SUMMARY REPORT FOR THE ATSDR EXPERT PANEL MEETING ON TRIBAL EXPOSURES TO ENVIRONMENTAL CONTAMINANTS IN PLANTS

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NOTE

This report was prepared by Eastern Research Group, Inc. (ERG), an ATSDR contractor, as a general record of discussion for the "ATSDR Expert Panel Meeting: Tribal Exposures to Environmental Contaminants in Plants." This report captures the main points of discussion among the expert panelists. This report is not a verbatim transcript of the meeting proceedings. Additionally, the report does not embellish, interpret, or expand upon matters or agenda topics that were incomplete, unclear, or not addressed. Except as specifically noted, no statements in this report represent analyses or positions of ATSDR or ERG.

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EXECUTIVE SUMMARY

The Agency for Toxic Substances and Disease Registry's (ATSDR's) Office of Tribal Affairs (OTA) convened a nine-member panel (five experts and four Ex Officio participants) to address issues related to the use of plant materials by native populations. The panel discussed the potential for contaminant exposure through traditional uses of plant materials by native populations (e.g., subsistence, ceremonial, and medicinal). This issue is relevant to ATSDR's ongoing public health assessments at hazardous waste sites where American Indian, Alaska Native, and other populations may have unique exposures.

During a 2-day meeting held December 4 and 5, 2000, the panelists discussed a variety of topics pertaining to the use of plant materials by native populations and the potential for plants to uptake metals and other contaminants which could contribute to human exposure. These topics included identifying: possible exposure scenarios, plants that are commonly used by American Indian and Alaska Native populations, important factors that influence the uptake of contaminants by plants, and the significance of various plant preparation and preservation methods in contributing to human exposure. In addition to the topics specified above, panelists discussed the extent to which the panel's conclusions and recommendations regarding plant uptake and absorption of metals can be applied to non-metal contaminants (e.g., pesticides) in plants, resources available for health assessors, data gaps, and issues regarding the sensitivity and confidentiality of information regarding the traditional uses of plants within native populations.

During the meeting, the panel identified a number of points related to the uptake and absorption of metals by plants and how traditional uses of plant materials by native populations may result in exposure to metals and other contaminants. This report provides an overview of the panelists' discussions and the key findings are listed below.

C *Factors that Influence the Uptake, Absorption, and Distribution of Metals By Plants.* The panel discussion focused on the relative importance of plant uptake of metals as a route of human exposure compared with exposure from aerosol deposition and soil splash onto plants. The plant experts on the panel agreed that people, in general, will have a greater potential for exposure to metals and other contaminants from the deposition of soil onto plants rather than the actual uptake of these contaminants from the soil into the plants. Experts noted that the potential for soil to adhere to plant surfaces is very high and it is very difficult to wash all soil particles from the plant materials before preparing and ingesting the foods. Root crops and portions of plants growing close to the soil pose a greater risk of exposure to metals or other contaminants than the aerial portions of plants, such as fruits and berries. The experts emphasized that the potential for contamination is generally only a concern if plants are collected from areas where heavy contamination is present. Exposure is most likely to be a health concern when contaminated soil or soil spray adhere to plant materials or the plants are among the small group of hyper-accumulators.

One panel member presented the most important factors that affect the uptake of metals by plants. These are:

– *The "soil-plant barrier."* The soil-plant barrier involves processes that prevent excessive plant uptake of potentially toxic elements. The extent to which this barrier prevents the uptake of metals is dependent on the solubility of the element that is present in the soil. Some elements (e.g., lead) are so insoluble they are not taken up into the edible parts of the plant.

– *Plant-specific characteristics.* Some plant species, referred to as "hyperaccumulators," (e.g., milkvetch or locoweed [*Astragalus]* and prince's plume [*Stanleya]*) can accumulate some elements such as selenium or nickel much more readily than other plants. If these accumulator plants are being harvested for human consumption, exposure to harmful concentrations of metals could occur through the uptake and translocation into the edible portions of the plant.

– *Soil properties.* The uptake of elements such as zinc, cadmium, and manganese are all very dependent on soil pH. As the soil becomes more acidic, the potential for metals to be absorbed by the roots of the plant increases.

– *Phytoavailability.* Many elements may be present in soil, but are not freely available for uptake by plants unless there is some deficiency (e.g., zinc) in the plant that allows the metal to be taken up more readily than usual.

– *Bioavailability.* Some plants uptake metals (e.g., mercury), however they tie them up in a form that is not readily bioavailable to animals and humans.

- *Indicators of contaminated or stressed environments.* Unusual changes in the coloring or growth pattern of plants may be a signal of phytotoxicity (e.g., arsenic poisoning) or a stressful growth environment (e.g., drought) resulting in plants potentially accumulating metals more readily than they would under normal non-stressful situations.

- *Plants Commonly Used by American Indians and Alaska Natives*. The panelists acknowledged the tremendous regional variation in the use of plants by native populations and emphasized that it was not possible to characterize the plant materials used by every tribe across the nation. The native panelists, especially, noted that plants will have many different uses; it is difficult to distinguish between the plants that are used exclusively for subsistence or medicinal purposes versus those used only for ceremonial or spiritual purposes.

  The panel discussed the variation in the frequency of produce that are grown locally among tribal communities. It was noted that some tribes continue to rely almost exclusively on subsistence agriculture. It was also noted that most tribes or native cultures have a main staple food like bread or soup which traditionally is eaten almost daily. A large variety of plants, including many root crops, are used to prepare these staple foods. In some regions these staple foods may be prepared with non-vascular plants such as mushrooms or moss.

- *Possible Exposure Scenarios*. The panelists discussed a number of common ceremonial or cultural activities that involve the use of plant materials. The activities identified by panelists as likely resulting in the greatest potential for contaminant exposure were those involving the inhalation of contaminants from burning plant materials (e.g., smudging), the inhalation of contaminants from smoking plant materials (e.g., tobacco or jimson weed), the volatilization of contaminants in plant materials in enclosed areas (e.g., sweat lodges or work areas), and the daily use of plant materials as tonics to promote health (e.g., ginseng or sage).

- *Effects of Plant Preparation and Preservation Methods on Potential Exposures.* The panel discussed plants used for traditional activities (e.g., basket weaving) and for ceremonial purposes (e.g., smudging). It was noted that the practice of making baskets involves certain activities that could result in ingesting or inhaling plant materials. For example, hand-to-mouth activities could result in the ingestion of contaminants and many of the ceremonies associated with basket weaving involve burning of plants which could result in inhalation exposures. Other preparations from plant materials such as dyes, paints, and topical ointments (e.g., poultices) may contribute to human exposure through dermal contact.

- *Additional Topics for Discussion.* Panel members discussed a variety of topics during the meeting, not all directly related to the use of plant materials. The native panel members and observers emphasized throughout the meeting that tribes view all aspects of their surroundings (i.e., water, air, soil, and biota) as being tied into one another and it was emphasized that this is an aspect of native culture that needs to be considered when assessing environmental exposures. They explained that although this meeting focuses on the use of plant materials as potential exposure pathways, it is not easy to isolate the issue of plant contamination from all other environmental concerns.

  The panel also discussed the value of building a foundation of trust with the tribes and having them be active participants in the public health assessment process. It also was explained that the plants used for cultural and medicinal purposes are a significant part of the traditional lifestyle and maintaining the confidentiality of this sacred knowledge is very important to the tribal nations. The panel members recommended improving the flow of information, both to the tribes and also out to the health agencies, so that accurate public health conclusions can be made.

1.0 INTRODUCTION

The Agency for Toxic Substances and Disease Registry's (ATSDR's) Office of Tribal Affairs (OTA) convened a nine-member panel (five experts, four Ex Officio participants) to discuss the potential for contaminant exposure from traditional uses of plant materials by American Indian and Alaska Native populations. A 2-day meeting of the expert panel took place at the Windmill Inn in Tucson, Arizona, on December 4-5, 2000. The primary goal of the meeting was to better understand the potential for exposure to environmental contaminants through the use of plant materials among native populations. This issue is relevant to ATSDR's ongoing public health evaluations at hazardous waste sites where native populations may have unique exposures.

This report summarizes the discussions among the panelists during the 2-day meeting. The remainder of this introduction describes the following in greater detail: ATSDR's purpose for convening the panel (Section 1.1), how ATSDR selected panel members (Section 1.2), the charge to the panel (see Section 1.3), the meeting format (see Section 1.4), and the organization of this summary report (see Section 1.5).

1.1 Background

Under Congressional mandate, ATSDR is required to conduct public health assessments for certain contaminated hazardous waste sites. As part of the public health assessment process, ATSDR characterizes all possible human exposure pathways associated with such sites. ATSDR's health assessors generally rely on environmental sampling data and population-based exposure factors (e.g., fish ingestion rates and plant ingestion rates) to quantify human exposures to site-specific contaminants. Native populations that live on or near contaminated lands may have very different types and rates of exposure due to the traditional lifestyles that they may practice. Furthermore, they may ingest or be otherwise exposed to plant materials not used by the general population.

ATSDR's OTA is responsible for helping to ensure that ATSDR appropriately evaluates environmental health issues facing American Indians and Alaska Natives. OTA has been actively involved in evaluating the potential health impacts of traditional lifestyles. These include hunting and growing food crops for subsistence, the use of plant materials for healing and promoting health, and utilizing the land and native plants for ceremonial and cultural purposes. These traditional activities are of great importance to these populations and contamination from both industrial activities and/or naturally occurring geologic processes (e.g., harmful concentrations of naturally occurring elements) may result in human exposure.

In order to identify and discuss the key issues related to exposure to environmental contaminants in plants, ATSDR convened an expert panel. The panel was charged with addressing specific questions related to unique activities that may result in exposures to metals and other contaminants specifically from the traditional uses of plant materials. The findings are to be used to assist health assessors in addressing public health questions and concerns among native populations living on or near contaminated lands.

1.2 Selection of Panelists

ATSDR identified candidates for the expert panel through literature searches; Web-based searches; and networking with research institutes and other academic centers (e.g., Alaska Native Science Commission and University of New Mexico). ATSDR also consulted with federal, state, and local government agencies (e.g., U.S. Environmental Protection Agency and Alaska Department of Environmental Conservation) and representatives of tribal nations from across the United States. Potential candidates were ranked based on their level of expertise (i.e., either high,

medium, or low) in each of the following categories:

- Plant physiology
- Metals toxicology
- Ethnobotany (specific knowledge of common plants used by American Indian and Alaska Natives for subsistence, medicinal, and ceremonial practices).
- Specific tribal knowledge (knowledge of customs and practices regarding the use of plants by American Indians and Alaska Natives).

Based on the specific criteria outlined and the recommendations from tribal representatives, ATSDR selected five individuals to serve on the expert panel. Each of the selected panelists had expertise in one or more of the categories listed above and the collective expertise of the panel covered all four categories.

In addition, ATSDR invited four others to participate in the panel discussion (considered to be Ex Officio participants). Two of these individuals offered specific expertise in toxicology and plant uptake of metals and non-metal contaminants. The other two Ex Officio participants provided additional perspective on the use of plant materials by tribal nations within certain geographic regions of the United States. In total, the nine panelists included representatives from federal agencies, universities, and four tribal nations.

The panel's collective expertise ranged from very specific scientific knowledge (e.g., plant physiology, the uptake of metals by plants, and the toxicology of exposures to metals and other contaminants via plant ingestion, dermal contact, or inhalation of toxic substances) to anthropological and ethnobotanical knowledge of traditional lifestyles. Appendix A lists the names and affiliations of the panelists who participated in the meeting.

1.3 Charge to the Panelists

To focus the discussions at the meeting, ATSDR prepared a "charge" for the panel, which included a number of questions, listed under the following five topic areas. A copy of the charge to the panelists is included in this report as Appendix B.

