community on the Diné reservation. This aim is meant to augment the health status of the Diné (Navajo) people and will include: increased knowledge and information about U and other heavy metals;

identification and prediction of U and heavy metal contaminate transfer; identification of environmental risks of U and other heavy metals; and the potential for developing partnerships with research and health care programs dedicated to increasing the community health status.

The Diné community will gain knowledge and information about U and other heavy metals in locally raised and harvested food sources at the individual and community level. Increasing knowledge about U, where U is found on the reservation, the routes of possible contamination, and the risk to health will allow for planned changes in behaviors to lower contamination exposure. Depending on the heavy metal examined, studies have shown that U may contribute to kidney toxicity (Gilman et al., 1998; Haley, 1982; Tracy et al., 1992), cadmium associated-cardiovascular disease (Prozialeck, Edwards, Nebert, Woods, Barchowsky, & Atchinson, 2008), Diabetes Mellitus associated to arsenic (DM; Rahman, Tondel, Ahmad, & Axelson, 1998), and mercury associated autoimmune disease (Gardner, Nyland, Silva, Ventura, de Souza, & Silbergeld, 2010). The Diné population has high rates of Type 2 DM (Will, Strauss, & Mendelein, 1997); Hochman, Watt, Reid, & O'Brien, 2007; Rahman et al., 1998), cardiovascular disease (Galloway, Goldberg, & Alpert, 1999) and Chronic Kidney Disease (CKD; Hochman et al., 2007).

Manageable or modifiable risk factors can be targeted to improve the health status of Diné people. Knowledge of risk awareness made known to participants will create opportunities to change dietary risk behaviors if they exist. Dietary risk factors are modifiable. Perhaps even modifying animal grazing patterns or modifying crop agricultural techniques can be beneficial.

Findings will be made available to the aforementioned community entities via written literature. For example, the current research will obtain geographical data which will be correlated with U and heavy metal concentrations. Forage and crop harvesting risk maps can be developed. These maps will be provided to the communities as they have the potential to determine the extent of contamination and inform communities to manage risk in relation to

food chain contamination. Similar risk maps for water and soil were developed by deLemos et al. (2009) and were well received by the current research area. The current study risk maps will be made accessible to community members at their local community chapters. It was identified by deLemos et al. (2009) that education materials or tools presented as pictorial representations were well received in this primarily monolingual community.

Mining Inception in Northwestern New Mexico

During World War II in the 1940's, the U.S. saw the largest harvest of uranium ore of 13 million tons in the Four Corners region (Brugge et al., 2006). From the 1940s to the 1980s, northwestern New Mexico alone contributed 40% of the U.S. uranium production (McLemore, 1983). The inception of mining on Navajo lands started in 1948 at the behest of the US. Atomic Energy Commission (Brugge & Panikkar, 2007). Even after the War ended, private companies continued mining on the reservation until 1988 (Brugge & Pannikar, 2007). In the aftermath, more than 1,100 abandoned and unreclaimed mines and associated waste features remain on the Navajo Nation (see Figure 1.1).

The European literature demonstrates a strong relationship of uranium mining and lung cancer and designated cancer compensable in 1932 (Brugge & Goble, 2002; Holaday, 1969; Lorenze, 1944). In fact, the U.S. Public Health Service started research examining the health effects related to mining two years after the inception of mining on Diné land. Yet, ventilation and protective equipment requirements were not enforced until the early 1960s (Brugge & Pankkar, 2007). Even in the 1940s and 1950s the Navajo culture was independent of mainstream American economic and social systems and there was a lack of awareness of the radioactive properties of the mined ores. Brugge et al. (2006) report that the Diné language lacked a word for radiological effects until recently.

The Diné Concept of *Leetso* or Uranium

The Diné concept of U is important to understand as it has a relationship to the four

related elements of atmosphere: land, water, and sunlight or fire (Woody, Jack, & Bizaholini, 1981). To the Diné, *Leetso* or uranium has its place in the natural order in the environment and possesses properties (Grinde & Johansen, 1995; Reichard, 1963). *Leetso* when translated means yellow dirt or yellow-brown. Uranium is considered to be the antithesis to the sacred corn pollen. Pollen is used in many Diné blessings. In origin stories, the *five-fingered* (human) beings emerged from the third world into the fourth and present world to face an important choice. The people were to choose between the two yellow powders. One was corn pollen from the fields, and the other was yellow dust from the earth and rocks. The people chose corn pollen, and the deities assented (Eichstaedit, 1994). In addition, the deities issued a warning. In choosing the corn pollen, the Navajos were to leave the yellow dust in the ground. If it was ever disturbed or removed, it would bring evil. The ultimate goal for Navajo is the maintenance of balance and harmony between humans and nature (Woody et al., 1981). Mining is regarded as disharmonious as there is a disruption in the balance of earth and sky and is disrespectful to the earth (Eichstaedt, 1994). It is this disturbance with nature that is believed to be the current source of disease, upheaval, and death among the Navajo people (Eichstaedit, 1994; Woody et al., 1981).

Elemental Uranium Properties

Uranium is the heaviest natural mineral, three tablespoons weigh close to two pounds (Cravens, 2007). Natural unprocessed U is a powdery yellow substance and refined U has a grayish-silver metallic hue and is malleable (Leggett, 1989). Isotopes with an odd number of neutrons and protons are more unstable and as they strive for balance the isotopes eject rays and particles known as radiation (Cravens, 2007). Alpha decay occurs in isotopes such as uranium, but other studies have shown beta (Archer, Brinton, & Wagoner, 1964), and gamma decay as well (Shields, Weise, Skipper, Charley, & Benally, 1992). Alpha particles are slow and heavy and have a low penetration power and can be stopped by a sheet of paper. Beta particles are fast

and light and have a medium penetrating power and can be stopped by a sheet of aluminum. Gamma ray waves have a high penetration power and a thick sheet of concrete or lead can reduce them significantly. There are three naturally occurring U isotopes, U-238 (the most abundant), U-235, and U-234 depending on the number of protons or neutrons the isotope possesses (Cravens, 2007). Although natural U is commonly found in the hexavalent form it occurs naturally in the +2, +3, +4, +5, or +6 valence state. The U-238 half-life decay is 4.5 billion years, U-235 is 704 million years, and U-234 is 247 thousand years (see Table 1.1). Hexavalent U is primarily associated with oxygen as the uranyl ion UO_2^{2+}.

In another part of the world, U ore had been mined for centuries in Jachimove, Czechosolvakia and Schneeberg, Germany (Brugge & Goble, 2002). A condition, *Bergkrankheit,* was strongly associated to mining. *Bergkrankheit* literally meant "mountain sickness" and was commonly attributed to mining. Many young miners developed symptoms of cough, difficulty breathing, and eventually died. One study reported 75% of all deaths among miners were a result of this disease (Arnstein, 1913). Modern medicine has identified the sickness with lung or bronchial cancer related to radioactive conditions (Zeman & Karlsch, 2008).

Alpha, beta, and gamma rays are known to cause untoward health effects and outcomes. Samet and colleagues (1984) have findings that support a significant association between lung cancer in uranium miners and alpha radiation. It is the radioactive daughters of radon associated with mining that are a strong indicator of lung cancer (Finkelstein, 1996).

Uranium affects multiple organ systems and is a known as a nephrotoxin (Zamora, Zilinski, Meyerhof, & Tracy, 2002). Animal and human studies demonstrate that those exposed to uranium show chemical toxicity manifested by functional and histologic changes in the proximal kidney tubules (Kurttio, Auvinen, Salonen, Saha, Pekkanen, Makelainen, Vaisanen, Penttila, & Komulanen, 2002; Zamora et al., 2002), altered renal filtration (Diamond, 1989), and

increased glucose and proteinuria (Diamond, 1989; Domingo, Llobet, Tomas, & Corbella, 1987). In addition, uranium can accumulate in the bones, muscle, adipose tissue, and lungs (Diamond, 1989; Foulkes, 1990; Kathern, McInroy, Moore, & Dietert, 1989). In a study by Gilman and associates (1998), histopathological lesions were observed in the liver, thyroid, and kidney in rats exposed to uranium. More so, several studies have demonstrated a well-established association between exposure to U progeny and lung cancer (Archer, 1988; Gottlieb & Husen, 1982; Roscoe, Deddens, Salvan, & Schnorr, 1995). In addition to lung cancer, other chronic diseases such as obstructive lung disease, silicosis, pulmonary fibrosis, emphysema, and silico-tuberculosis are common (Mulloy, James, Mohs, & Kornfeld, 2001).

Uranium Transport

Uranium can leach into the ground and aquifer as a result of mining, the presence of protore tailings, release of nuclear materials into the environment (Dreesen & Williams, 1982) Radionuclides such as U behave differently in water. Uranium is readily soluble in the presence of oxygen (Wirt, 1994). Due to its solubility, U is easily transported from its source of origin. Solid U can also attach itself to clay and mineral coatings on sand, silt, bacteria, precipitates, and rock fragments (Tricca, Wasserburg, Porcelli, & Baskaran, 2001). Uranium can be transported on sand and silt particles suspended in flowing water. Thus, adsorption into particles and surface deposition of airbourne materials are factors that influence transport. Drinking water is often supplied from groundwater or surface water sources which can be contaminated with heavy metals such as U. In addition, in surface water, contaminants are often stored in sediment and released slowly (WHO, 1999). After recharge (e.g. rain, snow melt, flooding), U can be transported great distances from the original source (Wirt, 1994). In groundwater, turnover rates are slow, (often hundreds or thousands of years) so that water remains contaminated for longer periods of time (WHO, 1999).

In northwestern New Mexico on the Diné Nation near mine waste disposal areas, deLemos and colleagues (2008) found a correlation between U concentration and mean particle

size. Specifically, higher concentrations of U were shown to be associated with very fine sand (deLemos et al., 2008). The sediment samples in close proximity (50m) to mine waste disposal sites were found to be highly soluble. In the same study, weathered surface sediment was shown to be depleted of soluble uranyl phases than deeper sediments which could potentially impact groundwater sources due to infiltrating precipitation.

Soil disturbance is another means of transport for U. deLemos and colleagues (2008) demonstrated that soil disturbance in unweathered mining waste sites increased exposure to humans and animals. deLemos et al. (2009) identified high, medium, and low levels of U soil contamination in the area of study and developed a *Soil Restriction Recommendation Map* to manage community risk. Soil disturbance via erosion and blowing dust is another exposure factor to consider. Blowing dust is an inhalation risk to humans and animals. In addition, soil ingested by grazing animals is a potentially important source of radionuclides. Soil adhesion to vegetation was highly seasonal (highest in autumn and winter) in a study by Beresford and Howard (1991). The authors reported that soil ingestion occurred as a result of ruminants licking soiled snouts, direct solid ingestion, and pulling up of roots. Settled dust on crops and local botanicals are other routes of exposure. Future research can implement air contaminant studies to determine the transport of U in the air.

Gaps in the Literature

A study that focuses on a thorough ecological assessment analyzing the human food chain and its resultant health effects is needed. Dietary risk factors related to traditional subsistence foods such as meats, crops, herbs, and medicinal plants need further examination in this high risk population. Existent studies have identified the various routes of food chain contamination but have recommended further examination. For instance, DiNEH cohort data indicated that the local community utilize regulated and unregulated water sources for personal consumption, watering of livestock, and locally grown crops. Thomas and Gates (1999) focused on a similar aboriginal community in Canada. The lichen-caribou-human food chain was

examined for U and other metals. Uranium transfer to caribou meat ranged from 1-16%. Predicting transfer from heavy metals to meat and plant food is essential for future studies to evaluate U impact on food chain. Studies near mining sites in Ambrosia Lake, New Mexico (near the reservation) have demonstrated uptake of radionuclides in animals (Lapham, Millard, Samet, 1989; Millard, Lapham, Hahn, & Jere, 1986) and will be discussed in detail in Chapter Two.

Direct contact with contaminated soil and water in relation to agricultural and harvesting food needs attention. In addition, contact with contaminated soil and activities when harvesting herbs should be studied and characterized. High risk maps that identify problem areas where harvesting activities occur need further development. The *Soil Restriction Recommendation Map* and *Water Hauling Map Recommendations* developed by deLemos et al., (2009) were well received in the Diné community. A risk assessment map would determine which high contamination areas are utilized by the community for grazing, agriculture, and other harvesting activities. What is the extent of community members grazing their sheep or harvesting crops in areas of high contamination? What other activities (hauling water, riding horses, building structures, etc.) related to land management or ranching occur in these areas of high contamination?

Studies have demonstrated that U tends to accumulate mainly in the roots of edible plant species (Shahandeh & Hossner, 2002), non-edible grass species (deLemos et al., 2008; Lapham, et al., 1989; Shahandeh & Hossner, 2002) with less translocation into the shoots. Uranium concentration and the amount of contaminated water irrigation is a main factor in plant uptake (Gulati, Oswal, Nagpaul, 1980). Shahandeh and Hossner (2002) identified solubility and mobility of U and the type of soil as influencing factors. Identifying local plants and delineating the nature of their uptake in terms of the plant parts consumed needs to be examined. The phytodegradation (plant uptake, metabolism, and degradation) of local plants needs further

characterization in this high risk area.

These above gaps in the literature are addressed in whole or partially by this current research dissertation. Literature gaps to be presented next are not the primary foci of the dissertation but are recommendations for future research.

There is a general lack of studies that have examined or addressed the psychological effects of U mining among the Diné people. The study by Markstrom and Charley (2003) found that psychological effects of human-caused disaster negatively impact grief and bereavement, environmental loss, feelings of betrayal and mistrust, fear about current and future effects, and cause prolonged psychological effects. In addition, marginalized populations whom are survivors of technological and human-caused disasters have compounded effects of psychological impacts (Green & Lindy, 1994). In other words, stress associated with lower socioeconomic status in which physical and economic restrictions are placed on access to resources, lead to adverse health outcomes for ethnically diverse populations (Flaskerud & Winslow, 1998). Psychological impact studies of U disaster have not been widely addressed. In addition, implementation of culturally-specific appropriate forms of healing or intervention are nonexistent . Further research needs to focus on environmental, socioeconomic, and cultural contexts of the Diné population to understand the contributing factors associated to environmental contamination.

Identifying and addressing the importance of other chronic debilitating illnesses related to chronic U and heavy metal exposures need attention. The bioeffects of U on organ systems such as the lymphatic, bone, gastrointestinal, genitourinary, and renal system need further study. Other inquires include whether chronic exposure makes one more susceptible to renal or pulmonary injury especially in a population that already has a high incidence of diabetes, renal failure associated to diabetes, and End Stage Renal Disease (ESRD). Hochman and associates (2007) reported that the prevalence of non-diabetic ESRD was higher in the Diné community

(0.63%) as compared to the U.S. population (0.19%) and all American Indians (0.36%) in the U.S. Studies by DiNEH researchers related to the association between U exposure and ESRD, Diabetes, HTN, and autoimmune disorders in the Diné population are currently underway.

There are other geographic areas on the reservation that have been exposed to U mining in Arizona and Utah. The majority of past and current studies have primarily focused on northwestern New Mexico regions. Extending U contamination studies and integrating the data with the rest of the reservation is needed. It cannot be assumed that one area of the reservation is similar to the rest of the reservation. There are many community, plant and animal life, geographical, geological, and climatic differences.

In 2010, the Navajo Nation Area Indian Health Services (IHS) implemented the Community Uranium Exposure Journey to Healing (CUEJTH) program. The CUEJTH program is intended to be a reservation-wide U surveillance program. There is research potential for prospective and retrospective studies examining the surveillance data in the near future. Data integration with 12 major IHS health care centers and their associated satellite clinics is anticipated. Examining additional associated heavy metals to the surveillance program may need further exploration.

Another priority for research includes the development of improved and more sensitive human biomarkers and diagnostic tools to identify perceived risk and actual uranium toxicity. Whether these changes can be detected at the subclinical toxic stage remains to be determined. The advantage of updated biomarker technology is the ability to detect subclinical and clinical stages of uranium toxicology at various sites (organ or bone) for appropriate intervention. As an example, Li and colleagues (2009) have data to support the use of human hair as a verifying determinant in conjunction with urinanalysis to evaluate chronic ingestion exposures.

In this chapter, a historical look at U mining, the associated health effects, and the literature gaps were presented. More recent attention has caused an increase in studies in this

community but as indicated by the literature gaps, more research questions emerge.

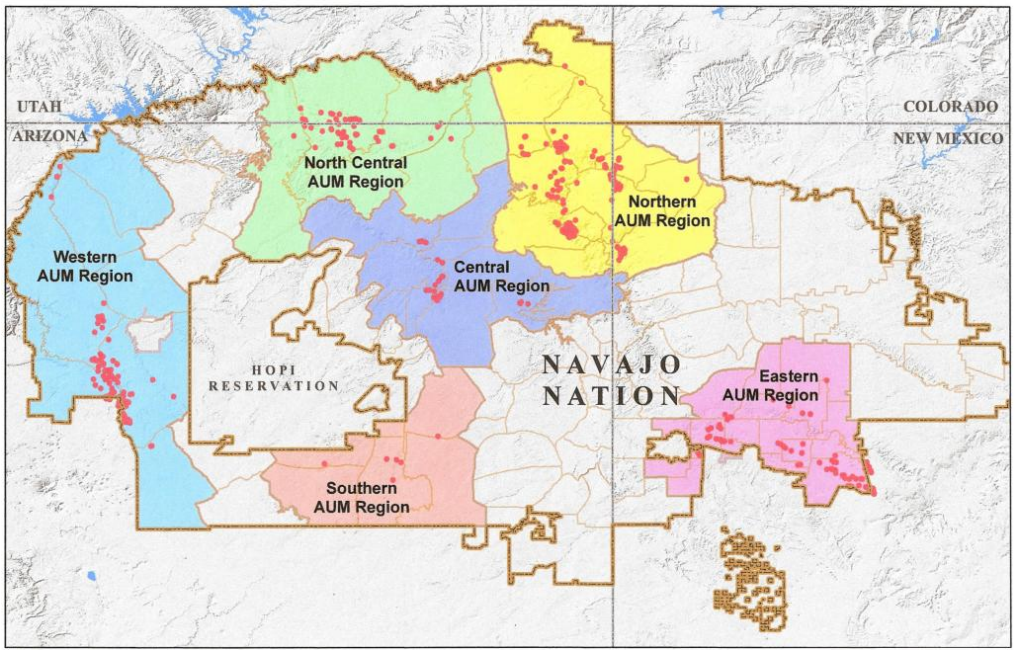

Figure 1.1 Abandoned Uranium Mines on the Navajo Nation. This map depicts the four corners region and includes Arizona, Colorado, New Mexico, and Utah. From. U.S. Environmental Protection Agency. http://yosemite.epa.gov/r9/sfund/

Nuclide	Abundance (%)	Number of Protons	Number of Neutrons	Half-Life
U-238	99.2745	92	146	4.5 billion years
U-235	0.7200	92	143	704 million years
U-234	0.0055	92	142	274 thousand years

Table 1.1 Summary of uranium isotopes U-238, U-235, and U-234. Isotopes with an odd number of neutrons and protons are more unstable and as they strive for balance the isotopes eject rays and particle known as radiation. The half-lives for each isotope is shown as above.

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Chapter Two

Review of the Literature

Introduction

The studies to be presented are reflective of the overall historical progression of U associated research. A portion of the studies presented were undertaken on Diné Lands. The first stages of the research began as U miners experienced first-hand direct mining exposure (via tunneling, transport, and milling processes,). The U miners reported or were diagnosed with major health effects such as lung cancer, pneumoconiosis, pulmonary fibrosis, silicosis, and other respiratory diseases. Next, the chronic environmental U exposure conditions created the impetus for studies related to birth outcomes and chronic ingestion of U contaminated water and its association to renal damage. Currently, focused studies evaluating various exposure routes while considering sociobehavioral and cultural aspects of a community have emerged. Contamination studies that evaluate the contribution of traditional subsistence harvesting are in the vast minority. The main driving force of this dissertation is to determine the extent and impact of uranium and other heavy metals on locally harvested food in a once heavily mined area in several northwestern communities in New Mexico. The outcome of this research could shape future studies on the health effects related to chronic U and associated heavy metal exposures and raise the necessary awareness of other harvesting communities exposed to mining contamination. The study could answer the community's concerns regarding heavily consumed locally harvested foods as well as augment further inquiry, prevention efforts, intervention, monitoring, and education. Importantly, the findings have the potential to support legislation, policy development, and advocacy.

A logical place to begin a historical analysis of the review of literature is from the inception of uranium (U) mining on the Diné reservation. Related or peripheral literature will be presented where relevant. Uranium research extending from the 1940s to the 21st century will be presented. The literature review will be divided into several eras: 1) the earliest studies

spanning from the 1940s to 1970s; 2) studies conducted between the 1980s and 1990s; and 3) the studies conducted after 1990. A separate section will be reserved for studies related to U and other heavy metal contamination and dietary risk factors associated to harvested animals and plants in various communities.

The 1940s to 1970s

European studies reported the ill health effects of radiation and U mining and had enforced a ventilation project by 1879. In 1942, the environmental cancer section of the National Cancer Institute (NCI) reported excess occupational risk of lung cancer in miners due to radon gas (Brugge & Panikkar, 2007). Despite the available scientific knowledge and information, little was done in terms of public health and safety measures at the inception of mining on Diné lands.

In 1964 a study by Archer and colleagues set out to determine the extent of loss of pulmonary function among U miners consisting of a study sample in which 20% were Navajo or Diné men. The study had many limitations. First, the four pulmonary function tests (PFTs) were unstandardized and were measured only on a short-term basis (less than one year). Also, the PFT measures of all Diné participants were excluded because the cooperation given by Navajo Indians was reported to be inconsistent (Archer et al., 1964, p.1184). The authors also commented that mining has a high turn-over rate, but failed to report the high attrition rate of the study. For future studies, Archer et al. (1964) recommended long-term follow-up of subjects and comparing the results of PFTs with those of chest x-rays for future studies.

A similar study by Soccomanno and associates (1964) focused on the incidence of lung cancer and types of histology in U miners in the Colorado plateau area. The demographics distribution supports that a large proportion of the participants were assumed to be of Diné descent or other American Indian (AI) individuals, but were not specifically identified. Therefore, the data results cannot be inferred to include Diné specifically. The results did show a

higher proportion (57 %) of undifferentiated varieties of tumors and the differences in cell types were not shown to be attributed to cigarette use or age. The information would have been valuable scientific information for the AI community. Earlier studies attempted to demonstrate an association between cigarette smoking and lung cancer in a population with history of low smoking levels. The same studies recommended that miners simply discontinue smoking to address the increasing incidence of lung cancer. Ceremonial smoking is a limited cultural practice among Diné people. Positive reporting of ceremonial smoking may have been misrepresented as "smoking on a regular basis" instead of reflecting limited usage for cultural practices. The amount of ceremonial tobacco smoke is likely significantly less than one pack per year over a lifetime for most Diné people (Brugge & Goble, 2002).

The 1980s to 1990s

Gottlieb and Husen (1982) aimed to examine a population with low smoking histories in Diné male miners who were hospitalized with a diagnosis of lung cancer from 1965 to 1979. The sample size was relatively small (N=17). Statistical analyses information was scarce. The authors reported distribution frequencies, proportions, and calculations of working level months by simple equation: one WLM (170 hours per month) = 1.3×10^5 MeV of alpha energy. The researchers concluded that 65% of the predominant cancer type was small-cell-undifferentiated carcinoma (SCUC). The low frequency of cigarette smoking among Diné men supports the view that radiation is the primary initiating agent of lung cancer among U miners and that smoking acts as a promoting agent.

In 1984, Samet and colleagues performed a population-based case control study to examine a relationship between lung cancer and U mining. The control group did not have data available regarding their smoking histories which made it difficult to assess the relative risk associated with cigarette smoking. The study was able to support a relative risk of 14.4 for lung cancer associated with U mining. The results demonstrated in a rural nonsmoking population

that the majority of lung cancer cases may be attributed to the hazardous occupation of U mining. The researchers recommended further follow-up for a complete characterization of the consequences of U mining in Diné workers. As the mortality rates of young (median age 44 years) Diné miners continued to climb from 1969 to 1981. More cases were anticipated.

Butler and colleagues (1986) investigated 26 cases of lung cancer occurring among Diné male miners between 1969 and1982. In depth, statistical analyses were reported in the article, but frequency distributions and few proportional data were reported. The authors stated that classification of cancer cell types was difficult with light microscopy and conflicting and confusing smoking classification and categorization were limitations of the study. The findings suggested that Diné miners who were heavy cigarette smokers combined with U exposure supported the development and predominance of SCUC. To a smaller extent, this was also supported in those participants who were "light smokers" to "non-smokers" .

The 1990s and Thereafter

A unique study by Shields and associates (1992) examined the birth outcomes on the Diné reservation in parents and children who lived near U tailings or mine dumps in Shiprock, New Mexico. The major limitation of this study was the presence of a local competing electronic assembly plant that employed the local population from 1969 to 1975. The assembly plant utilized various solvents and chemicals such as trichlorethylene, a well documented teratogen (Hayes, 2008). Another limitation included the exclusion of respiratory failure, spontaneous abortions , and low birth weight from the study. Contrarily, several studies support that respiratory failure can be a plausible symptom related to radiation effect (Gilliland, Hunt, Pardilla, & Key, 2000; Mapel, Coultas, James, Hunt, Stidley, & Gilliland,1997). The only statistically significant association was the maternal residence near tailings or mine dumps (OR 2.7, $p = .03$). Also, birth defects increased significantly if either parent (mother 2.56, $p = .02$; father 2.05, $p = . 01$) worked in the assembly plant . The researchers suggested further research

in dose-response analyses as related to genetic effects in U exposed people.

Roscoe and colleagues (1995) conducted a study to update mortality risk for Diné miners in the Four Corners region of the United States. Vital status was monitored from 1960 to 1990 in New Mexico and Arizona. A Cox regression-model was utilized and preferred over a log-linear model to evaluate an exposure-response relationship. The method was applied appropriately to control for confounding "time" variables when considering association with exposure (Jewell, 2003). A few of the limitations included reclassification of smoking criteria from previous data between 1950 and 1973 often without participant verification. In addition, during the study approximately five percent of the participants' tobacco information was categorized as "never having smoked" if they used chewing tobacco or snuff. Their findings showed elevated standardized mortality ratio for lung cancer (3.3), TB (2.6), pneumoconiosis and other chronic respiratory disease (0.4), and liver cirrhosis (0.5) in light-smoking Diné miners with a group average of 23 years from last-exposure (Roscoe et al., 1995). Standardized mortality ratios were estimated with combined Arizona and New Mexico non-White (all except Whites and Hispanics) mortality rates used for comparison. These results established mortality rates specifically for Diné miners for the first time.

A 1997 study (Mapel, Coultas, James, Hunt, Stidley, & Gilliland) evaluating criteria for compensation among ethnic minorities investigated the relationship between nonmalignant respiratory disease and underground U miners, underground miners were found to have higher exposure to silica dust, radon, and diesel fumes in underground mines. The underground mines lacked ventilation and workers were exposed to poor working conditions. This study utilized multivariate models for obstructive lung disease, radiographic pneumoconiosis, and Full Expiratory Volume in one second (FEV1) by ethnic group to evaluate for evidence of effect modification on the basis of ethnic differences which had not been undertaken in previous studies. One limitation was the use of an exposure self-identification process which may have

threatened the study construct validity due to recall bias. Step-wise regression was utilized to arrive at the most parsimonious models and non-significant covariates were eliminated ($p > .05$) . The intake information was self-reported and comprised of cigarette smoking, mining exposure, and health history. In addition, the relative small sample size for Hispanic and non-Hispanic whites may have reduced the power of effect via multiple linear regression and logistic regression models. In these cases, utilizing a mixed-methods model would have been more robust rather than analyzing smaller unequal sample sizes. Internal validity may have been violated as selection-historical bias may have occurred as many of the AI participants were working in the mines at the industry inception (1940s) and the non-AI participants primarily worked away from the reservation where the mining industry began in the l960s and 1970s. The AI workers had greater exposure in terms of time and physical exposure than the control group (Brugge & Panikkar, 2007). This study also recommended further development of screening tests and criteria and suggested utilizing high-resolution tomography versus the simple chest x-ray for screening and compensation purposes. This recommendation is consistent with findings from the Hnizdo and colleagues (1993) study which demonstrate that the routine chest x-ray was less accurate in identifying a large proportion of miners with moderate to severe silicosis verified by autopsy.

To determine lung cancer incidence and estimate risk, Gilliland and colleagues (2000) conducted a population based case control study from 1969 to1993 in Diné male miners. The statistical methods used include conditional logistic regression models for estimation of relative risk and the examination of smoking as a confounding variable. The publication also briefly mentioned the use of proportional hazards regression procedure which may have been chosen since time confounding effects at the time of risk were anticipated. If there are no confounding effects, the two approaches can be expected to yield similar results (Jewell, 2003). Gilliland and associates (2000) reported that tumors with squamous cell histology accounted for 40% of

tumors extending over the period from 1969 to 1993. In the same study, the relative risk for a history of mining was 28.6 (95% CI, 13.2-61.7) and 67% of the Diné subject cases had a positive work history as underground U miners. This is the first study to recommend case follow-up to document the full effects of U mining and suggested further research development in primary and secondary prevention strategies to reduce the burden of lung cancer in a high-risk population. No specific prevention or intervention strategies or plans were included in the publication.

Studies Related to Chronic Ingestion of Uranium Contaminated Water

The majority of animal studies have found structural changes in kidneys with chronic ingestion of U in water (Diamond, Morrow, Panner, Gelein, & Batt; 1989; Gilman Villenueve, Secours, Yagminas, Tracy, Quinn, Valli, Willes, & Moss, 1989; Haley, 1982; La Touche, Willis, & Dawydiak, 1987; Martinez, Cabrini, & Ubios, 2000). Damage to the renal system is one of the primary toxic actions of metals such as U (Mueller, Price, & Finn, 1998). The kidney can suffer considerable damage before losing function; 50% or more of renal capacity can be lost before serum creatinine levels become abnormal (Hook, 1981). The detection of renal damage at a reversible stage is necessary before effective measures can be implemented to ameliorate progression into the irreversible stage. Still little is known about the effects of chronic environmental U exposure in humans. In animal studies, regeneration of the proximal tubules may take up to eight weeks at which time significant interstitial renal damage remains (Haley, Bulger, & Dobyan, 1982). A major site of uranium accumulation is the skeleton (Wrenn, Durbin, Howard, Lipsztein, Rundo, Still, & Willis, 1985). Further, uranium is thought to be deposited on the bone surface and surfaces of bone mineral crystals (Leggett, 1994).

A peripheral research study conducted by Zamora and associates (1998) focused on chronic ingestion of U-contaminated drinking water. A comparative study was undertaken in Ontario, Canada. One group consisted of those with low-exposure (n=20, municipal water users)

and the second group was labeled high-exposure (n=30, private-well users). The primary aim of the study was to determine the renal changes associated with chronic ingestion of U contaminated water. Numerous renal biomarkers such as glucose, creatinine, glucose, total protein, alkaline phosphatase (ALP), and beta-two microglobulin (BMG) were studied. The findings showed an increase in urinary glucose, ALP, and BMG. In addition, kidney function at the proximal tubule was affected rather than the glomerulus as initially hypothesized. One major flaw in their investigation was excluding participants with a history of renal and heart disease, hypertension, and diabetes mellitus as these are known predisposing conditions for kidney disease. Essentially those at most-risk remained unstudied. Another weakness of the study was measuring U levels exclusively in food and water and not measuring the amount of U actually reaching the kidneys. This landmark study was of great interest as it focused on well-water (unfiltered) consumption as an important exposure variable in contamination studies which was largely ignored in the past. This study highlights the importance of assessing private water and livestock well (mostly un-regulated) use which are frequently utilized today on the Diné reservation for human consumption (deLemos, 2009).

Zamora et al. (1998) recommend studying U intake and excretion in urine to measure the fractional uptake of U in the gastrointestinal (GI) tract to determine if U uptake is primarily as a result of food or water intake. A study by Zamora and colleagues (2002) aimed to calculate an appropriate GI absorption factor (f_1) for humans to use in developing exposure guidelines for U. Fifty Novia Scotian subjects were grouped in low exposure (n = 20) and high exposure (n = 39) well water users and duplicate food and water samples were collected in conjunction with blood, urine, and feces for three days. The International Commission on Radiological Protection (1995) currently recommends f_1 values of 0.04 for infants to one year of age and 0.02 for all other age groups. The results of the study supported that U is absorbed equally from food and drinking water which is contrary to the 90% food absorption value reported by the World Health

Organization (WHO, 1998). The authors therefore recommended an amendment to WHO guidelines to be decrease U absorption to 20% in high exposure communities set by WHO (1998). The authors supported the f_1 ICRP recommendation of 0.02 for adults and children over one year of age as their best estimate was central f_1 value of 0.009. The authors also supported the conclusion that GI absorption of U appears to be independent of total U intake. The study would have increased statistical power by obtaining a larger sample size and collecting samples for greater than three days.

A study by Kurttio and colleagues (2002) aimed to evaluate the possible kidney effects of chronic exposure to U via drinking water. This was a Finnish study with 325 subjects consuming water from drilled wells. Uranium levels in water, urine, and blood, were collected for ten weeks. Urine and blood were collected by spot-sampling procedure. Crude and adjusted analyses were performed utilizing general linear regression models (one-way ANOVA). The study demonstrated an association between increased U exposures through drinking water and renal tubular function but, not between U exposure and biomarkers of glomerular injury (e.g., creatinine, albumin). The results were consistent with the previous study by Zamora et al., (1998) and other studies suggesting that consuming U contaminated water affects kidney tubular function, high doses of U cause acute renal failure (Haley et al, 1982; Pavlakis, Pollack, McLean, Bartrop, 1996), and lower exposure levels induce functional changes in kidneys (Diamond, et al., 1989; Gilman et al., 1989). One limitation of the Kurttio et al. study included utilizing a urine spot-sampling method which may be inadequate as U concentrations in groundwater vary over time; the study spanned only ten weeks and more frequent and prolonged sampling would have been beneficial. In addition, when utilizing participant self reports (water diaries) there is a risk to construct validity attributed by participant reactivity or misreporting. One way to address self-reported data would be to validate the information with participants or other public records. The study also failed to report the amount of water that was consumed away from the home if the

participants did not work in the home. Shimokura and colleagues (1997) found 33% of study participants consumed water away from the home at the workplace, restaurant, or at a friend or relative's residence. The researchers suggested using sufficiently sensitive functional biomarkers to detect latent kidney dysfunction which may have gone unobserved in other studies.

A more recent study (Selden, Lundholm, Edlund, Hogdahl, Ek, Bergstrom, & Ohlson, 2009) examined the association between drinking water from drilled and municipal water and measured kidney function biomarkers. The sample of 453 was divided into two groups: those who consumed drilled unfiltered water (n=301) and those who consumed municipal filtered water (n=152). The rural Arjäng municipality of Sweden was compared to central non-rural Arjäng community with access to public water supply. The recommended action level of U in the water is 15 µg/L (WHO, 2004). Three indices were measured including U levels in water, cumulative U exposure from drinking water, and U levels in urine samples. The study concluded that U levels in urine were strongly correlated to levels in drinking water from private drilled wells. The findings were supportive of past studies. Nephrotoxic effects were observed when U in urine was used as a measure of overall exposure. Limitations of the study include conflicting information in regards to the statistical analyses of the biomarkers using linear regression. It was reported that all the biomarker data were skewed except phosphate. A log transformation was performed with ratio of a grand mean. In the discussion section of the article, the authors mentioned considering a Bonferroni post-hoc test and opted against it as it would have exposed their study to falsely rejecting the null hypotheses. The control group varied in sample size and age (5.5 years) which might have been served better by a mixed-model versus a chi square test (Selden et al., 2009). In addition, the study reported that participants utilized filters or water quality improvement equipment, but there was no mention if they were appropriately controlled for in the study.

Studies of Dietary Risk Factors from Harvested Animals and Plants

In 1979 on Diné lands, America's largest U release occurred in Churchrock, NM where 1,100 tons of milling waste and 95 million gallons of mine effluent (Brugge & Panikkar, 2007) rushed from a broken dam. In one of the earliest exposure studies to that area, Ruttenber et al. (1984) implemented an *in vivo* examination of Diné people (N=6) who were exposed to the tailings and animal studies to determine radionuclide levels (Pb, Po, Ra, Th, U) in exposed sheep (n=4), goats (n=2), cattle (n=2) and non-exposed animals (n=3). The study did not comment on whether the six subjects ate from a representative Diné diet. For all radionuclides, higher bone concentrations were exhibited in exposed cattle and sheep than in controls. Estimated radiation doses were highest for ingestion of a single animal kidney and lowest for muscle meat. Estimated exposures did not exceed federal and state regulations at that time; the authors recommended further study of crops and bone marrow ingestion. This is an acute exposure study following a spill of mine effluent. A study in an adjacent mining community (Milan, New Mexico) reported Se levels of 26 to1800 mg/L in drinking water (Valentine, Kang, & Spivey, 1978).

Millard et al. (1986) investigated radionuclide levels (Pb, Po, Ra, Th, U) in Churchrock, NM (exposed area) and Crownpoint, NM (reference area) in sheep, cattle, and environmental samples (water, vegetation, and soil). Seven cattle and ten sheep were obtained from the Churchrock area. Ten cattle and 10 sheep were obtained in the reference area near Crownpoint. Animal muscle, kidney, liver, endosteum, and marrow samples were collected. In exposed sheep, U^{234} and U^{238} were found to be significantly higher in muscle, liver (Th), and kidney. Internal doses of radiation were calculated for three scenarios of tissue consumption. Consuming exposed animal meat was found to be negligible in hypothetical individuals as established by the Internal Commission on Radiological Protection (ICRP) as an acceptable risk to the general population (ICRP 77a). Cancer risk estimates were calculated for each scenario using the

effective dose equivalents of Dunning (1985). The ICRP risk coefficient of 125 x 10^{-6} per rem was used to estimate the expected number of cancer deaths (ICRP 77a, ICRP 77b).

Lapham et al. (1989) determined the radionuclide levels (Pb, Po, Ra, Th, U) in cattle in Ambrosia, NM which is an extensive U mining area adjacent to Diné lands. Exposed cattle (n=10) were compared to control cattle (n=10) from Crownpoint, NM. Radionuclide levels (including U) in the cattle were derived from liver, kidney, muscle, and bone tissue. Environmental samples included water, soil, and grasses. Radionuclides were elevated in all comparisons to controls, however U was highest in the liver and kidney. The authors concluded that consuming exposed cattle posed minimal health risk unless large amounts of liver and kidney were ingested. This investigation focused on cattle consumption, however, a common Diné dietary staple is mutton (Ballew, White, Strauss, Benson, Mendlein, & Mokdad, 1997). Further, in preliminary DiNEH data 76.5% exclusively raised and consumed sheep on their ranch.

In unpublished pilot data from Northern Arizona University (NAU), J. Ingram (personal communication, September 13, 2010) found that sheep exposed to high mining (n=1) areas had significantly higher U content than sheep in control areas (n=2). Tissues examined included muscle, rib, lung, and intestines. The NAU investigators also found elevated U in plant content in two grazing regions (out of three) near mining areas compared to two non-mining areas (A. Jauregui, personal communication, October 12, 2010). Using a macronutrient analysis, it was found that there was less plant uptake of U in phosphorus rich areas. It was hypothesized that the low pH in that part of Diné land (Arizona) did not allow large quantities of U uptake in plants. Currently NAU researchers are undertaking a greenhouse study utilizing high and low U containing soil; these results underscore the importance of local soil chemistry in influencing the degree of uptake. Greenhouse experiments are typically controlled environments requiring premeasured soil, irrigation, and U levels and often do not reflect important aspects of the

"natural" environment such as sunlight, rainfall, temperature, wind transference of heavy metals, etc. The current study will examine locally grown food in natural conditions and will provide valuable information in a high and low impact areas. Natural setting studies are preferred in environmental studies as they reflect sun exposure to plants, natural precipitation, livestock grazing conditions, human husbandry and harvesting techniques, and other important variables. Controlled greenhouse experiments often do not take into account such important natural variables. The NAU study focused exclusively on U concentration levels and would have benefited from evaluating other associated heavy metals. The current study will evaluate for additional heavy metals including U in a non-controlled natural environment.

Frohmberg et al. (2000) iterate that AI food chain assessment studies commonly do not characterize the Native lifestyle to include hunting, gathering, planting, or cooking cultural foods and recommended including the traditional dietary consumption pathways to mirror AI practices. For example, radionuclides (Cs, Pb, Po, Ra, U) were evaluated in the lichen-caribou-human food chain near mining operations in Saskatchewan by Thomas and Gates (1999) who examined muscle, kidney, liver, bone, GI samples, feces, and blood in 18 caribou. The authors determined the U concentration ratio in the crucial food chain in this population to be one to 16% in caribou muscle, one to two percent for Pb, and 260 to 370% for Cs. Transfer of U from lichen to caribou muscle was one to three percent and from rumen contents to muscle was five to 11%. For Pb, from lichen to muscle one to two percent and from rumen to muscle 0.5-1.3%. For Cs, only transfer from rumen to muscle was reported at 250-260% . This study highlights factors that each indigenous population can vary in diet due to availability, culture, and climate. Likewise, the bioavailability and bioconcentration ratios for metals can vary substantially based on local soil chemistry, micronutrient content, and climate (Adriano, 2001).

In a study by Tsuji et al. (2007), a Geographic Information System (GIS) was utilized to augment an integrative risk assessment in the traplines of Ouje-Bougoumou Cree indigenous

territories. The findings identified the potential routes of exposure from harvesting food in areas of mining. The authors found extensive overlap in areas of harvesting (various animals and birds) with mining operations. Other types of harvesting included medicinal plants, berries, firewood, and drinking water collection. Potential routes of exposure were identified to be from contaminated ingestion of game and drinking water. Similar studies have addressed the potential of using aboriginal land use data to document sites of environmental concerns. Kinney et al. (1997) examined the extent of contamination of local fish by polychlorinated biphenyls in the Mohawk Nation. Tsuji et al. (2005) also documented the need for food chain investigation by identifying receptors and routes of contaminant exposures. The study examined traditional activities such as harvesting and collecting terrestrial receptors (e.g. hares) and other media (e.g. spring water on the contaminated sites).