- Exposure scenarios: specific activities that are likely to cause native populations to be exposed to plant materials, either through ingestion, dermal contact, or inhalation.
- Plants commonly used by native populations.
- Factors that influence the uptake, absorption, and distribution of metals by plants.
- Effects of plant preparation and preservation methods on potential exposures.
- Additional topics for discussion.

As background information and to prepare for the meeting, ATSDR provided all panelists with five review articles on the uptake of metals by plants and plant materials commonly used by native populations (papers for panelists' review are provided in Appendix C) and a bibliography compiled by ATSDR that lists many relevant papers and resources pertaining to the topic areas of interest (Appendix D). In addition, depending on individual expertise, some experts were charged with compiling lists of the most important factors influencing the uptake of metals by plants or lists of the most common plant uses by native populations. Appendix E and F, respectively, contain the compiled lists provided by various panelists prior to the meeting.

1.4 The Meeting Format

The 2-day meeting took place at the Windmill Inn located in Tucson, Arizona, on December 4 and 5, 2000. The schedule of the meeting generally followed the agenda as shown in Appendix G. Leslie Campbell, representing ATSDR, opened the meeting and introduced Mr. Austin Nunez, who is the Chairman of the San Xavier District of the Tohono O'odham Nation. Mr. Nunez welcomed everyone and conducted the opening prayer, which is customary at tribal meetings.

Following the opening prayer, the meeting facilitator, Candace Shelton, a member of the Osage Nation, introduced herself and stated that the purpose of this meeting was to receive input from the panel members with expertise on the broad spectrum of plant uses by American Indian and Alaska Native populations. She explained that although it would be useful to identify the parts of plants or the types of plants used for ceremonies, it would not be necessary to go into detail about how plants are prepared or to describe ceremonies that may be sacred and confidential to the tribe. Ms. Shelton proceeded to introduce Donald Briskin, the panel chair and asked the panel members to introduce themselves and state their affiliations. On day two of the meeting, Mr Herman Shorty, a member of the Navajo Tribe, conducted the opening prayer as well as a ceremonial blessing later in the morning.

During the meeting, the panelists discussed a number of issues pertaining to plant physiology and metals toxicology as well as describing various methods of plant preparation. Some of the panel members were asked to lead these discussions based on their expertise of the subject matter. Donald Briskin led Topics 1 (Exposure Scenarios) and 2 (Plants commonly used by American Indians and Alaska Natives); Rufus Chaney led Topic 3 (Factors that influence the uptake, absorption, and distribution of metals by plants); Dolores Flores and Donald Briskin led Topic 4 (Effects of plant preparation and preservation methods on potential exposures); and Lauri Monti and Donald Briskin led Topic 5 (Additional topics for discussion). Discussion leaders generally presented an overview of their respective topic areas followed by a general discussion of the issues by the panel members.

In addition to the panelists, approximately 15 observers attended one or both days of the 2-day meeting. The observers included ATSDR staff; representatives from other federal and local government, academia and research centers; and representatives from at least seven tribal nations. A list of the observers who attended the meeting is included in Appendix H. Though the discussion at the meeting was largely among the panelists, comments from observers were often a very integral part of the dialogue. Observers were provided opportunities to comment or ask questions during the meeting (see agenda) and also were encouraged to write questions, comments, or thoughts on note cards and flip charts. These issues were recorded as "parking lot" issues for future consideration by ATSDR or the panel (see Appendix I).

1.5 The Report Organization

The organization of this report generally follows the list of topics outlined in the agenda and charge to the panelists. At several points during the discussions the panel members asked if key terms could be defined. Section 2 of this report, therefore, presents the panelists' definitions of key terms used throughout the meeting. Sections 3 through 7 summarize the panelists' comments and discussions related to the five topic areas outlined in the charge. Section 8 presents the panelists' conclusions and recommendations that resulted from the discussions. Observers' comments are generally presented in specific subsections within a topic area; however, in some cases observers comments are included as part of specific panel discussions.

Note: In subsequent sections, the panelists' initials used to attribute comments are as follows: RB (Rita Blumenstein), BB (Brenda Brandon), DB (Donald Briskin), RC (Rufus Chaney), DF (Dolores Flores, CL (Carol Locust), CM (Craig McFarlane), LM (Lauri Monti, and AS (Allan Susten).

Observers' initials used to attribute comments are as follows: LC (Leslie Campbell), GD (Greg De Bruler), EH (Earl Hatley), HS (Herman Shorty), and SWF (Sharon Williams-Fleetwood).

2.0 CLARIFICATION OF TERMINOLOGY USED DURING THE MEETING

During the course of the discussions, some of the panel members requested clarification of certain terms being used. Some of the terms were used so that people at the meeting could understand what processes are involved in preparing or using some of the plant materials. Other terms pertained to plant physiological or biological processes or to specific plant species. The definitions below are those that the panelists provided during the course of the meeting:

- *Astragalus.* Many North American species of this plant are poisonous (e.g., *Astragalus mollissimus* [locoweed]), especially to livestock and wildlife, a property due to the accumulation of selenium from soils (RC).
- *Bioavailable* refers to the fraction (or percentage) of the total amount of a substance (e.g., nutrients or toxic elements) that an individual is exposed to, regardless of the route, that enters the bloodstream or is absorbed by tissues and is available to aid or harm the body (AS, RC).
- *Decoctions* generally involve a vigorous process intended to boil down the plant materials (usually more durable parts of the plant (s) such as the roots or bark) resulting in volume reduction and extract (DB, BB).
- *Infusions* generally refer to steeping or soaking plant materials (usually softer or delicate parts of the plant(s) such as leaves, flowers, and blossoms) (DB,BB,DF).
- *Poultice* is a type of plant preparation described as a paste made from selected plant materials. It is often applied to the skin for medicinal purposes (DB, DF).
- *Phytoavailable* refers to the fraction of the total amount of a substance in soil which is in a form or position where the roots can absorb the element or compound (RC).
- *Precipitate* is the non-soluble (does not dissolve in water) portion of a mixture that settles out to the bottom (RC, AS).
- *Smudging* is a Native American traditional way of using smoke to purify a space. Smudging is a ritual burning of herbs, using the smoke that comes from the herb to alter the energy for one's self or another or to cleanse the energy of a specific area. Common herbs used for smudging include sage, sweetgrass, and cedar (BB,

CS). [*Note: the description of smudging was obtained from discussions between sessions with native panel members and dialogue during the meeting.*]

- *Stanleya pinnata (prince's plume)* is one of the few members of the mustard family (*Brassicaceae*) known to accumulate selenium, a poisonous element present in many western soils.

- *Translocation* is the process by which plants transport nutrients or chemicals throughout the plant (DB, RC).

3.0 TOPIC #1: EXPOSURE SCENARIOS

During the first session, Topic 1 (Exposure Scenarios), Dr. Briskin reviewed in general terms the common factors that may contribute to contaminant exposure in plant materials and the panelists discussed the types of activities that involve the use of plant materials by native people for subsistence, medicinal, and cultural or ceremonial practices. On the second day of the meeting, some examples of worst-case scenarios involving the use of plant materials were provided so that priorities could be established for additional research or information gathering.

3.1 Factors that Contribute to Exposure to Contaminants in Plant Materials

Dr. Briskin led off discussions by emphasizing that the main objective of this meeting from ATSDR's perspective was to generate useful information that will assist health assessors in public health assessments and help protect native populations from exposure to environmental contaminants. Dr. Briskin provided the panel with some perspective on the issue of plant use among native populations. He stated that the primary question to be considered for this meeting was: To what extent might plant materials used in the daily traditional lifestyles of native populations contribute to human exposures to environmental contaminants? Dr. Briskin emphasized that there are several important factors that can affect an individual's exposure to contaminants, all of which were considered throughout the meeting discussions. These factors include:

- The environment in which the plants are grown
- The types of plants that are harvested
- How the plant materials are used
- The quantities of plant materials used
- The part of the plant that is used
- How plant materials are prepared and/or preserved

Dr. Briskin explained that all these factors are important in understanding the potential for plants to contribute to human exposure to contaminants such as metals under different scenarios.

3.2 Common Activities That May Result in Exposure (Via Ingestion, Dermal, and Inhalation Routes)

The panelists' discussed various issues related to the use of plant materials, with a focus on scenarios where plant materials may be intentionally smoked or burned. For example, several of the native panelists and observers described the use of sweat lodges by their tribes and noted that the potential for exposure to contaminants was a concern. It was noted that it is important to distinguish between the inhalation of smoke as a direct result of smoking activities and the inhalation of smoke that is generated by other activities such as smudging or burning plant materials over red hot coals (DB). The panelists agreed that there are many activities that involve ceremonial or medicinal burning of plant materials other than smoking. The key activities identified by panelists are summarized below:

- *Uses of plant materials for smoking.* The tobacco plant is very important to the native people and is still used very extensively because of its cultural value. Other plants may be used for smoking as well, but they are not used as often as the tobacco plant (BB). The panel chair asked the panel to consider instances where materials may be intentionally smoked for medicinal purposes. One of the panel members responded by explaining that there are some plant materials (e.g., jimson weed [*Datura innoxia*]) that are smoked to relieve asthma (DF).

- *Use of plant materials in sweat lodges.* Two of the native experts and one of the observers at the meeting explained that the use of sweat lodges is a very important ritual (BB, GD, EH). During the "sweats," certain plant materials may be burned over the red hot lava rocks used to heat the room. The burning of sage, sweet grass, cedar, and other ceremonial plants in sweat lodges on rocks (lava rocks may reach temperatures above 200 degrees) can result in dermal exposures and inhalation of contaminants (BB). Another panelist suggested that particles from the burning of plant materials coming off of hot lava rocks may be large enough to be caught in the upper respiratory tract. Therefore, it is important to consider what is being burned and whether the materials that are burned generate aerosols, solid particles, or gaseous vapors (AS). It also was noted that, because children's lung size and skin surface area are proportionately much greater than adults, children may be a particularly susceptible population. Two panelists also noted that the elderly have very thin skin and may be more susceptible to dermal exposures (LM and DF).

- *Use of sage for smudging ceremonies and medicinals.* One panelist described the different varieties of sage used by native populations. She displayed two types of sage, big sage (also referred to as men's sage), commonly used for smudging, and women's sage, commonly used for medicinal purposes, and described the use of sage with respect to how it could lead to potential human exposure. The panelist emphasized that sage is used in abundance and each tribe uses it in different ways. For example, traditional members of the Oglala Lakota Sioux Tribe at Pine Ridge often use sage on a daily basis and sometimes several times a day; it is often used in teas, cooking, and in smudging ceremonies (BB).

- *Use of plants for ceremonial purposes.* Many native tribes in California (e.g., the Pomo Tribes) use the tule (e.g., *Scirpus lacustris or Scirpus acutus)*, a tall reed plant, for ceremonial purposes. The reeds, which are often wet, are used as matts. They are laid out on the ground and dermal contact can be very extensive during certain ceremonial practices[1] (BB).

- *The use of plant materials for preparing foods or herbal remedies.* Several panelists described infusion and decoctions as a process of extracting nutrients and flavors out of different parts of the plant (BB, RB, DF, CL). One of the native experts made the distinction between using delicate parts of the plant such as the blossoms compared with using the tougher parts of the plant such as the roots and barks (CL). The type of preparation method often depends on the level of difficulty in extracting the intended medicinal or nutritive substances from the plant material. Other preparation methods noted, but not specifically discussed during the meeting include extraction and tinctures (DB, DF).

- *Miscellaneous questions and concerns.* One panel member presented information regarding the presence of selenium and other heavy metals in plants at a bombing range which is near tribal ceremonial grounds. Two concerns were raised: 1) whether selenium accumulates in sage and 2) whether contaminants in plant materials consumed by animals

[1] In post meeting comments, Brenda Brandon noted additional concerns related to ceremonial use of plants. Potential dermal exposures might result from using freshly cut contaminated sage as matting in Sundance ceremonies. California Tribes use Tule, a type of reed in a similar manner in their ceremonies. Potential inhalation exposures are associated with use of certain medicinal plants. There is little scientific understanding about the use of sweetgrass, sage, and cedar in daily activities and even less known about their use in lodge ceremonies. These three medicines are widely used, but the degree of exposure is Tribal-specific and varies from one ceremony to another. Variables including temperature, use of water, and length of time spent in the lodge will lead to variations in inhalation exposures.

bioaccumulate into the milk of animals (BB).[2]

[2] In post meeting comments, Rufus Chaney provided additional information pertaining to the bioaccumulation of contaminants in milk. Most persistent lipophilic contaminants readily accumulate in milk and exposures to children are greater than to adults (per kilogram body weight). There are few elements from soil, however, that accumulate in milk via plants or soil ingested by humans. With the exception of organic forms of mercury, which like PCBs are lipophilic and are readily transferred to milk, no other trace element (e.g., cadmium and lead) readily accumulates in milk (Dowdy et al., 1983; Sansonet al., 1984; Vreman et al., 1986; Mueller 1987; Stevens 1991).

4.0 TOPIC #2: PLANTS COMMONLY USED BY AMERICAN INDIANS AND ALASKA NATIVES

This section describes panelist discussions regarding commonly used plants. Dolores Flores, a traditional healer affiliated with the Pascua Yaqui Tribe of Arizona, and Rita Blumenstein, an Alaska Native and Tribal Doctor, led the discussions. The purpose of these discussions was to provide some perspective on the large variety of native plants that are often used by tribal nations for subsistence, medicinal, and ceremonial purposes. Discussions were limited to the most common plants used by the individual or known to be used by their respective tribes. The panelists emphasized the tremendous variation in use of plants by tribal nations across different regions and they acknowledged that the discussion would not be representative of all tribes in all regions.

4.1 Common Plants Used by American Indians and Alaska Natives for Subsistence, Ceremonial, and Medicinal Purposes

The native panel members described many of the plants they used in their daily routines or for special ceremonies. Discussions of the plants and their respective uses were often accompanied by stories or anecdotal information which provided a historical perspective about the use of the plants. Panelists emphasized how a single plant can be used in many different ways. For many of the plants that are used by native populations, it is difficult to separate out plants that are used exclusively for medicinal purposes from those that are used for ceremonial purposes or for subsistence. It was explained that the whole plant is considered sacred and is an important part of the traditional culture (RB, BB, CL). Although during the discussions much of the focus of this charge question was on plants used for medicinal and ceremonial purposes, the meeting chair encouraged discussion of plants used for foods because foods will often represent a larger volume of intake than plant materials used for other purposes. A summary of the discussions regarding plant use follows:

- *Use of plants for healing practices.* One panelist provided information about the types of plants commonly used by traditional healers and by native people in the southwest part of the United States (DF). A list of the plants and their uses are provided below.

  – *Aloe vera.* The gel-like substance found within the aloe plant is often used for medicinal purposes (e.g., applying on skin for cuts and burns).

  – *Camomile.* Camomile is a very common and important herbal plant. Camomile is often used for making teas and can be used for medicinal purposes. The wild type of camomile is very difficult to find and most of what is used is purchased at local herbal shops.

  – *Cirrus cactus plant (commonly referred to as "night blooming").* This medicinal root crop is very important to tribes in the southwest.

  – *Mesquite.* The mesquite plant is a very important plant because the leaves, blossoms, and pods are used for a variety of purposes.

  – *Rue.* The leaves of the rue plant are used to produce an oil extract which can be applied to the joints to reduce inflamation.

  – *Other plants*. Other plants commonly used among the Pascua Yaqui Tribe and other tribes in the southwest include cedar (referred to as juniper in southwest), comfrey roots, dandelion roots, wild yams, and garbanzo beans.

- *Plants used for foods and for medicinal purposes.* One of the panelists talked about various parts of plants that are used for both foods and medicinal purposes. For example, the roots of certain types of plants (e.g., comfrey root) can be ground into powders and made into extracts. The stems, leaves, and flowers of many plants are used to make teas and other herbal medicines. The bark of certain plants (e.g., mesquite) are used for making soups and for medicinal or ceremonial purposes (DF, LM). One panel member asked about the frequency of using native plants for purposes of flavorings in foods and medicines (AS). Although no specific frequencies were provided, it was noted that plant materials (e.g., lemon juice, corn syrup, Mexican brown sugar, cloves, cinnamon, and lavender oil) are added to some preparations to improve the taste or add a pleasant aroma (DF).

- *Use of locally grown foods.* One panelist asked what fraction of the total diet of native populations is from locally grown crops? That is, what major food stuffs are grown locally rather than obtained from the general food supply? None of the panelists provided a precise fraction or percentage of the foods is grown locally. However, some of the panel members did respond to the question qualitatively. For example, one panel member stated that, although beans are very important to her people's diet, most come from the grocery store now (DF). Another panel member explained that in the case of the Hopi Tribe, they still grow quite a bit of their food crops such as corn, beans, and squash (LM).

- *Use of staple foods.* One panel member explained that most tribes or native cultures have a main staple food like bread or soup, which traditionally is eaten almost daily. It was noted that it would be useful to identify regionally what types of plant materials the tribe most often use in the preparation of these staple foods (CL). For example, some tribes eat the raw stalk of the tule plant, which is a type of bulrush (*Scirpus lacustris or Scirpus acutus*) or use cattails (*Typha angustifolia or Typha latifolia*) to produce flour to make breads (EH, BB). Panelists provided the following examples of plant materials commonly used in staple foods such as soups or breads:

    - Acorns
    - Agave Hearts
    - Cattails
    - Tule
    - Corn
    - Ferns
    - Hickory
    - Lichens
    - Moss
    - Mushrooms
    - Pine Nuts
    - Squash
    - Sunflowers
    - Wheat

- *Use of root crops.* During the course of the discussion the panel chair questioned whether root-based materials are frequently used to make a staple like breads (DB). Root crops are specifically relevant because they would likely pose a greater risk for metals exposure than the aerial portions of plants, such as fruits and berries (DB, RC). One panel member expressed concern that many of the traditional soups have barks and roots which may be higher in metal and other contaminants (LM). Another panel member responded by emphasizing that any contaminants on the bark or roots would more than likely be associated with residual soils that have not been washed off during the food preparation as apposed to those metal contaminants taken up by the plant.

  A number of different variations of the potato were mentioned as a common staple food among the native panel members. One panelist explained that root crops are very common in Alaska and are important staple foods (RB). Based on the discussions of the panel members and observers, the following root crops were identified as important staple foods for many native populations:

- *Angelica root.* This is a sacred plant that is used by the Pomo Indians and other tribes in the west (BB).

- *Devils club root.* This can be used in teas or ground into a powder for a variety of uses. The leaves and berries of the devils club root are poisonous and are sometimes used as natural pesticides (RB).

- *The Indian or Eskimo potato.* This potato is used for both subsistence and medicinal purposes (RB).

- *The stinging nettle.* This is a very important plant to the Alaska Native people; the root is a good source of protein and is used for medicinal purposes as well (RB).

- *Wild Potato*. The Seminole people of the southeast (i.e., Florida) use a variety of wild potato that resembles the palmetto (CL).

- *Wooly warts.* This is a starchy root crop that resemble potatoes (RB).

- *Unidentified root crop*. According to one of the meeting observers, there are two or three types of bulbous root crops that are harvested in the spring by native people in the Columbia River area. These are staple foods that are often eaten immediately after harvesting. The roots can also be ground into powder, dried and stored for later use (LC). The specific names of these crops were not known.

4.2 The Use of Non-Vascular Plants and Fungi by Native Populations

As part of Topic # 2 discussions, the panel was asked to consider the importance of non-vascular plants (e.g., mosses and liverworts) and fungi (e.g., mushrooms) in their traditional lifestyle. In general, the panel indicated that non-vascular plants are not generally found in the southwest or regions with very dry climates. However, in Alaska and parts of the Pacific Northwest, non-vascular plants are commonly used by native populations and could be an exposure pathway for the uptake of metals and other contaminants. The following is a summary of the discussions concerning non-vascular plants:

- *Extent of non-vascular plant use.* The Pascua Yaqui Tribe do not regularly consume locally grown non-vascular plants (e.g., mosses) or fungi (e.g., mushrooms) for foods or medicinal purposes since these types of plants are not native to their area (DF). Alaska

Natives collect different kinds of mushrooms, which are used for foods and medicinal purposes. The harvesting time for mushrooms is usually in August. The mushrooms are often dried so that they can be preserved for several months. In addition to mushrooms, Alaska Natives also collect mosses for many different uses. For example, some natives ferment the moss with seal oil. This can be used during times of famine to provide essential nutrients (RB).

An observer representing a tribe from the Pacific Northwest explained that mushrooms and lichens are used by native populations in the northwest. They are often consumed raw or boiled and used in a variety of ways for both a food source and for medicinal purposes. The lichen are used by some native populations and it serves as a food source for animals. The observer asked that ATSDR also consider whether radioisotopes are readily accumulated in these plants. One of the concerns among the people in the Pacific Northwest are the lichens, which may potentially be contaminated with cesium 137 and radioiodine 129, both of which have very long half lives (GD).

- *Potential contaminant uptake by non-vascular plants.* One of the panel members explained that since non-vascular plants usually grow near the ground, it is possible that soil contaminants may be deposited through soil splash or from aerosols (RC).

4.3 Comparisons of Relative Quantities of Plant Materials Used

One question asked by the non-native panelists was the frequency of plant use by native populations. For example, are some medicinals used on a daily basis as a means of promoting health or are they used periodically to heal acute conditions such as an injury or illness? The native panel members agreed that some plant materials are used on a daily basis to promote health (LM, RB, DF, CL). The panel concluded that for some medicinals taken on a daily basis, the quantity of plant materials ingested could be analogous to the amounts ingested for subsistence food ingestion exposures. Specific panel member comments are summarized below:

- *Medicinals used to promote health.* Panel members discussed whether scenarios exist where medicinals are used on a daily or continuous basis to promote health as a tonic (DB, RC, AS). According to one panel member, this type of continuous intake might parallel subsistence uses of plant materials with respect to potential exposure. One panelist provided the example of American Ginseng as a type of plant extract that is sometimes used every day or even several times per day (DB). The panel agreed that the distinction between the occasional use of plant based medicinals for an illness or treatment and the long-term daily use of plant materials to promote health was important.

Another panel member noted, for example, that the exposure from some of the ceremonial uses of plant materials that had been discussed would likely be of a relatively small magnitude and would not result in high enough doses to be harmful. However, if the plant materials were used as a tonic to promote health, it could potentially be a health concern. This point was followed up by the statement that "the dose makes the poison" (AS).

- *Current versus past use of medicinal plants.* With respect to the frequency of medicinals used, one panelist explained that in the past, medicines were used more for short durations rather than long-term use. However, many of the chronic illnesses that were not as common in the past, such as diabetes and cardiovascular or pulmonary diseases, have necessitated longer term use of some of the medicinal plants (CL).

4.4 Observers' Comments

Observer comments related to discussions from Topic #1 and #2 generally pertained to how native populations can better identify plant-use scenarios that may potentially lead to exposure. Two observers provided the following comments:

- *Herman Shorty, Office of Environmental Health, Navajo Nation*. Mr. Shorty explained that all the plants used by the native people are sacred and there is a prayer that is spoken when they are harvested. Mr. Shorty asked the panel to consider what relationship there is between plants that are used by native populations and documented exposures or illnesses resulting from environmental contamination.