In a greenhouse experiment, Shahandeh and Hossner (2002) found that sunflower (*Helianthus annuus*) and Indian mustard (*Brassica juncea*) of the 34 plant types examined had the most U accumulation. Wheat and ryegrass had the lowest U concentrations. Five categories of plants were examined: dicotyledonous plants (e.g. *Helianthus annuus*, *Brassica juncea*, saltbush), field crops (e.g. corn, oats, wheat), cool and warm season grasses (e.g. annual ryegrass, little blue stem, switchgrass), *Brassica* family (e.g. broccoli, collards, kale, cauliflower, turnip) and root crops (beets, sugarbeets, and Swiss chard). The dicotyledonous plants, the *Brassica* family plants, leafy vegetables, and root crops accumulated greater amounts of U than the grasses. Uranium accumulation was greatest in the roots (30 - 50x) rather than plant shoots (similarly in *Zea mays* or corn). The soil properties influenced the accumulation of U in plants. More acidic soils showed the lowest U shoot and root concentrations.

A study was undertaken in Mailuu Suu, Kyrgyzstan, by Vandenhove and colleagues (2006), an area known to be polluted with radionuclides from tailings dumps and heaps related to U mining. Inhabitants of the village of Kara Agach showed increased U exposure doses (~10-

30 mSv a^{-1}). In a nearby village of Maluu Suu, the ingestion dose was negligible. Further, Kara Agach is a food crop cultivating village with limited access to water treatment. In comparison, Maluu Suu had access to a water distribution plant and cultivated food crops to a lesser extent. The authors reported more than 90% of the ingestion dose was attributed to contaminated food crops in Kara Agach. The authors recommended that the people of Kara Agach to not use locally grown or reared foodstuffs; they provided no alternative solutions. They also recommended remediation in areas exceeding the U annual dose of 100 mSv a^{-1}.

Anke et al. (2009) investigated the U transfer in exposed food chains in East Germany. The study found cultivated and wild plants in the immediate waste dump vicinity stored normal to eight-fold U content. Leafy plants, tea and herbs accumulated more U, but less so in fruits, grains and stalks. Younger plants had greater U content than older plants. Further, fatty foods, sugar and starch were found to be U poor. Animal foodstuff accumulated lower U content. Of adult U ingestion, 33% was delivered via vegetables, 26% via animal meat, and 41% through beverages. Of the animal foodstuffs, cattle kidneys and livers, and hen's eggs accumulated the most U.

In an examination (Steenkamp, Stewart, Chimuka, & Cukrowska, 2005) of 30 different herbal remedies in the Transvaal region of South Africa, the samples consisted of leaves, stems, barks, and roots of various small herbaceous trees and plants. Adsorptive stripping voltammetry method found five samples greatly elevated above 40,000 ppb (parts per billion) and eight samples below the limit of detection. The remainder of the specimens had a mean U concentration in the order of 15,000 ppb. The authors hypothesized that the high U in the specimens were contributed by windblown dust. Tree bark was assumed to accumulate U via atmospheric aerosols. The plant species and therapeutic uses of the herbal remedies were not well described. The physical setting not well described; the authors reported that the area studied was an area with large uranium deposits. The area was initially examined due to soil and

groundwater contamination via natural leaching, dumping of mine waste or pollution from mineral processing.

It is undetermined if the Diné respondents identified in the studies undertaken in the study area utilize fertilizers or other agricultural soil amendments. If agronomic supplements are utilized, the extent of their usage in Diné agriculture needs to be examined. A study by McBride and Spiers (2001) examined the element content of fertilizers, lime, and dairy manures in the northeastern United States. They found that fertilizers with a phosphate component blend contained measureable levels of U (among cadmium, arsenic, and molybdenum). The concentrations of phosphate containing fertilizers and the rate of application would take decades to greatly increase soil concentrations above background. In one example, the authors calculated the U concentrations in the soil in Ithaca, New York (2.2 mg kg^{-1}) could double in 50 years (without controlling for leaching processes). Lime and dairy manures were found on average to have low U concentrations and other elements and heavy metals measured (excluding copper, zinc, and strontium).

Food chain contamination in locally harvested food in the Diné community in NM was reported as a plausible exposure pathway in a recent publication by deLemos et al. (2009). The deLemos et al. (2009) study was based on 1,304 residents in unreclaimed mining districts and non-mined areas in northwestern New Mexico. This cohort of study subjects referred to as the DiNEH (Diné Network for Environmental Health) cohort was enrolled in 2004 and continues to be followed today. The DiNEH survey was utilized to determine demographics, exposure route assessment, renal risk assessment, health and occupation history ascertainment, water and land use pattern determination, and cultural practices (deLemos et al., 2009). The study was initiated by the Eastern Agency Navajo Health Board on behalf of community concerns about health effects of U exposure in this area. deLemos and colleagues (2009) developed risk maps to characterize U exposure in the northwestern New Mexico mining district on the Diné

reservation. The GIS-based risk maps provided alternative safe sources of regulated municipal water and soil disturbance restriction areas. In a community with language-barrier issues, the maps were demonstrated to be effective, clear, and well-received.

Other Non-uranium Heavy Metal Studies and Harvested Foods or Products

Arsenic

Arsenic is a ubiquitious element and is a known teratogen which causes fetal malformation and death in mammals (Eisler, 1998a). In one study (Raab, Hansen, Zhuang, & Feldmann, 2002), two types of sheep wool were compared; sheep that exclusively ingested seaweed known for containing high levels of arsenic were compared to uncontaminated wool. The exposed sheep had greater mean As levels (5.2 ± 2.3 μg g^{-1}) whereas the non-exposed group contained about ten times less As. Inorganic arsenic was 11 to 17% easier to absorb in sheep wool fiber than organic arsenic species. Of adult sheep, five percent of total arsenic was contained in the lanolin of wool. For the current study, sheep wool will be evaluated to determine if there is significant uptake of U and other associated heavy metals. Sheep wool is utilized by the Dine community for creating textiles and in some instances as a sleeping implement. The question is whether wool handling and preparation is a risk to exposure of U and other heavy metals in this community.

Cadmium

In vertebrates, Cd accumulates in the liver and kidney and is known to increase in concentration in older individuals (Eisler, 1985a). Cadmium may cause kidney, ovarian, and prostate cancers in prolonged intake (Turkdogan, Kilicel, Kara, Tuncer, & Uygan, 2002; Trichopoulos, 1997; Feig, Reid, & Loeb, 1994). Further, Turkdogan and associates (1998), reported that one in eight persons that underwent a esophagastroscopy was diagnosed with cancer. In a subsequent study, they found two to fifty-fold elevated levels of Cd, Pb, Cu, and Co in the soil and three and a half to 340-fold higher amounts of Co, Cd, Pb, Mn, Ni, and Cu in fruit

and vegetable samples.

A medicinal herb and tea study (Barthwal, Nair, & Kakkar, 2008) compared plant samples in high traffic, residential, and industrial areas in a city in India and demonstrated that heavy metal levels (Pb, Cd, Cr, & Ni): were more elevated in soil than in plant parts, HM accumulation varied from plant to plant (even when the same plants were collected from three different locations), and the high traffic areas showed higher levels than the residential areas. Another herb study (Wu, Zou, Zhan, Chen, Lu, & Lai, 2008) reemphasized that many other factors may influence heavy metal uptake including ph, organic carbon content, and Zn elemental content. The researchers demonstrated this when they reported Cd concentrations was the greatest in herbs when there were low pH values and Zn elemental content in the soils.

Cesium

Cesium (Cs) is one of the naturally occurring radioactive nuclides. Mining and milling of certain ores can release Cs to the air, water, and soil. Cesium has been proposed for inclusion on the EPA National Priority List in at least eight of the 1,636 hazardous waste sites where Cs has been identified (Agency for Toxic Substances and Disease Registry, 2004).

In a hydroponic study (Soudek et al., 2011) of 20 different plants tested for uranium accumulation *Z. mays* or corn had the highest concentration at 0.16mg/g DW. In the same study, uranium was more localized in the root system and uptake was 3.9 or 4.5 times higher in the presence of phosphate deficiency. In a study site with a history of nuclear research development and testing, Fresquez et al. (1998) evaluated the concentrations of radionuclides (^{3}H, ^{137}Cs, ^{90}Sr, ^{241}Am, ^{238}Pu, $^{239,240}Pu$, ^{tot}U) and demonstrated significant differences between crop species with squash generally higher than beans or corn. Non-edible plant tissue had higher concentrations of radionuclides than edible portions of the crops. All radionuclide concentrations were significantly elevated in each crops except for ^{3}H and ^{tot}U than the background locations.

Lead

Lead is a neurotoxicant. Young mammals are the most sensitive to its toxicity (Eisler, 1998b). In humans Pb can be easily incorporated in the skeleton (Dokserv dissertation). Bunzl and Kracke (1984), demonstrated that domestic sheep livers had background concentration levels of Pb less than 1.5ppm (ww).

In a study by Brooks and Roberts (1978), lead levels tended to accumulate most in sheep livers, kidney cortex, and bones. The sheep were divided into flocks that were grazed near busy highways, those kept away from emissions but fed with contaminated grass, and sheep that were exposed to emissions but fed with uncontaminated grass. Lead burden was determined to accumulate directly by sheep inhalation and indirectly by consumption of contaminated forage.

Molybdenum

Various studies (Underwood 1979; Eisler, 1989) found domestic sheep Mo concentration levels starting at 2.4 ppm (DW) in lambs and adult sheep. In the same study, sheep fed a Mo rich diet were found to have concentrations of 12 to 33 ppm. Ruminant Mo poisoning has been reported all over the world but the sensitivity to Mo in domestic and wild sheep is unknown.

In a hydroponic study investigating the potential to stabilize tailings via native vegetation and the uptake of toxic trace metals (As, Co, Mo, Ni, Pb, ^{226}Ra, Se, U, and V) by native vegetation by Dreesen et al. (1978) found that *Bouteloua gracilis* (blue grama) grass and *Artemisia tridentata* (big sagebrush) shrub showed readily assimilation of Mo and Se from the tailings by both the grass and shrub when compared to the control. *B. gracilis* found somewhat elevated levels of U, ^{226}Ra, As, Ni, Co, and Pb. Above-ground shrub grown in tailings were highly enriched with U and ^{226}Ra, less so with As. *B. gracilis* did show increased germination and survival in leached tailings but, the total number of surviving on leached soil was small. *Bouteloua gracilis* (blue grama) is of particular importance as this is one of the forage species that will be evaluated in the current study.

Selenium

Selenium is known to be essential for animals (Pazurkiewicz-Kocot & Kita, 2003) and humans (Zhu, Wang, Li, Li, Su, & Liu, 2007). There is little Se data for domestic sheep and other ruminants. The role of Se in plants needs further characterization and investigation (Pazurkiewicz-Kocot & Kita, 2003). Domestic sheep fatalities occur when sheep eat 3.2 to 12.8 mg/kg Se in their diet (Eisler, 1985b). Chronic Se toxicity or selenosis can cause "blind staggers," anorexia, emaciation, and collapse, followed by death (Eisler, 1985b). Often with selenosis, conception is adversely diminished and the heart, liver, and kidney degenerate (Eisler, 1985b). Studies in high seleniferous areas have demonstrated a tolerance of certain plant species (Brown & Shrift, 1982). In a study by Zachara et al. (1993), of sheep fed supplemental Se in their diet, the highest Se concentrations were found in the kidney (1.3 μg/g (ww) and the lowest in the skeletal muscle (0.030 μg/g (ww). The other organs (heart, liver, lung, and spleen) were shown to have the same Se concentration levels (Zachara et al., 1993).

Thorium

In the environment, Thorium (Th) is one of the naturally occurring radioactive nuclides (Dang, Jaiswal, & Sunta, 1986). According to Agency for Toxic Substances and Disease Registry (1990), an increased chance of developing lung disease and cancer of the lung or pancreas upon prolonged exposure was demonstrated in several studies on thorium workers. Bone cancer is also a concern as Th is radioactive and may be stored in the bone for a prolonged time (ASTDR, 1990).

In a food study undertaken by Dang and colleagues (1986), the intake of Th was contributed most by food, followed by water, and then via air. The greatest concentration was contributed by cereals (especially rice). Green leafy vegetables tended to concentrate Th. Tea had high concentrations but only a small percentage contributed to the total concentration. Mutton meat did not reflect high uptake of Th.

Vanadium

Vanadium is one of the naturally occurring radioactive nuclides. Vanadium exposures primarily occur through oral ingestion or inhalation routes. Humans and animals exposed to higher concentrations than typically found in the environment reported adverse respiratory effects (ASTDR, 2012). Some evidence exists that suggests that V is an essential nutrient, but a functional role in humans has not been established (ASTDR, 2012). Studies are generally lacking in evaluating for V in harvested foods and products. A series of studies in the current study area examined forage plants and include *B. gracilis* (blue grama) grass and *Artemisia tridentata* (big sagebrush) shrub (Dreesen al. (1978). The study showed that V was not easily assimilated nor easily enriched in either the grass or shrub.

The above studies are related to the topic of this current dissertation research. The studies focus on examining local food chain contamination in cultures that are dependent on locally harvested foods and provide a research foundation to build upon. Previous studies were often limited in cultural consideration and utilized limited animal organs for sampling. The current study expanded on the types of tissues sampled and focused on the most commonly eaten foodstuff in this community. Crop plants have not been fully analyzed in the past, but are examined alongside animal tissue, herbs, forage, soil, and water in the current study. Further, plant species and nomenclature are identified; only a few of the earlier New Mexico studies identified the species of plants in the publications.

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Chapter Three

Theoretical Framework

Introduction

A theory is defined as a compilation of interrelated concepts that are defined to present a systematic view of a phenomenon (Burns & Grove, 2009). Chinn and Kramer (2008) define theory as a rigorous and creative structure of ideas used to describe, explain, predict or control phenomenon or segments of the empirical world. Further yet, a framework is defined as "the abstract, logical structure of meaning that guides the development of study and enables the researcher to link the findings to the body of knowledge used in nursing (Burns & Grove, 2009, p.155)." Theory and concept development support research to enhance development, refine, broaden, and guide nursing inquiry (Rodgers, 2005). Specifically, quantitative research is often implemented to test the accuracy of theory and serve to refine aspects of a theory as well (Chinn & Kramer, 2008). Nursing experts also point out that knowledge is often borrowed from other disciplines such as psychology, education, medicine, or physiology (McMurrey, 1982). Burns and Grove (2009) contend that two forms of borrowing exist, those that allow the integration of information with other disciplines (e.g. public health theories) and information that is taken from other disciplines and directly applied to nursing practice (e.g. technological advances, medical models or theories).

Theories of public health, epidemiology, concepts of disease prevention, and health promotion have roots in the environmental and occupational health model (Hunt, Lederman, Stoddard, LaMontagne, McLellan, Combe, Barbeau, & Sorenson, 2005). Health promotion is the key element to public health in the community and other service settings and includes three modes of prevention (primary, secondary, tertiary). Primary prevention focuses on efforts to protect the health of people who have not yet become ill (Murphy & Sauter, 1996). An example of primary prevention is education regarding avoiding ingestion of *O. aries* organ parts

demonstrating elevated levels of heavy metals or avoiding grazing in areas rich with heavy metals. Secondary prevention refers to early detection and effective efforts to correct the beginning stages of illness (Landsbergis, 2009). For instance, evaluating for pulmonary changes in a U mine worker and removing him or her from continued exposure as pulmonary function shows decline is an example of secondary prevention. According to Murphy and Sauter (1996), tertiary prevention involves measures to reduce or eliminate long-term impairments, disabilities and reduce suffering after illness has occurred. An example of tertiary prevention is providing experimental or palliative care for those with U occupational associated lung cancer or other chronic respiratory illnesses . Epidemiology has been called the basic science of public health and literally means the study of "what happens to people " (MacDonald, 2004, p. 381). In its common definition, epidemiology is seen as the study of the distribution of health and disease in populations. During the past century the focus shifted from acute infectious diseases to chronic diseases as well as a focus on environmental conditions, occupational concerns, and social conditions (Hanchett & Clarke, 1988).

The HAET Triad Model

The Host-Agent-Environment Triad (HAET) model (see Figure 3.1) which has its origins grounded in the public health and epidemiology framework is an ideal model for human and environment interaction research (Tulchinsky & Varavikova, 2009). Research inquiry that poses hypotheses about the long-term health consequences of ingesting a toxic environmental agent in a unique setting with an underrepresented population requires a solid and established theoretical model. Although the earlier version of the model was initially utilized primarily for infectious diseases, the latter half of the 20th century saw the evolving model to expand and include chronic diseases as well (Tulchinsky & Varavikova, 2009). The updated and expanded model emphasizes the importance of dealing with complex factors related to chronic disease which is the leading cause of morbidity and increased premature mortality. The framework identifies

a harmful *agent* in the environment coming into contact with a susceptible *host* to cause disease. The *host* is identified as a person(s) or communities at high risk for a specific disease, condition or exposure. For instance, the Diné community is at high risk for multi-system health effects due to chronic U and other heavy metal environmental exposures. The organism or direct cause of the disease is known as the *agent* (U and other heavy metal toxicants). The *environment* includes the external factors that influence the host, the individuals susceptibility to the agent, and a *vector* which carries the agent to the host from the environment (locally harvested foods or herbs). *Vehicles* are those that transmit the agent (e.g., soil, water, air, etc.).

In essence, the HAET model can be seen as congruent with the four domains of nursing (person, nursing, health, and environment). Hanchett and Clark (1988) argue one of the major limitations of the HAET model is that disease and its prevention are overemphasized and are rightfully eschewed from the concepts of the nursing metaparadigm. They argue that actual presence of illness or disease or the increased risk of disease is implied within the model. Hancett and Clark (1998) argue the nursing metaparadigm focuses more on implied health, the consideration of well-being, optimal or continued development and functioning. Pender (1982) defined health as an evolving concept with a focus on actualization, stability, and improved client health. A fundamental tenet underlying vulnerability is the concept of risk or the relative probability that an individual could become ill or is at risk to do so in a given period of time is consistent with the vulnerable populations conceptual model (Aday, 1994; Leight, 2003).

A limitation of HAET identified by Hanchett and Clark (1988) includes the lack of emphasis on the human aspect (e.g., human interaction) when considering environment. In other words, there is an overemphasis on the non-human environment with the triad model. On the contrary, the reformulated model specifically identifies social factors and psychology when discussing host and environmental factors (Tulchinsky & Varavikova, 2009). Nursing focuses on the immediate psychosocial environments of individuals and tends to ignore major social,

political, and physical environment issues in context with current environmental and economic globalization problems (MacDonald, 2004). The nursing metaparadigm is specifically discipline focused and therefore the domain concept of *nursing* is absent in the HAET model. Many of the limitations and shortcomings with the original HAET model have been updated and reformulated. Therefore, many of the aforementioned issues have been resolved and are no longer an issue. In fact, MacDonald (2004) argues that the arguments of Hanchett and Clark are based on outdated information and their arguments ignore the strides that social epidemiology has made. Evolving definitions of epidemiology have recently emerged to include the study of the determinants of health (not just illness; Kemm, 1993) and the application of study findings to address health problems (Last, 1995). Social epidemiology has played a crucial role in prevention and health promotion design, especially those interventions focused on setting-based and non-individual factors (MacDonald, 2004). Ecosociology has also made great strides recently. For example, the work of Urie Bronfenbrennen in social ecology has demonstrated that biological processes of environmental, social, and structural conditions can influence health and disease (Runyan, 2003). The premise of ecosocial theory is that health, disease, and well-being are socially interdependent within dynamically evolving biological and socially conditioned parameters that are intricately connected (MacDonald, 2004). The compatibilities and incompatibilities of the updated public health model (and its integration into Haddon's model) with those of the nursing metaparadigm have been updated and are more conceptually and theoretically congruent.

The Haddon Model

The father of the field of modern injury epidemiology, William Haddon Jr., drew upon the HAET model to develop the Haddon Model (Runyan, 2003). Though the model was developed for injury prevention, it can be applied to non-injury problems. In addition, the model can be used as a tool for guiding epidemiologic research and/or for developing interventions

(Runyan, 1998). Essentially, the Haddon Model (see Figure 3.2) reflects the HAET model and utilizes the concepts of host, agent, and environment in congruence with a few minor changes. The Haddon Model can readily be utilized as a theoretical model (see Figure 3.3) for addressing U and heavy metal exposures in four American Indian (AI) communities. Essentially, the definitions for agent, host, and vector mirror the HAET model. Again, *host* is the *person* or *persons* affected. The *agent* is energy transference to the person by an inanimate (e.g., wind carrying U and other heavy metals) or an animate *vector* (locally raised *Ovis aries* (sheep) meat, crops or herbs). *Vehicles* are those that transmit the agent (e.g., particles and silt carrying U and other heavy metals in moving water). The *environment* refers to physical elements in the surroundings that contribute to the occurrence of actual injury or those producing potential events (e.g., unsecured abandoned U mines, unregulated wells, exposed mine tailings or structures). In comparison, *social environment* includes sociopolitical aspects such as political environment (e.g., willingness to adopt regulatory interventions that restrict the freedom of mining companies), legal environment (e.g., enforcing environmental clean-up laws or statutes), and cultural norms (e.g., community beliefs related to U mining or the cultural concepts associated to the heavy metals).

Intrapersonal factors are an important consideration and include sociobehavioral, developmental, and biologic features of individuals. Some studies have shown increased uptake of U in neonatal animals which is inferred to mimic human biology and other mammals (Sullivan, 1980; Sullivan & Gorman, 1982; Zamora et al., 2002); this can be seen as an important developmental factor to consider. Other biologic features to consider are that younger plants have more U uptake than mature plants or that vegetables have a greater uptake than fruits and seeds as was demonstrated in a study by Anke and associates (2009). Further yet, being aware of the target populations' predisposition to illness, diseases or conditions are of significant importance. In the Diné population, renal risk factors such as diabetes, coronary artery disease,

and hypertension can predisposition one to Chronic Kidney Disease or End Stage Renal Disease. Nephrotoxicant heavy metals can compound an already compromised renal system. A study by Markstrom and Charley (2003) demonstrated social and behavior problems such as grief, guilt, bereavement, fear, mistrust, anxiety, depression, and PTSD as related to human-caused U contamination in several communities on the Navajo Nation. In a separate study, Dawson (1992) also determined the psychological effects of uncompensated occupational illnesses in U miners and the psychological repercussions of environmental degradation in the Diné community.

Interpersonal factors are referring to interactions between persons (e.g., employer/ employee, family interactions). These interactions can be viewed as either intentional or unintentional. For instance, intentional injuries may refer to knowingly exposing workers to underground mining activities without providing ventilatory protection. Unintentional injuries are unforeseen circumstances and include those not related to disciplinary practices or conflict resolution (Runyan, 1998). For instance, community members unknowingly exposing their family members to U and heavy metal contaminated crops or medicinal herbs is an example of unintentional exposure injury. Food sharing is common in AI communities (Tsuji, et al., 2007; Darby et al., 1956); this practice can unknowingly expose people to contaminated foodstuffs and products. Selling sheep wool (to make textiles) or meat contaminated with heavy metals may also unintentionally expose community members. Lack of community education and information are the main driving forces of unintentional injuries. In unpublished DiNEH data, 19 to 25% of participants incorrectly responded to a question of whether they lived within two miles of a mine site (geospatial analysis confirmation), indicating a lack of knowledge about these waste sites in the community. Providing and maintaining community appropriate updated information is essential to the success of education. For example, the DiNEH study (deLemos, Brugge, Cajero, Downs, Durant, George, Henio-Adeky, Nez, Manning, Rock, Seschillie, Shuey, & Lewis, 2009) provided risk maps for water use and soil restriction areas and

was well received in the community. The risk maps provided the communities a way to receive information and a means to make informed decisions about safer water options and promoting limiting activities in contaminated soil. The overall efficacy of the maps as assessed by a working group graded the maps with an average response of 4.3 (one being "ineffective" and five being "very effective").

Institutional components include the multiple organizations or agencies in which individuals or communities function. It is essential to determine if such organizations promote or control activities or deter or promote injury or illness. Many agencies and organizations exist on the reservation and have overlapping functions. For instance, various organizations may include community Chapter Houses, Agency Health Boards, Navajo Nation tribal agencies, Indian Health Services (IHS), federal agencies, grassroots activist groups, and joint (tribal and U.S. government) agencies such as the U.S. (USEPA) and the Navajo Nation Environmental Protection Agency (NNEPA). The presence or absence of reinforcement or fencing surrounding an abandoned U site that is not well maintained by regulatory agencies can determine injury risks. Further, uninformed groups may either encourage or discourage safe or unsafe practices. For example, communities unaware of alternate safe water options may continue to utilize unsafe wells due to lack of updated information. Likewise, local IHS clinics and hospitals are institutions that directly affect health outcomes and should be involved in environmental contamination issues. For example, maintaining community health surveillance programs and implementing seamless referral routes for high risk communities or those with occupational exposures are desirable. Improving and maintaining communication between these agencies is essential.

Cultural elements focus on social norms and values which also effect governing policies that regulate or guide behaviors of individuals and organizations. A few examples include values that are placed on individual freedom, well-being, and the environment. The cultural

element encompasses the physical and social environment as it has a direct effect on exposure and demographic characteristics. Cultural determinants such as harvesting and preparing food and the patterns of water and land use are important. deLemos and associates (2009) found that unregulated water was utilized as a primary water source by 46% of the Diné participants despite accessible municipal regulated water. In the same study, 60% of community respondents reported the belief that unregulated water was beneficial to their health (deLemos et al, 2009). There have been studies that have identified livestock and forage contamination (Lapham, Millard, Samet, 1989; Millard, Lapham, Hahn, & Jere, 1986) but there is a general lack of studies that have examined the use of medicinal herbs and plants for culinary, ceremonial or traditional health practices. Cultural elements also include social norms which may include addressing and providing alternative clean water sources especially for the elderly or disabled who are physically or mentally disabled.

The social-ecologic theory of Bronfenbrenner meets the needs of assessing the many variables that interact when assessing the extent and impact of heavy metal contamination in uranium impacted areas. The model addresses the unique characteristics of the individual (biologic, psychological, & developmental), the interpersonal interactions with their families, employers and other immediate community players, and various multi-faceted cultural factors. Further, it takes into consideration the complex factors of the communities affected in relation to the multiple agencies' and organizations' interactions. The social-ecologic theory of Bronfenbrenner includes a historical dimension that takes into account the changing relations among the variables over time (Runyan, 2003). For future studies in the same area of research, Haddon's integrated model can be relied upon to further evaluate the impacts of toxic agents and their possible connection with chronic health consequences in health disparate vulnerable individuals and their communities. The model can also be utilized to introduce and test interventions.

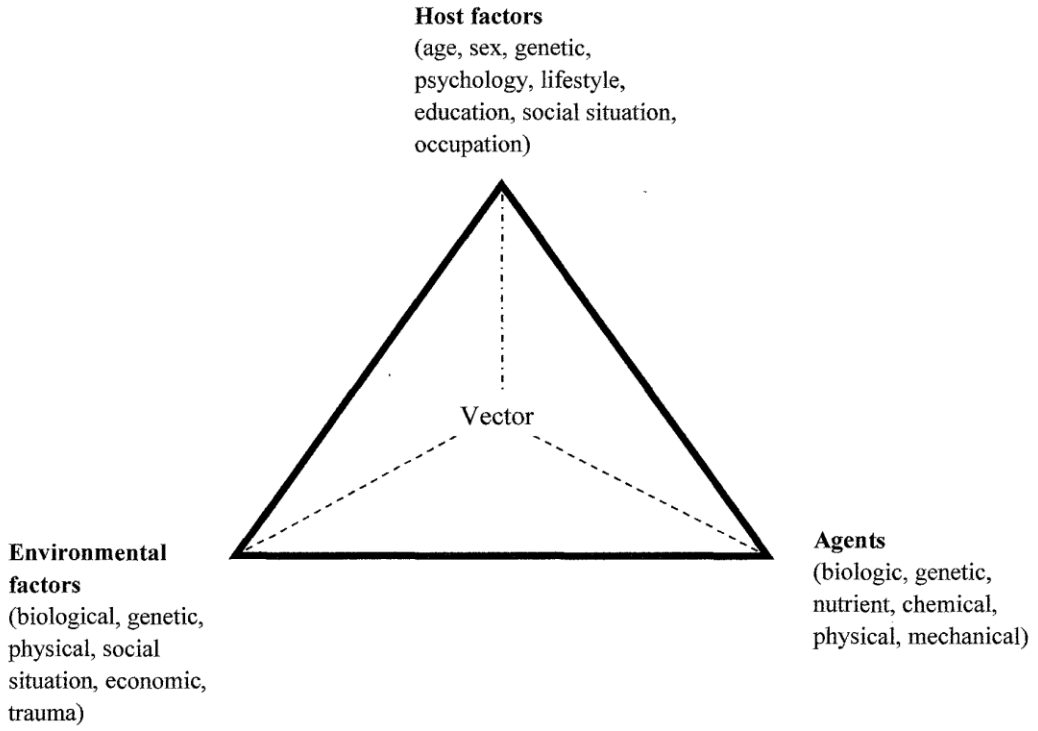

Figure 3.1. The expanded Host-Agent-Environment Triad. From Tulchinsky, T. & Varavikova, E.A. (2009). "*The new public health: An introduction to the 21st century*." (2nd ed.). Burlington, MA: Elsevier Academic Press.

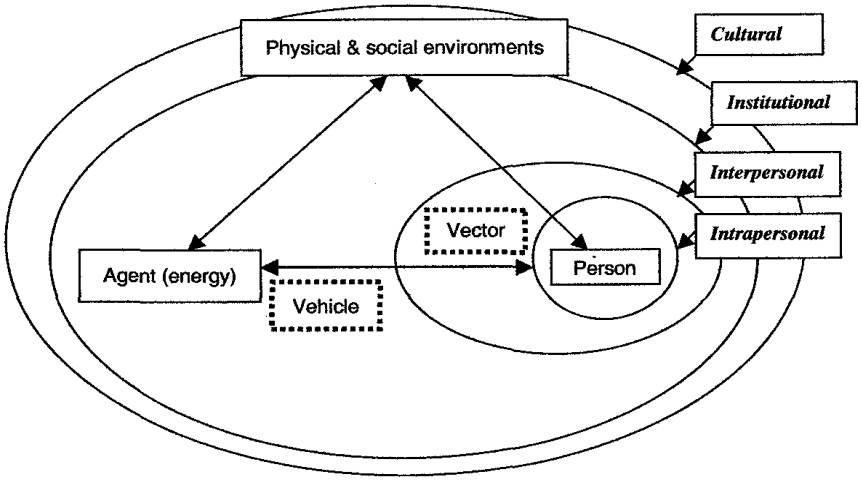

Figure 3.2. Integration of the public health model with Haddon's Model. From Runyan, C.W. (2003). "Introduction: Back to the future--revisiting Haddon's conceptualization of injury epidemiology and prevention." *Epidemiologic Reviews*, 25, 60-64.

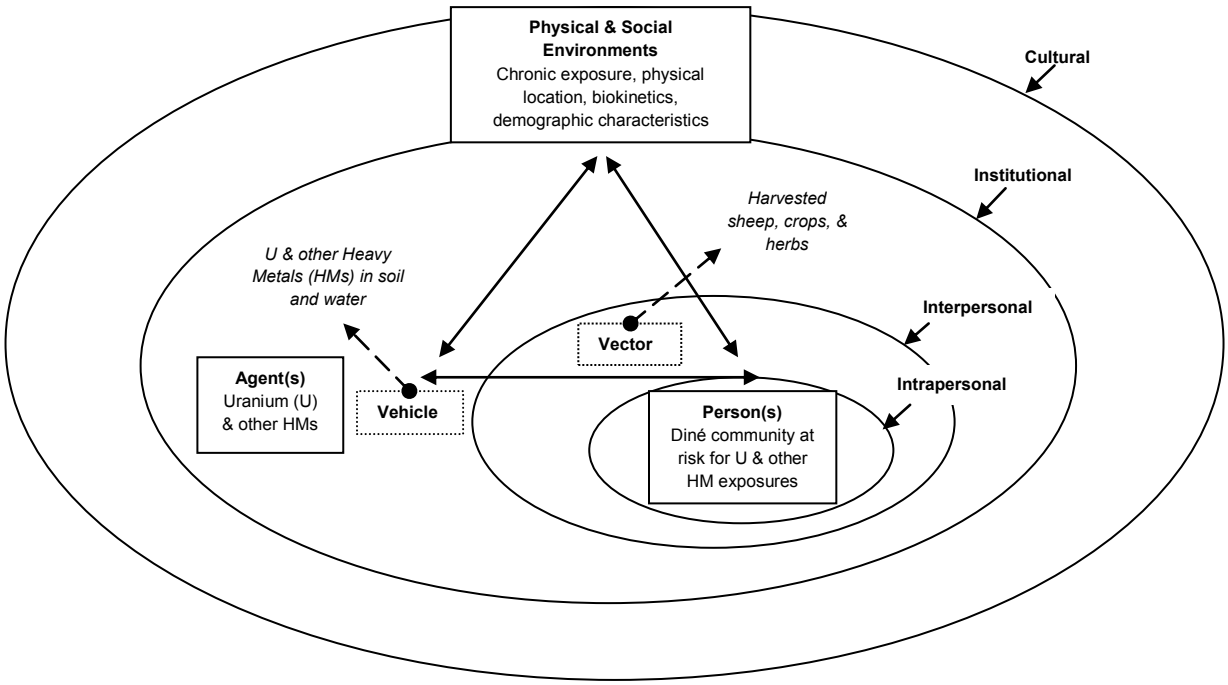

Figure 3.3. A theoretical model utilizing the Haddon Model. The model can readily be utilized as a theoretical model for addressing uranium and other heavy metal exposures in impacted Diné communities.

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Chapter Four

Methodology

This is a descriptive, comparative study that investigates uranium (U) and other heavy metal (HM; As, Cd, Cs, Mo, Pb, Se, Th, and V) levels in locally harvested dietary intakes of Diné people living in areas suspected to have a range of high and low environmental U contamination. The information will be used to educate the Diné community about the contamination levels of locally harvested food sources in relation to U and associated heavy metal contamination. The current research involved detailed sampling of a variety of crops and herbs harvested on plots and/or sheep and forage on grazing lands. These samples were provided by eight growers and harvesters who were partially nested in the DiNEH (Diné Network for Environmental Health) study. The study was conducted in collaboration with the study researchers (Lewis et al.). New participants were obtained via various snowball methods. The DiNEH study survey (Water and Land Use, Environmental and Health Survey, Revision No.11, 02/15/2008) was initially administered in 20 chapters (communities) on Diné lands in northwestern New Mexico (deLemos et al., 2009).

The goal of the present study is to determine if locally harvested animals and plants on the Diné lands are contaminated with heavy metals and to disseminate the study findings and educational information to the community. The specific aims of the study are to:

1: Describe dietary behavior of the Diné Nation residents who grow and harvest their own food specifically related to ingestion of locally harvested *Ovis aries* (sheep) and plants.

2: Compare U and other heavy metal levels in locally harvested *O. aries* and plants from areas suspected to have high levels of environmental U and heavy metal contamination and low levels of contamination.

3: Explore potential routes of U and other heavy metal exposures for locally harvested plants and sheep.

4: Disseminate study findings to the leadership and community on the Diné reservation.

Sample Size

Saying "yes" to raising and rearing locally harvested animals (see Figure 4.1 Items 20, 20a, & 20b) and crops (see Figure 4.2 Items 21, 21a) was one of the criteria for selection into the present study from the DiNEH study. New participants were also screened into the study with similar questions that correlate with the inclusion criteria. Therefore, four C*hapters* or communities across areas of expected high and low exposure served as the sample size. The *Chapters* contained both low and high impact areas.

Sample size calculations are based on differences in U previously reported for sheep, forage, and crops of Diné people. Sample size for *O. aries* are based on Northern Arizona University (NAU) pilot data (J. Ingram) that found tissue specific increases in U ranging three to 14 fold across different U exposure on different parts of the reservation. Using tissue demonstrating least uptake (muscle), 19 sheep each from the low and high exposure groups (total 38) should detect a two-fold difference at power of 80% and α of 0.05. NAU pilot data (A. Jauregui), found differences in U in 18 types of forage plants that ranged 1.1 to a 23 fold across high and low U exposure areas. Based on this, 13 different forage plants from both exposed and control areas (total 26) will be needed to show a two-fold difference at power of 0.80 and α 0.05. Data for crops that humans consume is based on Anke et al. (2009) although there is little overlap with crops eaten by Diné. Based on green beans, it appears that 49 different crop types from the high and low U areas should detect a 20% difference at power of 0.80 and alpha of 0.05. Three of the (*Zea mays* or corn, *Cucurbita pepo* or Squash, and *Phaseolus vulgaris* or beans) five main crops identified by the DiNEH parent study will be included in the current study. The human sample size is dependent upon the harvesting sample sizes and the extent of the harvesting overlap.

Sample

The sample of growers and harvesters consuming locally grown crops and *O. aries* was identified and partially drawn from the DiNEH parent cohort of 1,304 participants. The initial DiNEH study consists of 20 chapters in northwestern New Mexico. The purpose of these *Chapters* or *Chapter* Houses are to house community meetings, events, and often serve as voting facilities and main interaction centers for communities. The DiNEH participants were approached and recruited at chapter meetings, public events, water hauling locations, and by word of mouth (deLemos et al., 2009). Such methods were preferred due to the rural nature of the site. For example, more than 50% of Diné do not have telephones and a smaller percentage do not have access to cellular phones and/or internet services (U.S. Census, 2006). The DiNEH study was undertaken as a collaboration among the University of New Mexico Community Environmental Health Program (UNM-CEHP), Southwest Research and Information Center (SRIC), the Eastern Navajo Health Board, Indian Health Services (IHS) Crownpoint healthcare system, and in cooperation with several divisions of the Navajo Nation. The current study has Navajo Nation Human Research Review Board (NNHRRB) approval. In the DiNEH cohort, interview and sample collections were scheduled in community tribal *Chapter* houses, IHS hospitals, community facilities, or private homes. Geospatial coordinates were determined for all residences and compared with documented locations for all mine waste sites provided by the US Army Corps of Engineers and U.S. EPA. The current study contributed new information regarding dietary exposure by targeting a subsistence food harvesting community exposed to environmental U.

Inclusion criteria (see Table 4.1) for the growers and harvesters who provided crop and sheep samples in the present study include: 1) 18 years of age and older, 2) non-pregnant, 3) greater than 10 years of continuous residency in the community; 4) eat food grown and harvested locally, and 5) willing to participate. Additional oral screening questions for new participants

and participants from the original DiNEH study include: "Do you have your own sheep flock that you alone care for?" Do you eat the meat of the sheep you raise?" "Do the sheep eat winter fodder, if yes, at what yearly percentage? 0%, 25%, 50%, or 100%?" "Do the sheep eat non-winter fodder, if yes, at what yearly percentage? 0%, 25%, 50%, or 100%?" "Do you raise your own crops for eating purposes?" "Do you eat wild plants/herbs for medicinal purposes?" Low and high exposure populations were defined as in terms of distance from abandoned mines and structures.

Setting

This study setting focused on four *Chapters* on the Diné reservation innorthwestern New Mexico. The number of abandoned mines in the 20 C*hapters* range from 43 at the upper end to several chapters with no history of mining activity (Figure 4.3). Growers and harvesters were selected from four of the 20 *Chapters* participating in the DiNEH study and include: Baca/Prewitt/Haystack, Churchrock, Crownpoint, and Mariano Lake (Figure 4.4). The largest *Chapter* was about 204 square miles (mi^2) of land mass and the smallest *Chapter* was about 90 mi^2. Mine-related waste sites include abandoned U mines, structures, mills, and tailings piles as documented in GIS and the DiNEH spatial mapping (deLemos et al., 2009). These communities have expressed concern about the locally grown food. DiNEH utilized GIS to develop risk maps to inform the communities about high risk contamination areas based on soil and water analyses and information was integrated with existing environmental data sources (deLemos et al., 2009).

Study Procedures

Cooperation and collaboration from the Navajo Environmental Protection Agency (NNEPA) Public Water Division, and local community Chapters in northwestern New Mexico were ongoing with researchers who are following the DiNEH cohort. Institutional Review Board (IRB) for human research approval for the study described in this current application was obtained from UCLA and the NNHRRB before the study onset. Animal research approval was

provided by the UCLA Office of Animal Research Oversight (OARO). Voting consent was provided by each of the four participating *Chapters*, the Eastern Navajo Health Board (ENHB), and the Eastern Navajo Agency Council (ENAC). The growers or harvesters provided: a) consent for sampling, b) real or potential plant or animal harvesting activities, c) a schedule of harvesting activities at least two weeks in advance to allow the researcher to take samples, d) adequate field space and study protocol procedures to support sample field collection, storage, and e) data on harvested food through questionnaire and interview session. Populations within the Chapters have been identified to provide adequate sampling. This is an appropriate setting for the study.

Compensation for Participant Time and Provision of Sample Material

In total, each study growers and harvesters received a grocery gift-card worth $55. Five dollars were distributed at the completion of the general questionnaire and $50 upon sample collection. This compensation was a small amount of monetary compensation for participant time, expertise, and completion of a short interview at the time of food sample collection.

Study Intake Procedures

A subset of respondents from the original DiNEH study cohort who had given their prior permission to be re-contacted for further research served as a partial selection frame for this research. Once the subset was identified, participants were invited into the study by a letter, phone call, or home visit. New participants were recruited at Chapter and community meetings, or via home visits, via recruitment posters, radio announcements in (Diné and English languages) or other snowball methods such as referrals from previously approached potential or actual recruits. As part of the procedures with human subjects, the Principal Investigator (PI): a) described the study, b) completed the screening and enrollment, c) obtained informed consent, d) discussed incentive procedures, and e) answered questions and concerns. Dietary information was be collected from all participants who give consent. A random selection process was used to

select sheep, forage, and crops to be sampled among those who consented to the sampling. All procedures were conducted in English and translated into Diné as needed or vice versa. The PI is fluent in spoken/written English and Diné; participants can use either language. The PI was blinded during data collection as to whether the sites were in areas of expected high versus low U and heavy metal exposures.