- *Earl Hatley, Director of the Tribal Environmental Management Services, Tulsa University.* Mr Hatley stated that identifying the types or families of plants commonly used by native populations and studying whether these plants readily uptake contaminants would be most helpful to the tribes. If the discussion could be focused more on the general types of plants used, then the tribes would not have to divulge the specific reasons that they use the plants. One of the panel members added that discussions should focus as much as possible on plants that are already published or well known (LM).

  In response to the observer's comment, one panel member stated that it was his understanding that the phylogenetic classification of one type of plant and the knowledge of whether that plant is an accumulator of metals does not necessarily provide reliable information about another plant species within the same family. He questioned whether it is possible to talk about a family or a genus of plants and make generalities that would be applicable to other plants not specifically mentioned (CM).

Two other panel members agreed stating that many of the hyperaccumulators are species-specific (RC, DB). To illustrate this point, one panel member explained that within a genus that has about 100 different species maybe only 1 or 2 species might have the tendency to accumulate metals (RC). A lot of things are not known about many of the plants that are traditionally used by native populations. Most agricultural research is conducted using only a very small selection of agricultural plant species (RC, CM).

Another panel member responded by stating that if a native population is using a rare or uncommon plant that is very specific to the culture or region, it is very possible that the potential of that plant variety for accumulating contaminants will not be well documented. The only way to know whether specific plants are perhaps hyperaccumulators of metals, thus, would be to analyze them. However, it was emphasized that risks from using plant materials could be identified without necessarily having to divulge information regarding the traditional uses of the plants (RC).

5.0 TOPIC #3: FACTORS THAT INFLUENCE THE UPTAKE, ABSORPTION, AND DISTRIBUTION OF METALS BY PLANTS

This section summarizes panel discussions, led by Dr. Chaney, on the important factors that influence the uptake, absorption, and distribution of metals by plants. Much of the discussion focused on the relative importance of plant uptake of metals as a route of human exposure compared with exposure from aerosol deposition and soil splash onto plants. Dr. Chaney emphasized that, in general, people will have greater potential for ingesting, inhaling, or absorbing (via dermal contact) metals and other contaminants from the deposition of soil onto plants than from the actual uptake of these contaminants from the soil into the plants (Chaney 1985; Chaney et al. 1998; Chaney et al. 1999a; Chaney et al. 1999b).

5.1 Important Factors That Affect Plant Uptake of Metals

Dr. Chaney presented some of the important factors that influence the uptake of metals by plants. During the discussion, he emphasized that it is *how* people use the plant that will determine the extent of exposure to contaminants. For example, the food consumption patterns and nutritional interactions that are unique to a diet will dictate the extent of exposures associated with that diet. The following is a summary of the key points of his presentation:

- *Soil-plant barrier.* An important principle in plant physiology that strongly influences the potential for human exposure from plant uptake of metals is the soil-plant barrier. The soil-plant barrier involves processes that prevent excessive plant uptake of potentially toxic elements. The extent to which this barrier prevents the uptake of metals is dependent on the solubility of the element that is present in the soil. In most cases, elements that are not used by the plant (e.g., non-essential nutrients) are not readily taken up (Chaney and Ryan, 1994).

  Heavy metals such as lead and arsenic generally are not absorbed at all or may be absorbed into the roots but do not move through the plant body. These elements are so insoluble that under *most* conditions they do not get taken up into the edible parts of the plant, especially the leaves, berries, or fruits. Some elements such as iron, tin, silver, and fluoride may be absorbed at low concentrations, but usually do not enter the shoots of plants at levels that would be harmful to people.

Several elements can be phytotoxic (harmful to the plant) at levels well below those that would be toxic to people. Elements such as zinc, copper, nickel, and arsenic at high enough concentrations will kill the plant before it can be of harm to animals or humans consuming such plants. Also, some plants that accumulate metals from soil will limit the growth of the plant and results in smaller yields. This is true even though the concentrations of metals in the plants are not at toxic levels in animals.

- *Exceptions to soil-plant barrier.* An exception to the soil-plant barrier can be found under conditions where plants are phosphate-deficient. Without phosphates plants can take up certain elements (e.g., lead) into their tissues. All plants, however, need phosphates to grow properly and it is unlikely that under normal growing conditions a plant will be depleted enough in phosphates to uptake lead at levels of concern to humans (RC). Wild plants may be more likely to be deficient in phosphates because they are not being grown specifically for purposes of harvesting. Therefore, stressed vegetation could be a signal that conditions may be more favorable for the uptake of metals or other contaminants. There are also some elements that can be taken up by plants at levels that could be harmful to people. The two principle metals that can be taken up by plants under certain conditions are selenium and cadmium. If sufficient amounts of these contaminants were available in soil, it would be possible for certain plants to accumulate high enough concentrations to be harmful to people. In the case of cadmium, normal soil conditions (e.g., those with adequate concentrations of zinc in the soil) usually prevent excess uptake of cadmium by plants.

After Dr. Chaney finished his presentation he responded to questions and comments from the panel. Many of the questions pertained to specific types or species of plants that act as hyperaccumulators of certain metals. The following is a summary of the major points of these discussions.

- *Plant hyperaccumulators.* Some plant species (e.g., *Astragalus* [e.g., locoweed or milkvetch] and *Stanleya* [e.g., prince's plume]) may have a greater tendency to accumulate naturally occurring contaminants, but they are rare. These plants can readily take up some elements such as selenium or nickel. In these rare instances where a plant may act as an accumulator, it is possible for significant exposure to metals to occur by the uptake and translocation into the edible portions of the plant (RC). Panel members agreed that it would be worthwhile identifying whether any of the plants commonly used by native people are ones that readily accumulate metals (RC). One panelist noted that locoweed or milkvetch, a medicinal plant used by the Oglala Lakota Sioux Tribe, is one of the species that accumulates selenium (BB).

In order to provide some perspective on the metals that would be most likely to contribute to human exposure, one of the panel members wanted to know whether selenium and cadmium are the two toxic metals that would be of greatest concern (BB). Dr. Chaney replied by saying that some plants definitely accumulate selenium and to a lesser degree cadmium. With respect to cadmium, as long as people have a proper diet (i.e., adequate levels of zinc, iron, and calcium), this will usually prevent the absorption of harmful levels of cadmium.

- *Exposure considerations.* In terms of assessing health hazards associated with exposure to plant materials, it was reiterated that soil can be a potential vehicle for exposure to contaminants, either by skin contact with the surface of plant materials or by ingestion of contaminated soil. As a result of specific activities that bring people into contact with soil much more frequently than the general public, soil ingestion is a much more important risk to people who are dependent on the land and subsistence agriculture. For example, the risk from contaminants taken up by garden vegetables is about one-fifth as high as the risk from exposure to the soil that is brought into the house from being outside in the field harvesting crops or gardening (RC).

  Specifically, for heavy metals such as lead, cadmium, and arsenic, one should be primarily concerned about the roots and about soil contamination of the lower portion of the fruits or leaves that may be used (AS). Low lying plants (e.g., strawberries), leafy vegetables (e.g., spinach and lettuce), and root crops (e.g., potatoes and carrots) are particularly likely to contribute to human exposure to metals because soil can adhere to these plant surfaces quite readily. Since heavy metals are tightly bound to soil particles, soil is the primary vehicle for heavy metal exposure – not the uptake and translocation of metals from the root to the top of the plant.

  In general, soil contamination on the plants (e.g., small particles of soil that are on the skin of the crop) may be the most significant source of exposure to those elements that are insoluble in water. The soil that is attached to the plant, especially the unwashed portions, is likely to carry more contaminants than that actually taken up by the plant from the soil. For example, it is not uncommon for washed spinach leaves to contain 1 – 2 % soil. In fact, most of the iron that people get from eating spinach is actually from the soil and not the actual plant material (RC). Even after a thorough washing of many of these types of crops, a large number of soil particles continue to adhere to the plant materials. Peeling the skin of certain plants used for food, however, can remove much of the contaminated soil.

During the course of the discussions, the plant experts on the panel identified the following factors as important in determining the potential for plants to take up metals and translocate them throughout the plant body. Appendix E, prepared by Dr. Chaney and Dr. Briskin prior to the meeting, provides an expanded discussion of factors that affect the uptake of metals. The factors that were identified are summarized below:

- *Portion of the plant that is harvested.* In general, fruits and berries are less likely to accumulate soil metals and other contaminants because of plant processes (RC). Plants have physiological barriers in their structure which prevent contaminants from getting to the tops of the plants (DB, RC).

- *Influence of soil pH.* The pH of the soil plays a critical role in the extent to which certain elements such as zinc, cadmium, and manganese are taken up by plants. As the soil becomes more acidic, the potential for metals to be taken up by plants increases. For example, pyritic mine waste produces highly acidic soils which may allow metals to be translocated more readily through the plant. With few exceptions (e.g., cadmium), however, the concentrations of the elements would not be harmful to humans and animals without first killing the plants (i.e., soils with pH < 5.2 can prevent the growth of most plants) (RC).

  Two panelists asked whether there is a pH in soils where both plants can grow and where metals can be taken up at levels that could be harmful to animals and humans (BB, LM). In most cases the plants will not thrive in low pH soils and metal toxicity to the plants would likely occur before they harm animals or people. Although no specific pH was provided, it was noted that plants such as lettuce and spinach tend to be the most sensitive to metal accumulations whereas grasses tend to be least sensitive. The fact that only grass is growing in an open area could be an indicator that soil is contaminated or is otherwise in poor condition (e.g., low pH). It was also noted that in dryland or in land irrigated with high chloride waters, elevated chloride levels result in increased rates of cadmium uptake by plants (RC).

- *Phytoavailability of metals*. Many elements are not freely available for uptake by plants unless there is some deficiency that allows the metal to be taken up more readily than usual. Some plants (e.g., rice) are deficient in the necessary elements such as zinc, calcium, or iron to prevent the absorption of cadmium. Consequently, diets based almost entirely of rice would result in greater potential for human exposure to cadmium (RC). It was noted that the zinc-cadmium ratio in plants should be reported along with the concentrations of the metals being tested (RC, AS).

- *Bioavailability of metals*. One panelist explained that, although some wetland plants uptake metals (e.g., mercury), they store them in a form that is not readily bioavailable to animals and humans (AS). Another panelist added that the greatest potential for human exposure to mercury via plant materials is through the vaporization of the mercury and the deposition of the aerosol onto the leaves and shoots of the plant rather than through the uptake of mercury (RC).

- *Indicators of contaminated or stressed environments.* During the meeting, some panelists noted situations where unusual changes in the coloring or growth pattern of plants have occurred. One panel member explained that this could be a signal of phytotoxicity (e.g., arsenic poisoning) or a stressed growth environment (e.g., drought) resulting in plants potentially accumulating metals more readily than they would under normal conditions (RC). The panelists agreed that unusual plant appearance or animal behavior in areas where plant materials are being harvested may be important in identifying contaminated or stressed environments.

5.2 Observers' Comments

After considering the third charge question on factors that influence the uptake of metals by plants, observers were given the opportunity to comment on the panelists' discussions. A summary of observer comments and subsequent discussions follows:

- *Earl Hatley, Tribal Environmental Management Services, Tulsa University.* Mr. Hatley commented that at the Tar Creek Superfund site some of the plants that the native populations commonly use have been tested for heavy metal contamination. It was found that cattail flowers, which are located at the top of the cattail plant (*Typha angustifolia or Typha latifolia*), accumulated lead as readily as the mid-stem and roots. He also noted that the wild onions commonly harvested in northeast Oklahoma were found to be high in cadmium. Mr. Hatley also asked about the possibility of contamination in smaller plants like the dandelion where the flowers might be harvested.[3]

  One of the panel members asked whether the cattails grew in very dry barren soil, suggesting that it was possible that the windblown dust particles may be the agent contaminating the cattail flowers with lead (RC).[4] Mr. Hatley indicated that the cattails

[3]In post meeting comments, Dr. Chaney noted that dandelions are low growing leafy plants that tend to accumulate many elements more readily than other leafy crops such as lettuce.

[4]In post meeting comments, Dr. Chaney noted that cattails normally grow in swamps or wetlands. In areas with zinc-lead mining contamination (e.g., Oklahoma and Utah), mine wastes can become acidified if they are aerobic and soluble and, consequently, the zinc will be phytotoxic. However, within the general mine waste

were harvested from different areas with varying moisture content and said that the lead concentrations were similar in the different areas. He also noted that the cattails tended to grow where acid mine water at the site drained into. The panel member indicated that this was a scenario which may not be well reported in the literature. However, acid drainage does make the lead more soluble for plant roots to take up and it reduces the availability of phosphorus, the lack of which allows the lead to be mobilized throughout the plant. As noted previously, phosphates keep lead from moving beyond a plant's roots (RC).

Another panel member noted that it is important to consider air deposition, adsorption, and other special considerations such as metals contamination in cattail plants resulting from acid mine deposition. It might be that this is a new area of concern that has been identified and research should be conducted to evaluate the potential impact on people who use plant materials that grow near these sources of contamination (AS).

- *Greg De Bruler, Environmental Consultant for the Kalispel Tribe*. Mr. De Bruler suggested that it would be very beneficial if a tiered approach be developed such that the hyper-accumulator plants could be easily identified. For example, it might be useful to develop a list of the commonly used plants that have a low, medium, or high capacity to uptake metals via the roots and translocate them into other parts of the plant.

- *Maxine Ewankow, Environmental Programs Director for the Eight Northern Indian Pueblos Council.* Ms. Ewankow asked what happens to plants during extreme burn events (e.g., wildfires). Do plants take up contaminants differently? One panel member responded by explaining that in areas where smelters or incinerators are present, forests with tall trees tend to collect pollutants much more effectively than low-lying vegetation. During large forest fires more pollutants would be available to be volatilized. It was also noted that every fire volatilizes gases (e.g., mercury) and actually produces dioxins from the burning process (RC).

contaminated areas, wetland conditions can produce sulfide and raise soil pH which results in less phytotoxicity from zinc. If surrounding dry barren soils are wind blown, this may cause particulate contamination of wetland plants.

6.0 TOPIC #4: EFFECTS OF PLANT PREPARATION AND PRESERVATION METHODS ON POTENTIAL EXPOSURES.

Different native populations use various methods to prepare and preserve plant materials. Lauri Monti led the discussion on this topic shared the unique perspective of one who has worked with several native tribes including the Sonoran Desert Tribes, the Seri, and the Tohono O'odham Nations. Many of the panelists were concerned about plant preparation scenarios in which contaminants enter the lungs through inhalation or via dermal contact. For example, the harvesting of plant materials, the preparation of these materials, and the actual process of weaving baskets can present unique exposure scenarios that are not typically considered by public health agencies. This section summarizes Ms. Monti's presentation on native methods used to prepare and preserve plant materials and the panel discussions that followed.

- *Plant preparation and preserving methods: evaluating exposure and dose*. During the panel's discussion on plant preparation and preservation there was a great deal of emphasis placed on plants used for traditional activities (e.g., basket weaving or making matts and for ceremonial purposes such as smudging or face painting). There was also some discussion concerning the preparation and preservation of plants used as foods, but this was not the major focus of the panel discussions. In terms of relative importance of exposure and dose, the panelists were asked to consider whether the activities related to basket weaving, in particular, should be classified as an occupational exposure (LM). One panel member indicated that it is appropriate to consider such activities as occupational (RC). The panel members agreed that this could potentially be an important pathway for human exposure.

- *Basket weaving as an exposure pathway.* Ms. Monti described the art of basket weaving, providing examples of specific activities that could result in potential exposure to contaminants. She explained that gathering plants for basket weaving is a family event and exposures to sensitive populations (e.g., children and elders) need to be considered when assessing potential risk to human health. It was also noted that the practice of making baskets involves certain activities that could result in ingesting or inhaling plant materials. For example, hand-to-mouth activities could result in the ingestion of contaminants and many of the ceremonies associated with basket weaving involve burning of plants which could result in inhalation exposures.[5]

During this discussion, one of the observers explained that the cattail is also used for making baskets and in harvesting the plants the entire family will be in the field and come in contact with dust, soil, and possible contaminants. In addition, the storage root is often collected with the reeds and transport of these plant materials back to the living areas may result in potential ingestion exposure and mercury vapor exposure. Additionally, because much of the basket making takes place in an enclosed space, there is the potential for an inhalation exposure route, especially for the workers and children (EH).

- *The use of medicinals applied topically*. Many of the plant materials used by native populations are made into salves, ointments, or creams that can be used to reduce inflamation, itching, or help heal wounds (RB, DF). The plant materials are often made into a paste-like substance which can be applied as a poultice to the skin for medicinal purposes (DB, DF).

- *The use of plants for dyes, soaps, and other cosmetics.* One panel member questioned whether root crops were used to make soaps or dyes for cosmetic or ceremonial purposes (AS). Another panelist indicated that several root crops are used for such purposes. For example, the yucca root is used to make soaps and hair wash. Many plants, including root crops are used to make dyes for food coloring or coloring fabrics (DF).

- *Plant-related exposures from metals added during the preparation process (e.g., face and body paints)*. One panel member noted that mercury was intentionally added to some of the plant-based paints used for the face or other body parts (CL). Another panel member emphasized the importance of recognizing other forms of metal poisoning and exposure (e.g., occupational exposures or lead added to paints) other than what is actually found in soil or biota. The different exposures need to be put into perspective with respect to which pathway is resulting in the largest source of exposure (AS).

- *Practice of drying plants such as herbs*. It is common among many tribes to dry large quantities of aromatic herbs and other plants indoors which, under certain conditions, could result in potential exposure to volatile contaminants (BB).

[5] In post meeting comments, Dr. Chaney noted that natural soil processes during air drying (e.g., such as would occur when wet reeds are allowed to dry and brought into the basket making structure) cause reduction of mercury (Hg^{2+}) bound to soil to form mercury vapor which can be inhaled. This may allow mercury absorption from relatively little hand-to-mouth transfer of soil for both weavers and children who spend time in the basket making structure.

- *The use of seal oil to preserve plants*. It is common for Alaska Natives to use seal oil as a preservative for the long-term storage of plant materials for foods. The oil prevents spoilage and acts as an antibacterial agent (RB).

7.0 TOPIC #5: ADDITIONAL TOPICS FOR DISCUSSION

Additional topics discussed by panel members included: 1) the potential for exposure to other types of contaminants besides metals in plant materials (Section 7.1), 2) guidance from panel members regarding additional resources for health assessors (Section 7.2), 3) issues of sensitivity regarding the traditional use of plants (Section 7.3), and 4) identification of data gaps (Section 7.4). Some of the topics did not specifically relate to the use of plant materials (e.g., the potential exposure through the ingestion of contaminants in clays that are used in making pottery) and were included in Section 7.5 "other topic areas discussed." Although these topics did not relate directly to plant materials, many of the native panel members and observers emphasized that in their native culture, everything is tied into one another and one way or another all the discussion topics were interrelated.

7.1 Applicability of Conclusions and Recommendations Presented in this Meeting to Non-Metal Contaminants in Plants

During this session it was explained that people using plant materials are likely to be exposed to non-metal contaminants such as polychlorinated biphenyls (PCBs) and dichlorodiphenyl-dichloroethylene (DDE) in a similar way that they would likely be exposed to heavy metals. It is primarily deposition onto the plant surfaces versus the actual uptake of chemicals into the root systems that will result in the greatest potential for exposure. The following is a summary of the panelists' discussions:

- *Potential for plant uptake of non-metal organic chemicals (e.g., PCBs and DDE).* One panel member explained that for organic compounds such as PCBs and DDE, the deposition and adsorption onto plants is the most important route of exposure rather than the uptake by the roots and translocation into the other portions of the plant (RC, CM). For example, when PCBs are released into the environment in industrial areas, they typically volatilize in the air and are subsequently transported to the northern latitudes through the global atmospheric and oceanic circulation. This natural cycle of dispersion allows these contaminants to be adsorbed into vegetation in northern latitudes that are not industrial.

This is because the colder climates allows the contaminants to precipitate out and settle to the ground in the soil and on vegetation (RC).

- *Extent of deposition of organic compounds onto plant surfaces.* Many organic contaminants are soluble in the waxy layer of the cuticle. Even after washing the plant materials with the intent to remove the contaminants, substantial contamination often remains (CM). When questioned about whether these organic compounds would be bioavailable, a panel member responded by stating that they probably would be (RC).

- *Issues pertaining to plants that have tricomes or hairy cuticles.* One panel member asked whether hairy plants, plants with tricomes, or plants with waxy cuticles might be more likely to accumulate contaminants (LM). A panel member responded by stating that with respect to harvesting food and traditional uses of plants, plant leaves with tricomes will typically contain more soil. However, it is unlikely that people are being exposed to volatile organic compounds (VOCs) through this route because in the natural environment there will be very limited aerial deposition of VOCs. Exceptions to this conclusion exist in the northern most latitudes close to the arctic where VOCs are much more likely to settle down and be deposited on vegetation (RC).

- *Potential for exposure to radioactive contaminants in plants.* Two of the panelists asked whether any general conclusions can be drawn about radioactive contaminants (AS, DB). A panel member explained that the presence of radioactive cesium and iodine in plant materials, similarly to heavy metals and organic compounds, generally occurs from deposition or adsorption onto plant surfaces and not from uptake by the roots. For example, cesium, strontium, and iodine can bind to the leaves of plants through deposition. Certain trees with deep roots can accumulate cesium, but not vegetables such as lettuce or cabbage. It was also noted that for some reason barium (which is rarely accumulated in plants) is readily accumulated by brazil nuts. The actual reason for this is not fully understood, but, as noted previously, it is always important to consider the unusual plants that may hyperaccumulate a particular element (RC).

7.2 Guidance from Panel Members Regarding Additional Resources for Health Assessors

The panel member discussions focused on ways in which to improve the flow of information, both from health agencies to the tribes and vice versa, so that accurate public health assessments can be made. The following is a summary of these discussions:

- *Public health assessments based on tribal-specific diets.* One panel member explained that when ATSDR conducts a public health assessment it does not generally have information regarding the specific diet of the population living near the site. ATSDR tries to obtain site-specific information, but health assessors do not always know what questions to ask. In trying to understand total exposure from diet there should be some attempt at defining what a typical native diet is or, perhaps, at developing a model that can be used to apportion the different parts of the subsistence diet that could potentially lead to contaminant exposure (AS). Another panel member agreed and suggested that it seems reasonable to create a model specific to different diets and specific regions. The panelists can help identify the important plants used for foods and determine which of these plants are accumulators of metals (CM).

  Another panel member indicated that developing a model would be very difficult even though she acknowledged that it is important to do this. A model would be very dependent on how much each tribal culture relies on traditional lifestyles. Exposure routes also depend on the manipulation and preparation of plants and not just the frequency of ingesting plant materials (BB). One way of approaching this would be to identify the "risk levels" of the plants for uptake or absorption of contaminants (LM).