Data and Sample Collection and Testing Procedures

Sheep tissue and plant matter were collected at the participant's residence, prepared, and shipped overnight to the University of New Mexico (UNM) Analytical Chemistry Laboratory Earth and Planetary Sciences Department for analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). ICP-MS provides rapid and sensitive techniques for determining long-lived radionuclides and stable isotopes (Becker & Dietze, 2000; Goulle et al., 2005; Shen et al., 2002; Ting et al., 1999). Major advantages of ICP-MS include the tolerance of small sample sizes, high sample throughputs (Becker, 2005), and short measurement times with fewer sample preparation steps (Becker, 2005). Measurements will be performed on an PerkinElmer NexION 300D for analysis of trace metals in high matrix samples including environmental, clinical, and geological samples. The system detection limit for U (isotope 238) is two parts per trillion.

Data Collection Instruments and Measures

Harvested Food: Diné Plant-Animal-Human Questionnaire

There were four questionnaires. All growers and harvesters completed a general questionnaire known as the Diné Plant-Animal-Human Questionnaire (DPAHQ). For the DPAHQ see Figures 4.5 to 4.7 and 4.10. There were questionnaires for each type of food harvested: sheep, crops, and herbs or medicinal plants. The type of questionnaire form to be used was dependent on the type of food harvested. For example, some participants may have harvested animals and crops only and not herbs or medicinal plants; respectively the participant

completed the Diné Dibé Intake Questionnaire (DDIQ) and the Diné Crop Intake Questionnaire (DCIQ). Another participant may only have harvest crops and were required to complete the DCIQ. In it's entirety, the DPAHQ, was comprised of four pages with five sections. For ease of readability, the questionnaire was written in Calibri font face with an 11 size font (as all the subsequent questionnaires). In the literature, it is recommended that readability index computed for the source language be at the sixth or seventh grade level (Estrada, Hryniewicz, Higgs, Collins, & Bird, 2002). The Flesch-Kinkaid grade level score was calculated by Vista Microsoft Word 2007 version and found to be at the 5.3 grade level. The DPAHQ was completed within one hour. If the language preference of the participant was Diné, the English questionnaire was translated orally into Diné during the interview. The Diné language is historically an oral language. The majority of Diné speaking individuals do not read or write the Diné language (deLemos et al., 2009). The individual sections for the DPAHQ will be presented next.

Within the DPAHQ, personal information, demographic information, and a detailed exposure history was obtained. An exposure history is an important step in evaluating the respondents who may be at risk. The information regarding the source location, proximity, amount of time exposure, and amount of exposure (U and HM concentrations) to high risk activities were obtained. Other concurrent or past exposures may be significant and were assessed. For example, other activities associated to harvesting activities, including past and current activities in which the respondent may have been exposed directly or indirectly to U is relevant in evaluating personal exposure. For example does the respondent participate in outdoor activities such as land management or firewood collection that increase their risk of outdoor environmental heavy metal exposures.

Section I of the questionnaire (Figure 4.5) was dedicated to personal information such as name, date of birth, mailing address, telephone number, physical location of home, length of

residence in the current home, inquiry about the participant's place of voting, and language preference. The questionnaire data with home, address, and other personal identifying information were separated from the remaining sections (II-VIII) and filed separately under lock-and-key. Data was coded for subsequent sections. Physical "location of home" was identified via Global Positioning System (GPS) mapping and exposure data. Place of voting was important as it provided data about the growers and harvesters' *home* community or Chapter affiliation.

Section II (Figure 4.6) was developed to extrapolate grower and harvester demographic information. Demographic information sought included gender, ethnicity, language spoken in various settings, educational level, annual household income, number of residents in the participant's current home, and the availability of electricity in the home, and employment status. Language questions are similar to the DiNEH cohort study survey Item Six (Water and Land Use, Environmental and Health Survey, Revision No.11, 02/15/2008) which inquired about language preference within the home, work, and with friends (Figure 4.8). In comparison, the current DPAHQ inquires about the preference of language for the current interview.

Section III (Figure 4.7) gathered water exposure data for sheep and crops. Item Eight determined the water sources utilized specifically for sheep husbandry and is comprised of check-off boxes for: "Grocery store/trading post, Lake, NTUA, Pond, Private Well, Rain Water, Spring, Stream, Windmill" and an "Other" option and a blank space is provided to name the water source. Item 8 is similar to DiNEH survey Item 11 (Figure 4.9) except that cistern or tank has been removed for the current questionnaire. An "other" choice for types of water sources are provided in the current questionnaire dissimilar to the DiNEH survey. Item Nine is determining if the participant's home was connected to a community water system and if the answer was positive the name of the water system was to be provided. Item 9 was adapted from DiNEH survey (Revision No. 11, 02/15/08) Item 12 (Figure 4.9). Item Ten is asking if the participant

provided treated (filtered, disinfected, or boiled) water for sheep. Item 11 is a question regarding whether the participant hauled water for their crops. Items nine through 11 have five choice answers: "yes" or "no" or " I don't know" or "I do not have sheep/crops " or "I do not haul water."

Section IV (Figure 4.7) questions were directly related to grower harvester consumption of mutton and/or lamb. In this section the participant was allowed to skip to Item 17 if they did not consume sheep meat. Item 12 asked what part(s) of the sheep were personally consumed. Check-off boxes were provided (Brain, Heart, Liver, Intestines, Kidneys, and Muscle Tissue). After each tissue part(s), the number of years the part(s) consumed were provided. An "Other" option check-off box was provided with space provided for other parts consumed which were not listed. Item 13 is determining the overall amounts of meat intake from sheep alone and provided percentages of intake: "0%, 25%, 50%, 75%, or 100%." Question 14 determined the frequency of sheep meat eaten per meal in a typical week. A blank space is provided to accept a numerical amount. Item 15 is asking how many meals eaten out of a typical day was comprised of sheep or lamb meat. The answers provided were "1, 2, 3, or more". Space was provided after "more" to accept numerical quantities. The last question in Section IV (Item 16) was seeking the number of years the respondent has eaten sheep meat raised in the current location. A blank space is provided to accept numerical quantity years.

Section V (Figure 4.10) was developed to obtain relevant information regarding exposure to other types of associated harvesting activities. Item 17 asks participants to choose from an array of harvesting activities such as hunting small and large game (17a & 17b), firewood collection (17c), land management activities (17d), and animal herding (other than sheep, 17e), recreational activities (17f), and an "other" activities option is provided at the end of the list. The number of years the activity has been participated in by the interviewee is provided for each type of activity. For 17d and 17f the participant is asked to provide the top three activities they

participate in and the number of years each activity is participated in.

It was anticipated that each respondent might harvest sheep, crops, and collect herbs or plants but there was the possibility that some respondents might harvest sheep only and not crops or plants and herbs or vice versa. For these reasons, three detailed questionnaires were provided for each category of harvested food and they include: the Diné Dibé Intake Questionnaire (DDIQ), the DCIQ, and the Diné Wild Plant Herb Intake Questionnaire (DWPHIQ). Each individual questionnaire will be presented next.

Harvested Food: The Diné Dibé Intake Questionnaire

The Diné Dibé Intake Questionnaire (DDIQ) was a tool intended specifically to gather information regarding the sheep to be harvested. The Diné word for sheep is *Dibé*. The DDIQ was comprised of three pages. The Flesch-Kinkaid grade level score was calculated by Vista Microsoft Word 2007 version and found to be rated at the 5.9 grade level. This questionnaires was able to be completed within 15 minutes. Item 1 and Item 2 (Figure 4.11) were basic questions regarding the code number of the sheep owner and the date the questionnaire was populated. Photo(s) of the animal to be harvested were obtained for identification purposes and as an indicator of general health . Item 3 determined the type of sheep to be harvested including the gender of the animal, the approximate age of the sheep (Item 4), the length of time the sheep lived on the reservation (Item 4a), and the approximate weight of the sheep (Item 5). The approximate weight of the sheep was determined by measuring the circumference or heart girth just behind the shoulder and elbows (Figure 4.12). Heart girth *x* heart girth *x* body length ÷ 300 = weight in pounds (Pugh, 2002). Item 6 determined the food sources of the animal and requested the respondent to provide the type of forage and non-winter fodder the animal eats. If possible, the names or types of local forage that the sheep grazes on were listed here. Non-winter fodder refers to non-local forage supplemental types of feed (e.g. four-way, alfalfa hay, etc.) the animal feeds on during non-winter seasons. For the current study sheep tissue were

sampled only during non-winter months subsequently, sheep fodder eaten during winter months were not sampled. Item 6 also ensured that the inclusion criteria requirement for the harvested animal fodder consumption was indeed less than 25%. Of the sheep diet, 75% should have been attributed to local forage and plants. Item 7 asked where the harvester obtains water for the sheep to be tested. There were accommodations for three sites (Sites A, B, & C) on the chart provided. The interviewer was expected to provide location information and/or provide the well number for each site populated. The interviewer also entered GPS Latitude and Longitude data as well. For item 8, another chart was provided and the percentage of water used for livestock and the number of years the sheep drank water were provided for all sites identified in Item 7. In Item 9 a description of the sheep corral location was obtained, the interviewer also provided GPS Latitude and Longitude information. Items 10a, 10b, and 10c were seeking a description of the general grazing locations of the sheep flock and interviewer also provided GPS Latitude and Longitude information. Space was provided for three locations, a separate sheet of paper was added for additional grazing sites. For Items 11a, 11b, and 11c, (see Figure 4.13) the length of time (in weeks, months, or years) the sheep had grazed in the identified locations in the previous question were written down. Again space was provided for three locations, a separate sheet of paper was added for additional grazing sites. Item 12 asked whether the sheep to be tested had been recently evaluated by a veterinarian in the past three months. A response of "yes" or "no" was provided. If the answer was positive, the participant was asked to answer two questions: (Item 12a) the "reason" for the veterinarian visit and (Item 12b) the " medication(s)" the animal received. Question 13 was determining if the sheep had participated in a "sheep dip" or received any shots or treatments in the past three months. Item 14 asked whether the sheep to be tested was born with any abnormalities. The answers available are "yes" or "no." If the answer was "yes," the respondent was asked to provide: (Item 14a) a brief description of the abnormality or abnormalities, (Item 14b) whether similar abnormalities were seen in the sheep's parent(s),

sibling(s), or lambs(s) and if so, (Item 14c) a brief description of the abnormalities seen in the animal's parent(s), siblings (s), or lambs(s) are sought. Item 15 asked if the flock was moved to a different location during the various seasons of the year and if "yes", the participant was asked to provide (Item 15a) the season of movement (spring, summer, fall, winter). Item 15b, asked the interviewee to provide the location of the seasonal home. Item 15c sought directions or GPS locations to the sheep corral for the seasonal home identified. Item 15d asked the interviewee to identify two locations that the sheep grazed near the seasonal home identified. For Items 15b to 15d, a description of the location and/or providing a GPS location were needed. The researcher operated GPS instrument (2008 Trimble® GeoXT) was utilized to capture the information. As a fail-safe, a topographical map (1:24,000) was available on hand in the field. Item 16 questioned whether the sheep grazing area was considered to be rotated or not. If the answer was positive, Item 16a inquired about the amount of time the sheep were left in the paddock to graze. The participant was to provide the number of days or weeks or months the sheep were grazed in a particular paddock in the space provided. Item 16b asked the length of time the paddock was allowed to rest (in days or weeks or months); the numerical amount of time the grazing pasture was rested should be populated in the space provided. Item 17 asked the respondent if the sheep's wool was used for making textiles. If the answer was positive, the respondent was required to answer two questions with a "yes" or "no" response. For Item 17a (see Figure 4.14): (Item 17a) "do you and/or your family sell the wool?"; (Item 17b)"do you and/or your family sell the textiles?" Item 18 asked whether the harvester or their family sold live sheep to market. Further, 18a asks "do you and/or your family sell the mutton or lamb meat to the market?" Item 18b asked whether the harvester or family shared the sheep meat with other people. For Items 18c to 18e the participant asked to provide the last three community locations that sheep meat was shared with. Item 19 was an open-ended question and asked what other parts of the sheep were used in the home or for traditional or medicinal uses. Item 20 wad intended for the

researcher and inquires about the average pasture height. Pasture height was a reasonably reliable determinant of pasture quantity and available feed. Pasture height was measured by 50 random height measurements of representative grazing areas (Court, Ware, & Hides, 2010). A measuring stick was thrown in front of the measurer and the base of the thumb was run down the measuring stick until it touched the first green leaf (Court et al., 2010). Bare areas were recorded as zero, inedible plants were ignored and recorded as a zero. Table 4.2 demonstrates a sample recording sheet for pasture height. A space was provided at the end of the questionnaire to attach photos, if any exist (Figure 4.14).

Harvested Food: The Diné Crop Intake Questionnaire

The Diné Crop Intake Questionnaire or the DCIQ was intended to gather information on domestic agricultural crops. The DCIQ was comprised of three pages. The Flesch-Kinkaid grade level score was calculated by Vista Microsoft Word 2007 version and found to be rated at the 5.6 grade level. The questionnaire was completed within 15 minutes.

Contamination of plants by toxic heavy metals depends on species, cultivation, processing, harvesting time, level and duration of contaminant exposure, and geographical origin (Basgel & Erdemoglu, 2006). The DCIQ obtained information about types of crops harvested, cultivation techniques, harvesting time, and level and duration of possible U and other heavy metal exposures, geographical origin, and other relevant information.

Diné Crop Intake Questionnaire Item 1 and Item 2 (Figure 4.15) inquired about the date of planting and the anticipated harvest date. Item 3 was requesting the location of the crops and Item 3a was inquiring about an alternate crop plot location, if any. Location was provided on a map (provided by the respondent) or entered via GPS by the researcher. Item 4 asked how often the respondent planted crops. The answers provided were: "yearly" or "every two years" or "other." For "other" the interviewer wrote the time span the crop plot had been utilized in the space provided. Item 5 asked the respondent to report the overall number of years the crop

plot had been utilized. A blank space was provided for a numerical response of the number of years that planting had occurred. Item 6 asked where the harvester obtains water for crop irrigation for the crops that were tested. There were accommodations for three sites (Sites A, B, & C) on the chart provided. The interviewer was expected to provide location information and/or provide the well number for each site populated. The interviewee also entered GPS Latitude and Longitude data as well. For item 7, another chart is provided and the percentage of water used for crops to be tested and the number of years the crops were irrigated with water source were provided for all sites identified in Item 6. The responses to Item 8 were "yes" or "no" or "I don't know" for the question: "Were any insecticides, pesticides, or fertilizers used on the crops to be tested?" If the answer was positive, the respondent was asked to provide information as to the type(s) of product(s) (Item 8a) used on the crops. Item 9 asked about the type of planting technique utilized for the current planting. This is important to determine because the nature and extent of soil disturbance needed to be assessed. Item 10 asked what specific crops were grown at this site. A selection list was provided for the five most commonly planted crops (*Z. mays, C. pepo, P. vulgaris, Cucumis melo* or melon, and *Capsicum annum* or chili) reported by DiNEH data (deLemos et al., 2009.) An "other" option existed with a space for the interviewer to write in the "other" crops not listed. Item 11 asked the respondent where the crop seeds were obtained (e.g. purchased or otherwise). This was an open-ended question that determined if the seeds were local reservation (exposed) seeds or otherwise. Item 12 (Figure 4.16) was requesting how often the respondent was consuming the crops that he or she grows. A calendar was provided for the five most commonly planted crops and an "other" category for unlisted crops. The instructions for the crop calendar were: "Place the number "1 in each month where you eat the crop only once a week or less," place the number "2 in each month where you eat the crop two to three days a week," place the number "3 in each month where you eat the crop four to five days a week," and place an "X in the month where you do not eat any crops at

all." Item 13 determined the overall number of years each locally raised crop was eaten in the current location. Again, the five most commonly eaten crops (corn, squash, beans, melon, and chile) were listed with an "other(s)" option. The other option had a blank space provided for the interviewer to write in the type of crops not listed. Item 14 required a "yes" or "no" response. Item 14 asked which animals were typically given crops or crop parts as a feed supplement. A check off list was provided and include: sheep, goats, cattle, and "other(s). A space was provided for the "other" box to write the types of other animals that ate supplemental crop feed. If the answer was positive, the respondent answered two questions: (Item 14a) which "crops and crop parts" and (Item 14b) which animal(s) consumed the aforementioned "crops or crop parts." Item 15 asked the respondent if he or she and/or his and/or her family sell any of the crops? The responses to Item 15 were "yes" or "no" or "I don't know." If the answer was positive, the respondent was presented with a list (corn, squash, beans, melon, chili, and other) and was asked to check all the boxes that applied. The "other" check-off option had a blank space and was intended to take written information as to the types of crops that were not listed. Item 16 (see Figure 4.17) was interested in determining whether corn pollen ingestion was common for those that harvested corn crops. If the answer was positive, the number of years the corn pollen was ingested was to be provided for Item 16a. Corn pollen has great cultural significance (commonly used for blessings and traditional ceremonies) and determining whether it was a source of exposure needed to be determined. Question 17 asked the harvester to provide the location of storage for each crop that he or she harvested. The list included the aforementioned five crops and an "other" category. The last item (Item 18) in the DCIQ inquired to whether the harvester and/or their family share free crops with other people. The responses to Item 18a are "yes" or "no" or "I don't know." If the answer was "yes," the interviewee was to provide the last three locations where free crops were shared. A space was provided at the end of the questionnaire to attach photos, if any existed. A space was provided at the end of the questionnaire to attach

photos, if any exist (see Figure 4.17).

Harvested Plants: The Diné Wild Plant/Herb Intake Questionnaire

The Diné Wild Plant/Herb Intake Questionnaire (DWPHIQ) was a short tool that sought information regarding consumption of non-forage type wild plants, herbs, and medicinal plants. Information regarding sheep ingested forage grasses were included in the DDIQ and were not revisited. The DWPHIQ was a two page questionnaire. The Flesch-Kinkaid grade level score was calculated by Vista Microsoft Word 2007 version and found to be rated at the 4.4 grade level. The questionnaire was completed within 10 minutes. The use of herbs, wild plants, and medicinal plants were anticipated to be low for animals but, the use for human consumption was anticipated to be more frequent. At maximum, four different types of plants had the potential to be collected (two for human consumption and two for animal consumption or four for human consumption or four for animal consumption) per participant family.

Contamination of raw herbs by toxic heavy metals depends on species, cultivation, processing, harvesting time, level and duration of contaminant exposure, and geographical origin (Basgel & Erdemoglu, 2006). The DWPHIQ obtained information about the plant species, harvesting time, and level and duration of possible contaminant exposure, geographical origin, and other relevant variables.

Dine Wild Plant/Herb Intake Questionnaire Item 1 (Figure 4.18) determined the respondent's personal consumption of wild plants, herbs or medicinal plants. This was a question that had three response choices: "yes" or "no" or "I don't know." If the response was positive, the respondent was required to answer seven questions: (Item 1a) the location of the live plants (identified on a map or via GPS), (Item 1b) plants consumed were reported here by the respondent (photos will be taken), (Item 1c) how often (daily or weekly or monthly) the respondent utilized the live plants, (Item 1d) how many years the respondent utilized the plant, (Item 1e) what part of the plant or herb (root, stem, leaf, flower, or other) was utilized by the

respondent, (Item 1f) the therapeutic use of the plant or herb utilized, and (Item 1g) if the herb was rinsed with water or cleansed in any other way before use, (Item 1h) the preparation requirement of the therapeutic plant, and whether the use of the herb or plant was prescribed by a traditional healer (Item 1i). Item 2 determined whether the respondent's sheep consume non-forage type wild plants or herbs or medicinal plants. This was a question that had three response choices: "yes" or "no" or "I don't know." If the response was positive, the respondent was required to answer seven questions: (Item 2a) the location of the live plants (identified on a map or via GPS), (Item 2b) plants consumed by the sheep were reported here by the respondent (photos of the plant will be taken for identification purposes), (Item 2c) how often (daily or weekly or monthly) the sheep consumed the live plants, (Item 2d) how many years had the animal consumed the plant, (Item 2e) what part of the plant/herb (root, stem, leaf, flower or other) was consumed by the animal, (Item 2f) the therapeutic use of the plant or herb consumed by the animal, and (Item 2g) the preparation requirement of the therapeutic plant. Item 3 (see Figure 4.20) questioned the respondent if any insecticides, pesticides, or fertilizers were used on the non-forage type wild plants, herbs, or medicinal plants used for human or (3a) sheep consumption. Item 4 was a question that had three response choices: "yes" or "no" or "I don't know" to the question " Do you or your family sell any of the herbs, wild plants or medicinal plants?" If the response was positive, the respondent was required to answer which herbs, wild plants or medicinal plants were sold in Item 4a. Item 5 in the DCIQ inquires to whether the harvester and/or their family share free herbs or medicinal plants with other people. The responses to Item 5 are "yes" or "no" or "I don't know." If the answer was "yes," the interviewee was to provide the last three locations where free herbs and medicinal plants were shared in Items 6a through 6c. The last item on the questionnaire, Item 7, was intended for the researcher and was seeking almanac information regarding the average rainfall in that area for the year the study samples were collected. A space was provided at the end of the questionnaire to attach

photos, if any existed. A space was provided at the end of the questionnaire to attach photos, if any existed (see Figure 4.19).

For this current study, the sheep was the most appropriate animal from which to obtain harvested tissue. The age requirement for the ages of the sheep were those greater than six months of age but less than 10 years of age. These animals were sampled during normal harvesting times in the field on Diné lands which consist of Spring (March 20 to June 20), Summer (June 21 to September 21), and Fall (September 22 to December 21). The PI collected only what was required for sampling during a routine field harvest session. The remainder of the animal was harvested and consumed in its entirety to eliminate waste, respect the animal gift, and preserve the cultural sustenance life-cycle. Every effort was made to minimize the size of animals to be harvested. In addition, the wet weight equivalence of 1g dry weight for all *O. aries* tissue sampled was determined before field collection commenced. The sheep is the most commonly harvested animal in the Diné community and therefore the supply of sheep is abundant. The frequency of sheep harvesting is also common in this community for common meals and special occasions. The sheep is considerably smaller in size than cattle or horses. The physical effort and time needed to slaughter a sheep is considerably less than for other animals such as cattle or horses. In this cohort, the frequency of ingestion of smaller animals was considerably low. In this northwestern New Mexico community harvesting of chickens, pigs, and turkeys for food exclusively was nonexistent.

O. aries Tissue Samples

For sheep tissue samples, the researcher was summoned when a harvesting session was to take place by a study participant. From November 10 to December 13, 2012, one female lamb (eight months old) and two ewes (from 3 to 3.25 years of age) were contributed by community harvesters. The *O. aries* tissue samples were collected immediately after slaughter in the field and include bone, intestine, lung, liver, kidney, and wool. Upon collection, all samples were placed immediately on dry ice and shipped to the University of New Mexico (UNM) Analytical

Chemistry Laboratory Earth and Planetary Sciences Department and prepared for digestion and ICP-MS analysis. The 13th cortical rib bone samples were sheared from the proximal, middle, and distal portions and composited together after removal of excess tissue. The distal, medial, and distal portions of the small intestine were collected and composited. For lung tissue, samples were derived from the upper lobes, middle lobes, and lower lobes and composited. Liver sample composites comprised of the upper, middles, and lower lobes of the organ. Both kidneys were sampled and included both the cortex and medulla. All muscle samples were from the distal, medial, and distal portions of the gastrocnemius and composited. Of wool fiber samples, the anterior, medial, and posterior portions of the animal were sampled and composited appropriately. All tissues were weighed according to predetermined and pre-tested weights that were representative of 1 gram of dried tissue. For all coupled organs, tissues collected from the right side of the sheep were labeled as the sample and one duplicate was obtained for each tissue type from the left side of the animal. A composited duplicate or replicate was also obtained for non-dual type organs (liver and intestine). Visually abnormal tissue where present were noted, photographed, and collected as the sample and a duplicate was obtained from visually normal tissue. For more detail, see Appendix 1 for *O. aries* sampling Standard Operating Procedures (SOP).

Soil Samples

Soil samples were collected with a Teflon® lined soil auger from a depth of 0-15 and 15-91 cm and placed in polyethylene bags and kept on dry ice. 100g of soil will be composited and sent to the lab. One gram of sediment will be acid digested via HNO_3, hydrochloric acid (HCL), and hydrofluoric acid (HF) in Teflon containers. Elemental composition will be determined using ICP-MS. Soil analysis is not driven as a main hypothesis of this study, but will be collected to integrate with existing data and has the potential to be stored for future analyses. For more detail, see Appendix 2 for the soil, water, and plant sampling SOP.

All soil samples were collected using a stainless steel hand auger with a Teflon® coated-core sampler. All soil samples are obtained from the topsoil (0-15cm) excluding agricultural soil samples. Crop soil samples were taken from the topsoil (0-15 cm) and subsoil (15-91 cm) and composited. Crop soil samples were obtained by utilizing a topographic soil zone sampling pattern using a random zig-zag pattern. Two depths were obtained for agricultural soils to encompass or incorporate the plough zones The remaining soils (forage and herb) consisted of topsoil samples only. All soil samples were weighed at 100g. Physicochemical properties such as temperature, pH, Munsell color, depth, moisture were obtained. Each type of sample was paired with soil samples and water as appropriate.

Biota Samples

The entire live plant (edible portion and roots) were removed from the ground soil and handpicked with a latex gloved hand and stored in polyethylene plastic bags. The edible portion of each plant were harvested by removing the stem and root. The plant roots were harvested after a gentle wash with deionized water (ASTM II heavy metal grade) from the soil. The above-ground plants samples were not rinsed. The samples were weighed, photographed, bagged, and placed on dry ice for shipment to UNM.

Fresh crop samples were collected from August 8 to September 9, 2013. The crop plot size determined the number of soil to be composited ($\leq$ 0.4 ha (1 acre) = 6, 0.8 ha (2 acres) = 8). Sheep forage was collected from between November 10 to December 13, 2012.

For herbs, three topsoil samples were composited. The subsamples were mixed into one composite sample for each type of soil and analyzed in duplicate. The fresh herbs samples were collected between July 19 and October 10, 2012.

Plant Species Identification and Nomenclature

Live plants were collected in the field simultaneously as the food and forage samples were being collected. The plants were placed in a one gallon Ziplock® bag. The plants were

placed on dry ice to avoid excessive moisture or heat damage. The live plants were placed between newspapers and cardboard then placed in a plant press for several weeks with daily press tightening. For excessively moist or thick plants, the plants were removed from the press for one to two hours and repressed. The plant sample received the collector's initials and a Plant I.D. Code. A log accompanied the samples that had information such as date and time of plant collection, a precise location description or GPS location with Latitude and Longitude information, a plant description (color/abundance, type of sample, and the state and county the plant was collected and whether the plant is an annual, perennial, or unknown). The dried samples were sent to the UNM Herbarium for identification and archiving.

Water Samples

All water samples were collected as a composite grab sample except for those samples directly collected from a faucet or spigot; they were collected as first-draw samples. Lab grade appropriate for HM analysis polyethylene water bottles were used, volume of samples collected were 250mL. Chemical and physical characteristics data were collected (pH, moisture, color, and temperature). $HN0_3$ preservative was added to each water sample and immediately placed on dry ice. A duplicate for each sample was obtained. A blank for each sampling session was collected.

Uranium Levels in Ovis aries Tissue and Plants.

Metal analysis by ICP-MS was calibrated for U and the other heavy metals. Upon collection in the field, the samples were placed on dry ice and shipped overnight to UNM Analytical Chemistry Laboratory Earth and Planetary Sciences Department. When samples were received they were stored in a -20^{o}C freezer until sample preparation. Aqueous samples were prepared by acid digestion protocol in which 50 ml sample were transferred into digestion tube. Five ml nitric acid (HNO_3) and 2 ml hydrogen peroxide (H_2O_2) were added and samples were heated gradually up to 95^{o}C. At that temperature, the samples were digested for two hours. After

digestion was completed, digested water samples were transferred into 50 ml volume metric flasks and brought to volume using 18 mega ohm water. Solid samples (soils, plants, and bio tissue) were prepared by weighing about 2.000 grams (based on availability and amount of submitted sample) into digestion tube. Five ml nitric acid (HNO_3) and 2 ml hydrogen peroxide (H_2O_2) were added and samples were heated gradually up to 95^oC. At that temperature, the samples were digested for two hours. After digestion was completed, digested water sample were transferred into 50 ml volume metric flasks and brought to volume using 18 mega ohm water. With each bath of samples a reagent blank (3 ml HNO_3) was digested and after digestion completion, the reagent blank sample was transferred into 50 ml volume metric flasks and brought to volume using 18 mega ohm water.

Samples were then prepared for analysis using PerkinElmer NexION 300D ICP-MS by diluting the samples 100 times (100X D.F.) in glass culture tubes. Mixed standard (V, Cs, Pb, Th, U, Se, Mo, As, and Cd) were prepared using single element standards. Calibration standards range was 5, 10, 25, and 50 µg/l (ppb). Also quality control (QC) samples were prepared and used in the analysis of samples. The quality control samples included Initial Calibration Blank Verification (ICBV), Initial Calibration Verification (ICV), Continuing Calibration Verification (CCV), and Matrix Spike (MS), Matrix Spike Duplicate (MSD), and Matrix Spike Replicate (MSR).

A mixed internal standard (Sc, Y, In, and Bi) was used to match analytes mass range. Two percent HNO_3 was used as a carrier and rinse solutions. Elements were analyzed in three modes to minimize interferences, standard, Dynamic Reaction Cell gas A (Anhydrous Ammonia), and Dynamic Reaction Cell gas B (Oxygen) in groups. After analysis was completed, data were revised, validated, tabulated and concentrations were converted into mg/Kg material using instrument corrected concentration reading, sample digest final volume, and sample weight.

For further detail see the appendix for the Standard Operating Procedure (SOP) for the collection of *Ovis aries* tissue. The plants were separated in two for separate analyses: 1) edible parts of the plant (unrinsed) and 2) root samples (gently rinsed).

Statistical Analysis

Data were analyzed using SPSS statistical analysis version 21.0. Outliers were identified and addressed as necessary. Model assumptions were validated (normality, independency, linearity, and homoscedasticity).

Aim 1 Describe Dietary Habits

Descriptive statistics included frequencies for categorical data and range, means, SDs, and standard errors for continuous data. Length of exposure to *O. aries* (mean, SD, distribution) were determined from the DPAHQ questionnaire data and included the time duration that the sheep: 1) lived in the current sampling area 2) was grazed on or next to an abandoned U mine or milling sites (if any), 3) or exposed to U mine tailings or waste piles (if any), and 4) were watered with a particular water source site. Length of exposure to botanicals such as crops, forage, and herbs (mean, SD, distribution) was determined from DCIQ and DWPHIQ questionnaires. The percent of growers and harvesters who consumed harvested sheep, crops, and herbs was reported. The proportions of each type of sheep part, crop type, forage and herb type ingested were determined for the mammal.

Aim 2 Compare Heavy Metals in High Impact Areas to Low Impact Areas

Geographic Information Systems (GIS) mapping was chosen to provide a spatial analyses of the samples taken in the study setting. Data attributes were defined via Terrasync V4.1x Data Dictionary before field collection. All samples were marked in the field using a 2008 Trimble® GeoXT instrument. Rover files were uploaded to predetermined local base providers and underwent differential correction with GPS Pathfinder Office V5.30. Differential correction was completed within three days of data capture. Next, GIS export files were created. Several

cartographic maps were created using ArcGIS10. First, high impact area (HIA) and low impact areas (LIA) were created: a) HIA would be defined at < 2mi radius from mines and features and 2) LIA would be defined at >2mi radius from mines and features (data not shown). The researcher had been blinded to exposure areas when collecting data and as it turned out, all the samples except a few forage and water samples fell within the 2 mi radius zone. To discriminate exposure further, the proximity definition was stratified first into: 1- 2 mi and > 2 mi. Next further stratification into: 0.5 – 1 mi and < 0.5 mi (data not shown).

Independent t-test analyses were used to compare U and heavy metal levels across the exposure categories. The distance categories were then converted to dummy variables and used in linear regression to determine if distance categories predicted levels of heavy metals in the media tested. The relationship between U and As was explored through plots for sheep and Pearson correlation coefficient for selected crops and herbs.

Aim 3 Explore Routes of Heavy Metal Exposure for Locally Harvested Plants and Sheep

Correlations between U and other heavy metal levels and food transfer variables (e.g. proximity, sheep age, plant type or part, etc.) for food chain transfer were explored. The relationship between U in sheep and plants and potential exposure sources (water, water U concentrations, soil U concentrations, and other variables) were analyzed for associations using multiple regression. The dependent variable was U level in plants or animals and the exposure source potentials were the independent variables. A p of < 0.05 was considered significant.

Outcome Measures

The main outcome was to determine if locally harvested foods were contaminated with U and other heavy metals across heavily mined regions of the northwestern Diné reservation. The levels of U and other associated heavy metals in harvested sheep (and corresponding forage, soil, and water), crops, and herbs and medicinal plants were reported.

It was anticipated that certain ingestible parts or type of crop or plant parts would have

higher levels of U and HM concentrations. Depending on the ingestible sheep part or the type of plant, even moderate concentrations in food would provide an opportunity for education to develop or tailor eating behavior by providing safer alternative choices. Lapham et al. (1989) found that kidney and liver had the highest U concentration in cattle. The authors were able to support elimination or reduction of high risk organs to decrease one's internal radiation dose.

Harvesting and mining areas were anticipated to overlap significantly and would provide for educational opportunities. In this geographically limited population, eliminating ingestion of harvested food is not reasonable. Educational intervention regarding harvesting in low risk areas and avoiding high risk areas if it is feasible would provide alternative choices for the Diné. On the other hand, low U and heavy metal concentrations in sheep and plants will support local food selling and trading. Conceivably, GIS risk map can be developed for the harvested foods. Due to the low numbers of participants, developing risk maps to be shown to the rest of the community may be seen as a breach in confidentiality. The importance and relevance of risk maps can be used in the future with the other communities.

The outcome of this research could shape future studies on the health effects related to chronic U and heavy metal exposure and raise the necessary awareness of other harvesting communities exposed to mining contamination. The study could answer the community's safety concerns regarding heavily consumed locally harvested food as well as augment further inquiry, prevention efforts, intervention, monitoring, and education. The findings have the potential to support legislation, policy development, and advocacy.

Participant Number:_____ Revision No. 11, 2/15/08

Please tell us how much water you obtain from each of the water sources you just named for each of the following uses. Each site should add up to no more than "ALL".

	Site A	Site B	Site C	Site D	Site E
19a. Drinking water (includes water for cooking)?	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None
19b. Water for other uses like cleaning and bathing?	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None
19c. Livestock water?	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None
19d. Irrigation water?	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None	❑ All ❑ Most ❑ About Half ❑ Some ❑ None

terviewer Comments on water hauling:

_____

_____

_____

20. Do you eat the meat of the livestock you raise? 1 ❑ **Yes** 0 ❑ **No**

[If the participant does raise livestock but does not haul water for them, note that livestock is free range.]

Please tell us what animals you eat and the specific parts you eat, including the organs.

20a. ❑ Sheep/Goat 2 ❑ Cattle 3 ❑ Horse 4 ❑ Pig 5 ❑ Chicken 6 ❑ Turkey 7

20b. ❑ Muscle a ❑ Liver b ❑ Kidney c ❑ Brain d ❑ Intestine e ❑ Testicles f

❑ Tongue g ❑ Heart h ❑ Other i

Notes:

_____

_____

_____

Figure 4.1. The DiNEH (Diné Network for Environmental Health) survey Question 20 is evaluating whether participants raise and consume livestock and Item 20a. is determining the type of animal consumed and Item 20b is ascertaining the animal parts consumed.

Participant Number:_____ 1 0 Revision No. 11, 2/15/08

21. **Do you eat the vegetables or fruit you grow?** ❑ **Yes** ❑ **No**

21a. Please tell us what vegetables or fruits that you grow and eat:

❑ Apples ❑ Apricots ❑ Beans ❑ Bell Peppers ❑ Carrots ❑ Chile
❑ Corn ❑ Cucumbers ❑ Melons ❑ Onions ❑ Peaches ❑ Potatoes
❑ Squash ❑ Strawberries ❑ Tomatoes
❑ Other _____

Notes:

_____

_____

_____

22. **At other times in your life, have you drunk water hauled from other sites?** ❑ **Yes** ❑ **No**
[If "no," proceed to Question 23; if the answer is "yes," fill in the following table in Question 22a.]

22a. Please tell us the name of those other sites, their locations if you remember them, and the number of years you used water from those sites.

Other water hauling sites	Latitude	Longitude	Number of years water used
A.	N 3 _ . _ _ _ _ _ _	W - 1 0 _ . _ _ _ _ _ _	_____ years
B.	N 3 _ . _ _ _ _ _ _	W - 1 0 _ . _ _ _ _ _ _	_____ years
C.	N 3 _ . _ _ _ _ _ _	W - 1 0 _ . _ _ _ _ _ _	_____ years

Figure 4.2. The DiNEH (Diné Network for Environmental Health) survey. Item 21 is determining whether participants eat and grow their own fruit and vegetables. Item 21a. lists various crops that participants may harvest in addition to an "other" option is reserved for other options not listed.

	Inclusion	Exclusion
Humans	≥18 years of age and older, continual residence of >10 yrs in the community, and willing to participate in the study, eat food grown and harvested locally, and willing to participate in the study.	<18 years of age, inconsistent residence of < 10yrs in the community, those who do not eat food grown and harvested locally, and unable to consent to participate in the study.
Ovis aries or sheep	Domesticated male & non-pregnant female sheep > than 6 months of age but <10 years of age, without any visible physical defects to indicate acute injury (mauling, blunt trauma etc.), consumes local grass forage (<25% fodder), consumes water sources available on the reservation, and has lived all its life on the reservation, grazes regularly within 2 miles (3.2 km) of abandoned U structures or tailings/waste pile.	Undomesticated sheep, pregnant females, age < than six months of age of >10 yrs of age, with visible physical acute injuries (mauling, blunt trauma etc.), consumes a diet of > 25% of fodder, consumes water away from the reservation, and was born or lived away from the reservation, grazes further than 2 miles (3.2 km) away from abandoned U structures.
Crops/ Plants	Plants that are undamaged, non-wilted, non-wet or damp (from natural precipitation/frost), at the time of sampling, must be unfrozen, no visible insect or disease infestation, growth within 2 miles (3.2 km) of abandoned U mining structures or tailings/waste piles, watered by natural precipitation or irrigated via water sources from the reservation. Only fresh and un-cooked crops will be sampled.	Plants that have wetness/dampness at time of sampling, damaged, wilted, frozen, infested, watered (naturally) or irrigated by water sources away from the reservation, or growing outside 2 miles (3.2 km) of abandoned U mining structures. Non-fresh or cooked crops will be excluded.

Table 4.1. Detailed inclusion and exclusion criteria for humans and the sampling strategy for animals, crops, and plants.

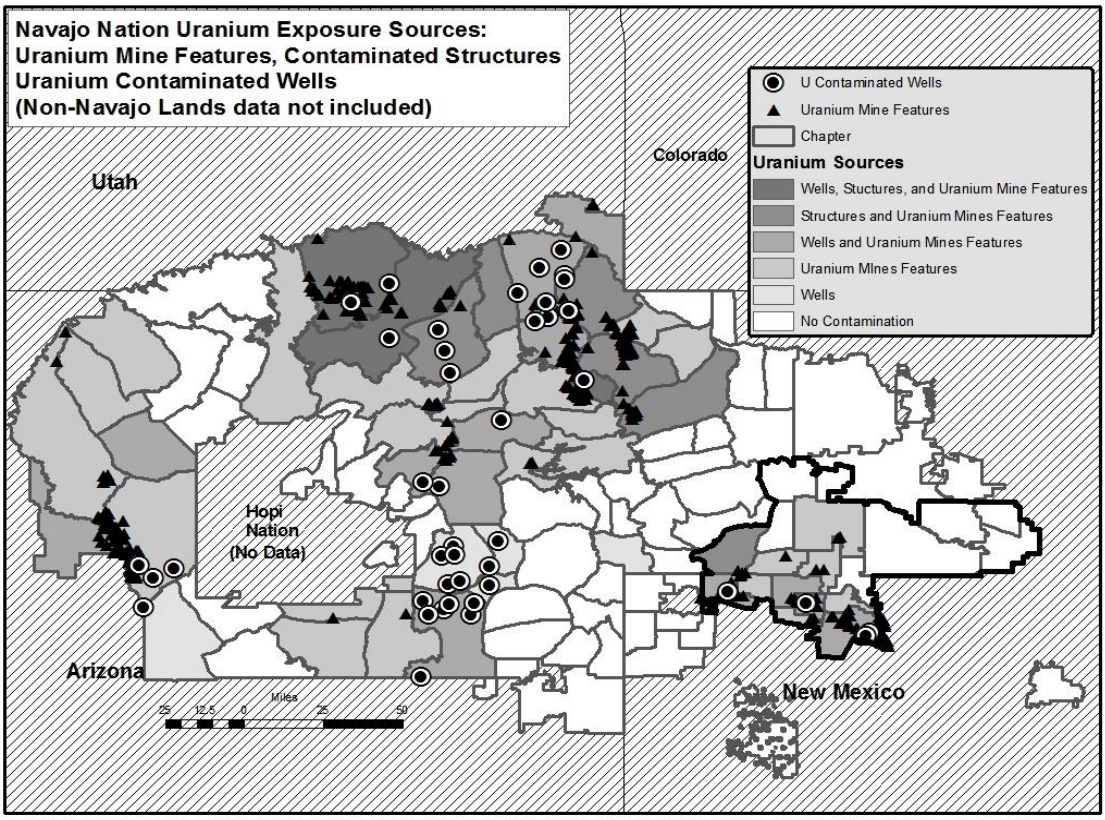
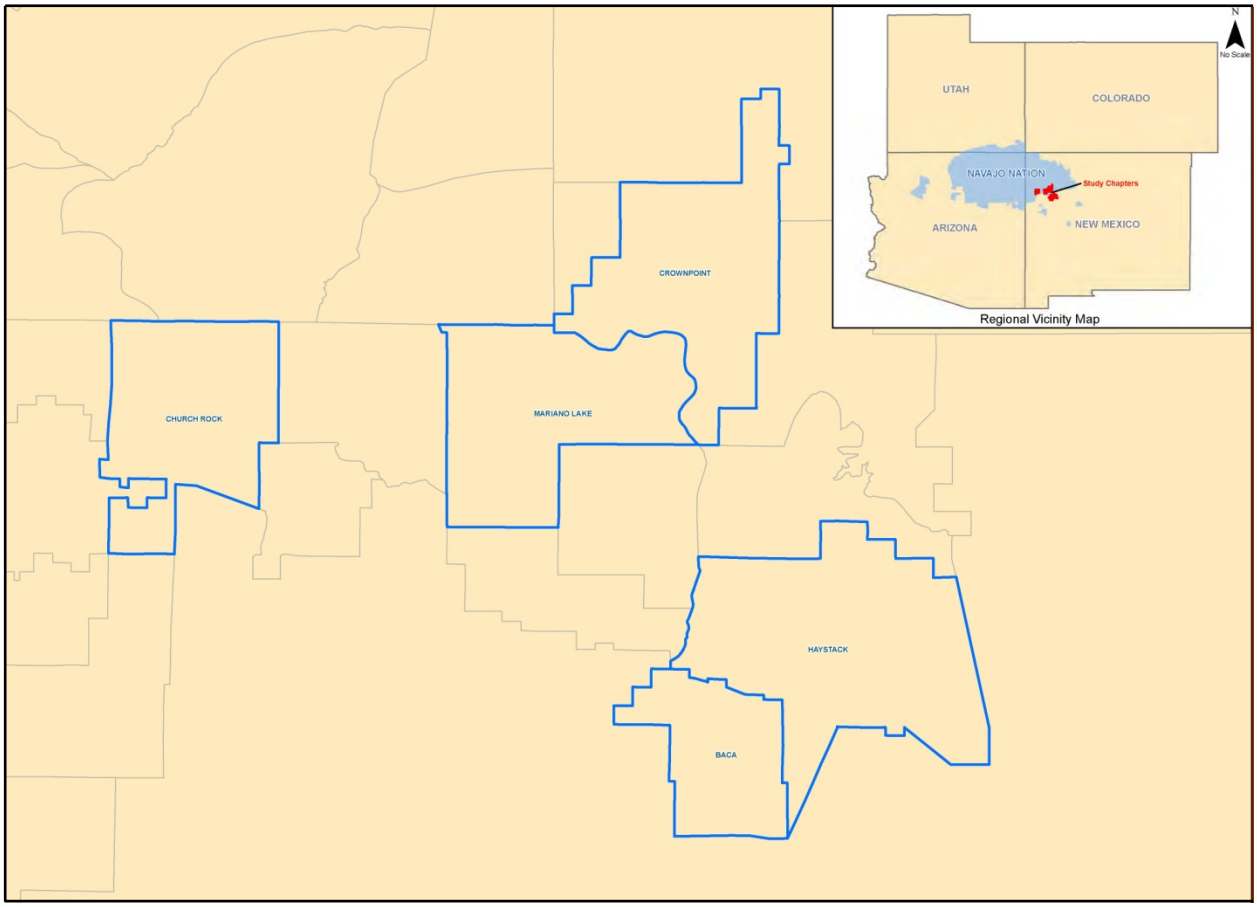


Figure 4.3. The study setting is outlined in black on the right lower corner of the Navajo Nation map. The majority of the chapters in the study setting area show characteristics of mine features or are areas with U contamination. Note also there are several chapters that are identified with "*No Contamination"* areas. The *No Contamination* areas will be the proposed study reference area. Representation prepared by Tommy Rock for the DiNEH Project. Courtesy of the DiNEH project.