- *Feasibility of analyzing a limited number of soil samples collected by tribes.* One panelist suggested having some tribal members collect soil samples for analysis in order to identify areas of contamination on tribal lands. He emphasized that whoever collects the samples will need to be properly trained so that the results are meaningful. He noted that an advantage of doing this is that the results would be confidential and used only for purposes specific to the tribes needs. The panel chair commented that he often analyzes samples blindly where the label is coded in a manner that can easily be identified only by the sender. This is a very common practice and can be done for tribal nations that are concerned about revealing the plants that they use.

- *Additional resources.* During the meeting the panelists identified possible resources or references that could help address some of the questions raised at the meeting. Most of the discussions centered around what information is available, either on the Internet, in government reports, or in the published literature, concerning the important factors that influence metal uptake by plants and the common names of those plants used by native people that also accumulate metals. One panel member provided a list of Web sites that could be useful in identifying information that may be available on the Internet (CM)(see Appendix J).

- *Indicators of soil contamination*. One panel member asked whether any information is available that has examined the poisoning of plants or animals as indicators of soil contamination (CL). A panel member responded by stating that a report has been released by the National Academy of Sciences that looks at sentinel animal species as possible indicators of potential human exposures and human effects (AS).

- *Review of technical information by tribes.* The panelists discussed whether highly technical papers in the published literature would be useful to those native people who do not have scientific training. The panel agreed that it would be most useful for ATSDR to generate summary documents that are not highly technical, and communicate information from the published literature on a practical level. It was also noted that some academic papers could be understood by native people without scientific training and it should not be assumed that no scientific literature would be suitable (LM). In order for technical documents to be most effective, it was suggested that they be prepared by an agency or organization that is both trusted by the tribes and accepted as credible by the scientific community (RC).

- *Development of "conceptual model" to address tribal environmental concerns.* One of the observers representing ATSDR stated that EPA and ATSDR have agreed to develop a conceptual model that would be designed by tribal nations to address environmental concerns. The tribes could use the model and adapt each nation's needs without having to provide confidential or sensitive cultural information to the agencies. This initiative will provide tribes with the framework for assessing risk that can be applied specifically to each tribe rather than on the standard risk assessment model. The proceedings from this meeting would be useful in helping ATSDR and EPA with this initiative (LC). There was a consensus among the panelists that this would be a very valuable tool for native populations.

7.3 Issues of Sensitivity and Confidentiality of Information Regarding the Traditional Uses of Plants

As noted previously, a major theme throughout the meeting was the value of building a foundation of trust with the tribes and including them as active participants in the public health assessment process. One observer noted that plants used for cultural and medicinal purposes are a significant part of many native traditions. Thus, it is very important to the tribal nations to maintain the confidentiality of this sacred knowledge (HS). One panel member was very concerned about any panel discussions pertaining to proprietary tribal information, emphasizing the widespread interest of pharmaceutical companies in medicinal plants (LM).

An observer representing ATSDR reemphasized that the focus of the meeting was not to obtain proprietary information, but rather to obtain guidance so that ATSDR can work more sensitively with tribal nations who request ATSDR's assistance in looking at public health questions related to contamination. Exposure can be very different for populations that subsist on the land and ATSDR wants to ensure that the questions that are asked are the correct ones. From this learning process, there can be dialogue with tribal nations as to what type of information can be shared (LC).

One of the observers commented that it has been a dilemma for agencies such as ATSDR and EPA to make sure they maintain the confidentiality of different plant uses but at the same time collect enough information to answer a public health question or address a concern that a particular tribe has. She noted that one strategy EPA has had some success with is obtaining coded samples from the tribe and also gathering information about how the plant might be used (e.g., smudging, food, herbal tea, etc.) so that some idea of the pattern of exposure can be ascertained (LC).

7.4 Identification of Data Gaps

In the course of the discussion, the panelists were not able to fully address all the questions they raised because of gaps in information in the current scientific literature. The panelists agreed that data gaps existed on topics where specific references could not be provided and it was unlikely that any published papers would be identified in further literature searches. The data gaps identified by panelists during the meeting are summarized below:

- *Common plants that hyperaccumulate metals.* Among those panelists most familiar with the literature on plant uptake of metals, there was a consensus that there is a lack of information about specific plants that hyperaccumulate metals (DB, RC, CM). It was acknowledged that only a limited number of accumulator species have been extensively studied and that many of the plants that are used by native populations have not been tested to determine their potential to accumulate certain metals (RC). One panel member offered to provide a list of the known accumulator species with references to specific papers and also indicated that he would try to identify the local names that are commonly used for those plants (RC)(Baker et al., 1999; Chaney et al., 1999). A bibliography of selenium phytoremedition is also provided in Appendix K.

- *Potential for grasses and herbs to accumulate metals.* The plant experts agreed that only limited information exists with respect to the potential for uptake by the numerous species of grasses that may be used by tribes (DB, RC, CM). As noted previously, because grasses may be used for making baskets or for other traditional uses, the various species of grasses used could be a possible exposure pathway if they readily accumulate metals (LM). One panel member noted that most of the research has focused on the common staple crops such as lettuce, wheat, rice, and soybeans. However, although some types of grass may accumulate metals, it was emphasized that contact with the soil from harvesting the plant materials would probably still be the greatest risk (RC).[6]

During this discussion it was emphasized that Native American scientists are trying to bridge the gap between their spiritual and traditional practices and the science that is necessary to identify potential health risks. It was pointed out that students and faculty at many of the tribal colleges throughout the country are valuable resources and that this knowledge base should be utilized. It was noted that some of these research efforts are being delayed because adequate resources are not available, thus preventing additional advances in identifying data gaps and conducting new research that is required to address the tribal concerns (BB).

7.5 Other Topic Areas Discussed

[6] In post meeting comments, Brenda Brandon added that many plants, such as sage, have never been studied and it is not known whether these plants also accumulate or sequester metals. Wetland plants that sequester metals tie them up in a form that is no longer toxic except possibly through burning. Most plants are sensitive to high concentrations of metals and can be used as indicators of the degrading ecology of an area. In other words, most plants are likely to die before they become too toxic for animals and humans to consume. However, sage is an important medicinal plant that is not understood. Sage is an opportunistic plant that is capable of growing in relatively contaminated areas. Until sage and its uses are studied on a site-specific basis, questions of metal exposure by this plant cannot be addressed.

This section summarizes other important issues that were discussed during the meeting, but were not specific to the four primary topic areas outlined in the charge. Many of these issues were not specifically related to the uptake of metals and other contaminants by plants. A common theme among many of the native people who attended the meeting was the holistic view that everything in the environment is interconnected. This message was communicated throughout the meeting and it was emphasized that this is an aspect of the native culture that needs to be considered when assessing environmental exposures. An overview of other issues that were discussed during the meeting follows:

- *The safety of topsoil.* One of the panel members asked about the general safety of topsoil sold for growing vegetables and other plants (RB). According to one panel member, most topsoils that are sold are largely unregulated and not well labeled (RC). In Alaska, the topsoils are probably free of harmful contaminants because of the vast area of uncontaminated land. However, in more industrialized regions, it is possible that some top soils that are sold could contain levels of lead and other contaminants above acceptable guidelines.

  Some follow-up discussion took place among the panelists concerning what is the most likely contributor to blood lead levels in the population (RC, AS). The three most significant sources of lead that were discussed included lead from gasoline emissions, lead from paint, and lead solder from the canning industry. Although lead is no longer added to gasoline and house paint, it continues to be a source of exposure because of its historical use.

- *Extent of contamination in clays used by native populations.* One panelist asked what levels of metals might be expected in the adobe clay (i.e., a dark, heavy soil containing clay) used by many tribes to make pottery. A panel member indicated that he had no definitive answer; however, if the clays are being collected from below the surface it is unlikely that the clay materials would contain metals above what are naturally occurring. The common clays from the river beds are generally comprised of aluminum, silicon, and natural materials that are rarely contaminated (RC).

- *Mercury poisoning from hazardous waste sites.* A panel member described how many Pomo tribal members of the Elem nation living near the Sulfur Bank Mercury Mine have developed kidney disease. She attributed this to mercury exposure and explained that mercury was detected at very high concentrations in soil, surface water, and in people's homes. It was also noted that due to high concentrations of mercury in the lake, advisories were issued warning people not to consume the fish. It was explained that many of the plant materials that are harvested for basket weaving are taken from the wetlands near the sources of contamination (BB).

A panel member communicated that he did not believe the mercury would be readily taken up by the plants. It, however, could be deposited on the surface of the plants. He explained that mercury gaseous vapors are volatilized and may bind on the first thing that comes in contact with it, which could be the stems or leaves on the plants (RC).

One of the panel members noted that it is important to consider what form the mercury is in and how people might be exposed. It was suggested that measuring the content of mercury and other metals in the reeds of the plants that are commonly used for basket weaving at or near the site would be useful. It is important to determine whether there is the potential for human health risk from this mercury exposure pathway compared with ingestion of fish or other sources (AS).[7]

A meeting observer, representing ATSDR, commented that the Sulfur Bank Mercury Mine site was considered a public health hazard for the consumption of fish. However, it may be necessary to revisit the potential for mercury exposure with respect to activities such as basket weaving and the uses of other plant materials by the native population (SWF).

7.6 Observers' Comments

After considering the last charge question addressing additional topics for discussion, observers were given the opportunity to comment on the panelists' discussions. Many of the discussions focused on the issues of sensitivity and how best to address important public health questions without asking tribes to divulge cultural practices. A summary of these discussion follow:

[7] In post meeting comments, Dr. Chaney noted that fish biomagnify methyl mercury formed in sediments and this highly toxic form of mercury bioaccumulates in the foodchain. In comparison, the exposure to mercury vapor would not be nearly as toxic because very little is absorbed by the lungs. If fish from contaminated water bodies are consumed, the potential for human exposure and adverse health effects is much greater than the worst case scenario of transporting sediments adhering to tule or other plants.

- *Herman Shorty, Office of Environmental Health, Navajo Nation.* Mr. Shorty cautioned the panel members that some tribes are going to be sensitive to exchanges of information and that it is essential to develop trust with the tribes. For example, he suggested that the Navajo people may be reluctant to share information until they see some evidence or data indicating that environmental contaminants are contributing to health problems. It is important not to give the impression to native populations that certain ceremonial or cultural practices that use these plant materials may be harmful. Instead, it would be very helpful if ATSDR could provide health statistics that show what, if any, relationship exists between plants that are used by native populations and exposures to contaminants and related health effects (HS).

  In response to Mr. Shorty's comment, another observer representing ATSDR commented that, in general, ATSDR does not have direct statistics regarding the health effects of these levels of contaminant exposure. However, the focus of the agency is to determine whether there are specific health actions that the agency needs to take in order to address the public health needs of the community. ATSDR has found that communities are very important in terms of helping ATSDR understanding all the aspects that are needed to fully identify an exposure scenario of potential concern (SWF).

  Mr. Shorty also expressed concerns about groundwater contamination on reservation lands. One panel member suggested that there may be geologic surveys on reservation lands that have characterized groundwater contamination (RC). He asked whether environmental surveys conducted by the United States Geological Survey (USGS) characterizing drinking water and irrigation wells have been reviewed to identify whether some of these wells contain unusually high levels of contaminants. Mr. Shorty stated that this has generally not been done in recent years and much of the information that tribal nations have collected in the past is outdated. He explained that more education and better training is necessary to conduct sampling and evaluate data from published reports.

- *Maxine Ewankow, Environmental Programs Director for Eight Northern Indian Pueblos Council.* Ms. Ewankow informed the panel of a bibliographical list of research that she is compiling which may be pertinent to some of the topic areas discussed at this meeting. She offered to share this with any of the meeting attendees upon request.