Figure 4.4. Study Area



Diné Nation Study Chapters: Baca/Prewitt/Haystack, Churchrock, Crownpoint, and Mariano Lake.

Figure 4.4. The study setting is outlined in red in the upper right corner of the Dine or Navajo Nation map. The approved study chapters are highlighted in blue in the enlarged map.

Code:_____

Diné Plant-Animal-Human Questionnaire (DPAHQ)

Date of Interview:_____**Interviewer:**_____

Location of Interview:_____

Section I - Personal Information

Name:_____ **Assigned Code #:**_____

DOB:_____

What is your mailing address?_____

Telephone and/or cell:_____ **I do not have a phone/cell**_____

What is the current location of your home? (*provide rural address with house number or nearest highway or distance from Chapter House).* _____Latitude N3___._____Longitude W-10___._____

How long have you lived in your current home? _____ **months OR years** *(please circle only one)*

Name other locations you have lived at previous to the current location (as indicated above) and indicate the number of years at each location? *(please provide location on a map or GPS location)*

Location 1:_____Latitude: N3__.____ Longitude: W-10__.____ ***x*** _____Years
Location 2:_____Latitude: N3__.____ Longitude: W-10__.____ ***x*** _____Years
Location 3:_____Latitude: N3__.____ Longitude: W-10__.____ ***x***_____ Years

Which Chapter House do you vote at for elections? _____ **I do not vote**_____

What language would you prefer to use for this interview? English **OR** Diné **OR** Both

[This page will be removed from the remainder of the questionnaire]

Figure 4.5. Diné Plant-Animal-Human Questionnaire (DPAHQ). This is the general questionnaire that comprises of Section I Personal Information. This is page 1 of 4.

Code:_____

Section II - Demographics

1. **Gender** ☐ male ☐ female *(mark the appropriate box based on your observation)*

2. **Are you Diné?** ☐ yes **OR** ☐ no. If no, what is your tribal affiliation? _____

3. **Currently, what is the highest grade in school you completed?**
   - ☐ No education
   - ☐ Less than a high school education
   - ☐ High school graduate/GED
   - ☐ Some college, no degree
   - ☐ Associate degree
   - ☐ Bachelor's degree
   - ☐ Master's degree or higher
   - ☐ Other_____

4. **Currently, what is your annual household income?**
   - ☐ less than $10,000 ☐ Refused
   - ☐ $10,000 -19,999
   - ☐ $20,000-29,999
   - ☐ $30,000-39,999
   - ☐ $40,000-49,999
   - ☐ $50,000-59,999
   - ☐ $60,000-69,999
   - ☐ $70,000-79,999
   - ☐ $80,000-89,999
   - ☐ $90,000-99,999
   - ☐ greater than $100,000

5. **How many people currently live in your household** *(including yourself)* ?_____

6. **What kind of heating do you currently have in your home?** *(please check all that apply)*
   - ☐ gas/electric
   - ☐ coal
   - ☐ woodstove
   - ☐ other:_____

7. **Are you currently:**
   - ☐ Self-employed
   - ☐ Out of work for > 1 year
   - ☐ Out of work < 1 year
   - ☐ A home-maker
   - ☐ A rancher
   - ☐ A student
   - ☐ Retired
   - ☐ Unable to work
   - ☐ Other_____

Figure 4.6. Diné Plant-Animal-Human Questionnaire (DPAHQ). This is Section II Demographics Items 1 through 7. This is page 2 of 4.

Code:_____

Section III - Water

8. Of the sheep to be tested, what water sources do the sheep currently drink from? *(check all that apply)*

- ☐ Grocery store/Trading post
- ☐ Rain water
- ☐ Lake
- ☐ Spring
- ☐ NTUA
- ☐ Stream
- ☐ Pond
- ☐ Windmill
- ☐ Private well
- ☐ Other:_____

9. Is your home currently connected to a public water system? yes **OR** no If yes, which water system?_____

10. Do you currently treat (filter, disinfect, or boil) the water that you haul for the sheep to be tested?

yes **OR** no **OR** I don't know **OR** I do not have sheep **OR** I do not haul water *(circle only one)*

11. Do you currently treat (filter, disinfect, or boil) the water that you haul for your crops to be tested?

yes **OR** no **OR** I don't know **OR** I do not have crops **OR** I do not haul water *(circle only one)*

Section IV – Sheep Foodstuffs

If you DO NOT eat the meat of the sheep you raise skip to question 17.

12. Currently, what part(s) of the sheep you raise do you consume and overall, how many years have you eaten each sheep part? *(check all that apply and indicate the number of years each part is eaten).*

- ☐ Bone and/or bone marrow ***x*** _____ years
- ☐ Intestines ***x*** _____ years
- ☐ Heart ***x*** _____years
- ☐ Kidneys ***x*** _____years
- ☐ Liver ***x*** _____years
- ☐ Muscle tissue ***x*** _____years
- ☐ Other_____***x*** _____years_____

13. Of the sheep you raise, how much of your overall meat intake is from sheep alone?

none **OR** 25% **OR** 50% **OR** 75% **OR** 100%

14. In a typical week, how many meals consumed consist of mutton/lamb that you raise?_____

15. Of the sheep you raise, how many meals out of the day do you typically eat mutton/lamb? 1 / 2 / 3 **OR** more___

16. In total, how many years have you eaten sheep meat you raised in the current location? _____years

Figure 4.7. Diné Plant-Animal-Human Questionnaire (DPAHQ). This is Section III Water and Section IV Sheep Foodstuffs Items. This is page 3 of 4.

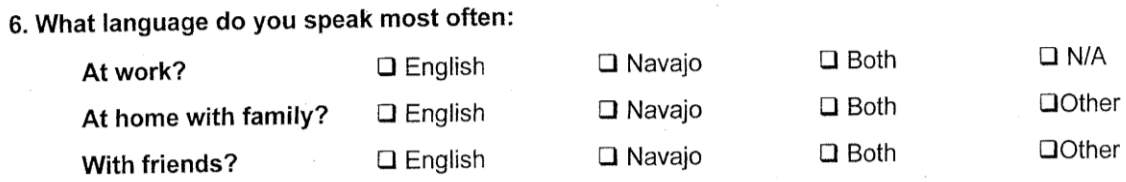


Figure 4.8. The DiNEH (Diné Network for Environmental Health, Revision No. 11, 02/15/2008) survey. Item 6 is ascertaining the use of language in the work setting, at home, and with friends.

11. Do you use water from any of the following sources for any purpose? *[Check all that apply.]*

- ❑ Cistern or tank
- ❑ Grocery or convenience store/ trading post
- ❑ Lake
- ❑ NAPI/Irrigation Water
- ❑ NTUA
- ❑ Pond
- ❑ Private well
- ❑ Rain Water
- ❑ Spring
- ❑ Stream
- ❑ Windmill or other well

12. Is your home connected to a community water system? ❑ Yes ❑ No *[If No, skip to #14]*

Figure 4.9. The DiNEH (Diné Network for Environmental Health, Revision No. 11, 02/15/2008) survey. Items 11 and 12. Items 11 and 12 are determining sources of water and the community water system.

Code:_____

Section V - Other Human Harvesting Exposure Activities

17. Check off all activities you participate in and the number of years you participated in these activities on the reservation:

17a.☐ Hunting small game animals (< than 10lbs) *x* _____years. Game type(s): _____

17b.☐ Hunting large game animals (> than 10 lbs) x _____years. Game type(s): _____

17c.☐ Collecting firewood for fuel or cooking *x* _____years

17d.☐ Land management/maintenance activities (e.g. building or mending fences) *x* _____years
Type(s) of activity (name top 3 activities):_____

17e.☐ Herding non-wild animals *(other than sheep)* *x* _____years.
Type of animals:_____

17f. ☐ Recreational activities (name top 3 activities):_____

17g.☐ Other activities:_____ *x* _____years

Figure 4.10. Diné Plant-Animal-Human Questionnaire (DPAHQ). This is Section V of the questionnaire regarding Other Human Harvesting Exposure Activities. This is page 4 of 4.

Code:_____

Diné Dibé Intake Questionnaire (DDIQ)

1. Participant owner code #:_____ **2. Today's Date:**_____

3. Sheep type/gender: Ewe **OR** Ram **OR** Wether **OR** if a Lamb: Male ☐ **OR** Female ☐

4. What is the approximate age of the sheep to be tested? _____ months **OR** years *(please circle one)*

4a. How long has this sheep to be tested lived on this reservation?_____ months **OR** years *(circle one)*

5. Approximate weight of the sheep? _____lbs.

6. Tested Sheep's Food Source: Local forage *(type if known)[photos]*:_____
_____**AND / OR** *(circle one)*
non-winter fodder type:_____

7. Of the sheep to be tested, where do you currently get water for your sheep? *(please fill in the table below according to the provided map of the local area or via GPS).*

Site	Map Site Location and/or Well Number	OR	GPS Latitude	GPS Longitude
A			N3____._____	W-10____._____
B			N3____._____	W-10____._____
C			N3____._____	W-10____._____

8. Currently, how much water used for your livestock do you get from each site you named in question 7? (*The total used for livestock should not be greater than 100%).*

Site	Percentage Used *(circle only one)*					Number Years the Sheep Drank the Water
A	0%	25%	50%	75%	100%	
B	0%	25%	50%	75%	100%	
C	0%	25%	50%	75%	100%	

9. Please provide the location of the sheep corral. *(please provide directions on this map or GPS)*_____ **OR**
GPS Latitude: N3____._____ Longitude: W-10____._____

10. Please provide the general locations of grazing. *(please provide directions on this map OR GPS)*

10a. Location 1:_____ **OR**
GPS Latitude: N3____._____ Longitude: W-10____._____

10b. Location 2:_____ **OR**
GPS Latitude: N3____._____ Longitude: W-10____._____

10c. Location 3:_____ **OR**
GPS Latitude: N3____._____ Longitude: W-10____._____

DDIQ Page **1** of **3**

Figure 4.11. Dine Dibé Intake Questionnaire (DDIQ). This is the sheep data to be collected and includes the type of sheep, age, gender, type of forage and fodder eaten, lifespan on the reservation, and the general health of the animal. Items 1 to 10c are shown here. This is page 1 of 3.

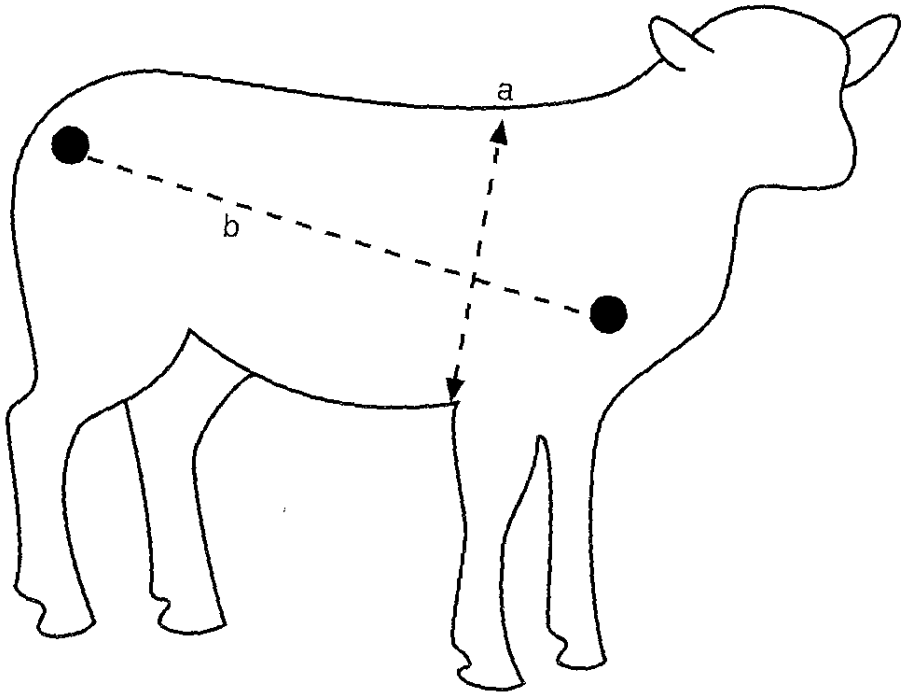


Figure 4.12. Approximate sheep weight. Approximate weight of the sheep will be determined by measuring the circumference or heart girth just behind the shoulder and elbows (a) and the body length (b). Heart girth *x* heart girth *x* body length ÷ 300 = weight in pounds. From Pugh, D.G. (2002). *Sheep and goat medicine*. Philadelphia: Saunders.

Code:_____

11. For the sheep to be tested, what is the length of time this sheep has grazed in these current locations identified in question 10.

11a. Location 1:_____Weeks **OR** Months **OR** Years *(please circle only one)*

11b. Location 2:_____ Weeks **OR** Months **OR** Years *(please circle only one)*

11c. Location 3:_____ Weeks **OR** Months **OR** Years *(please circle only one)*

12. Has the sheep to be tested been evaluated by a veterinarian in the past 3 months? yes **OR** no

12a. If yes, for what reason?_____

12b. If yes, what medication did the animal receive *(if any)*:_____

13. Of the sheep to be tested, list any sheep dip, shots or other treatments it received in the past 3 months *(If any)*_____

14. Was the sheep to be tested born with any abnormalities? yes **OR** no

14a. If yes, describe the abnormality(ies):_____

14b. If yes, any abnormalities in the: parent(s) OR sibling(s) OR lamb(s) *(circle all that apply)*

14c. If yes, describe the abnormality(ies):_____

15. Do you have another home that you relocate to? yes **OR** no

15a. If yes, which seasonal home is this? spring summer fall winter *(circle one)*

15b. If yes, please provide directions or locate this home on this map or GPS:_____**OR**
GPS Latitude: N3____._____ Longitude: W-10____._____

15c. If yes, please provide the location of the sheep corral on this map or GPS:_____**OR**
GPS Latitude: N3____._____ Longitude: W-10____._____

15d. If yes, please provide the general locations of sheep grazing areas on this map or GPS:_____**OR**
GPS Latitude: N3____._____ Longitude: W-10____._____ .
Location 2:_____**OR** GPS Latitude: N3____._____ Longitude: W-10____._____.
Location 3:_____**OR** GPS Latitude: N3____._____ Longitude: W-10____._____.

16. Of the sheep to be tested, do you rotate the sheep's grazing areas? yes **OR** no

16a. If yes, how long will the sheep be left in a paddock to graze? _____days **/** weeks **/** months *(circle one)*
16b. if yes, how long will the paddock pasture rest? _____days **OR** weeks **OR** months *(circle one)*

17. For the sheep you raise currently, do you use the sheep's wool for making textiles?

yes **OR** no **OR** I don't know *(circle one)*

DDIQ Page **2** of **3**

Figure 4.13. Dine Dibé Intake Questionnaire (DDIQ). This is the sheep data to be collected includes the sheep health status, seasonal relocation sites, grazing questions, and use of the sheep. Items 11 to 17 are shown here. This is page 2 of 3.

Code:_____

17a. Do you and/or your family sell the wool? yes **OR** no **OR** I don't know *(circle one)*

17b. Do you and/or your family sell the textiles? yes **OR** no **OR** I don't know *(circle one)*

18. Do you and/or your family sell live sheep to market? yes **OR** no **OR** I don't know *(circle one)*

18a. Do you and/or your family sell the mutton/lamb meat to market?

yes **OR** no **OR** I don't know *(circle only one)*

18b. Besides your family, do you share (free) sheep meat with other people?

yes **OR** no **OR** I don't know *(circle one)*

If yes, where do the people that you shared meat with live (list the last 3 locations):
18c._____**18d.**_____**18e.**_____

19. What other uses do you have for sheep parts in the home or for traditional purposes or medicinal uses?_____

Investigator Use Only:

20. Calculated average pasture height:_____ **cm**

PHOTO(S) HERE

Figure 4.14. Dine Dibé Intake Questionnaire (DDIQ). This is the sheep data to be collected and includes the versatility of sheep wool and other uses of sheep parts for home and medicinal purposes. Items 17a to 20 are shown here. This is page 3 of 3.

Code:_____

PASTURE HEIGHT RECORDING FORM

Height (cm) **A**	Place a mark besides the height whenever a measurement of this height is recorded	Number of recordings **B**	**A x B**
0			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
___			
___			
___			
	Totals		
	Average height=Total (A x B)/Total B		

Notes:_____

_____

_____

Version 04/21/2012

Table 4.2. Recording sheet for pasture height . Pasture height is a reasonably reliable determinant of pasture quantity and available feed. "Reproduced with permission from *Sheep Farming for Meat and Wool* (Eds: Jane Court, Sue Hides and John Webb-Ware). Copyright © Department of Primary Industries, Victoria. Published by CSIRO PUBLISHING, Collingwood, Victoria Australia - http://www.publish.csiro.au/pid/5853.htm."

Code:_____

Diné Crop Intake Questionnaire (DCIQ)

1. Date Planted:_____ **2. Harvest Date:**_____

3. Current location of crops to be tested *(provide directions on this map)*:_____ **OR**
GPS Latitude: N3____._____ Longitude: W-10____._____

3a. Alternate crop plot *(provide directions on this map or GPS)*:_____**OR**
GPS Latitude: N3____._____ Longitude: W-10____._____

4. How often do you plant crops at this current location? yearly **OR** every 2 years **OR** other:_____

5. Overall, how many years has this crop plot been used? _____ years

6. Of the crops to be tested, where do you currently haul water for crop irrigation? *(please fill in the table below according to the provided map of the local area or via GPS).*

Site	Map Site Location and/or Well Number	OR	GPS Latitude	GPS Longitude
A			N3____._____	W-10____._____
B			N3____._____	W-10____._____
C			N3____._____	W-10____._____

7. Currently, how much water do you get from each of the water sources you named in question 6 for crop irrigation? (*The total from each site should not be greater than 100%).*

Site	Percentage Used *(circle only one)*					Number of Years the Crops Irrigated
A	0%	25%	50%	75%	100%	
B	0%	25%	50%	75%	100%	
C	0%	25%	50%	75%	100%	

8. Were any pesticides or fertilizers used on the crops to be tested? yes **OR** no *(please circle one)*

8a. If yes, which type(s) and for which crop(s)_____

9. Current planting technique used: machinery *(type)*:_____hand: _____**OR**
a combination _____**OR** Other:_____

10. Currently, what crops did you plant? Corn / Squash / Beans / Melon / Chili / Others *(circle all that apply).* Others:_____

11. Where did you get the crop seeds *(purchased or otherwise)***?**_____

DCIQ Page **1** of **3**

Figure 4.15. Diné Crop Intake Questionnaire (DCIQ). This is the agricultural data to be collected and includes the date of planting and harvesting, planting technique, and an agricultural calendar. Items 1 through 11 are shown here. This is page 1 of 3.

Code:_____

12. On average, how often do you eat the crops that you raise? *(please fill in the table below)*

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Corn												
Squash												
Beans												
Melon												
Chili												
Other(s)												

-Place the number "4" in each month where you eat the crop daily
-Place the number "3" in each month where you eat the crop 4-5 days a week
-Place the number "2" in each month where you eat the crop 2-3 days a week
-Place the number "1" in each month where you eat the crop only once a week or less
-Place an "X" in the month where you do not eat any crops at all

13. Overall, how many years have you eaten the crops that you raise in the current location for the crops listed below: *(please check off all that apply)*

☐ Corn _____years
☐ Squash _____years
☐ Beans _____years
☐ Melon _____years
☐ Chili _____years
☐ Other(s)_____years_____

14. Which animals are typically given crops or crops parts as a feed supplement? *(check all that apply)*
☐ sheep ☐ goats ☐ cattle ☐ other(s):_____

14a. If yes, which crops and crop parts?_____

14b. If yes, which animals consumed the above crops and crop parts?_____

15. Do you and/or your family sell any of the crops? yes **OR** no **OR** I don't know *(circle one)*

15a. *If yes, which of the crops (check all that apply):*
☐ Corn ☐ Squash ☐ Beans ☐ Melon ☐ Chili and ☐ Other(s):_____

Figure 4.16. Diné Crop Intake Questionnaire (DCIQ). This is the agricultural data to be collected and includes the length of time crop were eaten, the use of crops as animal feed supplement, and the sale of agricultural produce. Items 12 through 15a are shown here. This is page 2 of 3.

Code:_____

16. Do you typically eat corn pollen from the corn that you raise? yes **OR** no **OR** I don't know *(circle one)*

16a. If yes, how many years have you eaten corn pollen from the corn that you raise? _____ years.

17. Where are the crops typically stored after harvest?

Corn_____

Squash_____

Beans_____

Melon_____

Chili_____

Other(s):_____

18. Do you share (free) the crops with other people? yes OR no OR I don't know *(circle one)*

If yes, where do the people that you shared the crops with live *(list last 3 locations)*:

18a._____**18b.**_____**18c.**_____

PHOTO(S) HERE

Figure 4.17. Diné Crop Intake Questionnaire (DCIQ). This is the agricultural data to be collected and includes questions regarding the ingestion of corn pollen, storage of crops, and sharing of crops. Items 16 through 18c are shown here. This is page 3 of 3.

Code:_____

Diné Wild Plant/Herb Intake Questionnaire (DWPHIQ)

1. Do you currently consume locally grown wild plants, herbs or medicinal plants? *(Please indicate only the two most frequently used plants by you). [Use a duplicate page of this questionnaire for plant #2]*

yes **OR** no **OR** I don't know *(please circle only one)*

1a. if yes, location of live plants *(provide directions on this map or GPS)*:_____ **OR**
GPS Latitude: N3_____._____ Longitude: W-10_____._____

1b. if yes, what plant do you consume at this site? *(Photos)*_____

1c. if yes, how often do you eat the live plant? _____**X** day **OR** week **OR** month *(circle only one)*

1d. if yes, overall how many years have you consumed this plant? _____ years

1e. if yes, what part of the plant do you consume? Root **/** Stem **/** Leaf **/** Flower **OR** Other_____
(circle all that apply)

1f. if yes, of the plant or herb consumed, what is it's therapeutic use? _____

1g. if yes, is the plant rinsed with water or cleansed in any other way before use?

yes **OR** no **OR** I don't know *(please circle only one)*

1h. if the plant requires it, how is it prepared before consumption (grinding/boiling etc.)?_____

1i. Is the treatment given under the direction of a traditional healer(s)? yes **OR** no **OR** I don't know

2. Of the sheep to be tested, did the sheep eat locally grown (non-forage) medicinal plants*? (Please indicate only the two mostly frequently administered plants). [Use a duplicate questionnaire page for plant #2]*

yes **OR** no **OR** I don't know *(please circle only one)*

2a. if yes, location of live (non-forage) plants *(provide directions on this map or GPS)*:_____ **OR**

GPS Latitude: N3_____._____ Longitude: W-10_____._____

2b. if yes, what non-forage plant does the sheep consume? *(Photos)*_____

2c. if yes, how often does the sheep eat the live plants? _____X day **OR** week **OR** month

2d. if yes, overall how many years has the sheep consumed this plant? _____ years

2e. if yes, what part of the plant is given to the sheep to eat? Root **/** Stem **/** Leaf **/** Flower **/ OR** Other
(circle all that apply)

2f. if yes, of the plant or herb consumed, what is it's therapeutic use? _____

2g. if yes, If the plant requires it, how is it prepared for consumption (boiling/grinding)?_____

DWPHIQ Page **1** of **2**

Figure 4.18. Diné Wild Plants/Herbs Intake Questionnaire (DWPHIQ). The purpose of this questionnaire tool is to gather data on wild plants, herbs, or medicinal plants and include: plant location, consumption frequency, and plant parts consumed. Items 1 through 2g are shown here. This is page 1 of 2.

Code:_____

3. Of the plants to be tested, were any insecticides or fertilizers used on the wild plants/herbs or medicinal plants for human use? yes **OR** no **If yes, which type?**_____

3a. what of those for sheep use? yes **OR** no **If yes, which type?**_____

4. Currently, do you or your family sell any of the herbs, wild plants or medicinal plants?

yes **OR** no **OR** I don't know *(circle only one)*

4a. If yes, which herbs, wild plants or medicinal plants are sold?_____

5. Besides your family, do you share (free) herbs or medicinal plants with other people?

yes **OR** no **OR** I don't know *(circle only one)*

6. If yes, where do the people that you shared plants with live (list the last 3 locations)?

6a._____**6b.**_____**6c.**_____

Investigator Use Only:

7. Almanac Information: Average rainfall for year 20____:_____

PHOTO(S) HERE

Figure 4.19. Diné Wild Plants/Herbs Intake Questionnaire (DWPHIQ). The purpose of this questionnaire tool is to gather data on wild plants, herbs, or medicinal plants and include pesticide use, and the sale of and sharing of herbal plants. This is page 2 of 2.

Figure 4.20. Overview map of sampling areas witing the 2 mile buffer zone

Figure 4.21.Overview map of sampling areas within the 1-2 mile, >2 mile & 0.5-1 mile, <0.5 mile buffer zones

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Chapter Five

Results

Eight growers and harvesters provided the animal and plant material evaluated for this study. The specimens included three sheep (n=24 samples), three types of crops (n=20 samples), seven species of forage (n=33 samples), three species of herbs (n=18 samples), each coupled with soil and water samples (if any). The growers or harvesters are described in Table 5.1. Their average age was 57 years (SD=10.9) and 63 percent were female. Half had less than a high school education or GED equivalent, while 38% had a high school education, and 13% had "some college." Most of the participants reported that they held "other" types of occupations such as working with the city or tribe (35%), 25% reported being "unable" to work, 12.5% identified themselves as "ranchers," 10% were self-employed, six percent reported being out of work for greater than a year, four percent reported being out of work for less than a year, and/or six percent were retired. Thirty eight percent reported having income less than $10,000 per year and an equal percentage reported earning $10,000 to $19,999 per year. Another 12.5% each reported earning $20,000 to $29,999 and $30,000 to $30,999 per year. All of the growers and harvesters identified themselves as Diné for their tribal affiliation. The preference for language during the study interactions indicated that most preferred using both English and Diné (63%), 25% exclusively used the Diné language, and 13% preferred the English language.

To heat their homes, most of the growers/harvesters utilized a wood stove, followed by gas, then "other" means of heating (e.g. pellet stove), and coal (see Table 5.2). Most homes had two, three or four persons living in the home (25% each) and 12.5% of those interviewed indicated they had one to five persons living in the home including themselves. Of those contributing sheep samples (n=3), 33% indicated using the public tribal water system (Navajo Tribal Utility Authority, NTUA) followed by pond water (17%), then equal use between rainwater (when available), windmill, and private wells (eight percent each). Others report

using a livestock dam when water was available (25%). Details on sheep and crop water sources are shown in Table 5.3. Seventy-five percent of the growers/harvesters reported not having access to a community water system.

The survey inquired about activities that may expose participants to heavy metals encountered in the outdoors. Thirty-one percent of participants reported firewood collection activities, 20% participated in land management activities, an equal percentage participated in herding animals, and 18.1% participated in outdoor recreational activities (such as hiking, horseback riding, etc.). A smaller percentage hunted small game weighing less than 10 pounds (two percent) and nine percent hunted large game weighed more than 10 pounds (refer to Table 5.2).

Of all harvesting activities, 44% consisted of herb samples, 33% of sheep tissue, and 22% were crop samples (see Table 5.4). The samples of food and water consumed by *O. aries* were equally split between forage and water samples. There was no crop or herb consumption by sheep. All human participants that contributed sheep tissue reported consuming all the sheep parts listed (bone marrow, intestine, leg muscle, liver, lung, and kidney) for a combined total average of 58 consumption years (SD=1.73). Of the two contributing crop samples, 67% reported harvesting squash, and 17% each was reported for corn and bean planting. Of herbs collected, the majority were Diné tea samples (75%), 13% each for juniper and sage samples. Details on soil sources collected for forage, crop, and herb areas are shown in Table 5.5.

Water

For crop water, the most frequently used water source was rainwater (75%) and public water (25%). One crop harvester used public water exclusively to water squash only (see Table 5.2). For sheep water consumption the water source most utilized was seasonal livestock dams (43%), followed by public water (29%), and 14% being equally split between windmill water and well use (Table 5.4).

Of sheep and crop water samples, crop water had higher concentrations of heavy metals . Molybdenum (Mo) occurred at highest concentration (M=1358.16, SD= 98.40 followed by Cd (M=44.73, SD= 0.38), V (M=26.44, SD= 0.19), Se (M=21.81, SD 12.38), Pb (M=16.18, SD= 3.11), U (M=7.03, SD=5.44), As (M=3.80, SD=0.59), and lastly, Cs (M=0.46, SD=0.12). For both sheep and crop water there were negligible Th levels.

Water was collected for the purpose of determining sheep intake and was not collected for the assessment of forage watering practice. No sheep harvesters reported watering forage for sheep consumption. For sheep water use, the greatest in heavy metal concentrations were found were for Pb (M=8.10, SD=2.39), then V (M=7.71, SD=5.87), Se (M=5.58, SD=1.19), U (M=5.29, SD= 3.48), Mo (M=3.93, SD=2.80), As (M=0.95, SD=0.23), Cs (M=0.37, SD=0.40), and lastly Cd (M=0.33, SD=0.20, see Table 5.6). For the sheep tested, two water sites (Site A and B) were used for 2.3 mean years (SD=1.4) and a third water site (Site C) was used for 1.1 mean years (SD=1.9). The lowest heavy metal concentrations were found in water samples of two of three sheep herders who reported utilizing public water 75% of the time or a regulated well for watering their sheep. One sheep herder utilized a livestock windmill, several seasonal livestock dams, and well water.

Water samples were not collected in herb harvesting or sheep forage areas. Of the tea harvesters, watering wild tea was not practiced. Forage was not watered by harvesters.

Soil for Crops, Sheep Grazing Forage Areas, and Herb Harvesting Areas

Near the current study area, the de Lemos et al. (2009) study reported mean U soil concentrations of 3 to 8 mg/kg which was near typical background concentrations from downstream abandoned uranium mine sites. The soil data was comparable to National Uranium Resource Evaluation (NURE) sampling that was undertaken in the late 1970s. In the same study, vegetation grass samples ranged from below the detection limit 0.5 mg/kg to 7.7 mg/kg with more uptake in the roots (5.0 mg/kg) than the plant blade (2.4 mg/kg). Mean soil levels for

As (3.42 ppm), Cd (-4.93 ppm), Cs (3.35 ppm), Pb (9.91 ppm), Se (-4.97 ppm), Th (9.48 ppm), and V (48.53 ppm) by Delayed Neutron method from 1975 to 1979 (Mo not evaluated).

There exist recommended limits for heavy metals in soil in parts per million (ppm). The limits for heavy metals will be described here for crop and other food harvest areas and compared to the current study findings. The Regional Screening Level for Superfund Sites or Residential Soil (U.S. EPA Region 9) recommends 390 ppm for Mo, 390 ppm for Se, and 390 ppm for V; the use of The Human Health Screening Level (HSSL) is recommended for As (0.07 ppm), Pb (39 ppm), and Cd (300 ppm). In the current study, crop harvesting area topsoil (0-15 cm) and subsoil (> 15-91 cm) were collected to adequately reflect the plow zone. In the soil samples collected, the heavy metals with the highest concentrations in topsoil were V (M=20.69, SD=10.88), Se (M=7.48, SD=2.95) and Pb (M=6.77, SD=1.14). None of these were elevated above those found in the NURE sampling conducted in the 1970s (Mo not evaluated by NURE). The other heavy metals (As, Cd, Cs, Th, & U) in soil were also not elevated above the NURE data (see Table 5.7). For subsoil, the highest concentrations found were V (M=21.84, SD=10.88), Se (M=4.48, SD=1.31) and Th (M=3.96, SD=0.61). These were not elevated above the data collected by USGS (Mo not evaluated by USGS). Of the crop soils, V and Th were higher in the subsoil than the topsoil. Concentrations of the remaining heavy metals (As, Cd, Cs, Pb, and U) in subsoil did not exceed the NURE data values. In crop harvesting areas the CA HSSL for As (0.07 ppm) was exceeded for topsoil (M=1.95, SD=0.68) and subsoil (M=1.98, SD=0.69). In sheep grazing forage areas, all heavy metal levels fell below those reported by USGS. Nevertheless, the higher levels seen were in V (M=10.33, SD=2.41), Pb (M=7.43, SD=2.69), Th (M=4.59, SD=2.13), and As (M=2.86, SD=1.34). For sheep grazing areas, the CA HSSL for As was exceeded.

In herb harvesting areas, all heavy metal levels were well below the NURE data levels. On the contrary, the CA HSSL for As was exceeded in herb harvesting areas (M=1.65,

SD=0.56).

Crops

All crop harvesters utilized two different sites to water their plant crops. The mean number of years Site A and Site B were used was 17 mean years (SD=19.80). At the onset of harvest season (September to October), *C. pepo* or squash, *Z. mays* or corn, and *P. vulgaris* or beans were eaten two to three times a week or less until February or April (depending on the crop type, bounty, and consumption). The months where crops were not available (dependent on crop type) were from March to September or October. Of all crop growers, corn and beans were eaten on an average of 15.5 mean years (SD=21.92) and squash consumption was 17.5 mean years (SD=19.09) at the locations of sampling. For all crops, topsoil and subsoil were collected. In general, the uptake of heavy metals was greater in the roots than the above ground edible crop except for squash (Mo & Se), bean (As, Se, & Mo), and corn (As & Se) samples (see Table 5.8). Only a few samples of squash leaves were collected (n=6), of those there was greater heavy metals levels in Cd, Cs, Mo, Se, and Th than in the edible parts of the squash and its roots. In topsoil, Cs, Se, Mo, and Cd had the highest levels and V, Pb, Th, U, and As were highest in the subsoil. For squash root, Pb had the greatest uptake when compared to edible parts of the squash, its root, topsoil, and subsoil samples (See Figure 5.1). Corn root had the greatest uptake in V and the least uptake in U.

Sheep Forage

Seven types of native forage were collected and their species identified (see Table 5.9). There were some forage consumption overlap among the three sheep grazing areas. The most abundant forage in the research area was blue grama (see Table 5.10). Of all the forage samples, *Bouteloua gracilis* (blue grama) comprise the most numerous samples at 46%, 15% was contributed to *Pascopyrum smithii* (western wheatgrass) at 15%, and 8% split equally between five local forages (*Achnaterum hymenoides* or Indian ricegrass, *Aristida purpurea* or purple

threeawn, *Muhlenpergia replens* or creeping muhly, *Pleuraphis jamesii* or galetta, and *Sporobolus cryptandrus* or sand dropseed). All sheep forage were collected within a two mile proximity of mines and features. Various forage demonstrated a propensity for certain heavy metals (see Table 5.11). Purple threeawn contained elevated levels of V, Pb, Se and As. While, creeping muhly showed more uptake in V, Pb, and Se. Galleta showed elevated levels of V, Pb, and As. Also, V and Se showed the greatest uptake in sand dropseed. Compared to the other heavy metals, U consistently had relatively low levels but for U the greatest uptake was demonstrated in western wheatgrass and the least in galetta. Most often heavy metals were found in greatest amounts in forage soil and then in descending order, root soil, forage roots, and above-ground forage parts. Above-ground forage parts contained the least amount of heavy metals except for Mo and Cd.

Ovis aries Tissue

Case Study Sheep1

For Sheep 1, heavy metal water toxicity levels were not exceeded (see Table 5.12). The highest concentrations of heavy metals in water were V (M=9.95, SD=11.80), Pb (M=8.42, SD=3.07), and Se (M=7.52, SD=3.62). The highest concentrations of heavy metals in forage were V (M=2.76, SD=0.49), Se (M=2.54, SD=0.40), and Pb (M=1.52, SD=0.26). All heavy metals did not exceed the toxicity levels set for sheep forage except for Se. Se toxicity 2.2 mg/kg orally (Pugh, 2002) or chronic ingestion of 0.25 mg/kg of body weight (Pugh, 2002; Garry, Chew, Rings, Tarr, & Hoffsis, 1990). The absorption for dry matter diet in sheep for V is 1.6% (Pugh, 2002).

This is an eight month old lamb. This sheep grazed within a two mile radius of two to three mines depending on the area grazed. The wool heavy metal levels were greater in Sheep 3 and Sheep 2, for Sheep 1 the higher heavy metal levels occurred in wool for V (1.45 mg/kg) and Pb (1.12 mg/kg).

When you examined the sheep organ heavy metal levels collectively, wool samples were consistently elevated most heavy metals with exception of Se, Mo, and Cd. For Se, the higher heavy metals occurred in the liver (Sheep 3: 3.28 mg/kg; Sheep 2: 3.24 mg/kg; Sheep 1: 3.93 mg/kg), medulla (Sheep 3: 2.62 mg/kg, Sheep 2: 2.83 mg/kg; Sheep 1: 2.04 mg/kg), in some cases the lung (Sheep 3: 0.79 mg/kg & Sheep 2: 1.12 mg/kg). The Mo levels were elevated all sheep. No statistically significant correlations were found between HMs in specific sheep tissue.

Case Study Sheep 2

Sheep 2 is a three year old sheep. Sheep 3 forage grazed within a two mile proximity of one to two mines. The sheep heavy metal water toxicity levels were not exceeded. For Sheep 2, the higher concentrations of heavy metals in water were demonstrated in Pb (M=6.51, SD=none), V (M=5.63, SD=none), and Se (M=5.82, SD=none). The higher levels of heavy metals in forage were found in V (M=3.83, SD=1.48), Pb (M=1.90, SD=0.58), and Se (M=1.56, SD=0.53). All heavy metal levels for forage did not exceed toxicity levels. In general, of the three sheep, Sheep 2 had the lesser heavy metal levels with Cs (0.19 mg/kg), Pb (0.07mg/kg), Th (0.23 mg/kg), As (0.56 mg/kg), and Cd (0.04 mg/kg). The higher heavy metal levels in organs occurred in Pb for both the bone (1.09 mg/kg) and wool (1.07 mg/kg). The vanadium level in wool was 1.63 mg/kg (the second highest level, the greatest occurred in Sheep 1).

Case Study Sheep 3

Sheep 3 is a 3.25 year old sheep. This sheep grazed within a two mile radius of one to two mines depending on the grazing area. The Se levels for the liver was 3.28 mg/kg, for the kidney medulla 2.62 mg/kg, and for remainder of the organs the levels ranged from between 0.39 mg/kg (wool) to 0.78 mg/kg (liver). Vanadium levels for wool was 3.14 mg/kg. The majority of the wool, heavy metal levels were more elevated in Sheep 3 rather than Sheep 2 (except for Se) or Sheep 1 (except for Mo). In general, for most organs sampled, Pb levels were greater in Sheep 3 than Sheep 2 (except for bone) and Sheep 1.

Herbal tea

Tea was the most commonly harvested herb in this study group. Of tea harvesters, one person drank tea once a day and the others did not report consuming it on a daily basis. The average mean times per week the herbal tea users drank tea were once a week. In total, the average number of years all the participants consumed wild tea was 34.7 years (SD=26.4). The greatest uptake in tea samples were in the roots rather than the above ground edible part of the plant (see Table 5.13). In all soil samples, the greatest uptake was demonstrated in V, Cs, and U. In tea root soil the greatest uptake in order of concentration levels were V, Pb, and As. When comparing herb roots and soil only As and Pb had greater uptake.

Riparian areas refer to areas associated with water courses and in the Western U.S., riparian areas often refer to desert arroyos or washes (Fisher, Martin, Ratti, & Guidice, 2001). In the Southwest U.S. riparian areas are important for providing seasonal sources of water. For tea in riparian areas, Mo (M=6.45, Se=0.79) and Se (M=0.61, Se=0.32) had the greatest uptake. In non-riparian areas Mo (M=9.01, Se=9.42) and Th (M=2.75, Se=0.35) had the greatest uptake. For tea root samples, the riparian areas showed greater uptake in V (M=3.75, Se=1.34), Mo (M=0.94, Se 0.12), Pb (M=0.91, SE=0.46), in non-riparian roots the greater uptake occurred in Mo (M=20.21, SE=21.50), V (M=1.83, SE=1.53), and Se (M=1.27, SE=0.51). In tea soil, there were more elevated heavy metals in the non-riparian areas in Cd, Cs, Mo, U, and V. For tea soil in riparian areas, the greatest concentration levels were seen in V

(M=15.69, SE= 5.22), Mo (M=8.10, SE=6.39), Pb (M=5.60, SE=1.60). Vanadium (M=16.91, SE=6.77), Mo (M=13.03, SE=2.20), and Pb (M=4.81, SE=0.78) were elevated in non-riparian tea soil.

When the proximity area was defined as: a) HIA < 1 mi and b) LIA as 1 - 2 mi (see Map 1) three herbal tea areas could be compared. Further stratification (HIA = 0.5 mi and LIA = > 0.5 - 1 mi) allowed the researcher to compare two crop areas growing squash. Independent t-tests were undertaken and found that squash had less V in HIA (M=0.054, SE=0.026) than in LIA (M=0.055, SE= 0.004) $t(10)= 0.074$, $p<0.05$. Lead in HIAs was greater in squash (M=0.278, SE=0.101) than to LIAs (M=0.192, SE=0.027) $t(10)= -1.62$, $p<0.05$. Comparing Diné tea across the areas, t-tests for Cs in LIAs was greater in (M= 0.075, SE=0.083) HIAs (M= 0.033, SE=0.035). This difference was significant $t(12)= 0.98$, $p<0.05$. Mo in LIAs was greater in (M=11.004, SE=9.359) HIAs (M=0.193, SE=0.034). This difference was significant $t(9)= 2.25$, $p<0.05$. Cd in LIAs was greater in tea (M=0.458, SE=0.302) than for HIAs (M=0.067, SE=0.027). This difference was significant $t(12)= 2.52$, $p<0.05$.

Next, linear regression was undertaken to fit an appropriate model for the data to predict values of the dependent variables (heavy metal concentration levels) from the independent variable (proximity for which a categorical dummy variable was created 0, 1, 2). For herb samples, proximity made a significant contribution to predicting heavy metals, Mo ($R^2= 31.4$, $p <.001$) and Cd ($R^2= 35.8$, $p <.001$). Proximity did not make a significant contribution to predicting heavy metals for the categories: crops, crop water, sheep tissue, and sheep water.

The relationship between As with U was explored. To test this relationship, correlation studies were undertaken with several media and they included the most abundant sample of crop (squash), crop water, the most abundant forage (blue grama), and the most abundant herb samples (Diné tea). As was positively correlated with U in edible squash (r = 0.543, n=18, $p<0.05$) and in blue grama forage (r = 0.879, n=8, $p<0.01$). As and U had strong negative

correlation in tea leaf (tea) ($r = -0.617$, $n = 4$, $p<.05$). As and U were not statistically significantly correlated in crop water ($r = 0.371$, $n=6$, $p>0.05$).

The sheep harvesting, forage, soil, and water samples all fell within the two mile radius and could not be stratified into smaller areas. The grazing areas of sheep were difficult to map as the sheep were not stationary. Sheep have a varying grazing region depending on season, terrain, and availability of forage. The non-stationary sheep harvesting associated samples (forage, soil, and some water samples) all fell within the two mile buffer zone.

Table 5.1. Demographics of Growers & Harvesters Who Provided Sheep, Crop, Herb, Forage Soil, and Water Samples, N=8

CHARACTERISTIC	***PERCENTAGE***
GENDER	
Female	62.5
Male	37.5
AGE median 57 years (SD ±10.94)	
40-59	62.5
50-69	25.0
>70	12.5
EDUCATION	
Less than high school/GED	50.0
High School/GED	37.5
Some College	12.5
OCCUPATION	
Self-employed	10.4
Out of work <1 yr	4.2
Out of work > 1 yr	6.3
Rancher	12.5
Retired	6.3
Unable to work	25.0
Other (tribal programs, city services)	35.4
INCOME	
<10K	37.5
10K-19,999	37.5
20K-29,999	12.5
30K-39,999	12.5
CURRENT RESIDENCE in YEARS	
30-39 yrs	12.5
40-49 yrs	37.5
50-59 yrs	25.0
>60 yrs	25.0
TRIBAL AFFILIATION	
Diné	100
Other	0.0
LANGUAGE PREFERENCE	
English	12.5
Diné	25.0
Both	62.5

Table 5.2. Demographics of Growers and Harvesters, continued, N=8

CHARACTERISTIC	*PERCENTAGE*
HEATING HOME	
Gas	35.4
Coal	4.2
Wood	41.7
Other (pellet stove)	18.8
NUMBER OF PEOPLE IN HOUSEHOLD	
1	12.5
2	25.0
3	25.0
4	25.0
5	12.5
PUBLIC WATER	
No community water system	75
Community water system	25
OUTDOOR ACTIVITY PARTICIPATION	2.1
Hunting small game (< 10 lbs)	8.7
Hunting large game (> 10 lbs.)	30.6
Firewood collection	20.2
Land management activities	20.2
Herding animals	18.1
Recreational activities	

Table 5.3. Uranium in Water for Crop and Sheep Samples.

Grower/Harvester	U µg/L	Proximity*	Water Source & Percentage of use	Sample Type	Comment
001	15.94**	< 0.5 mi from plot	>2 mi away Catholic School (25%) Rainwater (75%)	Squash Water	H_20 stored plastic tank Rainwater not sampled
012	2.18**	< 0.5 mi from plot	Public NTUA water from house <0.5 mi away from plot (25%) irrigate squash only. Beans & corn (rainwater, 75%).	Squash, corn, beans Diné Tea Water	Public water source for squash only, other crops or herbs not watered. Rainwater not sampled
003	2.01± 1.49 Range: 0.62-3.58	< 0.5 mi from juniper and sage	Cistern & windmill #00K000 Rio Puerco wash is seasonal near sage & juniper < 0.5 mi	Sage & juniper	#003 does not water herbs
007				Diné Tea	No water samples
013				Diné Tea	No water samples
004	5.25 ± 3.64 Range: 0.35-9.67 5.25 ± 3.64 Range: 0.35-9.67	0.5 - 1.5 mi from sheep grazing areas	-Windmill #00T000 (50%) 0.5 to 1 mi from sheep grazing areas. -Dams, seasonal (25%)0.5 to 1 mi from sheep grazing areas. -Well 2.5 mi w of Ch House (25%)	Sheep tissue Sheep water	Metal trough near windmill Livestock dams dry, not sampled Well water sampled
014	4.70* *	0.5 - 1.5 mi from sheep grazing areas	Public NTUA water from house 0.5 - 1.5 mi from sheep grazing area (75%) Dam, seasonal (25%)	Sheep tissue Sheep water	Metal spigot to metal trough hose runs from house
015	5.25 ± 3.64 Range: 3.85-9.32	0.5 - 1.5 mi from sheep grazing areas	Water from NTUA from home 0.5 - 1.5 mi away from grazing area (75%) Dam, seasonal (25%)	Sheep tissue Sheep water	Two water troughs sampled (metal vessels) Livestock dams dry, not sampled

*Proximity refers to the distance between the water samples to the other samples collected (crops, herbs, sheep grazing areas). ** Based on one sample, no range.

Table 5.4. Demographics of Growers and Harvesters, continued.

Participation of Human Harvesting Activities	**n**	**Proportion**
Sheep/forage	3	33.3
Crops	2	22.2
Herbs	4	44.4
Sheep Consumption		
Local forage	3	50.0
Crops	0	0.0
Herbs	0	0.0
Water	3	50.0
Human Consumption		
Sheep part		
Bone marrow	3	16.6
Intestine	3	16.6
Leg muscle	3	16.6
Liver	3	16.6
Lung	3	16.6
Kidney	3	16.6
Crop type		
Bean	1	16.6
Corn	1	16.6
Squash	2	66.6
Herb type		
Diné tea	4	75
Juniper	1	12.5
Sage	1	12.5
Water Use		
Crop		
Rainwater	2	75
Public water/ NTUA	2	25
Sheep Water type		
Public water/ NTUA	2	28.6
Dam/seasonal	3	42.9
Windmill	1	14.3
Well	1	14.3

Table 5.5. Uranium in Crop, Herb, and Sheep Forage Soil.

Grower/Harvester	U mg/kg	Proximity*	Sample Type
001	Topsoil 0.990 ± 0.609 Range: 0.420-0.585 Subsoil 1.020 ± 0.601 Range: 0.460-1.790	< 0.5 mi from mine	Squash soil
012	Topsoil 0.990 ± 0.609 Range: 0.420-0.585 Subsoil 1.020 ± 0.601 Range: 0.460-1.790 1.071 ± 0.505 Range: 0.226-1.490	0.5 - 1 mi from mine	Squash, corn, bean soil Diné Tea soil
003	0.314** 0.226**	1 – 2 mi from mine	Sage soil Juniper soil
007	1.071 ± 0.505 Range: 0.226-1.490	1 – 2 mi from mine	Diné Tea soil
013	1.071 ± 0.505 Range: 0.226-1.490	1 - 2 mi from mine	Diné Tea soil
004	0.740 ± 0.332 Range: 0.353-1.240	> 1.5 mi	Sheep forage soil
014	0.741 0.740 ± 0.332 Range: 0.353-1.240	> 1 mi	Sheep forage soil
015	0.742 0.740 ± 0.332 Range: 0.353-1.240	> 1 mi	Sheep forage soil

*Proximity refers to the distance between the soil samples to the other samples collected (crops, herbs, sheep forage). **Based on one sample.

Table 5.6. Concentration of Arsenic, Cadmium, Cesium, Lead, Molybdenum, Selenium, Thorium, Uranium, and Vanadium in *Ovis aries* Water (Mean ± S.D. µg/L, Range) Consumption and Crop Water Use.

Average values across all study areas.

Sample Type	As µg/L	Cd µg/L	Cs µg/L	Pb µg/L	Mo µg/L	Se µg/L	Th µg/L	Total U µg/L	V µg/L
O. aries Consumption n=4	0.95 ± 0.23 0.34 - 2.15	0.33 ± 0.20 0.05 - 1.15	0.37 ± 0.40 0.02 - 1.19	8.10 ± 2.39 5.14 - 11.80	3.93 ± 2.80 1.70 - 6.14	5.58 ± 1.91 2.27 - 10.04	ng	5.29 ± 3.48 0.35 - 10.22	7.71 ± 5.87 1.32 - 23.39
Water for Crops n=12	3.80 ± 0.59 2.99 - 4.67	44.73 ± 0.380 39.75 - 48.72	0.46 ± 0.12 0.36 - 0.61	16.18 ± 3.11 13.21 - 19.09	1358.06 ± 98.40 1243.63 - 1535.65	21.81 ± 12.38 8.40 - 34.80	ng	7.03 ± 5.44 2.18 - 15.94	26.44 ± 0.19 25.87 - 27.15

ng=neglible

Table 5.7. Concentration of Arsenic, Cadmium, Cesium, Lead, Molybdenum, Selenium, Thorium, Uranium, and Vanadium in Soil (Mean ± S.D. mg/Kg, Number of Samples, Range).

SOIL	As mg/Kg	Cd mg/Kg	Cs mg/Kg	Pb mg/Kg	Mo mg/Kg	Se mg/Kg	Th mg/Kg	Total U mg/Kg	V mg/Kg
Crop harvesting areas topsoil	1.95±0.68 1.13-2.99	0.54±0.31 0.12-0.85	1.86±1.38 0.80-4.78	6.77±1.14 5.20-8.26	8.73±6.04 0.16-14.27	7.48±2.95 4.48-10.37	3.73±0.68 3.04-4.90	1.00±0.61 0.42-2.01	20.69±10.88 9.33-37.75
Crop harvesting areas subsoil	1.98±0.69 1.29-2.90	0.52±0.29 0.09-0.77	1.70±0.76 0.81-2.72	7.00±0.50 6.41-7.72	8.12±5.78 0.12-13.84	4.48±1.31 3.57-5.99	3.96±0.61 3.32-4.78	1.01±0.60 0.46-1.89	21.84±12.39 9.29-39.11
Herb harvesting soil areas	1.65±0.56 0.87-3.12	0.40±0.28 0.04-0.74	0.98±0.35 0.42-1.61	4.94±1.27 3.38-8.75	7.94±6.49 0.05-16.29	1.12±0.11 0.99-1.29	2.64±0.90 1.41-5.25	1.04±0.49 0.21-1.55	13.63±6.89 5.24-26.24
Herb root soil	2.32±1.17 1.49-3.14	0.05±0.03 0.03-0.70	0.49±0.26 0.30-0.68	5.78±3.70 3.16-8.40	ng	ng	2.64±1.95 1.27-4.02	0.83±0.42 0.53-1.13	9.20±4.01 6.40-12.04
O. aries forage areas	2.86±1.34 1.15-5.20	0.08±0.03 0.04-0.17	1.12±0.39 0.67-2.02	7.53±2.69 3.66-13.80	0.10±0.032 0.04-0.17	1.81±0.72 0.85-3.49	4.59±2.13 1.27-4.02	0.73±0.32 0.33-1.46	10.33±2.41 7.08-15.10
O. aries forage root soil	2.31±0.17 2.19-2.43	1.71±0.01 0.17-0.18	1.02±0.28 0.83-1.22	8.35±0.63 7.90-8.79	0.18±0.09 0.12-0.24	1.89±0.43 1.58-2.19	3.47±0.28 3.27-3.66	0.74±0.07 0.69-0.79	8.94±0.35 8.91-8.96

ng=negligible

Table 5.8. Concentration of Arsenic, Cadmium, Cesium, Lead, Molybdenum, Selenium, Thorium, Uranium, and Vanadium in Crop Samples (Mean ± S.D. mg/kg, Range).

Average values across all study areas.

CROP Scientific & common name	As mg/kg	Cd mg/kg	Cs mg/kg	Pb mg/kg	Mo mg/kg	Se mg/kg	Th mg/kg	Total U mg/kg	V mg/kg
Cucurbita pepo or Squash (n=12)	0.116 ± 0.086 0.014 - 0.282	0.020 ± 0.008 0.008 - 0.068	0.069 ± 0.039 0.002 - 0.107	0.250 ± 0.092 0.086 - 0.363	0.170 ± 0.041 0.120 - 1.197	0.354 ± 0.133 0.295 - 0.365	0.049 ± 0.042 0.008 - 0.141	0.006 ± 0.006 0.001- 0.025	0.053 ± 0.021 0.020 - 0.098
Cucurbita pepo or Squash root (n=3)	0.328* 0.038	0.029* 0.029	0.099+0.130 0.017-0.248	11.773+19.803 0.210-34.599	0.141* 0.141	0.096* 0.096	0.106 + 0.067 0.034 - 0.166	0.029 ± 0.021 0.013-0.053	0.911 + 0.799 0.286 - 1.810
Cucurbita pepo or Squash leaves (n=6)	0.243 ± 0.014 0.224 - 0.266	0.100 ± 0.362 0.049 - 0.149	0.112 ± 0.910 0.045 - 0.252	0.450 ± 0.165 0.319 - 0.662	0.203 ± 0.056 0.157 - 0.276	1.256 ± 0.523 0.631 - 1.981	0.246 ± 0.110 0.147 - 0.457	0.023 ± 0.010 0.014 - 0.036	0.607 ± 0.325 0.317 - 1.034
Phaseolus vulgaris or Bean (n=4)	0.516 ± 0.165 0.329 - 0.713	0.020 ± 0.008 0.012 - 0.029	0.004 ± 0.002 0.002 - 0.006	0.188 ± 0.014 0.176 - 0.205	0.293 ± 0.102 0.154 - 0.380	0.670 ± 0.045 0.637 - 0.701	0.069 ± 0.059 0.010 - 0.139	0.004 ± 0.001 0.003 - 0.005	0.047 ± 0.002 0.044 - 0.049
Phaseolus vulgaris or Bean root (n=1)	0.360* 0.360	0.039* 0.039	0.770* 0.770	0.417* 0.417	0.262* 0.262	0.473* 0.473	0.201* 0.201	0.039* 0.039	1.511* 1.511
Zea Mays or Corn (n=4)	0.647 ± 0.271 0.488 - 0.941	0.012 ± 0.002 0.011 - 0.014	0.054 ± 0.059 0.004 - 0.111	0.210 ± 0.029 0.187 - 0.223	0.147 ± 0.032 0.122 - 0.188	0.331 ± 0.157 0.114 - 0.490	0.010 ± 0.010 0.004 - 0.022	0.003 ± 0.006 0.002 - 0.003	0.049 ± 1.005 0.043 - 0.056
Zea Mays or Corn root (n=2)	0.557 ± 0.088 0.47 – 4.311	0.530 ± 0.021 0.008 – 0.680	0.241 ± 0.098 0.003 - 0.310	0.584 ± 0.135 0.242 - 0.679	0.764 ± 0.612 0.120 – 1.197	0.295** 0.295-364	0.287 ± 0.094 0.184 - 0.353	0.101 ± 0.033 0.008 - 0.124	3.391 ± 1.300 0.047 - 4.311

*Based on one sample, ** based on two samples.

Figure 5.1. Heavy Metal Concentration Levels (mg/kg) in Various Crop Part Samples.

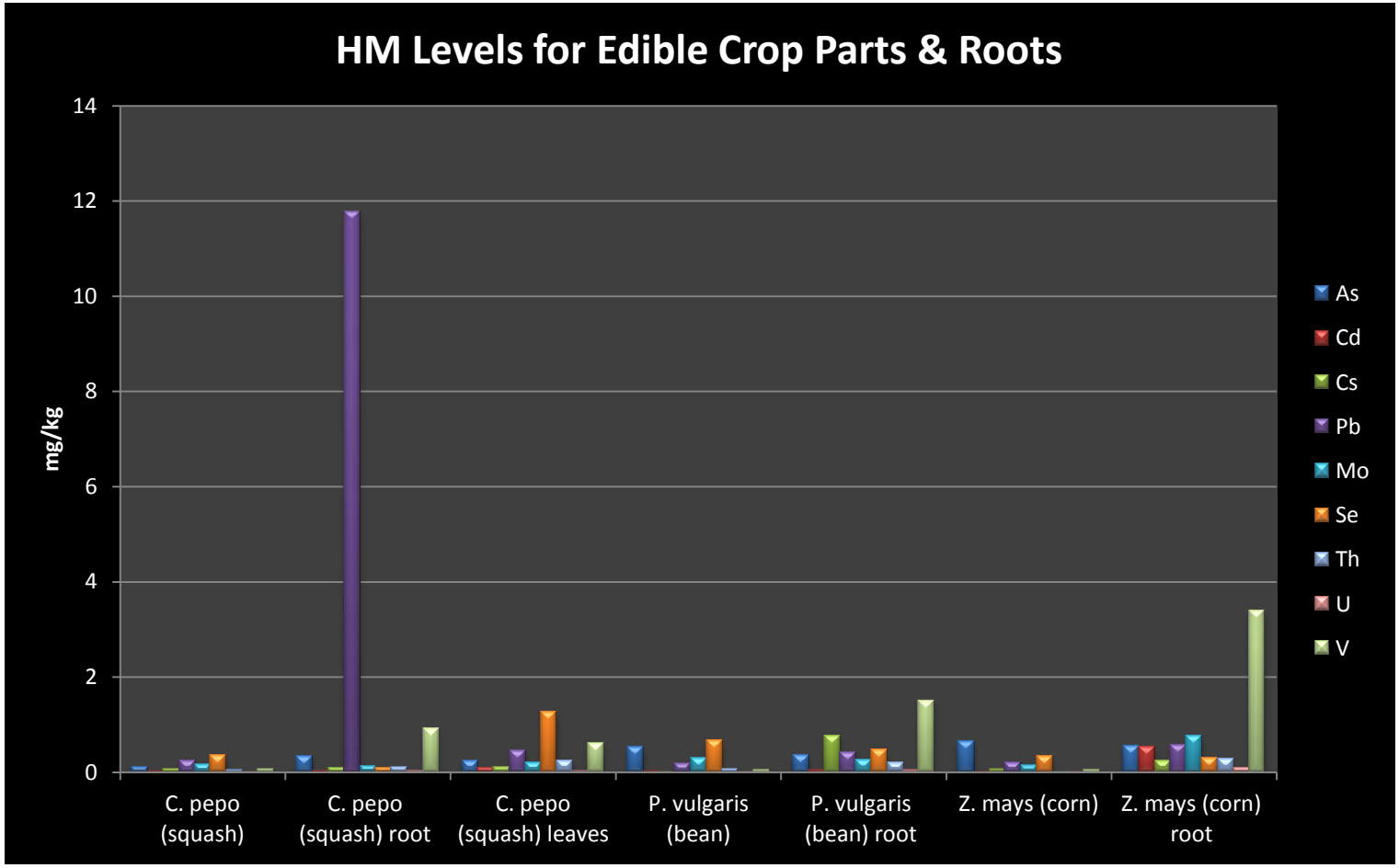

Figure 5.1. The greatest uptake of lead was reported in the squash roots. For each crop type, the majority of the heavy metals were found in root samples (including U) rather than the edible squash parts and leaves.

Table 5. 9. Scientific Nomenclature and Common Names of Plant Species

	Scientific Nomenclature	Common Name(s)
CROP		
	Cucurbita pepo	Squash
	Phaseolus vulgaris	Bean
	Zea mays	Corn
HERB		
	Artemisia tridentate	Big sagebrush
	Juniperus monosperma	One-seed juniper
	Thelesperma megapotamicum	Greenthread, Diné tea, Hopi tea, cota
FORAGE		
	Achnaterum hymenoides	Indian ricegrass
	Aristida purpurea	Purple threeawn
	Bouteloua gracilis	Blue grama
	Muhlenpergia replens	Creeping muhly
	Pascopyrum smithii	Western wheatgrass
	Pleuraphis jamesii	Galetta
	Sporobolus cryptandrus	Sand dropseed

Table 5.10. Proportions of Sheep Forage Samples

Sheep Consumption	**n**	**Proportion**
Forage type		
A. hymenoides or Indian ricegrass	1	7.7
A. purpurea or purple threeawn	1	7.7
B. gracilis or blue grama	6	46.14
M. repens or creeping muhly	1	7.7
P. smithii or western wheatgrass	1	15.38
P. jamesii or galleta	1	7.7
S. cryptandrus or sand dropseed	1	7.7

Table 5.11. Concentration of Arsenic, Cadmium, Cesium, Lead, Molybdenum, Selenium, Thorium, Uranium, and Vanadium in *O. aries* Forage (Mean ± S.D. mg/kg, Range).

Average values across all study areas.

FORAGE scientific and common name	As mg/kg	Cd mg/kg	Cs mg/kg	Pb mg/Kg	Mo mg/kg	Se mg/kg	Th mg/kg	Total U mg/kg	V mg/kg
Achnaterum hymenoides or Indian ricegrass (n=4)	1.31 ± 0.19 1.05 - 1.50	0.07 ± 0.02 0.05 - 0.10	0.39 ± 0.96 0.25 - 0.45	1.86 ± 0.35 1.39 - 2.22	0.73 ± 0.51 0.28 - 1.29	1.32 ± 0.46 0.87 - 1.95	1.23 ± 0.28 1.02 - 1.63	0.43 ± 0.13 0.29 - 058	4.27 ± 0.66 3.32 - 4.84
Aristida purpurea or purple threeawn (n=4)	1.22 ± 0.32 1.02 - 1.70	0.22 ± 0.10 0.14 - 0.35	0.67 ± 0.26 0.46 - 0.98	2.66 ± 0.65 1.96 - 3.41	0.76 ± 0.17 0.66 - 1.01	2.31 ± 1.39 1.09 - 3.78	1.05 ± 0.55 0.31 - 1.63	0.37 ± 0.07 0.28 - 0.44	5.82 ± 1.03 4.61 - 6.89
Bouteloua gracilis or blue grama (n=9)	1.08 ± 0.47 0.19 - 1.96	0.10 ± 0.06 0.00 - 0.22	0.35 ± 0.12 0.11 - 0.53	1.98 ± 0.66 0.63 - 2.99	0.77 ± 0.44 0.24 - 1.65	1.76 ± 0.60 0.81 - 2.99	1.04 ± 0.70 0.36 - 3.26	0.16 ± 0.66 0.06 - 0.25	1.54 ± 0.34 0.00 - 5.69
Muhlenpergia repens or creeping muhly (n=4)	1.01 ± 0.28 0.68 - 1.33	0.14 ± 0.07 0.06 - 0.21	0.32 ± 0.17 0.15 - 0.50	1.96 ± 1.24 0.78 - 3.40	0.96 ± 0.26 0.60 - 1.21	1.33 ± 0.10 1.23 - 1.45	0.97 ± 0.80 0.32 - 2.00	0.18 ± 0.12 0.07 - 0.32	3.20 ± 1.87 1.37 - 5.19
Pascopyrum smithii or western wheatgrass (n=4)	1.19 ± 0.41 0.81 - 1.62	0.08 ± 0.04 0.04 - 0.11	0.41 ± 0.18 0.25 - 0.58	1.93 ± 0.91 1.13 - 2.76	0.33 ± 0.47 0.28 - 0.39	1.16 ± 0.34 0.86 - 1.54	1.04 ± 0.61 0.51 - 1.78	0.57 ± 0.42 0.20 - 1.08	3.45 ± 1.58 2.02 - 4.89
Pleuraphis jamesii or galleta (n=4)	1.40 ± 0.28 1.06 - 1.75	0.87 ± 1.42 0.12 - 3.00	0.34 ± 0.06 0.32 - 0.45	2.15 ± 0.14 2.05 - 2.36	1.07 ± 0.48 0.53 - 1.44	2.41 ± 1.77 0.02 - 4.16	0.61 ± 0.37 0.18 - 0.82	0.12 ± 0.04 0.11 - 0.14	3.89 ± 0.39 3.62 - 4.45
Sporobolus cryptandrus or sand dropseed (n=4)	0.85 ± 0.26 0.55 - 1.12	0.19 ± 0.03 0.16 - 0.22	0.54 ± 0.21 0.35 - 0.75	2.00 ± 0.73 1.36 - 2.72	1.09 ± 0.26 0.82 - 1.33	2.28 ± 2.23 0.22 - 5.30	1.50 ± 1.30 0.41 - 3.07	0.17 ± 0.08 0.10 - 0.26	4.35 ± 1.52 2.90 - 6.03

Table 5.12. Heavy Metal Levels for Each Individual Sheep*

	V mg/kg	Cs mg/kg	Pb mg/kg	Th mg/kg	U mg/kg	Se mg/kg	Mo mg/kg	As mg/kg	Cd mg/kg
Sheep 1									
LM	0.044	0.056	0.116	ng	ng	0.474	0.015	0.057	0.007
KM	0.094	0.105	0.205	ng	0.019	2.038	0.443	0.090	ng
KC	0.103	0.057	0.150	ng	ng	0.526	0.049	0.077	0.007
BO	0.047	0.037	0.197	ng	0.001	0.484	0.041	0.082	ng
LI	0.048	0.051	0.183	ng	0.001	3.934	1.196	0.071	0.063
LU	0.055	0.037	0.134	ng	ng	0.388	0.081	0.058	ng
I	0.061	0.059	0.134	ng	0.001	0.772	0.022	0.074	0.009
W	1.449	0.222	1.122	0.275	0.062	1.300	0.080	0.659	ng
Sheep 2									
LM	0.026	0.037	0.144	ng	ng	0.755	0.049	0.098	0.009
KM	0.053	0.047	0.214	ng	0.001	2.834	0.660	0.103	1.020
KC	0.074	0.040	0.245	ng	ng	0.675	0.089	0.062	0.005
BO	0.053	0.039	1.092	ng	0.003	0.752	0.238	0.088	0.016
LI	0.050	0.027	0.181	ng	0.004	3.236	0.734	0.061	0.112
LU	0.107	0.034	0.244	ng	0.005	1.116	0.249	0.066	0.021
I	0.049	0.024	0.145	ng	0.004	0.540	0.075	0.054	0.002
W	1.631	0.186	1.065	0.230	0.078	1.880	0.232	0.557	0.036
Sheep 3									
LM	0.055	0.044	0.395	ng	0.004	0.452	0.022	0.086	0.005
KM	0.091	0.034	0.344	ng	0.002	2.623	0.474	0.087	0.630
KC	0.083	0.035	0.353	ng	0.001	0.595	0.110	0.070	0.018
BO	0.047	0.037	0.700	ng	0.006	0.611	0.110	0.081	0.003
LI	0.068	0.047	0.545	ng	0.001	3.278	1.468	0.135	0.226
LU	0.090	0.034	0.402	ng	0.005	0.788	0.104	0.110	0.018
I	0.059	0.042	0.463	ng	0.003	0.464	0.052	0.142	0.005
W	3.137	0.354	1.895	0.367	0.092	0.385	0.232	0.714	0.054

*All reports based on one sample. LM (leg muscle), KM (kidney medulla), KC (kidney cortex), BO (bone), LI (liver), LU (lung), I (intestine), and W (wool) .

Table 5.13. Concentration of Arsenic, Cadmium, Cesium, Lead, Molybdenum, Selenium, Thorium, Uranium, and Vanadium in Herbal Samples (Mean ± S.D. mg/kg, Range).

Average values across all study areas.

HERB scientific & common name	As mg/kg Range	Cd mg/kg Range	Cs mg/kg Range	Pb mg/kg Range	Mo mg/kg Range	Se mg/kg Range	Th mg/kg Range	Total U mg/kg Range	V mg/kg Range
Artemisia tridentate or big sagebrush (n=2)	0.498±0.012 0.489-1.323	0.091±0.016 0.079-0.325	0.217±0.396 0.189-0.484	0.510±0.081 0.452-1.817	0.530±0.381 0.261-1.083	1.547±0.021 1.532-2.978	0.042±0.008 0.036-0.711	0.012±0.003 0.010-0.127	0.259±0.233 0.242-3.719
Artemisia tridentate or big sagebrush root (n=2)	1.134±0.267 0.945-1.323	0.313±0.177 0.300-0.325	0.475±0.134 0.465-0.484	1.660±0.221 1.504-1.817	0.995±0.125 0.906-1.083	2.672±0.433 2.366-2.978	0.627±0.119 0.543-0.711	0.115±0.017 0.103-0.127	3.446±0.386 3.173-3.719
Juniperus monosperma or one-seed juniper (n=2)	0.742±0.033 0.489-1.323	0.042±0.171 0.027-0.61	0.218±0.009 0.211-0.474	0.504±0.023 0.488-0.914	0.342±0.027 0.261-1.083	1.102±0.679 1.026-1.168	0.068±0.006 0.063-0.200	0.018±0.001 0.017-0.236	0.386±0.013 0.376-2.084
Juniperus monosperma or one-seed juniper root (n=2)	0.732±0.154 0.623-0.841	0.057±0.006 0.052-0.061	0.380±0.133 0.289-0.474	0.845±0.976 0.776-0.914	0.602±0.037 0.576-0.628	1.089±0.883 1.026-1.151	0.173±0.038 0.146-0.200	0.215±0.030 0.194-0.236	2.074±0.134 2.065-2.084
Thelesperma megapotamicum or greenthread (n=14)	0.423±0.103 0.207-1.182	0.346±0.311 0.043-1.680	0.063±0.074 0.008-0.550	0.304±0.731 0.178-1.305	7.916±9.291 0.164-54.240	0.738±0.393 0.120-1.788	0.200±0.248 0.021-0.792	0.019±0.012 0.004-0.197	0.244±0.096 0.115-5.160
Thelesperma megapotamicum or greenthread root (n=14)	0.758±0.238 0.433-1.182	0.634±0.658 0.059-1.720	0.213±0.169 0.036-0.550	0.809±0.286 0.252-1.305	18.304±21.563 0.822-54.240	1.242±0.557 0.120-1.802	0.268±0.162 0.021-0.471	0.114±0.041 0.062-0.197	2.342±1.589 0.710-5.160

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Chapter Six

Discussion and Conclusions

An environmental study was undertaken to evaluate the impact of heavy metal contamination in the local food chain in uranium impacted communities. Generally, in the biota samples, there was greater heavy metal levels in the plant roots than the above-ground parts. Further, the soil samples showed greater heavy metal levels than the food, herb, and forage samples. The heavy metal levels in various sheep organs (wool, separation of the kidney cortex and kidney medulla) provided new data that is lacking on the subject. New data on the commonly used greenthread herbal tea not previously reported in literature will be presented.

The concentrations of U and other heavy metals found in the food samples, forage, soil, and water samples evaluated in this study depended on many factors. For crops, the metal concentrations were different in squash, bean, and corn, both above-ground parts and root although the majority of heavy metals concentrations were significantly higher in roots than in the above-ground parts. This is consistent with several other studies that found similar results of U accumulating in greater amounts in the roots rather than plant shoots (Soudek, Petrova, Benesova, Dvorakova & Vanek, 2011; Anke, Seeber, Muller, Schafer, & Zerull, 2009; Stojanovic, Stevanovic, Iles, Grubisic, & Milojkovic, 2009; Shahandeh & Hossner, 2002). Other published data found non-edible plant tissue had higher concentrations of radionuclides (^{3}H, ^{137}Cs, ^{90}Sr, ^{241}Am, ^{238}Pu, $^{239,240}Pu$, ^{tot}U) than edible portions of the crops (Fresquez, Armstrong, Mullen, Naranjo, 1998). Similar to the current study, Soudek et al. (2001) reported that U was more localized in the corn root system (Anke et al., 2009). Where U uptake was found to be 4.5 or 3.9 higher in the presence of phosphate deficiency in the Soudek et al. (2001) study. Fresquez et al. (1998) found that the concentrations of radionuclides were significantly different across crop species with squash generally higher than bean or corn. In a hydroponic study (Soudek et al., 2011) of 20 different plants tested for uranium accumulation corn had the

highest mean concentration at 0.16 mg/g DW or 160 mg/Kg. This current study showed increased uptake of U in squash roots compared to above ground parts but there was difficulty in comparing corn and bean because of the small sample size.

Nascent and more mature plants and animals also have varying heavy metal uptake. Various studies have found that uptake of U in young plants was greater than in older plants (Anke et al., 2009; Laroche, Henner, Cameilleri, Modelko, Garnier-Laplace, 2005). In addition, studies of young mammals demonstrated that uptake of U was greater in infant mice than older animals (Leggett & Harrison, 1995). In the current study, only one lamb of eight months of age was donated for testing. Cesium, As, Th, and U levels were greater in the lamb compared to older sheep sampled whereas the three year old sheep had greater elevations in Mo, V, Pb, and Cd levels. Selenium levels were highest in the oldest animal (3.25 years) sampled. All the sheep were female.

In the samples provided, the kidney medulla rather than the kidney cortex showed increased uptake of U, Se, Mo, and As which is a new finding that has not been previously reported in the literature. Although the renal toxic effects of heavy metals are well supported in the literature, the uptake of the various heavy metals in the sheep kidney needs further exploration. Elevated levels of Se, V, and Pb in sheep wool was an interesting finding. Further, even though Th was negligible in all other sheep tissue it was detected in sheep wool. This finding may indicate that Th (as well as other heavy metals) may be accumulating across time in sheep wool. Direct dirt and dust aerosol capture and the effects of lanolin may be contributing exposure factors. In a study (Raab, Hansen, Zhuang, and Feldmann, 2002) examining the uptake of As in wool, the study demonstrated that 11 to 17% of inorganic As was absorbed in the fiber and more so with a longer incubation time (indicating exogenic contamination). The lanolin in the wool contained about 5% of total arsenic where the fat is about 20% of the total weight of the wool. The study by Raab et al. (2002) confirmed that As species in the fiber were

easy to extract with water. Future studies should focus on determining the speciation of heavy metals which may have a greater affinity to wool and which metals are easily extractable in water. The current study community relies on wool to create textiles. It is common practice to place local plants in hot water (drawn locally) to pigment the wool. The wool is handled often by weavers once the wool is removed from the animal, hand-carding the wool, hand-spinning, dyeing, and weaving the textile. The entire process often takes weeks to months suggesting a potential lengthy human exposure to heavy metals. Although this small study of three sheep provided interesting insight, further investigation with a larger sample size containing both lambs and adult sheep across high and low impact areas is a future research need.

The most abundant native forage in the study area was blue grama. The greatest uptake of U occurred in western wheatgrass and the least in galetta. Further work needs to evaluate the uptake of the seven species of forage presented here and other like forage with a larger sample size. Both sheep tissue and forage were sampled at the same time in one fall season. Collecting nascent samples and comparing them to mid and late season plants in relation to heavy metal uptake is needed. Laroche et al. (2005) reported that plants concentrated more U in their tissues more during the seedling stage than the flowering stage.

In unpublished data regarding native forage plants undertaken by Northern Arizona University (NAU, A. Jauregui) was performed in a different region of the Diné reservation in Arizona. The results showed that most native plants sampled did not contain large concentrations of U uptake. Plants in the current study that were evaluated previously in the NAU study reported the concentration levels of Indian ricegrass to be 0.61 (SD= 0.15) ppb of U (or 0.00061 mg/kg of U) and Artemisia spp. (Sagebrush) 1.62 ppb (S.D=0.14) of U (or 0.00162 mg/kg of U). The current study evaluated a small sample size of sage brush. However, in the NAU study the sagebrush subspecies was not well identified and should be applied to big sagebrush with caution.

Near the current study area, the deLemos et al. (2009) study reported mean U soil concentrations of 3 to 8 mg/kg which was near typical background concentrations from downstream abandoned uranium mine sites. The soil data was comparable to National Uranium Resource Evaluation (NURE) sampling that was undertaken in the late 1970s. In the same study, vegetation grass samples ranged from below the detection limit 0.5 mg/kg to 7.7 mg/kg with more uptake in the roots (5.0 mg/kg) than the plant blade (2.4 mg/kg).

In a hydroponic study investigating the potential to stabilize tailings via native vegetation and the uptake of toxic trace metals (As, Co, Mo, Ni, Pb, ^{226}Ra, Se, U, and V) by native vegetation by Dreesen and colleagues (1978) found that blue grama grass and big sagebrush shrub showed ready assimilation of Mo and Se from the tailings by both the grass and shrub when compared to the control. Similar to this study across all metals the mean for Se (M=1.76, SE=0.60) in blue grama was the second highest next to Pb (M=1.98, SD=0.66). Again, across all metals the mean for Se (M=1.547, SD=0.021) and Mo (M=0.530, SD= 0.381) was greatest for big sagebrush. Except for Pb, the Dreesen et al. study (1978) found elevated levels of U, ^{226}Ra, As, Ni, Co, and Pb in blue grama.

Sheep dietary toxicity can occur in excess intakes of Cd, Pb, Mo, and Se. Cadmium toxicity levels are reached at 10 mg/kg of dry matter diet (NRC, 2005). The lead toxicity level is 100 mg/kg of dry matter diet (NRC, 2005). The Mo sheep diet requirement is 0.1 to 0.5 mg/kg of dry matter (NRC, 1985 & 1975). Molybdenum toxicity occurs at 5,000 mg/kg (Dreesen & Williams, 1982). The sheep Se dietary requirement is 0.10 to 0.20 ppm. Selenium toxicity was reported at 2.2 mg/kg orally (Pugh, 2002). The mean of purple threeawn, galetta, and sand dropseed forage exceed the toxicity level for Se. Mean Se levels reflected in the sheep tissue were demonstrated in the liver (M=4.381, SD=1.383), wool (M=2.865, SD=1.846), and kidney medulla (M=2.500, SD=0.413). Other supplementary sources of forage were minimal during the time of sampling, sheep harvesters reported relying on alternative fodder sources for their sheep

in the winter months. Further work with the seven species of forage presented here and other like forage with a larger sample size are areas of future research need.

For crop use, most harvesters utilized rainwater followed by public water. See Table 1 for comparing existing water studies from the current study. Interestingly, the levels of heavy metals were greater in crop water than sheep water. In fact, the mean averages for Cd (M=44.73, SD=0.38) and Pb (M=16.18, SD=3.11) exceeded the National Primary Drinking Water Regulations (NPDWR, 2009). The U.S. EPA maximum for U as well as the Navajo Nation EPA (NNEPA) of 30 µg/L was not exceeded but the EPA daily dose limit (0.07 to1.1 µg/D has the potential to be exceeded depending on the daily intake of water. The NPDWR (2009) regulations were not exceeded for As (10 µg/L), Cd (5 µg/L), and Se (50 µg/L). National Primary Drinking Water Regulations guidelines do not exist for the other heavy metals. The heavy metal levels in rainwater and other water sources need to be evaluated and compared.

For the heavy metals that have standards or toxicity levels, none were exceeded. For sheep water the upper limits of As toxicity in water is 0.2 ppm or 200 µg/L (Pugh, 2002). For Cd the toxicity range is from 0.01 to 0.05 ppm or 10 µg/L to 50 µg/L (Pugh, 2002). For lead, the toxicity limit ranges from 0.05 to 1 ppm in water or 50 µg/L to 1,000 µg/L (NRC, 2005). The limits of potential Se toxicity for sheep in water is 0.05 ppm or 50 µg/L. For V, the recommended standard for the upper limit of V toxicity in water is 0.1 ppm or 100 µg/L (Paterson, Hansard, Ammerman, Henry, Zech, & Fisher, 1986).

Fresh greenthread plant samples also demonstrated a greater heavy metal uptake in herb root than the parts of the herb that are infused. For the Diné, only the tea leaves, stem and flowers are used to make tea. The root is not used as an infusion. Usually when tea is harvested only the above ground parts are pinched off to preserve the plant for use later. The tea can be boiled in water fresh or as a dry bundle. Some families will use the tea to dye wool, in this study only one out of three tea herb harvesters reported past use of tea as a pigment. An interesting

research question to consider is what other local tea infusion plants are utilized and what their uptake of heavy metals are. For example, in this study only a few samples of big sagebrush and one-seed juniper were collected; both plants may also be used as a tea concoction for stomach problems or an emetic (juniper) and colds and/or nasal congestion (sage). For the current study 60% of the herb harvesters reported that they did not wash or rinse their herbs before boiling the herbal teas. One harvester reported using juniper ash as a dry cleanse to remove impurities from her freshly harvested tea plants.