8.0 CONCLUSIONS AND RECOMMENDATIONS

In general, the panel members agreed that the important outcome of this meeting was to identify pathways or routes of potential contaminant transfer that are applicable to native populations who are consuming plants for food (especially through subsistence practices) and using plant materials for medicinal purposes (e.g., for the promotion of health or to treat chronic ailments). The panel also considered the list of plants for cultural and ceremonial purposes. This section presents the panelists' conclusions and recommendations as recorded by the facilitator throughout the 2-day meeting.

8.1 Overall Conclusions

- *Exposure scenarios* from use of plant materials

  — The highest potential of risk at sites contaminated with heavy metals (e.g., lead, arsenic) is from soil ingestion. Eliminating carryover soil from plant materials as well as from clothing and hands is an important step in preventing exposure to these contaminants.

  — Contaminants from plant materials (e.g., sage) used in sweat lodges, which typically contain red hot lava rocks to heat the room, may volatilize into the air (e.g., mercury, PCBs).

  — Workers (e.g., basket weavers and/or plant harvesters) who spend most of the day in an enclosed environment may inhale the contaminated dust and small soil particulates bound to plant materials.

  — Because some of the medicinal plant materials are used by native populations, either daily or on a regular basis, to promote health, the potential for exposure to metals or other contaminants could present concerns similar to those connected with consuming plants for subsistence purposes.

  — If dyes or paints, especially cosmetics or face paints, are made from the roots of plants, this use of the plant should be considered as a potential metal exposure scenario.

- *Factors that influence the uptake, absorption, and distribution of metals by plants*

  — When evaluating potential exposure pathways via plant materials, it is important to make the distinction between the elements that accumulate in plants (e.g., zinc) versus those that are not readily taken up (e.g., lead).

  — From an exposure standpoint, it is important to consider which parts of the plants are used. Root crops (e.g., potatoes) and low lying plants (e.g., strawberries) are more likely to be harmful when grown in contaminated soils than are parts of the plants that are higher from the ground.

  — In general, the use of fruits and berries that grow higher from the ground will not be a significant source of exposure to metals or other contaminants from the soil. In most cases these plant materials will not present an exposure pathway unless there is evidence of significant aerial deposition.

  — With respect to plant uptake of metals through the soil, the focus should be on the root harvested materials or low growing leaves or fruits (e.g., strawberries).

  — Unless the soil is contaminated or in the unlikely event of elements occurring naturally at toxic levels, the consumption of soil attached to plant materials is not a problem.

8.2 General Recommendations

- The list of "hyperaccumulator" plants should be expanded to account for plants that are commonly used by Native populations. If accumulator plants are identified, some mechanism should be in place to effectively communicate this information to appropriate tribal groups.

- With respect to lead, native populations should identify where lead paint was used on the lands and where it may be contaminating the soils. The potential for exposure through soil ingestion is a more significant problem than the actual uptake of lead by plants (which does not occur to any great extent).

- It is important to look at the zinc to cadmium ratio in the plants being sampled to assess the cadmium's phytoavailability.

- The levels of mercury and other metals in the reeds of the plants commonly used for basket weaving near hazardous waste sites should be tested to determine whether there is the potential for human health risk from these exposure pathways in comparison with ingestion of fish or other sources.

- ATSDR should communicate information from the meeting so that the conclusions and recommendations will be easily conversed to the tribes.

- In future ATSDR-or other government- sponsored meetings concerning tribal issues, emphasis should be placed on trying to include more regional representation.

8.3 Specific Recommendations for Health Assessors

Panelists suggested that when evaluating potential plant-related exposures, health assessors should:

- Consider unusual (e.g., drought) conditions and soil type (e.g., pH) that may contribute to the uptake of contaminants in plants.

- Obtain additional information from tribes about physiological changes (e.g., coloring or growth patterns) in plants that may indicate contamination or a stressful growth environment.

- Pay closer attention to low-growing plant materials (e.g., fruits or berries) that may come in direct contact with soil.

- Develop a list of documented symptoms or conditions within tribal communities that may be attributed to contamination from metals or other chemicals.

- Examine indicators of toxicity (e.g., changes in animal behavior or patterns of illness) in areas where plant materials are being harvested and there is suspected contamination.

- Evaluate exposure from plant materials that may be associated with non-ingestion exposure pathways (e.g., basket weaving and face painting).

- Ask about any workers who spend most of the day in enclosed areas where plant materials may contain metals or other soil contaminants.

- Assess the use of root-crops among the tribe when considering the potential for soil transfer into or onto foods or other plant preparations.

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APPENDIX A

EXPERT PANELISTS

Agency for Toxic Substances & Disease Registry
Division of Health Assessment & Consultation

Expert Panel Meeting: Tribal Exposures to Environmental Contaminants in Plants

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Ex Officio Participants

APPENDIX B

CHARGE TO THE PANELISTS

Charge to the Expert Panel

Tribal Exposures to Environmental Contaminants in Plants

The Agency for Toxic Substances and Disease Registry's (ATSDR's) Office of Tribal Affairs (OTA) is convening an expert panel to address one of the many unique environmental exposure scenarios associated with traditional, subsistence, medicinal, and ceremonial practices among American Indian and Alaska Native populations. The ultimate goal of this workshop is to better understand the potential for exposure among these populations to environmental contaminants via the plant ingestion pathway. This issue is relevant to ATSDR's ongoing public health evaluations at hazardous waste sites where American Indian, Alaska Native, and other populations may have unique exposures.

Background. ATSDR evaluates the public health implications of exposure to environmental contamination by characterizing all possible human exposure pathways at hazardous waste sites. ATSDR's health assessors generally rely on environmental sampling data and population-based exposure factors (e.g., fish ingestion rates, plant ingestion rates) to quantify human exposures to site-specific contaminants, but we realize that sampling and population studies do not always reflect the different subsistence and traditional practices by Tribal populations. As a result, ATSDR's OTA continues to research unique exposure scenarios for Tribal populations and to inform health assessors of how to address such scenarios in public health assessments. Convening this workshop is just one example of how OTA helps ensure that ATSDR appropriately evaluates environmental health issues facing American Indian and Alaska Native populations.

Charge to Panel Members. The workshop discussions will focus on answering questions that pertain to five topics, listed below. The questions primarily address the potential for human exposure to metals through the traditional use of plants, for either subsistence, medicinal, or ceremonial practices, by American Indian and Alaska Native populations. ATSDR has charged this panel with primarily considering the uptake of metals in plants rather than looking at all contaminants. This focus on metals is largely due to the fact that plants uptake metals much more readily than plants uptake most organic compounds. Therefore, most of the workshop will address issues pertaining to metals. However, ATSDR recognizes that deposition of organic compounds may also contribute to potential exposure and, therefore, the uptake and deposition of other types of contaminants will be considered as part of the final topic of discussion.

Topic #1: Exposure Scenarios. To evaluate the public health implications of environmental contamination, ATSDR conducts detailed evaluations, which often start with characterizing how individuals might come into contact with contaminants. When plant materials used by American Indian and Alaska Native populations are contaminated, it is important for us to understand all possible ways that people may be exposed to the contaminants in the various parts of the plants. One goal of this workshop is to get input from the panel members with expertise on the broad spectrum of plant uses by American Indian and Alaska Native populations. The following questions address the uses of plants that can lead to exposure (and the questions under Topic #2 consider the specific plant species most often used):

- Prior to the workshop, please consider common American Indian and Alaska Native subsistence, ceremonial, and medicinal practices, and prepare and submit three lists using the form that ATSDR provides:

  —Provide a list of the activities that are likely to cause American Indian or Alaska Native populations to *ingest* contaminants from plant materials (e.g., direct consumption of plants as food). Please note the parts of plants (e.g., seeds, leaves, roots) that are typically used for the various activities, if such generalities can be made.

  —Provide a list of common activities of American Indian or Alaska Native populations that involve *dermal application* of plants or plant products (e.g., smudging inks derived in part from plants onto the skin). Please note the parts of plants that are typically used for the various activities, if such generalities can be made.

  —Provide a list of common activities that can lead American Indian or Alaska Native populations to *inhale* contaminants in plants (e.g., smudging). Please note the parts of plants that are typically used for the various activities, if such generalities can be made.

  ATSDR will compile the lists submitted by the individual panelists into one master list and distribute that to the panelists for review prior to the workshop. At the workshop, we ask that you offer any additions or revisions to the list and be prepared to discuss how the frequency and magnitude of potential exposures varies among the listed activities.

- ATSDR has provided a list of articles and resources that we have identified relating to the uptake of metals by plants and the use of plants for various purposes. Please review this resource list and provide any additional resources for characterizing how American Indians and Alaska Natives use plants and plant products that are not included on ATSDR's list?

Topic #2: Plants commonly used by American Indians and Alaska Natives. ATSDR recognizes that the plants used by American Indians and Alaska Natives vary from tribe to tribe, with each tribe having a history of using hundreds, if not thousands, of individual plant species. However, to guide our health assessors in their evaluations of exposure to plant contamination, we seek your input on the following issues:

- List some of the most common plants that American Indians and Alaska Natives use for subsistence purposes (e.g., corn, potatoes, rice), ceremonial practices (e.g., sage, tobacco, corn), and medicinal practices (e.g., birch tar oil, geranium root, dogwood bark).

- To what extent and in what activities are non-vascular plants used by American Indians and Alaska Natives? (Note, non-vascular plants are plants without specialized tissues for conducting nutrients; non-vascular plants are primarily algae, lichens, mosses, and fungi.)

- Can any general statements be made about the relative quantities of plant materials used for subsistence, ceremonial, and medicinal purposes? Are there scenarios (either over the long or short term) in which the quantity of plant materials used for medicinal purposes exceeds that consumed in the diet?

Topic #3: Factors that influence the uptake, absorption, and distribution of metals by plants. ATSDR would like the experts to consider general patterns in plant uptake that might serve as a guide to our health assessors. For example, if our health assessors know that certain plants commonly used by American Indians and Alaska Natives take up metals much less readily when grown in highly acidic soils or in very arid climates, then they can make more informed public health decisions in specific situations. Please answer the following questions pertaining to the extent to which plants uptake and store contaminants:

- The extent of plant uptake clearly varies from species to species and from metal to metal, but ATSDR's health assessors would benefit from knowing general information on the differential uptake of metals by various plants. Please outline any consistent trends on the differential uptake *of metals* across different groups of plant species. For instance, does the uptake of metals vary considerably between vascular and non-vascular plants? Can general guidance be given on how metals uptake vary across grasses, shrubs, vines, and trees? Are certain metals readily taken up by a broad range of plant species, and are other metals rarely taken up by plants?

- List the factors (e.g., soil pH, climate, plant characteristics) that have the greatest impact on the amount of contaminants that plants uptake.

- Can you recommend any general guidelines for estimating reasonable upper bound metals concentrations in plants when plant sampling data are not available (e.g., plant concentrations of most metals likely do not exceed corresponding soil concentrations by more than a factor of X)?

- Is there a relatively uniform distribution of metals in plants (e.g., highest concentrations in roots and the lowest concentration in seeds)? Can general statements be made about how contaminants distribute in other compartments (bark, fruit, leaves) of plants? Do plants primarily uptake metals from contaminants deposited in soil or is absorption of contaminants deposited on their leaves an important contributor?

- What published studies are the best resources for documenting how plant uptake varies with soil conditions, type of contaminants, plant species, and so on? What are the most important data gaps in the current state of science of plant uptake of metallic contaminants?