A medicinal herb and tea study (Barthwal et al., 2008) compared plant samples in high traffic, residential, and industrial areas in a city in India and demonstrated that heavy metal levels (Pb, Cd, Cr, & Ni) were more elevated in soil than plant parts (similar to the current study), heavy metal accumulation varied from plant to plant (even when the same plants were collected from three different locations), and the high traffic areas showed higher levels than the residential areas. Anke et al. (2009) also demonstrated that leafy plants, tea and herbs accumulated more U than in fruits, grains and stalks. Two of three tea samples collected were from low traffic areas. A future study to consider would be to compare heavy metal concentrations in greenthread from high and low traffic areas near uranium impacted areas.

The results for this study support that there may be an increased uptake of Mo, As, and Se in greenthread. Uranium and Cs had the least uptake in the tea plant species. Further studies related to this phenomenon need to be explored. For this plant comparing the uptake of heavy metals between high and low impact areas would be recommended.

Unpublished data from Northern Arizona University (NAU) showed that riparian areas showed greater uptake in U in soil with phosphorus deficient areas. The researchers reported that the increased uptake may have been a result of water leaching phosphorus which created an environment for greater U uptake. The NAU study only evaluated for U in their study. uptake of U. Shahandeh and Hossner (2002) *Helianthus annuus* (sunflower) and *Brassica*

juncea (Indian mustard), of the 32 plant types examined had the most U accumulation. The soil properties influenced the accumulation and tolerance of U in plants with more acidic soils which showed the lowest U shoot and root concentrations (similar to the NAU study).

In the current study, greater U uptake was not demonstrated in the riparian areas and there may be several factors associated to this phenomenon. Further research is needed to explore other factors that may influence heavy metal uptake such as salinity and the geochemical makeup of the soil. A study by Laksmanan and Venkateswarlu (1988) showed that phosphorus competes for plants with U. Soudek and colleagues reported increased uranium uptake with phosphate deficient soil while Laroche et al. (2005) did not demonstrate influences by phosphate on uranium uptake in bean.

Geographic Information Systems (GIS) mapping was not a good surrogate in the present study for evaluating contamination for various reasons. One reason may have been that water was carried into harvesting areas from outside the two mile proximity areas. All water that was hauled in was mostly public water. Not all public water showed low contamination. Some of the higher concentration levels were collected in public water systems (notice Mo, Cd, and Pb in Chapter 5, Table 7). The small sample sizes also reduced the incidence of properly comparing high and low impact areas. More sensitive and accurate assessment of grazing patterns may have been achieved with animal collars in combination with harvester verbal information. The cost of GPS or satellite tracking animal collars can be cost prohibitive ($1985 to $3000 per collar, not including data transmission fees). An informal pilot test of canine collars was not environmentally robust, was battery time deficient, and provided poor signals over great distances and was therefore abandoned. However, GIS was the more precise and accurate than a traditional paper map for documenting and mapping exact Global Positioning System (GPS) locations for all samples.

There exist recommended limits of heavy metals in soil in part per million (ppm). The

limits of heavy metals will be referred to for crop and other food harvest areas. The Regional Screening Level for Superfund Sites or Residential Soil (U.S. EPA Region 9) recommends 390 ppm for Mo, 390 ppm for Se, and 390 ppm for V; the use of The Human Health Screening Level (HSSL) is recommended for As (0.07 ppm), Pb (39 ppm), and Cd (300 ppm). For herbal medicines, guidelines do not exist in the U.S. but for levels of safe exposure for Cd (7μg or 0.007 mg), Pb (25 μg or 0.025 mg), and inorganic As (15 μg or 0.015 mg) the guidelines set by the world Food and Agriculture Organization/World Health Organization in terms of Provisional Tolerable Weekly Intake values for body weight in kg (Joint Expert Committee on Food Additives; JECFA; 1988, 1999, 2005) were utilized as a guide.

Dust transference or atmospheric aerosols to plants is another important route of exposure to examine (Bellis, Ma, Bramall, McLeod, Chapman, & Satake, 2001; Steenkamp, Stewart, Chimuka, & Cukrowska, 2005). In this study, harvested food, water, soil, and sheep forage were examined; air contaminant exposure needs to be evaluated in the near future. The elevated levels of the samples may have been due to windblown dust. Air studies should be implemented during all seasons over an extended length of time to evaluate inhalation, ingestion, as well as dermal exposure. Further, the type of plant and its surface area characteristics (leaf, flower, or stem capture), its maturation stage, and how it is prepared for use are only a few variables that may influence the uptake of heavy metals; their influences need to be explored and characterized. This community relies on plants for food or drink, medicinal purposes, and for use in implements (wool, basketry, tools). Considering their impact on the community, additional studies are needed.

Food and herb selling and sharing was common among the participants in the study. For those that provided sheep samples, two of three participants reported using the wool of the sheep they raised to create textiles. Of the same group, the textiles created were sold. Sixty-six percent of the sheep harvesters reported selling live sheep to market and 33% reported selling mutton or

lamb meat cuts to market. Further, all participants reported sharing free mutton or lamb meat with neighbors living on the Diné reservation. On average, each sheep harvester distributed free meat to two households. For crops, one out of two participants sold squash plants. Both crop participants reported sharing free crops with families out-of state and on the Diné reservation. On average, crop harvesters provided free crops to three households. For herbs, 25% of the participants sold Diné tea. All the participants verbalized sharing free herbs with other families out-of-state and across the Diné reservation. An average of 2.25 households received free herbs from Diné tea harvesters. Emphasis should be placed on determining the incidence and frequency of food selling and sharing when assessing food chain studies. Harvesting can overlap in impacted areas, it is important to consider consumption of contaminated food not only by individuals and their families, but potentially the whole community and beyond.

The current study data is suggesting that uranium impacted areas should not only be concerned with U but with other heavy metal contaminants. In the majority of the various samples collected, U levels consisted of lower levels when compared to the other heavy metals evaluated. A more comprehensive evaluation would be recommended in community harvesting areas. Once evaluated, recommendations as those provided below can be explored.

The sample size was not robust but if similar results are found in future studies, elevated heavy metals in crops can be addressed by educating the community in minimizing intake of crops that demonstrate elevated levels of the various heavy metals. For example, with the current study, corn demonstrated higher As levels when compared to bean and squash. Similarly, beans had greater levels of Se than squash or corn. Also minimizing basic risk by contact such as encouraging that vegetables avoid contact with the dirt during harvesting season and storage can be implemented. Further, if feasible, eliminating or rotating crop use to areas that are less contaminated can be encouraged. For forage, rotating grazing areas may also be an

option. Some harvesters reported having access to other crop plots but were not often used due to distance or the impact of the drought. Although this practice was not reported in this study group but evaluating whether harvested animals are also given live or dried crop parts as fodder needs further characterization and study. For instance, the majority of crop root heavy metal levels were greater than the edible crop parts, whether animals consume root as fodder needs to be explored.

deLemos et al. (2009) created safe drinking water educational information for the current community. Encouraging the safe practices recommended by that study should reemphasized. Unregulated water or those for livestock use should used discouraged for crop irrigation or emphasis should focus on using clean water for human consumption. The Navajo Nation Environmental Protection Agency also has literature for the currently community regarding safe water practices such as using clean human drinking water grade containers. This is mentioned as containers themselves may contain heavy metal or other contaminants.

Phytoremediation may be an option for areas contaminated with As, Cd, Se, and U. Phytoremediation is the use of biota to detoxify, extract, or minimize environmental pollutants (Alkorta, Hernandez-Alliea, Becerril, Amezaga, Albizu, & Garbisu, 2004). The plant *P. vittata* shows some potential to hyper accumulate As (Alkorta et al., 2004; Fitz & Wenzel, 2002). Other plants that uptake heavy metals U for sunflower, Cd for *T. caerulescens*, Se for *Astralgalus racemosa*, and As for *P. vittata* (Alkorta et al., 2004; Fitz & Wenzel, 2002). Further, native forage that demonstrate low uptake of various heavy metals may be grown in heavy metal rich areas to minimize plant uptake. Depending on the soil and other important environmental and plant factors, drought resistant plants would be desirable for this current study area. Further study would be needed to explore the potential of this type of remediation.

A food chain study requires comparison groups with long term studies. Future studies in the same community need to focus on more extensive recruitment time, implement creative

incentives, and develop long-term relationships with community spokespersons and leaders. Advanced and creative recruitment technique must be relied upon to increase participation to improve statistical power. For the current study, low participation rates were an issue and may have been an issue for several reasons. Although community members publicly expressed interest in the need for the current study very few people participated. One noticeable trend was that highly impacted uranium areas had the greatest study participation and low impact areas had little to no participants (one low impact "Chapter" had no participants). The PI did spend a considerable time in attending community events, chapter meetings for each study area, provided study flyers, advertised on radio stations (was interviewed on a radio show), and obtained support and referrals from other researchers in the community. All interactions with the community were done in the Diné and English languages to avoid exclusion based on language. Harvester time and money constraints might have limited participation. Most community members spend a considerable time obtaining their basic needs (water, food, supplies) and spending a considerable amount of time to participate in a study may have been too time constraining. Further, even though the study provided a grocery gift card incentive, the amount may not have been an appropriate exchange for the time needed to participate in the study. Harvesters also may have shunned participation because untoward results may negatively impact the value of their products (sheep meat, wool, herbs, etc.) and those of their community neighbors.

Some new information was brought forth by this study but, as with all research with new findings, come new questions. Areas for future research have been highlighted as well as ways to refine methods for the work. The lower levels of heavy metal contamination in this study should be generalized to the community with caution due to the limited sample size. Further study is needed to provide more information about the local food chain status in the community of focus. The findings from this study and future research recommendations will be shared with

the communities as well as their leaders. The research findings also have the capacity to reach other impacted areas outside the study community.

Table 6.1. Existing Water Quality Data for the Current Study

Well Name or No*/ Date of Collection	Heavy Metal	Concentration/ Units	Collecting Agency	Other Collection Dates	Heavy Metal	Concent./ Units
M. Lake Residence Well						
05/02/08	As	ng	DiNEH			
05/02/08	Cd	ng	DiNEH			
05/02/08	Pb	ng	DiNEH			
05/02/08	Mo	0.65 µg/L	DiNEH			
05/02/08	Se	ng	DiNEH			
	Th	nr				
05/02/08	U	0.30 µg/L	DiNEH			
05/02/08	V	ng	DiNEH			
00T000						
08/30/07	As	2.40 µg/L	DiNEH			
08/30/07	Cd	ng	DiNEH			
08/30/07	Pb	1.20 µg/L	DiNEH			
08/30/07	Mo	5.90 µg/L	DiNEH			
08/30/07	Se	6.30 µg/L	DiNEH			
	Th	nr				
08/30/07	U	10.00 µg/L	DiNEH			
08/30/07	V	28.00 µg/L	DiNEH			
00K000						
10/19/10	As	11.00 µg/L	USEPA	10/04/09	As	5.10 µg/L
10/19/10	Cd	1.00 µg/L	USEPA	10/04/09	Cd	1.00 µg/L
10/19/10	Pb	3.58 µg/L	USEPA	10/04/09	Pb	2.10 µg/L
10/19/10	Mo	nr	USEPA	10/04/09	Mo	5.20 µg/L
10/19/10	Se	10.20 µg/L	USEPA	10/04/09	Se	0.55 µg/L
10/19/10	Th	-0.78 µg/L	USEPA		Th	nr
10/19/10	U	0.58 µg/L	USEPA	10/04/09	U	8.00 µg/L
10/19/10	V	1.00 µg/L	USEPA	10/04/09	V	ng µg/L

* Data not available for Catholic School ng = negligible, nr = not reported

Reference Values:
USEPA/NNEPA U:30 µg/L
The National Primary Drinking Water Regulation (2009): As (10 µg/L), Cd (5 µg/L), and Se (50 µg/L).

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APPENDIX 1

STANDARD OPERATING PROCEDURE FOR: HARVESTING OVIS ARIES TISSUE

SOP 2011B

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Revision Log SOP for COLLECTION of OVIS ARIES TISSUE (SOP 2011B)		
Revision and Date	Page Reference	Revision Description
12/09/2011	P.4 (Sect. 2.0); P.5-6 (Sect. 4.0 -7.0); P.7 (Sect. 9.0); P. 8 (Sect. 9.2.1)	Liquid nitrogen LN2 tissue flash-freezing procedure removed; only flash-freezing via dry ice will be utilized.
12/21/2011	P.9 (Sect 13); P.10 (Sect 14).	Added both FedEx® and UPS® to shipping companies to be utilized.
03/25/2012	P.6 (Sect. 7.0)	Added to supply list: bone rib shears
03/25/2012	P.15 (Appendix)	Added sheep corral site to form, removed "punch biopsy" from last row and column and added "bone shear"
03/25/2012	P.7 (Sect. 7.0)	Added decontamination supplies for reusable bone shears.
04/02/2012	P.6 (Sect); P16 (Appendix)	Remove disposable tweezers, and 12mm punch biopsy. The scalpel sizes 10, 11, 22 and forceps are adequate.
04/02/2012	P.9 (Sect. 11)	Added procedures for decontamination of rib shears.
04/08/2012	P. 7 (Sect. 8.0)	Added Section 8.0 *Ovis aries* Handling Biohazardous Tissue and corresponding sections corrected.
04/08/2012	P.8 (Sect. 10.2)	Procedure for collecting composite tissue sampling added.
04/08/2012	P.12 (Sect. 16); P22 (Appendix)	Added Figures for shipping labels.
04/12/12	P.7 (Sect. 7.0)	Added MSDS for chemicals to equipment list.
04/15/2012	P.16 (Appendix, Figure 2)	Updated form. Added elevation, composite weights, total wet weight, Rib shears for Bone category, & photo check off categories.
04/15/2012	P. 18 (Appendix, Figure 4)	Update form. Added fourth set of rows, added notes section.

Standard Operating Procedure

HARVESTING OVIS ARIES TISSUE

Contents

Standard Operating Procedure

1.0 Scope and Application

The purpose of this standard operating procedure (SOP) is to describe the procedures for the collection of *Ovis aries* (*O. aries*) or sheep tissue samples. Analysis of tissue samples may determine whether the concentrations of uranium (U) and other heavy metals (HM) exist or if the concentrations present a risk to public health, welfare or environment.

Mention of trade names or commercial products does not constitute University of California at Los Angeles (UCLA) endorsement or recommendation of use.

2.0 Method Summary

Metal analysis will be done via Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) which will be calibrated for uranium and other heavy metals. Wet weight samples equivalent to a minimum dry weight (DW) of 1g of sheep muscle, bone, organs, and wool samples will be tissue-harvested, identified, and labeled in the field.

Tissue samples will be harvested and placed on dry ice before shipping. Snap freezing is the process by which samples are lowered to temperatures below -70°C very rapidly using dry ice (Biocision, 2011). Snap freezing will provide sample vessel stability, organization, consistent freezing parameters while eliminating lost or contaminated samples. Snap freezing is the use of very low temperatures to preserve structurally intact living tissue. Mammalian tissues are snap frozen to avoid loss by contamination or other temporal factors.

All locations where samples will be taken will be marked on a paper map and/or 2008 Trimble GEO XT® Global Positioning System (GPS) unit. Areas outside of the four preapproved "*chapter*" or community areas will not be sampled or areas fenced-off by regulatory agencies (unless permission is granted). These areas will be omitted from sampling.

3.0 Rationale for the Use of *O. aries* or Sheep Tissue

Every effort has been made to minimize the sampling size of animals to be harvested. Statistical analyses have been utilized to determine the smallest sample size to find an effect. The sheep is the most commonly harvested animal in the Diné community. Therefore, the supply of sheep is abundant and has been verified by preliminary data (Lewis et al.). The physical effort and time needed to slaughter a sheep is considerably less than for larger mammals (cattle or horses). In this cohort, the frequency of ingestion of smaller animals was considerably low. In this northwestern New Mexico community harvesting of chickens, pigs, and turkeys for food exclusively was nonexistent. In addition, mutton is a staple of the Diné diet (Ballew et al., 1997). In preliminary data from the parent DiNEH (Diné Network for Environmental Health) cohort, 76.5% exclusively raised and consumed sheep on their ranch, 2.4% raised cattle exclusively, and the remaining 2.1% were all other categories of meat combined.

The sheep cannot be replaced with a phylogenetically lower species because the transfer of U

and other HMs into sheep tissue is unique and needs further characterization. In addition, the chosen species and its ruminant digestion system is unique to this species and the gastrointestinal uptake of heavy metals in sheep need to be characterized. Animal studies have demonstrated changes in the renal morphology of male Sprague-Dawley rats (Haley, 1982) as well as atrophy of the proximal tubules of rats (Haley et al., 1982) and the lack of renal regeneration after U exposure in beagles (Stevens et al, 1980). Animal bone studies have found the retention half-time in rabbit bone is substantially longer than for humans (Tracy et al., 1992), Wistar rat bone was shown to be the major long-term organ for soluble U (Neuman, 1948). In a serial multiple animal study (monkey, dog, and rat), after massive inhaled doses of insoluble uranium compounds (UO_2) was retained longer in the lungs and pulmonary lymph nodes with little translocation to kidney or bone (Leach et al., 1970; Leach et al., 1973). Studies in phylogenenetically lower species have been undertaken, but sheep studies are lacking in terms of U uptake, metabolism, and bioeffects. A study examining the uptake of U and other HMs in sheep within its natural environment ingesting local forage and water is needed.

4.0 Sample Preservation, Containers, Handling, and Storage

Sheep tissue samples will be snap frozen at a very rapid rate to temperatures below -70°C using dry ice and protected from significant temperature rises. The amount of sample to be collected and proper sample container type are discussed in Section 9.0.

5.0 Interferences and Potential Problems

Two primary problems are associated with tissue sampling: 1) cross-contamination of samples and 2) improper sample collection. Cross contamination can be eliminated or minimized through the use of dedicated sampling equipment for each sample and using sterile technique. Improper sample collection can involve using contaminated equipment thereby contaminating the tissue samples.

6.0 Reagents

Reagents are not used for the preservation of sheep tissue samples.

7.0 Equipment

Tissue sampling equipment include the following:

SAFETY

- Nitrile gloves
- Waterproof rubber work boots, steel toe
- Field first-aid kit including eyewash
- Safety goggles
- N95 Mask, Kimberly Clark regular PFR95
- Tyvek® arm sleeves and disposable plastic apron
- Cryo safety gloves to handle dry ice
- Medical grade waterless hand sanitizer (e.g. Purell®)

- Ear plugs
- Rain gear
- UCLA Radiation Safety Program issued whole body and exposed area dosimeters

TISSUE SAMPLING HARVESTING EQUIPMENT

- Maps and/or Trimble GEOXT® GPS instrument
- Sterile disposable stainless steel (SST) scalpel blade numbers 10, 11, and 22.
- Sterile disposable plastic forceps with jaw grips (cryo-appropriate)
- Laser cryo-labels (7.5 cm x 5 cm) and cryomarkers for sampling bags & bottles
- Polyethylene sampling 4"x6" bags
- Ziplock® bags, quart size
- Disposable dissection trays, lids, and pads
- Sheep Tissue Sampling Forms, including the Diné Dibé Intake Questionnaire (DDIQ) and Chain-of-Custody Form
- Laminated sheep age dentition card
- Portable table and plastic sheeting cover
- Denver Instrument® portable weight balance and extra batteries
- Balance draft shield
- Low nitrogen weighing paper or parchment paper
- Shipping boxes, sizes 10"x10"x10" & 8"x8"x8"
- Omega® thermometer to monitor shipping temperature, soil, and water. Probes (2), Thermocouplers (4), extra batteries.
- Bone rib shears (3)
- Duct tape
- Field logbook
- Waterproof pen ink or marker
- Clipboard
- Measuring tape
- Camera, memory, and extra batteries
- Ice cooler dedicated to sheep tissue only
- Dry ice
- Trash bags
- Paper towels (regular)
- Plastic sheeting

DECONTAMINATION SUPPLIES

- DOT approved 19 liter drums (in compliance with Title 49, Code of Federal Regulations and UN approved). Acid waste, 5 gallons white. Flammable corrosive, 5 gallons red.
- Potable water
- Plastic rinse bottles
- Nitric Acid rinse (10%), trace heavy metal grade
- Acetone, pesticide grade
- Non-phosphate-based detergent (Alconox® Liqui-nox®)
- MSDS Acetone, Nitric Acid, Alconox Liqui-nox®
- Decontamination fluids (ASTM grade II reagent grade deionized water and distilled water)
- White nylon cleaning brush and white nylon scouring pads
- Trash bags, regular and biohazard bags
- Paper towels (lint-free and regular)

8.0 *Ovis aries* Handling Biohazardous Tissue

Ovis aries may carry infections or diseases that can be transmitted to humans. Such diseases include Q-fever, Orf virus, Tularemia, Chlamydia, E. Coli, and Brucellosis. Brucellosis primarily is transmitted from ingestion of raw milk. Safety measures such as utilizing Personal Protective Equipment (PPE) will be utilized to avoid or minimize infection transmission. The PI will not sample animals that are visibly acutely ill. *O. aries* afterbirth or placenta will not be handled by the PI. There will be no exposure to lambing animals. Q-fever is a primary risk during lambing season. PPE will include N95 mask, Tyvek® sleeves, disposable apron, rubber boots, and goggles. There will be no eating in the field during sample collection. Dr. Joanne Sohn of UCLA Clinical Veterinarian agrees to consultations in the field as needed and provided her on-call pager at 1-800-233-7821. Dr. Sohn provided the PI with PPE training.

9.0 Ovis aries or Sheep Sampling Eligibility

Of eligible households, sampling will occur only once per household. Based on sample size calculations, 19 male and non-pregnant domesticated sheep from the high and 19 male and non-pregnant sheep from the low exposure groups (total of 38) will be utilized for the current study. The harvester owner, not the Principal Investigator (PI), will randomly select the sheep to be sampled. Only one sheep (and one correlating owner harvester's information) will be selected from combined flocks to minimize unnecessary replication of samples and data questionnaire information. Typical *O. aries* behavior is to flock together (Pugh, 2002) and significant solitary deviations from the flock is thought to be minimal thereby minimizing sampling bias. *O. aries* will be selected randomly from high U and low U impact (exposure) areas. The eligibility criteria for sheep are: (1) greater than six months of age but less 10 years of age, (2) without any visible physical defects to indicate acute injury (mauling, blunt trauma etc.), (3) consumes mostly local grass forage (<25% non-winter fodder) available on the reservation, (4) consumes water sources available on the reservation, and (5) has lived all

its life on the reservation.

10. Procedures

10.1 Preparation

1. Determine the extent of the sampling effort, the sampling methods to be utilized, and the types and amounts of equipment and supplies required.

2. Obtain necessary sampling and temperature monitoring equipment.

3. Examine equipment and ensure that it is properly functioning.

4. Prepare harvesting schedule and coordinate with harvester owner.

5. Use pre-labeled polyethylene bags and bottles before presenting to all sampling locations. Preassemble a sampling kit enclosed in large Ziploc bags beforehand.

10.2 Tissue Sample Harvesting

10.2.1 *Ovis Aries* or Sheep Tissue

Each sheep will receive only a one time collection. The owner will contact the PI when the time arrives to sacrifice the sheep. The animal will be sacrificed by the harvester owner and the PI will present at the sampling location (before the onset of the sacrifice) to collect the tissues only. The selected sheep is exsanguinated, therefore, there will be little to no bleeding when the samples are collected. The PI will collect the tissues and leave the owner's home. The PI will not be responsible for disposal of the animals as the remainder of the sheep tissue will be consumed by the owner and/or owner's family.

The following procedure is used to collect *O. aries* tissue:

1. Protective equipment will be worn (e.g. nitrile gloves, long pants, rubber work boots, safety glasses, N95 masks, and Tyvek® sleeves or apron).

2. Determine the approximate weight of the animal (see Figure 1) and record on the *Dibé (Sheep) Tissue Sampling Form* (see Figure 2). Ask the harvester owner the approximate age of the animal and verify by the dentition of the animal (see Figure 3) and record on the *Dibé Tissue Sampling Form.*

3. The sheep will be photographed with the sample ID.

4. Record GPS location obtained from a map or the Trimble GEOXT® GPS unit on the *Dibé (Sheep) Tissue Sampling Form.*

5. Place organs onto dissection tray. When excising tissue, avoid areas that have been cut by non-sterile blades/knives or have been handled by non-research persons. Change nitrile gloves, scalpels, and handling tools (e.g. forceps) between organs to avoid cross-contamination. Rib or costal shears will be utilized for collecting bone.

6. Excise tissue onto disposable dissection tray. The organ and/or muscle sheath will be incised, removed, and underlying tissue biopsied. Abnormal tissue may be excised (where present). Three areas per organ will be excised and composited for sampling. All samples will be collected from the right side of the animal and duplicate samples will come from the left side of the animal. Of non-coupled organs, three areas per organ (liver, intestine) will be composited for sampling. All tissues are weighed according to predetermined and pre-tested weights that are representative of 1 gram of dried tissue.

7. Collection or sampling will follow the typical order of sheep dissection in this culture:

- Excise approximately 4g total wet weight (WW) of wool (from the anterior, middle, and posterior) from the right and left aspect of the sheep,
- 5 $\pm$ 0.5g (WW) total from the proximal, middle, and distal small intestine,
- 4 $\pm$ 0.5g (WW) total from the proximal, middle, and distal gastrocnemius,
- 5 $\pm$ 0.5g (WW) total from the right, middle, and left lobes of the lung,
- 4 $\pm$ 0.5g (WW) total from the right, middle, and left lobes of the liver,
- 5 $\pm$ 0.5g (WW) total from cortex tissue and 4 g (WW) of medulla will be excised from each kidney (superior, middle, and supra cortex and medulla),
- 2 $\pm$ 0.5g (WW) total from the right proximal, middle, and distal 13th rib bone

8. The tissue will be weighed and recorded as WW. The weighing paper and/or vials and/or bags will be tared from the tissue weight and recorded. Place dry wool in Ziploc® bag. Large intestine, arm muscle, lung, liver, and kidney tissue will be placed in polyethylene sampling bags. All tissues will be handled with a remote sterile handling tool(s) (e.g. disposable forceps) for each specimen collection.

9. The bags/containers will be appropriately labeled for identification. Secure the zip-tops and bag tightly. A leak-proof outer-bag will be utilized.

10. The samples will be packaged on dry ice and shipped to the University of New Mexico (UNM) Geo/Analytical Chemistry Laboratory for sample preparation, digestion and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analyses via overnight shipping. Storage below -78° $\pm$2°C in the cooler is acceptable on dry ice. Ideal temperatures for shipping of the samples are 4$\pm$2°C.

11. Rib Shear Decontamination

Sample collection tools must be decontaminated prior to reuse. Three stainless steel bone shears will be available to minimize time loss decontaminating and to maximize time use. Most instruments are one use items. The procedure is based upon the American Society for Testing and Materials (2010), Standard Practices for Decontamination of Field Equipment Used at Low Level Radioactive Waste Sites, number D5608-10.

1. Wash and scrub the rib with tap water using a hand pressurized spray rinse bottles. A white nylon brush and/or nylon scouring pad will be used to remove adhered blood and tissue.

2. Wash with laboratory grade detergent and water to remove all visible particulate matter and residual oils and grease. Discard contaminated solvent by pouring into a waste container for disposal later.

3. A generous tap water rinse and a distilled and deionized water rinse to remove the detergent follows this.

4. An nitric acid (10%) rinse, provides a low pH media for trace metals removal.

5. Follow with another distilled water rinse.

6. Follow with an Acetone rinse using a wash bottle. Acetone will be utilized to remove adhered organic matter.

7. Rinse with distilled and deionized water.

8. Air dry the equipment and use lint-free paper towels.

9. Package in plastic bags. Date, time, and initial the plastic bag of the sampling device and document on the *Equipment Decontamination Record* form (see Figure 4).

To capture solvent, a funnel will be used as a collector below the tools during washing. Wash bottles will be used in the field to spray the solvents onto the tools.

12. Documentation

For each tissue field sample collection, documentation will be completed to record the location, time, and type of tissues collected. When filling out the field forms, the following procedures will be followed for each residence:

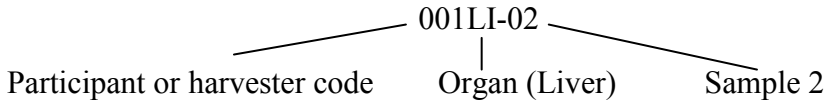
1. All entries for each sample collected will be completed.

2. All entries will be made in ink.

3. Time entries will be made using military time.

4. Site identification will be coded to preserve confidentiality.

5. Sample name will incorporate site identification (Code ID, type of organ sample: Wool Dorsal (WD); Wool Ventral (WV); Intestine (I); Arm Muscle (AM); Lung (LU); Liver (LI); Kidney Cortex (KC); Kidney Medulla (KM); Bone (B); Leg Muscle (LM); and sample number). For the ending numerical designations: "01" denotes a sample and "02" denotes a duplicate.



6. The type of equipment used for the sample procedure will be noted. The SOP will be readily available in the field for the PI to reference.

7. GPS coordinates and/or paper mapping will be noted for each sampling.

8. The PI will sign data forms upon departure from the residence.

13. Field Logbook Documentation

Field logbooks will be maintained by the PI and used to record episodic observations or activities. In addition to the minimum requirements discussed in the Documentation (Section 10), the field logbooks should document those sampling characteristics specific to this SOP. Additional notes will be taken or noted on the Field Logbook as appropriate. Additional notes may include:

1. Non-study personnel on-site.

2. Conversations with homeowners, regulatory personnel, visitors, tribal officials etc.

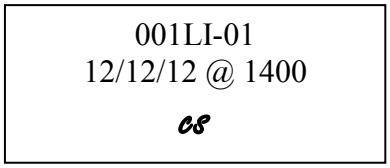
3. Deviations from intended scope of work.

14. Labeling

1. The sample label will be pre-printed with a coded ID.

2. The sample label will be completed using indelible waterproof marking cryomarker and will include:

- Tissue sample identification code (reflecting Code ID, type of organ sample : Wool Right (WR); Wool Left (WL); Intestine (I); Lung (LU); Liver (LI); Kidney Cortex (KC); Kidney Medulla (KM); Bone (B); Leg Muscle (LM); and sample number).
- Date sampled,
- Time sampled, and
- Name or initials of person who collected sample.



3. The polyethylene bags and bottle containing samples will be checked to ensure that they are tightly sealed. All samples will be place in a sturdy outer packaging leak-proof bag. An absorbent pad will be placed between the primary bag and secondary leak-proof bag.

15. Packing Procedures

1. The samples will be shipped to UNM via Distribution Management Corporation, Inc. (DMC) or UPS or FedEx overnight shipping.

2. Dry ice will be placed at the bottom and sides of the shipping cooler.

3. An insulation divider will be placed between the dry ice and samples.

4. The remaining space in the cardboard shipping box will be filled with cushioning material.

5. The UNM *Chain-of-Custod*y forms (see Figures 5, pg. 1-4) will be placed in a large Ziploc® bag and placed on top of the cushioning material.

6. The cardboard shipping box will be closed and fastened with packaging tape. The temperature of the boxes' contents will be monitored by direct application of thermometer thermocouples until the packages relinquished to the shipping vendor.

16. Shipping Procedures

1. Samples for heavy metal determination will be shipped from the field to UNM via Distribution Management Corporation, Inc. (DMC) (primary) or UPS or FedEx (secondary) overnight shipping. DMC courier services will be utilized during regular weekday hours 0800 to 1700. DMC does not provide services on weekends or major holidays. UPS shipping is available only during regular weekday hours from 1430 to 1700. UPS does not ship packages on the weekends. When UPS shipping hours are unavailable, FedEx shipping services will be utilized. Samples for analysis will be shipped according to Biological Substance Category B UN3373 (Figure 8A) and in accordance with 49 CFR 173.426 and current International Air Transport Association (IATA), International Civil Aviation Organization (ICAO) regulations, and applicable D.O.T standards. The FED-EX UN3373 Pak will be utilized. The package or shipping label will identify the content of Dry Ice (Figure 8B) upon the shipping box.

2. The following chain-of-custody procedures will apply to sample shipping:

a) Relinquish the samples to the laboratory via express carrier. The signed and dated forms should be within the cardboard shipping box. The express carrier will not be required to sign the chain-of-custody forms.

b) When the samples are received by the laboratory, the lab personnel shall complete the chain-of-custody forms by signing, dating, and initialing to acknowledge receipt of samples. The internal temperature of the shipping container is measured and recorded. The sample identification numbers on the samples are then checked to insure that they are consistent with the chain-of-custody forms.

17. Quality Assurance and Quality Control

There are no specific quality assurance (QA) activities which apply to the implementation of these procedures. However, the following QA procedures apply:

1. All data must be documented on field data forms or within field notes.

2. All instrumentation must be operated in accordance with operating instructions as supplied by the manufacturer, unless otherwise specified in the work plan. Equipment calibration and check activities must occur prior to sampling/operation, and they must be documented.

3. Collection of duplicate samples will provide for the evaluation of the laboratory's and field sampling team's performance by comparing analytical results of two samples from the same sampling location. One of every 20 samples will be submitted as a "blind" sample.

4. The temperature of shipped samples will be monitored and documented on the *Chain of Custody Forms*. The internal temperature of the shipping container will be measured and recorded upon receipt of the package from the field. Ideal temperatures for shipping of the samples are $4 \pm 2^{\circ}C$. At the beginning stages of the sample collections, the temperature of the shipping samples will be monitored more frequently and less frequently thereafter on a monthly basis. If for whatever reason the samples cannot be shipped overnight, the PI will monitor the internal temperature of the shipment with a tolerance of $4 \pm 2^{\circ}C$. The temperature monitoring will be documented on the "Temperature Specimen Monitor Sheet (see SOP 2011A Soil, Plant and Water Sampling Figure 12)."

18. References

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STANDARD OPERATING PROCEDURE

HARVESTING *OVIS ARIES* TISSUE

Appendix A

Figures

SOP#2011B

September 2011

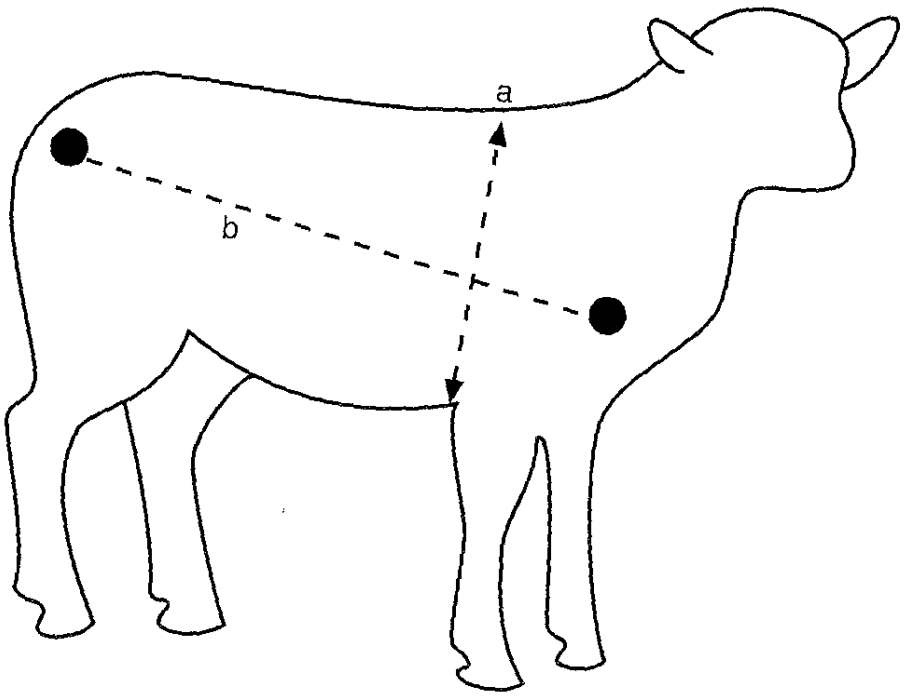


Figure 1. Approximate sheep weight. Approximate weight of the sheep will be determined in inches by measuring the circumference or heart girth just behind the shoulder and elbows (a) and the body length (b). Heart girth ***x*** heart girth ***x*** body length ÷ 300 = weight in pounds. From Pugh, D.G. (2002). *Sheep and goat medicine*. Philadelphia: Saunders.

DIBÉ *(SHEEP)* TISSUE SAMPLING FORM

Participant owner code #:_____ **Today's Date:**_____ **Time:**_____

What is the approximate age of the sheep to be tested? _____ months **OR** years *(please circle one)*

Approximate weight of the sheep: Body length: _____" Heart girth: _____" = _____lbs.

Breed:_____ **OR** Unknown **OR** Mixed. **Flock:** Single **OR** Combined:_____

General Harvesting GPS site *(via map or GPS)*:_____**OR**

GPS Latitude: N3____._____ **Longitude: W-10**____._____ **Elevation:** _____m

Sheep corral site *(via map or GPS)*:_____**OR**

GPS Latitude: N3____._____ **Longitude: W-10**____._____ **Elevation:** _____m

Wind speed: Gale ☐ Breezy ☐ Calm to Light ☐ **Humidity:**_____ **Air Temperature:** _____°C

Tissue Sample No.	Composite Weights	Total Wet Weight	Sampling Equipment Used
1. _____WR_____	1._____g ant.	1._____g	☐ Scalpel size: 10 11 21
2. _____WR_____	2._____g mid	2._____g	☐ Forceps
3. _____WR_____	3._____g post.	3._____g	
1. _____WL_____	1._____g ant.	1._____g	☐ Scalpel size: 10 11 21
2. _____WL_____	2._____g mid	2._____g	☐ Forceps
3. _____WL_____	3._____g post.	3._____g	
1. _____I_____	1._____g prox.	1._____g	☐ Scalpel size: 10 11 21
2. _____I_____	2._____g mid	2._____g	☐ Forceps
3. _____I_____	3._____g dist.	3._____g	
1. _____LU_____	1._____g RUL	1._____g	☐ Scalpel size: 10 11 21
2. _____LU_____	2._____g mid	2._____g	☐ Forceps
3. _____LU_____	3._____g LLL	3._____g	
1. _____LI_____	1._____g RUL	1._____g	☐ Scalpel size: 10 11 21
2. _____LI_____	2._____g mid	2._____g	☐ Forceps
3. _____LI_____	3._____g LLL	3._____g	
1. _____KC_____	1._____g super	1._____g	☐ Scalpel size: 10 11 21
2. _____KC_____	2._____g mid	2._____g	☐ Forceps
3. _____KC_____	3._____g supra	3._____g	
1. _____KM_____	1._____g super	1._____g	☐ Scalpel size: 10 11 21
2. _____KM_____	2._____g mid	2._____g	☐ Forceps
3. _____KM_____	3._____g supra	3._____g	
1. _____LM_____	1._____g prox.	1._____g	☐ Scalpel size: 10 11 21
2. _____LM_____	2._____g mid	2._____g	☐ Forceps
3. _____LM_____	3._____g dist.	3._____g	
1. _____B_____	1._____g prox.	1._____g	☐ Scalpel size: 10 11 21
2. _____B_____	2._____g mid	2._____g	☐ Forceps
3. _____B_____	3._____g dist.	3._____g	☐ Rib shears No. 1 2 3

WR: wool right; WL: wool left; I:intestine; LU: lung; LI: liver; KC: kidney cortex; KM: kidney medulla; LM: leg muscle; B: bone; RUL: right upper lobe; LLL: left lower lobe

Describe animal or tissue abnormalities *(if any)*:_____

_____

Additional remarks:_____

_____

_____

_____

Attach photos *(if any)*: Close ☐ Wide ☐ Extra ☐ _____

Figure 2. *Ovis aries* or Sheep Tissue Sampling Form. Page 1 of 1.

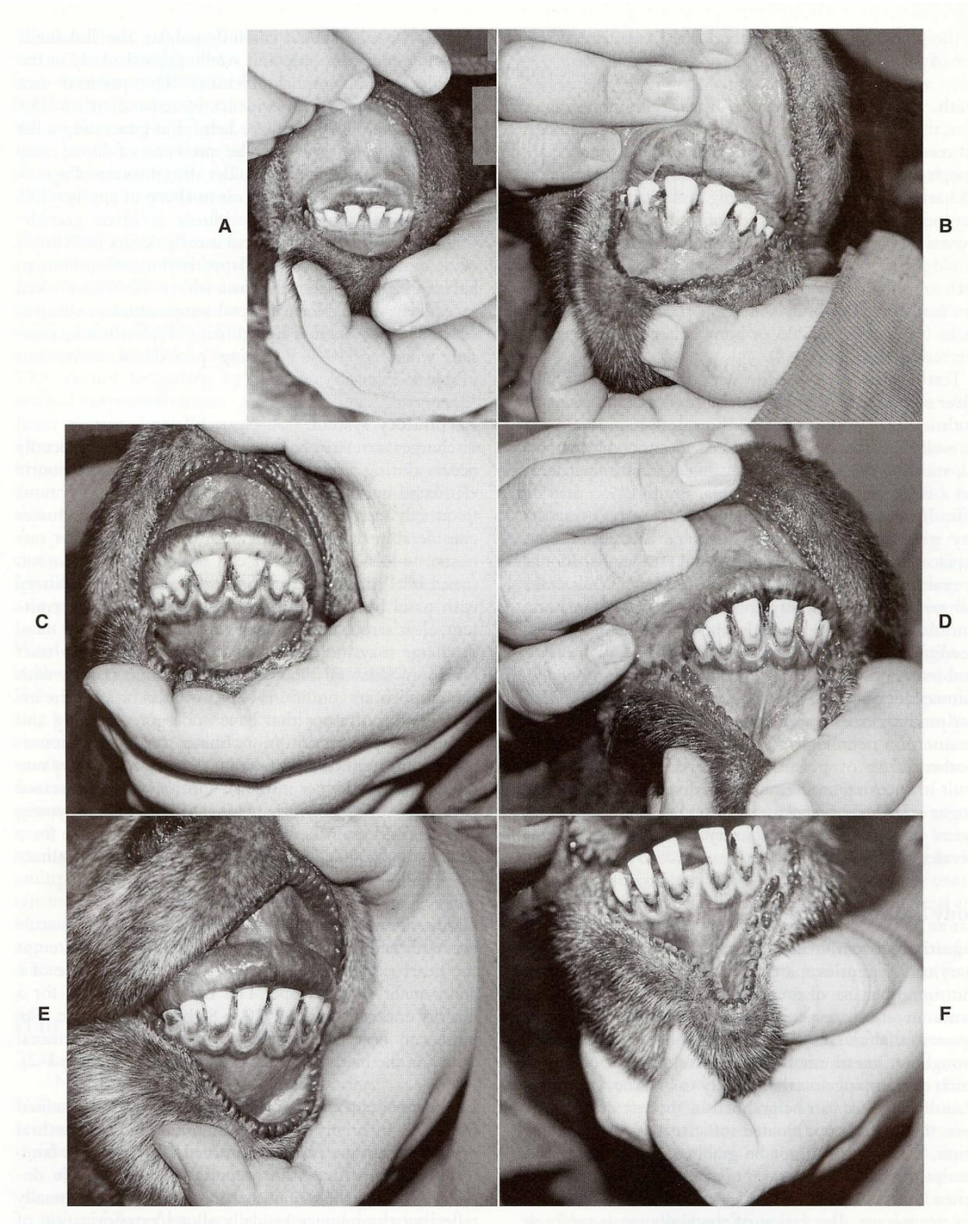


Figure 3. The age appropriate dentition of *O. aries* or sheep. A. Dentition at six months to one year, B. Dentition at one to 1.5 years, C. Dentition of a two year old, D. Dentition of a three year old, E. Dentition of a four year old, and F. Dentition of an aged or a broken-mouthed ewe. From Pugh, D.G. (2002). *Sheep and goat medicine*. Philadelphia: Saunders.

EQUIPMENT DECONTAMINATION RECORD

Equipment Decontaminated	Date & Time Decontaminated	Location Decontaminated	Decontamination Performed by *(initials)*
Rib shears No._____			
Rib shears No._____			
Other			
Other			
Other			
Other			
Rib shears No._____			
Rib shears No._____			
Other			
Other			
Other			
Other			
Rib shears No._____			
Rib shears No._____			
Other			
Other			
Other			
Other			

Version 04/15/2012

Notes:_____

_____

Page _____ of _____

Figure 4. Equipment decontamination Record form. This is page 1 of 1.

Filename: 0

Sample Information:

				Preparation								Parameters																						
Sample Group(s) (Lab Number(s))	Sample Type(s)	Number of Samples	Preservation																															

Total Number of Samples: 0

Title: Chain of Custody
Filename: Report Certificate.xls

Create Date: 10/05/2004
Update Date: 10/05/2004
Revision # 0

Figure 5. UNM chain-of-custody form. This is page 2 of 4.

No.	Lab#	Client Sample I.D.	Lab#	Client Sample I.D.	Lab#	Client Sample I.D.	Lab#	Client Sample I.D.
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								

Figure 5. UNM chain-of-custody form. This is page 3 of 4.

Northrop Hall Building # 24
MSC03-2040
Albuquerque, NM 87131
Contact: Mehdi Ali, PhD
E-mail: mehdiali@unm.edu
Phone: 505-277-1637

Chain of Custody Record
Geo/Analytical Chemistry Laboratory
Earth and Planetary Sciences Dept.
University of New Mexico (UNM)

Project Name:
Submission Date:
FileName:

SET SEQUENCE #'S: _____ -- _____

Comments:

	Client Information	Billing Information
Client Name:		0
Address:		0
		0
		0
Telephone:		0
Client Code		

Account or P.O.#

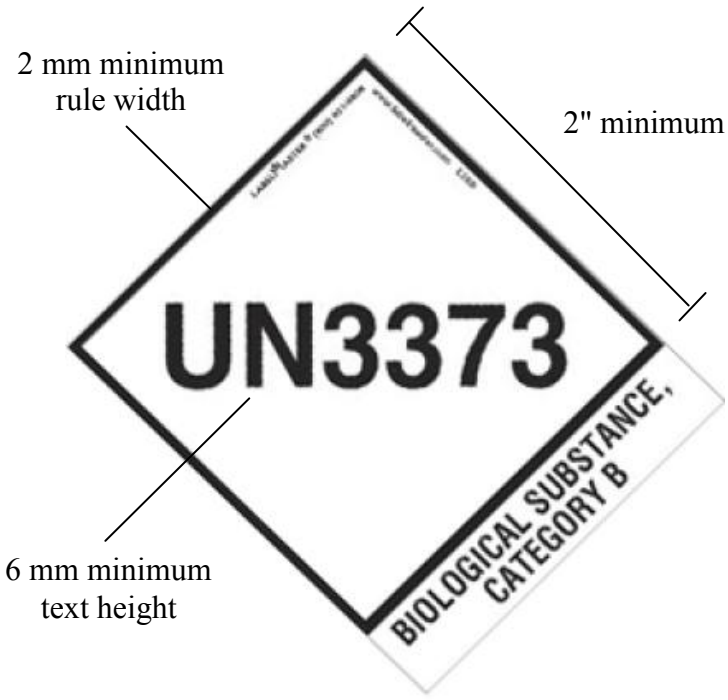
Email :-

	Collector	Sample(s) Condition
Signatures:		
Name:		

	Relinquished by	Received by
Signatures:		
Name:		

Figure 5. UNM chain-of-custody form. This is page 4 of 4.

A.



B.

Figure 6. A: Biological Substance Category B UN3373 shipping label. B: Dry Ice shipping label.

APPENDIX 2

STANDARD OPERATING PROCEDURE FOR:

SOIL, PLANT, and WATER SAMPLING

SOP 2011A

Prepared by

Christine Samuel-Nakamura, MSN, Ph.Dc

from

The University of California Los Angeles

School of Nursing

Revision Log SOP for SOIL, PLANT, and WATER SAMPLING (SOP 2011A)		
Revision and Date	Page Reference	Revision Description
12/12/2011	P.3; P.5 (Sect. 5.0); P.12 (Sect. 7.2.4); P.30-31.	Added water sampling procedures. Added water sampling to *Chain-of-Custody* form.
12/12/2011	P.11 (Sect.7.2.3); P.26.	Pasture height measurement protocol and Table 1 *Pasture Height Recording* form added
12/21/2011	P.14 (Sect.12.0); P.14 (Sect.13.0).	Added both FedEx® and UPS® to shipping companies to be utilized.
01/05/2012	P.5 (Sect. 5.0); P.12 (Sect. 7.2.4)	Water sampling preservative and protocol added
03/25/2012	P.5 (Sect. 6.0); P.13 (Set 7.2.3)	Added use of pH paper to assess water.
03/25/2012	P.5-6 (Sect. 6.0)	Added other supplies: soil moisture meter, shipping thermometer, soil and water thermometer, and white nylon, Plastic sieve with nylon screen, rubber mallet, and wooden cleaning brush.
03/25/2012	P.21-22; P.23-24; P.27-28 (Appendix)	Added soil temperature measurement to each form.
04/02/2012	P. 4 (Sect. 2.0) P.21-22; P.23-24; P.27-28 (Appendix)	Removed horizon assessment. Pit examination is the best method to evaluate horizon assessment; It is difficult to evaluate soil horizons with an auger.
04/02/2012	P.13-14 (Sect. 8.0).	Originally had procedure for decontaminating direct contact equipment. Added decontamination procedure for in-direct contact equipment.
04/02/2012	P.30 (Appendix)	Decontamination List name changed to Record. Added decontamination location and added most commonly used equipment.
04/09/2012	P.3-15(Sect. 7.2.4-7.2.6)	Added water sample collection for faucet/spigot waters, still waters, and moving waters.
04/12/12	P. 7 (Sect. 6.0)	Added MSDS.

Standard Operating Procedure

SOIL, PLANT, and WATER SAMPLING

Table of Contents

15.0 References

16.0 Appendix

Standard Operating Procedure

1.0 Scope and Application

The purpose of this standard operating procedure (SOP) is to describe the procedures for the collection of representative soil and plant samples. Sampling depths will be those that can be reached with the use of Art's Manufacturing and Supply, Inc. (AMS), Core Sampling Mini-Kit®. Analysis of soil samples may determine whether the concentrations of uranium (U) and other heavy metals (As, Cd, Cs, Mo, Pb, Se, Th, & V) exist or if the concentrations of pollutants present a risk to public health, welfare, or environment.

Mention of trade names or commercial products does not constitute University of California at Los Angeles (UCLA) endorsement or recommendation of use.

2.0 Method Summary

Soil samples will be collected using an AMS Core Sampler® (see Figure 1). Top soils (0-15 cm) may be easily sampled using this device. Sampling at greater depths (15-91 cm) may also be performed using the same AMS Core Sampler®. The AMS Core Sampler® consists of stainless steel (SST) core tubes with interlocking, recessed channels and male square threaded ends, SST Regular Auger, SST Core Cup, plastic basket retainer with flexible leaves, SST extension rods in three foot lengths, rubber-coated cross handle, slide hammer, 5.1 cm x 15.2 cm core tube plastic liner, and plastic end caps. The AMS regular and mud auger, core cups, and core cups are entirely coated in Teflon® and was completed by a specialist company.

All locations where samples will be taken will be marked on a paper map and/or 2008 Trimble GEO XT® Global Positioning System (GPS) unit. Areas to be avoided include old fertilizer bands or areas that have lime or fertilizer applied within 30 days (if any), dead furrows, end rows, where livestock congregate (current and past), 15 m away from barns, roads, and fence (old and new) lines; these areas will be omitted from sampling. Areas outside of the four preapproved "c*hapter*" or community areas will not be sampled or areas fenced-off by regulatory agencies (unless permission is granted). These areas will be omitted from sampling. Soil horizons or soil strata and pit examination will not be undertaken. Soil horizons or soil strata are difficult to determine with augers because the exact location of the sample are difficult to implement with these devices (Mason, 1992). Although pit examination are the ideal method to examine the top two meters of soil and provide the both lateral and vertical views of soil

horizons (Que Hee, 1999), it is often difficult to obtain due to practicality and time constraints.

3.0 Sample Preservation, Containers, Handling, and Storage

Samples will be air-dried and protected from sunlight to minimize any potential reaction. The amount of sample to be collected and proper sample container type are discussed in Section 7.0.

4.0 Interferences and Potential Problems

Two primary problems are associated with soil sampling: 1) cross-contamination of samples and 2) improper sample collection. Cross contamination can be eliminated or minimized through the use of dedicated sampling equipment. If this is not possible or practical, then decontamination of sampling equipment is necessary. Improper sample collection can involve using contaminated equipment, or inadequate homogenization of the samples where required resulting in non-representative results.

5.0 Reagents

Reagents are not used for the preservation of soil or plant samples. Nitric acid will be added to the water samples to a pH of 2 and will be frozen via dry ice in polyethylene sample containers that are laboratory graded for heavy metal analyses. Once placed on dry ice the temperature will be maintained at -15.55°C or 4°F (Que Hee, 1999).

6.0 Equipment

Soil sampling equipment includes the following:

SAFETY

- Nitrile gloves
- Waterproof rubber work boots, steel toe
- Field first-aid kit including eyewash
- Safety goggles
- Cryo safety gloves to handle dry ice
- N95 Mask, Kimberly Clark regular PFR95
- Tyvek® arm sleeves and disposable plastic apron
- Ear plugs
- Rain gear
- UCLA Radiation Safety Program issued whole body and exposed area dosimeters

SEDIMENT/PLANT SAMPLING EQUIPMENT

- AMS Core Sampling Mini-Kit® (Teflon®- coated SST regular auger, Teflon® coated SST core sampler, four 3 foot SST extensions, rubber-coated handle, 2x6 plastic liner,

two plastic liner end caps, universal slip wrench, and slide hammer).

- AMS SST Mud Auger® (Teflon®-coated)
- AMS Core Catcher®
- Extra AMS SST Core Sampler Cup® (powder-coated), plastic liners, and end caps.
- Crescent wrenches
- Paper maps and Trimble GEOXT® GPS instrument
- Kelso® handheld soil moisture meter
- pH paper
- Plastic sheeting
- Plastic spades
- Disposable plastic sampling scoops/spoons
- Laboratory supplied polyethylene sample containers (8.45oz)
- Ziplock® quart size bags for soil samples
- Disposable STT scalpels, sizes 10, 11, 21
- Soil and Crop Sampling Forms including Diné Crop Intake Questionnaire (DCIQ) and Diné Wild Plant Herb Intake Questionnaire (DWPHIQ).
- Portable table and plastic sheeting cover
- Denver Instrument ® portable balance and extra batteries
- Balance draft shield
- Kodak® hipsometer
- Dyer® windmeter
- Disposable polyethylene weighing pour boats
- Shipping boxes and tape
- Field logbook
- Shovel
- Rubber mallet
- Plastic bucket to mix the cores of soil in or XL(10g) or XXL(20gal) Ziplock® Bags
- Waterproof pen ink or marker
- Clipboard
- Munsell® soil color chart
- Working gloves
- String, premeasured 1 m length
- Measuring tape
- Survey flags
- Camera, memory storage, and extra batteries
- Cooler specific to soil/plant/water samples
- Dry ice
- Duct tape
- Omega® thermometer to monitor shipping temperature, soil, and water. Probes (2),

Thermocouplers (4), and extra batteries.

DECONTAMINATION SUPPLIES

- DOT approved 19 liter drums (in compliance with Title 49, Code of Federal Regulations and UN approved). Acid waste 5 gallons white. Flammable corrosive 5 gallons red.
- White pressurized polyethylene fruit tree sprayer
- Polyethylene rinse bottles
- Potable water
- Nitric Acid, trace metal grade (1%)
- Acetone, pesticide grade
- Non-phosphate-based detergent (Alconox Liqui-nox®)
- MSDS for Acetone, Nitric Acid, Alconox Liqui-nox®
- Decontamination fluids (ASTM grade II reagent grade deionized water and distilled water)
- White nylon cleaning brush and white nylon scouring pads
- White plastic buckets
- Trash bags regular and biohazard
- Paper towels (lint-free and regular)

7.0 Procedures

7.1 Preparation

1. Determine the extent of the sampling effort, the sampling methods to be utilized, and the types and amounts of equipment and supplies required.

2. Obtain necessary sampling and monitoring equipment.

3. Decontaminate or pre-clean equipment and ensure that it is properly functioning.

4. Prepare schedules and coordinate with harvester owner.

5. Use stake flags to identify and mark all sampling locations. If necessary, the proposed locations may be adjusted based on site access, property boundaries, and surface obstructions.

7.2 Sample Collection

7.2.1 Crop Areas

Depending on the crop plot size and shape, the crop plot will be divided into sampling areas. Composite samples using a grid sampling pattern will be utilized

(see Table 1). The soil sample collection will follow a random zig-zag pattern (see Figure 2). Obtain photographs of the crop plot. A hipsometer will be utilized to verify plot size.

Size of the Plot	Number of Composite Samples
≤0.1 ha (0.25 acre)	2
0.2 ha (0.50 acres)	3
0.3 ha (0.75 acres)	4
0.4 ha (1 acre)	5
0.5 ha (1.25 acres)	6
0.6 ha (1.50 acres)	7
0.7 ha (1.75 acres)	8
0.8 ha (2.00 acres)	9
0.9 ha (2.25 acres)	10
1.0 ha (2.50 acres)	11
1.2 (2.75 acres)	12
>1.3 (3.00 acres)	13-15

Table 1. Recommended number of samples to encompass composite samples

For the crop areas, soil samples will be obtained at two depths: the top soil from 0 to 15 cm and the subsoil 15 to 91cm.

Surface material is removed to the required depth. If difficulties occur with the planned sample collection (e.g. rocks or other obstructions) then check to make sure that these have been documented properly.

The crop samples will be collected from a 1 m radius sampling ring from the pre-augered soil sampling areas. A premeasured 1 m white string will designate the area the crop(s) will be measured from.

The following procedure are used to collect top and subsoil soil samples in crop areas:

1. Protective equipment will be worn (e.g. latex gloves, long pants, rubber work boots, safety glasses, dust masks as needed).

2. GPS coordinates of the crop plot and each sample point will be taken via paper map or by the Trimble GEOXT® GPS unit.

3. Measure the soil pH with pH paper. Measure moisture using a handheld soil meter.

4. Carefully remove and discard a thin layer of soil or debris to the desired

sample depth with a pre-cleaned plastic spoon or spade.

5. For the topsoil composite samples, place samples from all sampling intervals or locations into the homogenization container (plastic bucket) and mix thoroughly (breaking up all the cores). The homogenization bucket will be double bagged to avoid contamination between sampling sites. The first plastic bag will line the bucket and a 2.5 gallon Ziplock® bag (holding the soil) will sit in the prelined bucket. New liner plastic and Ziplock® bags will be used for each sampling site. The topsoil can be obtained via utilizing the AMS Core Sampler® and slide hammer if the soil is penetrable. If the soil is not penetrable with the AMS Core Sampler® and slide hammer, a regular auger will be utilized to access the soil.

6. For the subsoil samples an "AMS Core Sampler®" will be utilized up to a depth of 15 cm. The soil probe will be permanently marked to each depth for consistency between samples. With the AMS Regular Auger® a new depth of 15 to 91 cm will be attained. A clean AMS Core Sampler® will be reinserted into the same hole as the topsoil sample and another core will be attained (up to 91cm). The subsoil samples will be paced into another plastic composite bucket. The Ziplock® bags and forms will record the depth of each sample for future reference.

7. When compositing is complete, place the sample into appropriately labeled Ziplock® bags consisting of 100g of soil. The plastic pour buckets will be tared from the soil weight and recorded. The soil will be labeled in the field with a sample ID on the bag and the sampling form and the *Soil and Crop Sampling Form* (see Figure 3).

8. The crop samples and all other samples will be photographed with sample ID.

9. Depending on each crop type sample, approximately 2.5-30 g (WW) will be collected from a sampling area within and around a 1 m radius sampling ring from the pre-augered soil and stored in a Ziploc® bag. The edible crop part(s) will not be washed in the field. The crops will be segmented into edible fruit and roots using sterile disposable scalpels. For each crop, three similar crops (in height and appearance) and it's various segments will be composited together. Depending on the type of crop, each crop segment will be placed in a polyethylene bag separately weighing approximately 2.5 -30 g (WW). Root samples will be attained via utilizing an AMS Mud Auger®. The roots will be placed in a Ziploc® bag and stored at 4˚C$\pm$2˚C until they can be washed non-vigorously

using deionized water in the laboratory.

All crop tissues are weighed according to predetermined and pre-tested weights that are representative of 1 gram of dried tissue. Collection of sampling weights are as follows:

- 2.5 $\pm$ 1 g (WW) *Phaseolus vulgaris* or Beans
- 5 $\pm$ 1 g (WW) *Zea Mays* or Corn
- 30 $\pm$ 1g (WW) *Cucurbita pepo* or Squash
- 15 $\pm$ g (WW) *Capsicum L*. or Chile pepper

10. The soil sample and crop samples will be weighed and recorded on the *Soil and Crop Sampling Form*. The Ziplock® bags and forms will record the depth of each soil sample for future reference.

11. Duplicate samples are to be included in each matrix at a minimum rate of one of every 20 samples (5% total) and be submitted to the lab as "blind" samples. If less than 20 samples are collected per episode, one duplicate will be performed.

7.2.2 *Ovis aries* Grazing Areas

The grazing areas will be divided into sampling areas by zones. For grazing areas only the top soil (0 to 15 cm) will be sampled.

Grazing areas will be determined or verified by the sheep harvester owner's information. Photographs of the grazing areas will be obtained.

Composite samples will be obtained by utilizing a topographic soil zone sampling pattern (see Figure 4). Each sample will represent 4 hectares (10 acres) or less per sample. Each sample zone will be composed of between 10 to 16 cores depending on area size.

Surface material is removed to the required depth. If difficulties occur with the planned sample collection (e.g. rocks or other obstructions), the Principal Investigator (PI) will check to make sure that these have been documented properly.

The following procedure is used to collect surface soil samples in *O. aries* grazing areas:

1. Protective equipment will be worn (e.g. latex gloves, long pants, rubber work boots, safety glasses, and N95 masks as needed).

2. GPS coordinates of the general grazing area and each sample point will be taken via paper map or Trimble GEOXT® GPS unit.

3. Measure the soil pH with pH paper and moisture with a hand-held soil meter.

4. Carefully remove and discard a thin layer of soil or debris at the desired sample depth with a pre-cleaned plastic spoon or spade.

5. For the subsoil samples an "AMS Core Sampler®" will be utilized up to a depth of 15 cm. The soil probe will be permanently marked to each depth for consistency between samples. The Ziploc® bags and forms will record the depth of each sample for future reference.

6. For the composite samples, place a sample from another sampling interval or location into the homogenization container (plastic bucket) and mix thoroughly (breaking up all the cores). The homogenization bucket will be double bagged to avoid contamination between sampling sites. The first plastic bag will line the bucket and a 2.5 gallon Ziplock® bag (holding the soil) will sit in the prelined bucket. New liner plastic and Ziplock® bags will be used for each sampling site. When compositing is complete, place the sample into appropriate, pre-labeled Ziplock® bags consisting of 100 g of soil. The soil will be labeled in the field with a sample ID on the bag and sampling form or the *Soil and Forage Plant Sampling Form* (see Figure 5).

7. Photographs of the forage plants (with sample ID) will be obtained.

8. Approximately 2 to 12g of each forage type sample will be collected from the area within a 1 m radius sampling ring from the center of the pre-augered soil and stored in a Ziploc® bag. The forage plant part(s) will not be washed in the field. The forage will be segmented into above ground samples and below-ground samples (roots). For each type of forage, three similar plants (in height and appearance) and it's various segments will be composited together. Each forage segment will be placed in a polyethylene bag separately weighing approximately 2 to12g. Root samples will be attained via utilizing an AMS Mud Auger®. The roots will be rinsed non-vigorously with DI water and be placed in a Ziploc® bag and stored at $4 \pm 2^{\circ}C$.

Depending on each forage type, all foliar samples will weigh between 14.5 g and 1.5 ± 0.5g (WW). There are numerous native foliar plants and their abundance will vary greatly from region to region. Foliar will be collected to weigh approximately 1g dry weight. The roots of the plants to be collected will also vary according to each plant and will weigh between 0.46 to 0.65 ± 0.5 g (WW). As these are mostly drought resistant plants, the roots will

typically weigh less than the foliage.

9. The soil sample will be weighed and recorded on the *Soil and Forage Plant Sampling Form*. The Ziplock bags and forms will record the depth of each sample for future reference.

10. Duplicate samples are to be included in each matrix at a minimum rate of one of every 20 samples (5% total) and be submitted to the lab as "blind" samples. If less than 20 samples are collected per episode, one duplicate will be performed.

7.2.3 Herb and/or Plant Harvesting Areas

Depending on the size and shape of the herb/plant area, the protocol for sampling is illustrated in Figure 6. Photographs of the plant harvesting area will be obtained. Three composite samples, near the herb/plant to be harvested will be collected. At maximum, four different types of plants have the potential to be collected (two for human consumption and two for animal consumption or four for human consumption or four for animal consumption) per participant family. The soil samples will be collected using an AMS Core Sampler®. The soil probe will be permanently marked to depth for consistency between samples. The sampling method will allow for direct sample collection in the tube (with a plastic liner), and minimization of cross-contamination between samples. The soil samples will be collected by laying out a 1 m radius sampling ring around the plant to be harvested and collecting equally spaced samples around the perimeter of the ring. A premeasured 1 m white string will be attached to the plant and the sampling ring will be scribed on the soil. Each soil sample will be collected from the surface to a depth of 15 cm. Vegetation will also be collected from within and around the sampling ring.

Surface material is removed to the required depth and a plastic spade is then used to collect the sample.

If difficulties occur with the planned sample collection (e.g. rocks or other obstructions), then the PI will check to make sure that these have been documented properly.

The following procedure is used to collect topsoil samples in herb/plant harvesting areas:

1. Protective equipment will be worn (e.g. latex gloves, long pants, rubber work boots, safety glasses, dust masks as needed).

2. Location will be identified by paper map or Trimble® GPS unit for each

sample point taken for herb and/or plant samples obtained.

3. Measure the soil pH with pH paper and moisture with a handheld soil meter.

4. The average pasture height will be measured by 50 random height measurements of representative grazing areas. A measuring stick will be thrown in front of the measurer and the base of the thumb will be run down the measuring stick until it touches the first green leaf. Bare areas will be recorded as zero, inedible plants should be ignored and recorded as a zero (Court et al., 2010). See Table 1 for the *Pasture Height Recording Form*.

5. Carefully remove and discard a thin layer of soil or debris to the desired sample depth with a pre-cleaned plastic spoon or spade.

6. A 1 m radius sampling ring will be scribed on the ground in the location where the sample is to be procured. A premeasured 1 m white string will be attached to the plant and the sampling ring will be drawn on the soil.

7. For the topsoil composite samples, place a sample from another sampling interval or location into the homogenization container (plastic bucket) and mix thoroughly (breaking up all the cores). The topsoil will be sampled to a depth of at least 15 cm. The homogenization bucket will be double bagged to avoid contamination between sampling sites. The first plastic bag will line the bucket and a 2.5 gallon Ziplock® bag (holding the soil) will sit in the pre-lined bucket. New liner plastic and Ziplock® bags will be used for each sampling site.

8. Photographs of the herbs (with sample ID) will be obtained.

9. Approximately 7-16g of each herb type sample will be collected from the area within and around the sampling ring and stored in a Ziploc® bag. The edible herb part(s) will not be washed in the field. The crops will be segmented into edible parts and roots. For each type of herb, a similar plant (in height and appearance) will be collected for duplicate. Depending on the type of herb, each herb segment will be placed in a polyethylene bag separately weighing approximately 7-16g. Root samples will be attained via utilizing an AMS Mud Auger®. The roots will be rinsed non-vigorously with DI water and be placed in a Ziploc® bag and stored at $4\pm2^{\circ}C$.

All herb tissues are weighed according to predetermined and pre-tested weights that are representative of 1 gram of dried tissue. Collection of sampling weights are as follows:

14 (WW) $\pm$ 1 g *Thelesperma megapotamicum* (greenthread) or Diné Tea

16 (WW) ± 1 g *Juniperus monosperma* or one seed juniper

5 (WW) ± 1 g *Artemisia tridentata or big s*agebrush

10. Three cores at each herb/plant site will be collected.

11. The sampling locations will be refilled to surface ground with the original soil that was removed.

12. When compositing is complete, place the sample into appropriately labeled Ziplock® bags consisting of 100g of soil. The soil and plant samples will be labeled in the field with a sample ID on the bag and sampling form or the *Soil and Non-Forage Plant Sampling Form* (see Figure 7).

13. Weigh the soil and plant samples and record on the *Soil and Non-Forage Plant Sampling Form*. The Ziplock® bags and forms will record the depth of each sample for future reference.

14. At the UNM Laboratory the soil samples will be archived as sub-samples and will be stored at the UNM Laboratory. The archived laboratory preserved samples will be stored dry and contamination free at -20°C at a pH < 2. Duplicate samples are to be included in each matrix at a minimum rate of one of every 20 samples (5% total) and be submitted to the lab as "blind" samples. If less than 20 samples are collected per episode, one duplicate will be performed.

7.2.4 Plant Identification and Nomenclature

1. Live plants will be collected in the field simultaneously as the food and forage samples are being collected. The plants will be placed in one gallon Ziplock® bag. The plants will be placed on dry ice to avoid excessive moisture or heat damage.

2. The live plants will be placed between newspapers and cardboard then placed in a plant press for several weeks with daily press tightenings. For excessively moist or thick plants, the plants will be removed from the press for one to two hours and repressed. "*Collecting Plant Specimens for New Mexico Herbaria*" provided by the UNM Herbarium will be referred to for specimen collection procedures. Field collection trainings were provided by UNM Herbarium by Dr. Bob Sivinski.

3. The dried samples will be sent to the UNM Herbarium for identification and archiving. See Figure 8 for the "UCLA Food Chain Study Plant ID Log" sheet.

The plant collection log will obtain information such as date and time of plant collection. A precise location description or GPS location with Latitude and Longitude information, a plant description (color/abundance, type of sample (fruit, seed, foliage, flower, stem, root, tree, shrub, plant or other), and the state and county the plant was collected and identification information on whether the plant is an annual, perennial, or unknown. A form will accompany the sample.

4. The plant sample will receive the sampler's initials and a Plant I.D. Code:

cs 001NF0-01

7.2.5 Water Sampling from Faucet, Spigot, Private Well Faucet, or Hand-pump

The water sampling will evaluate for heavy metals (including Pb). Therefore, faucet and spigot type water delivery systems will comprise of first-draw samples. The water will not be run before collecting the sample. Some water sources are used publicly and true first-draw sampling will depend on circumstances.

The following procedure is used to collect water samples in harvesting areas:

1. Protective equipment will be worn (e.g. latex gloves, rubber boots, safety glasses, and N95 masks as needed).

2. Location will be identified by paper map or Trimble® GPS unit of each sample point for each water sample obtained.

4. The polyethylene bottle lid will be removed, placed under the faucet or spigot and 250 mL of water will be collected.

5. Measure the water pH (with pH paper) and temperature. Record the findings

on the *Water Sampling Form* (see Figure 8).

6. Add NHO_3 to pH of < 2 for each water sample.

7. Screw the lid on tightly and place on dry ice. Monitor temperature of contents in shipping box prior to shipping.

7.2.6 Water Sampling from Standing Waters (lakes, ponds, rainwater vessels, or livestock Dams, open wells, open windmill water tank).

The water sampling will evaluate for heavy metals. Composite water grab samples will be collected using a Weighted Water Sampler. When sampling

stagnant waters, it is important to collect a “vertical” sample of the water because still waters have a greater tendency to stratify than rivers or streams. Samples will be collected without disturbing the sediment. Three 250mL samples will be collected, poured into a bucket, mixed thoroughly, and 250mL will be collected and sent for analysis. If the water is shallow (less than 25cm deep), the water will be sampled at one depth at 0-10 cm (0-4"). If the water is greater than 25cm deep, the water will be sampled at two depths: 0-10 cm (0-4") and 25-76.2 cm (10-30"). At the shallow depths, the water will be collected by direct method or a dipper with a handle. Deeper waters will be sampled using a Weighted Water Sampler.

The following procedure is used to collect standing-water samples in harvesting areas:

1. Protective equipment will be worn (e.g. latex gloves, rubber boots, safety glasses, and N95 masks as needed).

2. Location will be identified by paper map or Trimble® GPS unit of each sample point for each water sample obtained.

3. Two samples will be drawn from each end of the standing-water and one sample from the middle of the standing water (conditions permitting). The samples will be collected in a double disposable plastic lined homogenization bucket. If the middle sample is unobtainable, the PI will flip a coin to select the right or left side of the standing water (heads = left, tails = right).

4. The polyethylene bottle lid will be removed and 250 mL of water will be collected from the homogenization bucket and poured into the polyethylene bottle.

5. Measure the water pH (with pH paper) and temperature for each sample.

Record the findings.

6. Add NHO_3 to pH of < 2 for each water sample.

7. Screw the lid on tightly and place on dry ice. Monitor temperature of contents in shipping box prior to shipping.

7.2.7 Water Sampling from Moving Waters (rivers, streams, rain or snow run-off water)

The water sampling will evaluate for heavy metals. Sampling areas should be characterized by well mixed water laterally and vertically and where there is fast

moving or turbulent waters. Sampling will start the furthest downstream and work upstream (to minimize the disturbances of bottom sediments and potential downstream sample locations).

The following procedure is used to collect water samples in harvesting areas:

1. Protective equipment will be worn (e.g. latex gloves, rubber boots, safety glasses, and N95 masks as needed).

2. Location will be identified by paper map or Trimble® GPS unit of each sample point for each water sample obtained.

3. Collect a single sample (250 mL) at mid-depth 25-76.2 cm (10-30") and the mid-point of the main current (conditions permitting). When capturing water point the polyethylene sampling bottle upstream. Avoid sediment. The water samples will be composited into in a double disposable plastic lined homogenization bucket.

4. The polyethylene sampling bottle lid will be removed. 250mL of water will be drawn from the homogenization bucket with disposable polyethylene cup and poured in to the polyethylene bottle.

5. Measure the water pH (with pH paper) and temperature for each sample. Record the findings.

6. Add NHO_3 to pH of < 2 for each water sample.

7. Screw the lid on tightly and place on dry ice. Monitor temperature of contents in shipping box prior to shipping.

8.0 Field Decontamination

Sample collection tools must be cleaned prior to use. There are two types of decontamination

procedures: (1) direct-contact equipment and (2) non-direct contact equipment. The procedure is based upon the American Society for Testing and Materials (2010), Standard Practices for Decontamination of Field Equipment Used at Low Level Radioactive Waste Sites, Number D5608-10. The direct equipment decontamination procedure will be discussed first:

1. Wash and scrub tools with tap water using a hand pressurized funnel top fruit tree sprayer. A white nylon brush and/or white nylon scouring pad will be used to remove adhered soil.

2. Wash with laboratory grade detergent and water to remove all visible particulate matter and residual oils and grease. Discard contaminated solvent by pouring into a waste container for

disposal later. Washing buckets are to be white and of polyethylene material.

3. A generous tap water rinse and a distilled and deionized water rinse to remove the detergent follows this.

4. An nitric acid (10%) rinse, provides a low pH media for trace metals removal.

5. Follow with another distilled water rinse.

6. Followed with an Acetone rinse using a wash bottle. Acetone will be utilized to remove adhered organic materials.

7. Rinse with distilled and deionized water.

8. Air dry the equipment and use lint-free paper towels.

9. Package in plastic bags. Date, time, and initial the plastic bag of the sampling device and document on the *Equipment Decontamination Record* form (see Figure 9).

For non-direct equipment decontamination:

1. Wash and scrub tools with tap water using a hand pressurized funnel top fruit tree sprayer. A white nylon brush and/or white nylon scouring pad will be used to remove adhered soil.

2. Wash with laboratory grade detergent and water to remove all visible particulate matter and residual oils and grease. Discard contaminated solvent by pouring into a waste container for disposal later. Washing buckets are to be white and of polyethylene material.

3. A generous tap water rinse and a distilled and deionized water rinse to remove the detergent follows this.

4. The equipment will be allowed to air dry and/or use regular paper towels.

5. Package in plastic bags. Date, time, and initial the plastic bag of the sampling device and document on the *Equipment Decontamination List* form (see Figure 9).

To capture solvent, a funnel will be used as a collector below the tools during washing. Wash bottles will be used in the field to spray the solvents onto the tools.

9.0 Documentation

For each type of field sample collection, documentation will be completed to record the location, time, and depth of each soil core collected. When filling out the field forms, the following procedures will be followed for each residence:

1. All entries for each sample collected will be completed.

2. All entries will be made in ink.

3. Time entries will be made using military time.

4. Site identification will be coded to preserve confidentiality.

5. Sample name will incorporate site identification (Code ID, type of sample (C:crop; S: soil; F:forage; NF: non-forage; and sample number). See Labeling Section 11.

6. The type of equipment used for the sample procedure will be noted. The AMS Core Sampling Mini-Kit® user's manual and SOP will be readily available in the field for the PI to reference.

7. GPS coordinates and/or paper mapping will be noted for each crop plot, grazing area, each plant and or herb sampling point, and water sampling points.

8. The PI will sign data forms upon departure from the residence.

10. Field Logbook Documentation

Field logbooks will be maintained by the PI and used to record episodic observations or activities. In addition to the minimum requirements discussed in the Documentation (Section 9.0), the field logbooks should document those sampling characteristics specific to this SOP. Additional notes will be taken or noted on the Field Logbook as appropriate. Additional notes may include:

1. Non-study personnel on-site.

2. Conversations with homeowners, regulatory personnel, visitors, tribal officials etc.

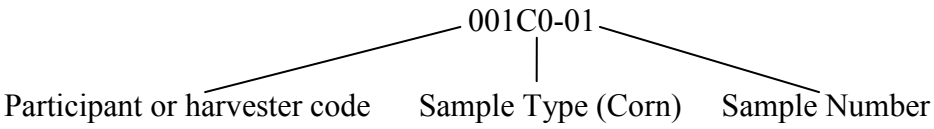
3. Deviations from intended scope of work.

11. Labeling

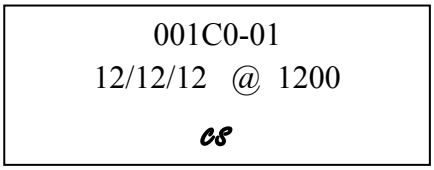
1. The sample label will be pre-printed with a coded ID.

2. The sample label will be completed using indelible waterproof marking pen and will include:

    - Sample identification code (reflecting Code ID, type of sample (CO: crop; CS: soil; F:forage; NF: non-forage; and sample number) and number of samples. For the ending numerical designations: "01" denotes a sample and "02" denotes a duplicate.



- Date sampled,
- Time sampled, and
- Name or initials of person who collected sample.



3. The sample bags will be checked to ensure that they are tightly sealed. Parafilm® will be fastened with clear tape around the bottle cap to avoid leakage. All samples will be place in a sturdy outer packaging leak-proof bag. An absorbent padding will be placed between the primary bag and secondary leak-proof bag.

12. Packing Procedures

1. The samples will be shipped to UNM via Distribution Management Corporation, Inc. (DMC) (primary) or UPS or FedEx (secondary) overnight shipping. DMC courier services will be utilized during regular weekday hours 0800 to 1700. DMC does not provides services on weekends or major holidays. UPS shipping is available only during regular weekday hours from 1430 to 1700. UPS does not ship packages on the weekends. When UPS shipping hours are unavailable FedEx shipping services will be utilized.

2. Dry ice will be placed at the bottom of the cooler.

3. An insulation divider will be placed between the dry ice and samples.

4. The remaining space in the cardboard shipping box will be filled with cushioning material.

5. The UNM C*hain-of-Custod*y forms (see Figures 10) will be placed in outer-bag and placed on top of the cushioning material. An absorbent pad will be placed between the primary bag and secondary leak-proof bag.

6. The cardboard shipping box will be closed and fastened with packaging tape and appropriate shipping labels.

13. Shipping Procedures

1. Samples for heavy metal determination will be shipped from the field to UNM Geo/Analytical Chemistry Laboratory via DMC or UPS/FedEx via overnight shipping. Samples

for analysis will be shipped according to 49 CFR 173.426 and in accordance with current and applicable D.O.T. standards and current International Air Transport Association (IATA) and International Civil Aviation Organization (ICAO) regulations. On weekends or holidays the PI will make arrangements with UNM laboratory for private vehicle delivery by PI. The package or shipping label will identify the content of Dry Ice (Figure 11) upon the shipping box.

2. The following chain-of-custody procedures will apply to sample shipping:

a) Relinquish the samples to the laboratory via express carrier. The signed and dated forms should be within the cardboard shipping box. The express carrier will not be required to sign the chain-of-custody forms.

b) When the samples are received by the laboratory, the lab personnel shall complete the chain-of-custody forms by signing, dating, and initialing to acknowledge receipt of samples. The internal temperature of the shipping container is measured and recorded. The sample identification numbers on the samples are then checked to insure that they are consistent with the chain-of-custody forms.

14. Quality Assurance/Quality Control

There are no specific quality assurance (QA) activities which apply to the implementation of these procedures. However, the following QA procedures apply:

1. All data must be documented on field data forms or within field notes.

2. All instrumentation must be operated in accordance with operating instructions as supplied by the manufacturer, unless otherwise specified in the work plan. Equipment calibration and check activities must occur prior to sampling/operation, and they must be documented.

3. Collection of duplicate samples will provide for the evaluation of the laboratory's and field sampling team's performance by comparing analytical results of two samples from the same sampling location. A "blind" sample will be obtained for each sampling session in the field.

4. The temperature of shipped samples will be monitored and documented on the *Chain of Custody Forms*. The internal temperature of the shipping container will be measured and recorded upon receipt of the package from the field. Ideal temperatures for shipping of the samples are $4 \pm 2^{\circ}C$. At the beginning stages of the sample collections, the temperature of the shipping samples will be monitored more frequently and less frequently thereafter on a monthly basis. If for whatever reason the samples cannot be shipped overnight, the PI will monitor the internal temperature of the shipment with a tolerance of $4 \pm 2^{\circ}C$. The temperature monitoring will be documented on the "Temperature Specimen Monitor Sheet (see Figure 12)."

15. References

American Society for Testing and Materials (2010). *Standard Practices for Decontamination of Field Equipment Used at Low Level Radioactive Waste Sites*, Number D5608-10.

Barth, D.S. & Mason, B.J. (1989). *Soil sampling quality assurance user's guide.* EPA-600/8-69/046.

Billets, S. (1999). *Innovative technology verification report: Art's manufacturing and supply, Inc., split core sampler for submerged sediments*. EPA 600/R-01/009.

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Court, J., Ware, J.W. & Hides, S. (2010). *Sheep farming for meat and wool.* Collingwood, Victoria: Csiro Publishing.

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Provin, T.L. & Pitt, J.L. (1999). *Testing your soil: How to collect and send samples.* Texas A& M University System. L-1793, 3-99.

Que Hee, S. (1999). *Hazardous waste analysis*. Rockville, Maryland: Government Institutes, ABS Group Inc.

Standard Operating Procedure

SOIL AND PLANT SAMPLING

Appendix A

Figures

SOP #2011A

December 2011

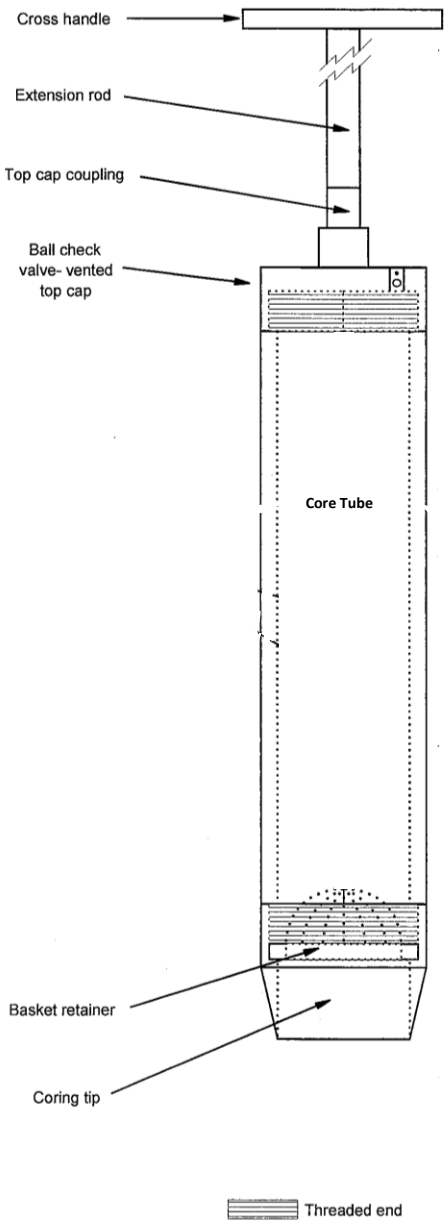


Figure 1. AMS Core Sampler ®. From Billets, S. (1999). Innovative technology verification report: Art's manufacturing and supply, Inc., core sampler for submerged sediments. EPA 600/R-01/009.

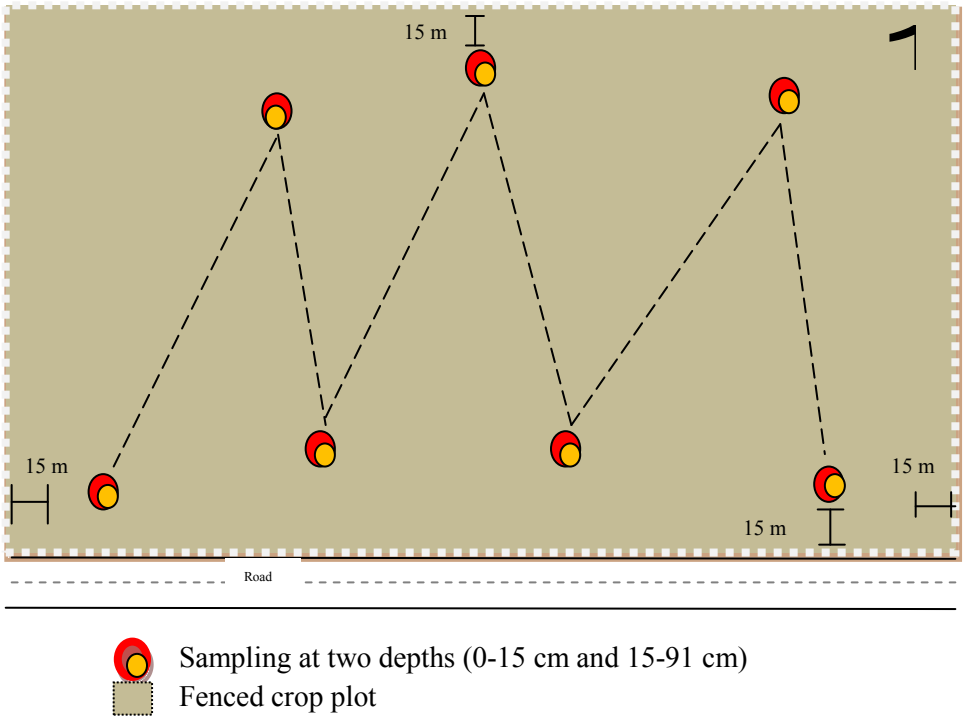
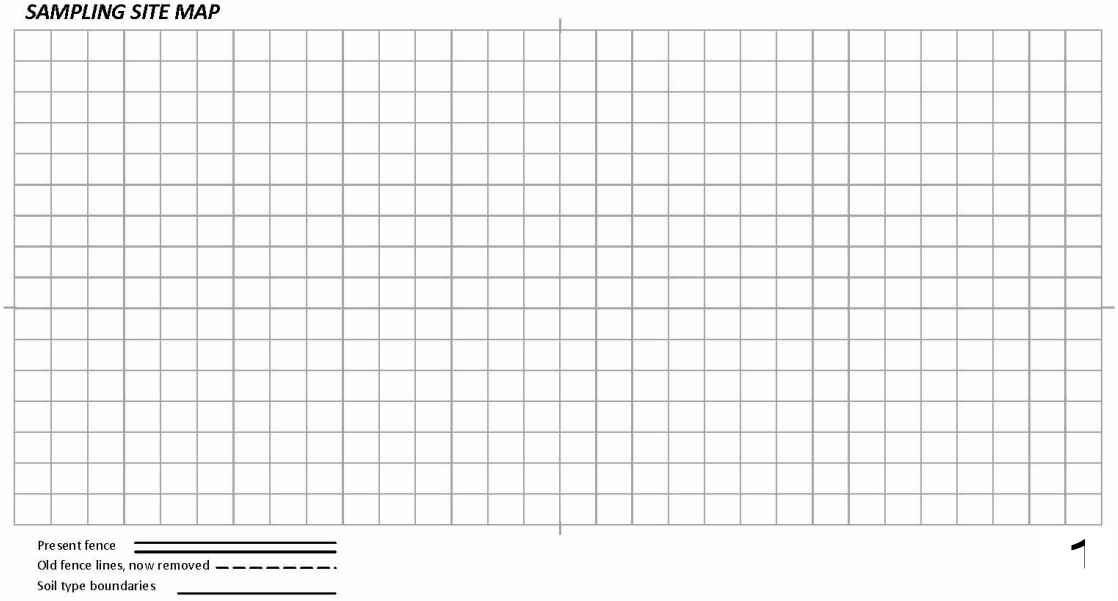


Figure 2. Crop Sampling Diagram. The soil sample collection will follow a random zig-zag pattern.



SOIL and CROP SAMPLING FORM

Name(s) of Sampling Staff: ____**Code:**__________________________

Date of Sampling: __________________________________**Time of Sampling:** _________________________________

Crop Plot Geographical position *(via map or GPS)*: ____**OR**

GPS Latitude: N3___.___________ Longitude: W-10___.___________. Plot Acreage:_________ Elevation: ______m

SAMPLING SITE MAP

Present fence
Old fence lines, now removed
Soil type boundaries

WIND SPEED: Gale ☐ Breezy ☐ Calm to Light☐ **Humidity**:_____________ **Air Temperature**: ________˚C

Potential sources of contamination *(if any)*: ____

SAMPLING DEVICES: Auger Regular ☐ Mud ☐ Core sampler ☐ Slide hammer ☐ Plastic liner/caps ☐ Core catcher ☐

Soil Sample No.	GPS Lat/Long	Soil Color	WW	Depth	pH	Temp.	Moist.
_____TC_____			g	cm		˚C	%
_____SC_____	N3__.__________/ W-10__.__________		g	cm		˚C	%
_____TC_____			g	cm		˚C	%
_____SC_____	N3__.__________/ W-10__.__________		g	cm		˚C	%
_____TC_____			g	cm		˚C	%
_____SC_____	N3__.__________/ W-10__.__________		g	cm		˚C	%
_____TC_____			g	cm		˚C	%
_____SC_____	N3__.__________/ W-10__.__________		g	cm		˚C	%
_____TC_____			g	cm		˚C	%
_____SC_____	N3__.__________/ W-10__.__________		g	cm		˚C	%
_____TC_____			g	cm		˚C	%
_____SC_____	N3__.__________/ W-10__.__________		g	cm		˚C	%
_____TC_____			g	cm		˚C	%
_____SC_____	N3__.__________/ W-10__.__________		g	cm		˚C	%

WW: Wet Weight

Soil Sample Crop Page **1** of **2**

Figure 3. Soil and crop sampling form. This is page 1 of 2.

Code: __________________

Analysis site history: ____

SLOPE: Steep ☐ Moderate ☐ Flat ☐ : ______________________________________

SURFACE RUNOFF: Rapid☐ Medium☐Slow☐ Ponded ☐ **SURFACE EROSION:** Severe☐ Moderate☐ Slight to None☐

VEGETATION: Dense ☐ Scattered/Sparse☐ Absent☐ **ROOTS:** Many☐ Common☐ Few☐

MACROFAUNA: (#/m2): Many ☐ Common ☐ Few ☐ None☐ **MICROFAUNA:** Many☐ Common☐ Few☐ None☐

For crops sampled at this site, please draw on "Sampling Site Map" on page 1 where samples were obtained.

Types of crops sampled at this site: Corn ☐ Squash ☐ Beans☐ Melon☐ Chili ☐ Other(s)☐ : __________________

Crop Sample No. & WW	GPS Lat/Long N3/W-10	WW	Root Depth	Crop Ht.	Crop Description (including root size)	Soil pH / Temp	Soil Moist.
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%
	__.________/__.________	g	cm	cm	mm	°C	%
__________	__.________/__.________	g	cm	cm	mm	°C	%
__________g	__.________/__.________	g	cm	cm	mm	°C	%

WW: Wet Weight, CO: Corn, SQ: Squash, BE: Beans, M: Melon, CH: Chili, R: Root, P: Pollen (e.g. CO, COR (root), COP (pollen), SQ, SQR, BE, BER, M, MR, CH, CHR). Version04/25/2012

Other Sampling Device(s) Used: pH meter☐ Moisture meter ☐Thermometer ☐ Other☐ ____________________

Additional remarks: ____

____

____

Attach photos ***(if any)*****.** **Close☐ Wide☐ Extra☐** ____

Soil Sample Crop Page **2** of **2**

Figure 3. Soil and crop sampling form. This is page 2 of 2.

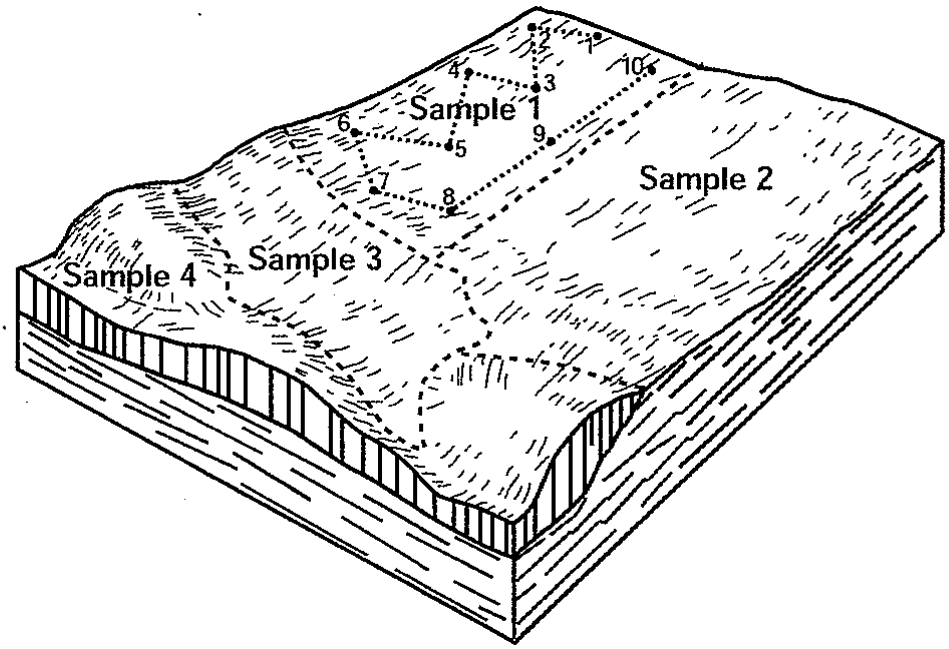


Figure 4. *Ovis aries* grazing area sampling diagram. Grazing fields should be subdivided into sampling units as needed as a composite sample should be collected from each unit. From Provin & Pitt (1999). Testing your soil: How to collect and send samples. Texas A& M University System. L-1793. 3-99.

SOIL and FORAGE PLANT SAMPLING FORM

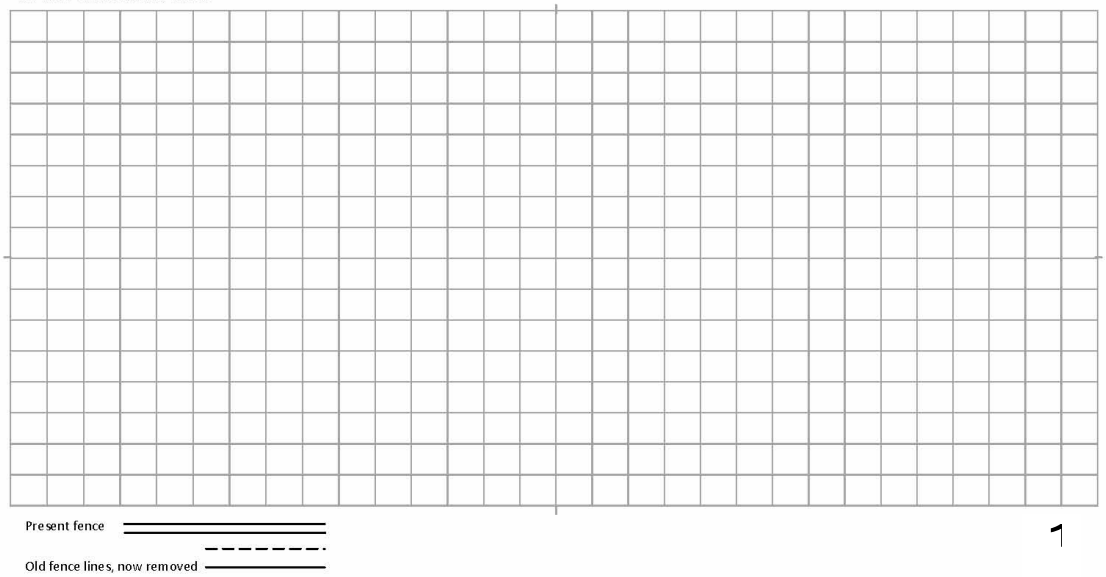
Name(s) of Sampling Staff: _____ **Code:** _____

Date of Sampling: _____ **Time of Sampling:** _____

General Geographical Position *(via map or GPS)*: _____ **OR**

GPS Latitude: N3___.____ **Longitude: W-10**___.____. **Plot Acreage:**_____ **Elevation:** _____m

SAMPLING SITE MAP



WIND SPEED: Gale ☐ Breezy ☐ Calm to Light ☐ **Humidity:**_____ **Air Temperature:** _____˚C

Potential sources of contamination *(if any)*: _____

SAMPLING DEVICES: Auger Regular ☐ Mud ☐ Core sampler ☐ Slide hammer ☐ Plastic liner/caps ☐ Core catcher ☐

Soil Sample No.	GPS Lat/Long	Soil Color	WW Depth	pH Temp.	Moist.
_____FS1_____			g cm	˚C	%
_____FS1_____	N3__.____/ W-10__.____		g cm	˚C	%
_____FRS1___			g cm	˚C	%
_____FRS1___	N3__.____/ W-10__.____		g cm	˚C	%
_____FS2_____			g cm	˚C	%
_____FS2_____	N3__.____/ W-10__.____		g cm	˚C	%
_____FRS2____			g cm	˚C	%
_____FRS2____	N3__.____/ W-10__.____		g cm	˚C	%
_____FS3_____			g cm	˚C	%
_____FS3_____	N3__.____/ W-10__.____		g cm	˚C	%
_____ NFRS3___			g cm	˚C	%
_____ NFRS3___	N3__.____/ W-10__.____		g cm	˚C	%
_____ FS4____			g cm	˚C	%
_____ FS4____	N3__.____/ W-10__.____		g cm	˚C	%
_____ FRS4___			g cm	˚C	%
_____ FRS4___	N3__.____/ W-10__.____		g cm	˚C	%

WW: Wet Weight

Soil Sample Forage Page **1** of **2**

Figure 3. Soil and forage sampling form. This is page 1 of 2.

Code:_____

Analysis site history: _____

SLOPE: Steep☐ Moderate☐ Flat☐: _____

SURFACE RUNOFF: Rapid☐ Medium☐ Slow☐ Ponded☐ **SURFACE EROSION:** Severe☐ Moderate☐ Slight to None☐

VEGETATION: Dense☐ Scattered/Sparse☐ Absent☐ **ROOTS:** Many☐ Common☐ Few☐

MACROFAUNA: (#/m2): Many☐ Common☐ Few☐ None☐ **MICROFAUNA**: Many☐ Common☐ Few☐ None☐

For non-forage plants sampled at this site, please draw on "Sampling Map" on page 1 where samples were obtained.

Name type(s) of forage plants sampled at this site: _____

_____

Forage No. & WW	GPS Lat/Long N3/W-10	WW	Root Depth	Plant Height	Plant Description (flowers, root size etc.)	Soil pH & Temp.	Soil Moisture
_____F1___	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
	__._____/__._____	g	cm	cm	mm	˚C	%
_____F1___	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
_____FR1__	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
	__._____/__._____	g	cm	cm	mm	˚C	%
_____NFR1__	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
_____F2___	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
	__._____/__._____	g	cm	cm	mm	˚C	%
_____F2___	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
_____FR2__	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
	__._____/__._____	g	cm	cm	mm	˚C	%
_____FR2__	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
_____F3___	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
	__._____/__._____	g	cm	cm	mm	˚C	%
_____F3___	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
_____FR3__	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%
	__._____/__._____	g	cm	cm	mm	˚C	%
_____FR3__	__._____/__._____	g	cm	cm	mm	˚C	%
_____g	__._____/__._____	g	cm	cm	mm	˚C	%

WW: Wet Weight Version 04/25/2012

Other Sampling Device(s) Used: pH meter☐ Moisture meter☐ Thermometer☐ Other☐ _____

Additional remarks: _____

_____

_____

Attach photos *(if any)*. **Close☐ Wide☐ Extra☐** _____

Soil Sample Forage Page **2** of **2**

Figure 3. Soil and forage sampling form. This is page 2 of 2.

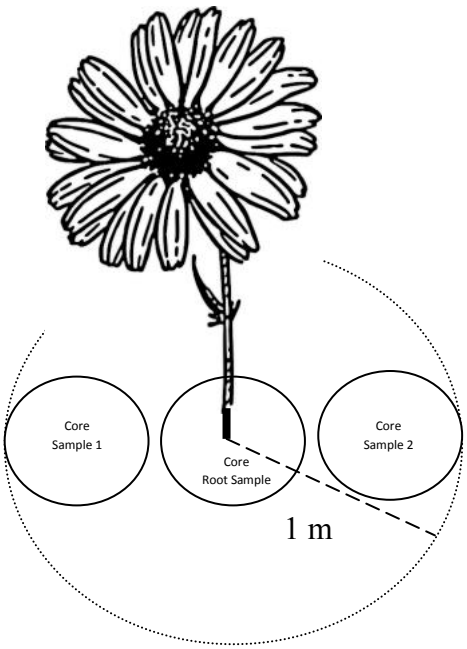


Figure 6. Non-forage or herb sampling diagram. The soil samples will be collected by laying out a 1 m radius sampling ring around the plant to be harvested and collecting equally spaced samples around the perimeter of the ring.

Code:_____

PASTURE HEIGHT RECORDING FORM

Height (cm) **A**	Place a mark besides the height whenever a measurement of this height is recorded	Number of recordings **B**	**A x B**
0			
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
___			
___			
___			
	Totals		
	Average height=Total (A x B)/Total B		

Notes:_____

_____

Table 1. Recording sheet for pasture height. Pasture height is a reasonably reliable determinant of pasture quantity and available feed. "Reproduced with permission from *Sheep Farming for Meat and Wool* (Eds: Jane Court, Sue Hides and John Webb-Ware). Copyright © Department of Primary Industries, Victoria. Published by CSIRO PUBLISHING, Collingwood, Victoria Australia - ttp://www.publish.csiro.au/pid/5853.htm."

SOIL and NON-FORAGE PLANT SAMPLING FORM

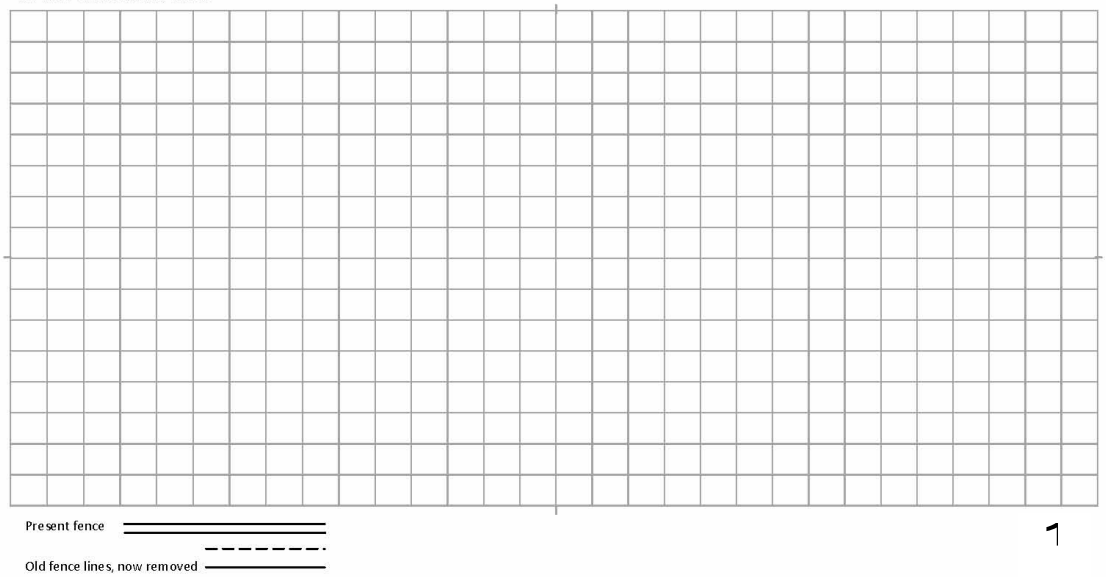
Name(s) of Sampling Staff: _____ **Code:** _____

Date of Sampling: _____ **Time of Sampling:** _____

General Geographical Position *(via map or GPS)*: _____ **OR**

GPS Latitude: N3___.___ **Longitude: W-10**___.___. **Plot Acreage:**_____ **Elevation:** _____m

SAMPLING SITE MAP



WIND SPEED: Gale ☐ Breezy ☐ Calm to Light ☐ **Humidity:**_____ **Air Temperature:** _____˚C

Potential sources of contamination *(if any)*: _____

SAMPLING DEVICES: **Auger** Regular ☐ Mud ☐ Core sampler ☐ Slide hammer ☐ Plastic liner/caps ☐ Core catcher ☐

Soil Sample No.	GPS Lat/Long	Soil Color	WW Depth	pH Temp.	Moist.
_____NFS1_____			g cm	˚C	%
_____NFS1_____	N3__._____/ W-10__._____		g cm	˚C	%
_____NFRS1___			g cm	˚C	%
_____NFRS1___	N3__._____/ W-10__._____		g cm	˚C	%
_____NFS2_____			g cm	˚C	%
_____NFS2_____	N3__._____/ W-10__._____		g cm	˚C	%
_____NFRS2___			g cm	˚C	%
_____NFRS2___	N3__._____/ W-10__._____		g cm	˚C	%
_____NFS3_____			g cm	˚C	%
_____NFS3_____	N3__._____/ W-10__._____		g cm	˚C	%
_____NFRS3___			g cm	˚C	%
_____NFRS3___	N3__._____/ W-10__._____		g cm	˚C	%
_____NFS4_____			g cm	˚C	%
_____NFS4_____	N3__._____/ W-10__._____		g cm	˚C	%
_____NFRS4___			g cm	˚C	%
_____NFRS4___	N3__._____/ W-10__._____		g cm	˚C	%

WW: Wet Weight

Soil Sample Non-Forage Page **1** of **2**

Figure 7. Soil and non-forage sampling form. This is page 1 of 2.

Code:_____

Analysis site history: _____

SLOPE: Steep☐ Moderate☐ Flat☐: _____

SURFACE RUNOFF: Rapid☐ Medium☐ Slow☐ Ponded☐ **SURFACE EROSION:** Severe☐ Moderate☐ Slight to None☐

VEGETATION: Dense☐ Scattered/Sparse☐ Absent☐ **ROOTS:** Many☐ Common☐ Few☐

MACROFAUNA: (#/m2): Many☐ Common☐ Few☐ None☐ **MICROFAUNA**: Many☐ Common☐ Few☐ None☐

For non-forage plants sampled at this site, please draw on "Sampling Map" on page 1 where samples were obtained.

Name type(s) of forage plants sampled at this site: _____

_____

Forage No. & WW	GPS Lat/Long N3/W-10	WW	Root Depth	Plant Height	Plant Description (flowers, root size etc.)	Soil pH & Temp.	Soil Moisture
_____NF1___	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
	__.______/__.______	g	cm	cm	mm	˚C	%
_____NF1___	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
_____NFR1__	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
	__.______/__.______	g	cm	cm	mm	˚C	%
_____NFR1__	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
_____NF2___	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
	__.______/__.______	g	cm	cm	mm	˚C	%
_____NF2___	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
_____NFR2__	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
	__.______/__.______	g	cm	cm	mm	˚C	%
_____NFR2__	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
_____NF3___	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
	__.______/__.______	g	cm	cm	mm	˚C	%
_____NF3___	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
_____NFR3__	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%
	__.______/__.______	g	cm	cm	mm	˚C	%
_____NFR3__	__.______/__.______	g	cm	cm	mm	˚C	%
_____g	__.______/__.______	g	cm	cm	mm	˚C	%

WW: Wet Weight Version 04/25/2012

Other Sampling Device(s) Used: pH meter☐ Moisture meter☐ Thermometer☐ Other☐ _____

Additional remarks: _____

_____

_____

Attach photos *(if any)*. **Close☐ Wide☐ Extra☐** _____

Soil Sample Non-Forage Page **2** of **2**

Figure 7. Soil and non-forage sampling form. This is page 2 of 2.

Figure 8. UCLA Food Chain Study Plant ID Log. This is page 1 of 1.

UCLA FOOD CHAIN STUDY PLANT ID LOG

Code:__________________

Collection Date/Time	Plant I.D. No	Latitude	Longitude	Plant Description	Notes
		N3__.__________ **Elev.:**____________**m / ft**	**W-10__.__________**	Color/Abundance: Fruit Seed Foliage Flower Stem Root Tree Shrub Plant **or** Other:______________	State/County: **New Mexico** **McKinley** or **Cibola** County Location (if no GPS):___________ ______________________ Annual Perennial Unknown
		N3__.__________ **Elev.:**____________**m / ft**	**W-10__.__________**	Color/Abundance: Fruit Seed Foliage Flower Stem Root Tree Shrub Plant **or** Other:______________	State/County: **New Mexico** **McKinley** or **Cibola** County Location (if no GPS):___________ ______________________ Annual Perennial Unknown
		N3__.__________ **Elev.:**____________**m / ft**	**W-10__.__________**	Color/Abundance: Fruit Seed Foliage Flower Stem Root Tree Shrub Plant **or** Other:______________	State/County: **New Mexico** **McKinley** or **Cibola** County Location (if no GPS):___________ ______________________ Annual Perennial Unknown
		N3__.__________ **Elev.:**____________**m / ft**	**W-10__.__________**	Color/Abundance: Fruit Seed Foliage Flower Stem Root Tree Shrub Plant **or** Other:______________	State/County: **New Mexico** **McKinley** or **Cibola** County Location (if no GPS):___________ ______________________ Annual Perennial Unknown
		N3__.__________ **Elev.:**____________**m / ft**	**W-10__.__________**	Color/Abundance: Fruit Seed Foliage Flower Stem Root Tree Shrub Plant **or** Other:______________	State/County: **New Mexico** **McKinley** or **Cibola** County Location (if no GPS):___________ ______________________ Annual Perennial Unknown

Notes:

This form was created by C.Samuel-Nakamura, Ph.Dc

Plant ID Log 07/31/12 Version Page ____ of ____

WATER SAMPLING FORM

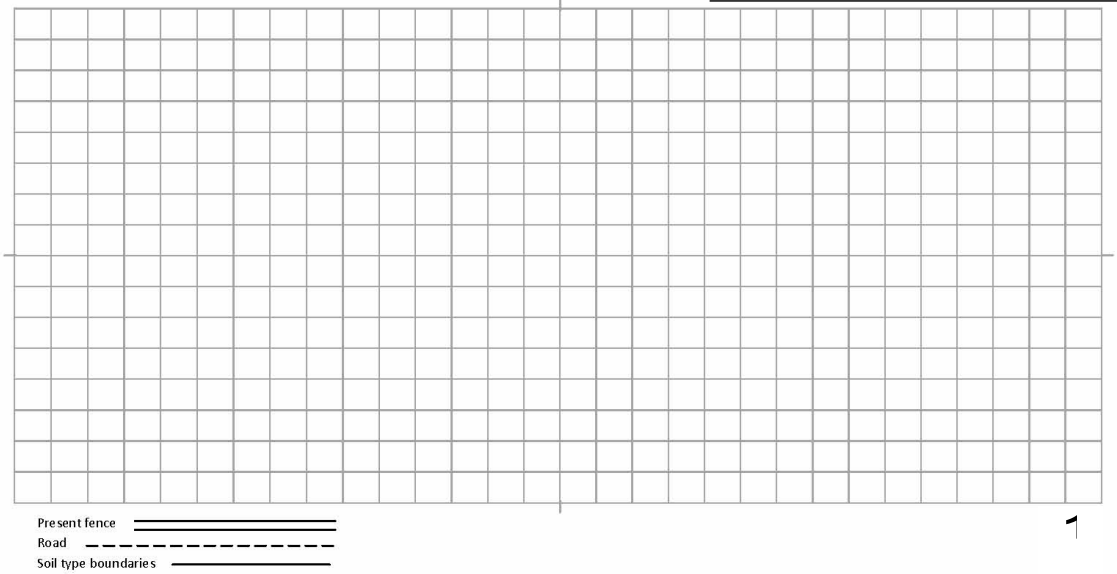
Name(s) of Sampling Staff: _____ **Code:** _____

Date of Sampling: _____ **Time of Sampling:** _____

Water Source Geographical position *(via map or GPS)*: _____ **OR**

GPS Latitude: N3___._____ **Longitude: W-10**___._____. **Elevation:** _____m

SAMPLING SITE MAP **Well No.** _____



Wind Speed: Gale ☐ Breezy ☐ Calm to Light ☐ **Humidity:** _____ **Air Temperature:** _____˚C

Potential sources of contamination *(if any)*: _____

Analysis site history: _____

Water Sample No. & Type	GPS Lat/Long	Water Sample Type* & Description	Amt.	Depth	pH	Temp
____W____			ml	cm		˚C
☐ Moving			ml	cm		˚C
☐ Still	N3__._____/ W-10__._____		ml	cm		˚C
☐ Spigot/faucet			ml	cm		˚C
☐ Other_____		☐ Added HNO_3 (to pH <2)	ml	cm		˚C
____W____	N3__._____/ W-10__._____		ml	cm		˚C
☐ Moving			ml	cm		˚C
☐ Still	N3__._____/ W-10__._____		ml	cm		˚C
☐ Spigot/faucet			ml	cm		˚C
☐ Other_____	N3__._____/ W-10__._____	☐ Added HNO_3 (to pH <2)	ml	cm		˚C
0____W____	N3__._____/ W-10__._____		ml	cm		˚C
☐ Moving	N3__._____/ W-10__._____		ml	cm		˚C
☐ Still	N3__._____/ W-10__._____		ml	cm		˚C
☐ Spigot/faucet	N3__._____/ W-10__._____		ml	cm		˚C
☐ Other_____	N3__._____/ W-10__._____	☐ Added HNO_3 (to pH <2)	ml	cm		˚C

*GTP: Grocery/Trading Post; L: Lake; N: NTUA; P: Pond; R: Rainwater, SP: Spring; ST: Stream, WE: Well; WI: Windmill; O:Other Version 07/28/12

Figure 8. Water sampling form. This is page 1 of 2.

Date:_____Code:_____

Water Sample No.	GPS Lat/Long	Water Sample Type* & Description	Amt.	Depth	pH	Temp
____W____			ml	cm		˚C
☐ Moving			ml	cm		˚C
☐ Still	N3__._____/ W-10__._____		ml	cm		˚C
☐ Spigot/faucet			ml	cm		˚C
☐ Other_____		☐Added HNO_3 (to pH <2)	ml	cm		˚C
____W____	N3__._____/ W-10__._____		ml	cm		˚C
☐ Moving			ml	cm		˚C
☐ Still	N3__._____/ W-10__._____		ml	cm		˚C
☐ Spigot/faucet			ml	cm		˚C
☐ Other_____	N3__._____/ W-10__._____	☐Added HNO_3 (to pH <2)	ml	cm		˚C
____W____	N3__._____/ W-10__._____		ml	cm		˚C
☐ Moving	N3__._____/ W-10__._____		ml	cm		˚C
☐ Still	N3__._____/ W-10__._____		ml	cm		˚C
☐ Spigot/faucet	N3__._____/ W-10__._____		ml	cm		˚C
☐ Other_____	N3__._____/ W-10__._____	☐Added HNO_3 (to pH <2)	ml	cm		˚C
____W____	N3__._____/ W-10__._____		ml	cm		˚C
☐ Moving	N3__._____/ W-10__._____		ml	cm		˚C
☐ Still	N3__._____/ W-10__._____		ml	cm		˚C
☐ Spigot/faucet	N3__._____/ W-10__._____		ml	cm		˚C
☐ Other_____	N3__._____/ W-10__._____		ml	cm		˚C
	N3__._____/ W-10__._____		ml	cm		˚C
	N3__._____/ W-10__._____		ml	cm		˚C
	N3__._____/ W-10__._____		ml	cm		˚C
	N3__._____/ W-10__._____		ml	cm		˚C
	N3__._____/ W-10__._____	☐Added HNO_3 (to pH <2)	ml	cm		˚C

*GTP: Grocery/Trading Post; L: Lake; N: NTUA; P: Pond; R: Rainwater, SP: Spring; ST: Stream, WE: Well; WI: Windmill; O:Other (Specify) Version 07/28/12

SAMPLING DEVICES: Weighted sampler☐ H_20Sampling holder/rod☐ Nalgene® sampler☐ Storm mount®☐ Manual ☐

OTHER SAMPLING DEVICES USED:_____

Comments:_____

_____

_____

_____

_____

Attach photos (if any): Close ☐Wide☐ Extra☐ _____

Figure 8. Water sampling form. This is page 2 of 2.

EQUIPMENT DECONTAMINATION LIST

Equipment Decontaminated	Date & Time Decontaminated	Location Decontaminated	Decontamination performed by: Initials
1. Regular auger			
2. Mud auger			
3. Core sampler cup and cap No._____			
4. Core sampler liner and caps No._____			
5. Core catcher No._____			
6. Other			
7. Other			
8. Other			
9. Other			
10. Other			
1. Regular auger			
2. Mud auger			
3. Core sampler cup and cap No._____			
4. Core sampler liner and caps No._____			
5. Core catcher No._____			
6. Other			
7. Other			
8. Other			
9. Other			
10. Other			

Page ____of _____

Figure 9. Equipment decontamination list form. This is page 1 of 1.

Analytical Chemistry Laboratory
Earth and Planetary Sciences Department
The University of New Mexico

Memorandum of ~~Sample(s)~~ Submission Agreement:

From: _____
_____

File Name : _____

Sample(s) Sequence No. (_____ - _____)

I, _____, have been informed that the Analytical Chemistry Laboratory at the Earth and Planetary Sciences Department, the University of New Mexico is not an accredited laboratory. Also, the submitted samples are not related to any regulatory program(s) that may require the analysis of such samples using specific approved procedures and Quality Assurance and Quality Control (QA/QC) measures. I understand that the Analytical Chemistry Laboratory implements basic QA/QC measures with each sample batch (20 samples) that may include, but not limited to:

- Four calibration points including calibration blank, where applicable.
- Initial Calibration Blank Verification (ICBV), if applicable.
- Initial Calibration Verification (ICV), if applicable.
- Continuing Calibration Verification (CCV), if applicable.
- Duplicated sample, if applicable.
- Replicated sample, if applicable.

Additional QA/QC measures can be added at extra cost only if requested by the client. My signature below indicates that I have read, understood, and agreed with the above statement.

_____ _____
Signature Date

Figure 10. UNM chain-of-custody form. This is page 1 of 4.

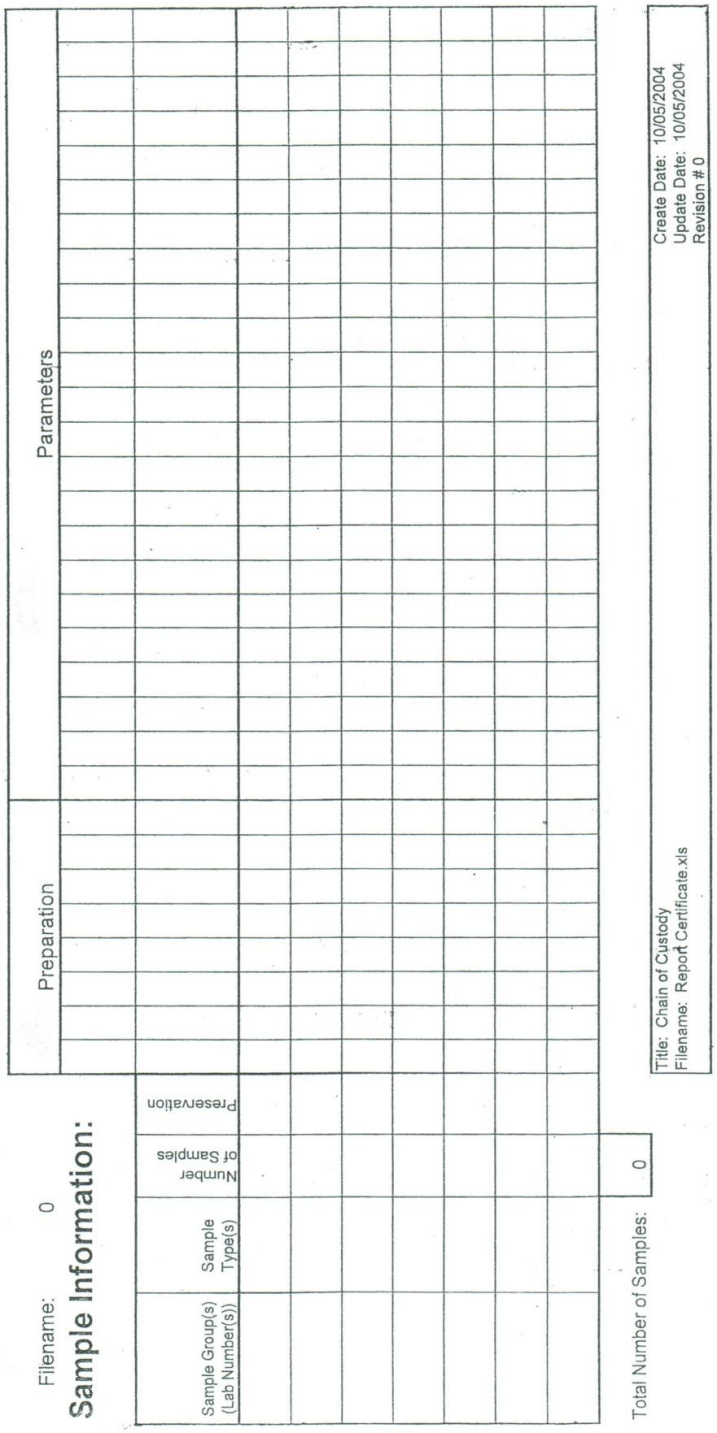


Figure 10. UNM chain-of-custody form. This is page 2 of 4.

No.	Lab#	Client Sample I.D.	Lab#	Client Sample I.D.	Lab#	Client Sample I.D.	Lab#	Client Sample I.D.
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								

Figure 10. UNM chain-of-custody form. This is page 3 of 4.

Northrop Hall Building # 24
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Chain of Custody Record
Geo/Analytical Chemistry Laboratory
Earth and Planetary Sciences Dept.
University of New Mexico (UNM)

Project Name: _____
Submission Date: _____
FileName: _____

SET SEQUENCE #'S: _____ -- _____

Comments:

	Client Information	Billing Information
Client Name:		0
Address:		0
		0
		0
Telephone:		0
Client Code		Account or P.O.#

Email:-

	Collector	Sample(s) Condition
Signatures:		
Name:		

	Relinquished by	Received by
Signatures:		
Name:		

Figure 10. UNM chain-of-custody form. This is page 4 of 4.

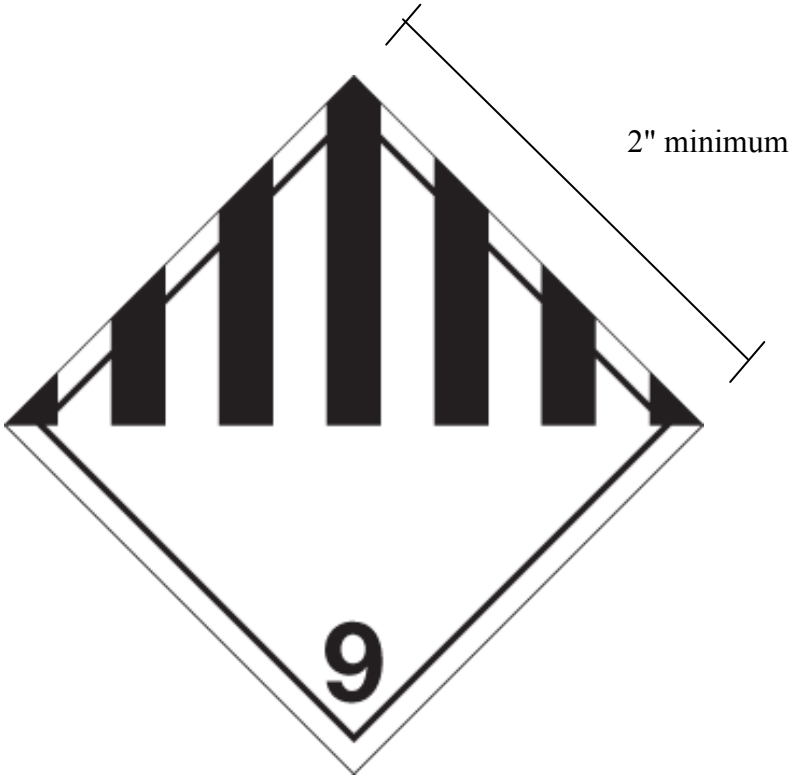

Figure 11. Dry ice shipping label.