Topic #4: Effects of plant preparation and preservation methods on potential exposures. Many American Indian and Alaska Native populations use various methods to prepare and preserve plant materials. For instance, preparation methods include frying, boiling, and curing, and preservation methods include drying and salting. To evaluate exposures, ATSDR's health assessors seek guidance on the extent to which these preparation and preservation methods affect the concentrations and bioavailability of contaminants. As an example, if a specific food preparation method causes a metallic contaminant in plant tissue to change into a considerably less bioavailable form, then our health assessors might overestimate exposure doses by basing them on measured levels of contamination in plant tissue. Please respond to the following questions:

- Prior to the workshop, please submit a list of the various food preparation methods (e.g., steaming, curing, boiling) most commonly used by American Indian and Alaska Native populations and what portion of the plant is used for each of the methods of preparation. ATSDR will compile the panelists' lists and distribute a comprehensive list at the workshop. Do any of the food preparation methods lead to significant increases or decreases in the concentrations of metals in the plant materials? Do they affect the bioavailability of the metals? How should ATSDR consider the effects of food preparation methods when evaluating exposures?

- Prior to the workshop, please submit a list of the various techniques used for preserving plant materials. ATSDR will compile the panelists' lists and distribute a comprehensive list at the workshop. Do any of the preservation methods lead to significant increases or decreases in the concentrations of metals in the plant materials? Do they affect the bioavailability of the metals? How should ATSDR consider the effects of preservation methods when evaluating exposures?

- Do any of the preparation or preservation methods cause contaminants in plants to convert to considerably more toxic forms? If so, please provide specific examples.

- American Indians and Alaska Natives routinely use and prepare some plants for non-ingestion purposes, such as basket making, dying, and weaving. Explain how metals in these plants may be available for ingestion exposure (perhaps by users engaging in hand-to-mouth activity) or inhalation exposure. How does the magnitude of such exposures compare to that of food ingestion exposures?

- What published studies are the best resources for documenting how food preparation and preservation techniques affect the concentration and bioavailability of metals in plants? What are the most important data gaps relevant to this topic?

Topic #5: Additional topics for discussion. In addition to the four specific goals for this workshop outlined in the previous topics, ATSDR also asks the panelists to address the following general topics:

- What are the best resources for health assessors to identify plant use on a site-specific basis? Please provide specific references/resources?

- Can the expert panel's conclusions and recommendations regarding the uptake of metals by plants also be applied to other contaminants such as PCBs, pesticides, and other organic compounds?

- ATSDR recognizes that ceremonial, medicinal, and other uses of plants by American Indians and Alaska Natives are rooted in centuries of tradition and that we must address the issue of contamination of plant resources with great sensitivity. What guidance can the panelists give in this regard?

- Please bring to ATSDR's attention any other topics relevant to plant uptake of metals and other contaminants that are not addressed by the other questions in this charge.

APPENDIX C

PAPERS FOR PANELISTS' REVIEW

ATSDR

Agency for Toxic Substances & Disease Registry
Division of Health Assessment & Consultation

Expert Panel Meeting: Tribal Exposures to Environmental Contaminants in Plants

Papers for Panelists' Review

December 4-5, 2000

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APPENDIX D

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Tribal Exposures to Environmental Contaminants in Plants

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APPENDIX E

LIST OF FACTORS
INFLUENCING THE UPTAKE OF METALS BY PLANTS

Donald Briskin

Factors that influence the uptake of metals and other contaminants by plants

Plant Factors:

(1) Life cycle of the plant (annual vs.perennial)-with perennials being around longer there could be a greater possibility for metal accumulation in tissues.

(2) Age of plant at point of harvest-again very likely important as in (1) above.

(3) Rate of transpirational water flux through the plant-High rates of water flux could lead to greater movement of heavy metals via bulk flow in the soil solution to the roots.

(4) Extent of root development-Plants with large and/or bulkly root systems (and especially where the root system is used for human purposes) could potentially have a greater extent of heavy metal contamination. This could also involve heavy metal ion binding to the "apoplast" region of the cell walls which could act in a similar manner to an ion exchange resin.

(5) Plant tolerance to heavy metals--Plants can also find heavy metals to be toxic. Those plants that do survive in heavy metal contaminated soils may do so by actually taking the metals up and then sequestering the metals in vacuoles of root cells. Hence, harvest of these plant materials could lead to heavy metal ingestion if the root systems are used.

(6) Capacity of plant to transport heavy metals to the top of the plant--Some plants not only take heavy metals into root cells but then transport them to the leaves. Again this could lead to heavy metal accumulation in the plant. These types of plants are often termed as being "hyper-accumulators" and have been considered in phytoremediation.

(7) Extent of mycorrhizal association with roots--There is some limited evidence from the literature that association of vesicular-arbuscular mycorrhize with the roots may decrease uptake of heavy metals in some plants.

Soil Factors:

(1) Soil cation-exchange capacity--Heavy metals are polyvalent cations and the extent of soil binding and availability to plant roots would likely be determined by the cation-exchange capacity of the soil.

(2) Soil pH--Together with the cation exchange capacity of the soil this could determine the extent of heavy metal availability to roots.

(3) Level of inorganic mineral nutrients--Some evidence from the literature that nitrogen level can influence the extent of heavy metal uptake.

(4) Soil compaction and porosity--This would determine the extent of the root system and also the extent of bulk water flow to the roots.

Environmental Factors:

Any factors that influence the rate of water flow to the roots could impact the extent of heavy metal uptake--this would include air temperature and relative humidity.

Rufus Chaney

Element, soil, and plant characteristics affect the potential for plant contamination which would be relevant to food, medicinal, or ceremonial use of the plants.

Over the decades, I have built a model to assist in understanding soil element risk to animals, the Soil-Plant Barrier. Because of natural processes, very few elements in soil result in risk to food or feed chains through plant uptake. Further, interactions between elements can prevent risk or induce risk, but few food-chain poisoning risks result from such interactions – rather interactions prevent risk.

The following processes affect the potential for soil elements to be accumulated in plant tissues.

1) *Insolubility.* For most elements, the insolubility of the element in soil, or the insolubility of the element within the fibrous root system, prevents appreciable uptake and translocation of the element from soil to plant tissues, especially storage tissues. Examples include Pb^{2+}, Cr^{3+}, Ag, Sn^{4+}, Zr, Ti, and most of the periodic chart. These are the stable oxidation form of the element in the soil environment.

   Pb accumulation by plants can be significant under conditions of phosphate deficiency and strong soil acidity, the conditions which allow Zn to be phytotoxic. Most Pb rich mining wastes/soils which could be a source of concern also contain Zn and Zn phytotoxicity is much more important than Pb phytotoxicity. When normal phosphate fertilization is practiced to achieve higher crop yields, Pb uptake is decreased. As noted below, Zn-Pb mine and smelting wastes are commonly highly phytotoxic, so that no yield is obtained. Remediation of such soil/mine wastes to allow growth of cover crops or even garden or food plants requires that Zn phytotoxicity and Pb-induced phosphate deficiency be alleviated by limestone plus phosphate fertilization from many sources. And in all cases, for Pb uptake to be appreciable, soil Pb much be so high that ingestion of the soil would result in much higher risk than eating garden foods. If soils are contaminated, one may limit consumption of leafy and root vegetables to minimize soil transfer to diets.

2) *Phytotoxic before zootoxic*. Another group of elements are readily absorbed from soil and translocated to plant tissues, but the concentration of element in edible plant parts is not dangerous to humans (lifetime chronic exposure) because the element harms the plant at lower element concentration in the plant than required to result in risk if consumed. Plants suffering "phytotoxicity" have visible symptoms (yellowing of leaves; necrosis of leaf tips and leaf margins; stunting; death) and low yields. If the visibly harmed plant (say at 50% yield reduction) does not result in risk to consumers, no risk occurs from plant absorbed elements. Much higher potential risk could result from soil ingestion than from garden foods (see below). The "phytotoxic before zootoxic" elements include: Zn, Cu, Ni, Co, As, F, B, and Mn.

For the cations among the phytotoxic elements (Zn, Cu, Ni, Co, Mn), soil pH and soil adsorption strongly affect plant uptake; plant species also differ in uptake from the same soil. These elements are usually adsorbed or precipitated in the soil, commonly bound to Fe and Mn oxides or organic matter in the soil. Protons compete with the metal cations for the specific sorption by these surfaces, so as pH is reduced, metal ion solubility is increased and plant uptake is increased. Strongly acidic soils allow Zn and the other elements to be phytotoxic, while adding enough limestone to make the soil alkaline prevents phytotoxicity. We have used these principles to revegetate mine wastes in western U.S. by applying or incorporating a mixture of biosolids and wood ash on the contaminated soil. The site on Bunker Hill, ID, was barren before treatment. Treatment allowed rapid and effective revegetation, and the plants would be safe for chronic ingestion by livestock and wildlife. The high phosphate and pH of the amended soil allows the metal cations to be strongly bound by the soil or precipitated in the roots, so that Zn, Cd, and Pb in edible plant tissues did not comprise a chronic ingestion risk.

Among food crops, the spinach/beet family and lettuce are known to accumulate Zn more strongly than other foods. For Zn, Cu, Ni, and Mn, there is simply no evidence that plant accumulated element can cause risk to consumers. Plants suffering Zn phytotoxicity could be a useful source of the micronutrient in plant-based foods.

3) *Zootoxic before Phytotoxic*. Several elements can be hazardous to humans, wildlife or livestock through plant uptake and translocation to edible plant tissues at lower soil concentrations than required to cause phytotoxicity. These include Se and Mo, commonly toxic to livestock or wildlife because plants accumulate higher concentrations than present in soil (plants bioaccumulate soil Se and Mo if soil pH is high and soil is enriched or contaminated). This group includes Cd, which can cause chronic human disease at least through subsistence rice production. The anions (Se and Mo) have very different soil-plant relationships from Cd and will be discussed separately.

Selenium (Se) and Molybdenum (Mo)

For both these elements, soil pH is very important in adsorption to soil vs. uptake; at lower pH, these anions are sorbed to Fe and Al oxides in soil and uptake is low. But if soil is calcareous, the anion is not sorbed to soil and can more freely be absorbed by crops.

Both Se and Mo are readily translocated to plant shoots and storage tissues; that is, both forage, grain, and vegetable crops can cause Se poisoning of humans, or Se or Mo poisoning of livestock or wildlife.

Because many of the tribal lands in western US are calcareous soils, the high soil pH would promote Se and Mo transfer into plants. Se and Mo are common veterinary toxicity problems is parts of the western US. Se is accumulated by particular weeds (often locally called locoweed) of a few plant families and ingestion of these plants could comprise very important risk. The plants obtain advantage against insects and perhaps against plant disease organisms by accumulation of high Se levels in their shoots, and their occurrence is often limited to Se mineralized soils. Several well known Se-poisonous plants from SD, WY, CO, and other western US states include *Astragulus bisulcatus*, *Astragalus racemosus*,

The Se accumulator species are not food plants. Others may know if the plants had medicinal or ceremonial uses. The Se would be a chronic poison, not an acute poison, so occassional use would not likely cause risks. But deliberate use of such plants as food, or growing of food plants on Se contaminated alkaline soils could readily cause human disease.

Mo is also bioaccumulated from alkaline soils, but humans are resistant to much higher levels of Mo than are ruminant livestock. Soil Mo limits are developed based on Mo-induced Cu deficiency in cattle, the most sensitive species commonly exposed to these crops. Among crops, leafy vegetables could transfer the highest amounts from soil to diets. Human poisoning from soil Mo through garden food plants has not been reported.

An example of food-chain Se poisoning of humans can illustrate the case. In China, Yang et al. (1983) reported on a village which burned Se-rich coal and disposed the coal ash on their rice fields. During a period of drought, they had to grow wheat rather than rice, and wheat accumulated higher Se than did rice. Both livestock and humans suffered Se toxicity over time. The poisoning involved contaminated alkaline soils, and the Se-rich plants comprised a high fraction of their diets.

Cadmium (Cd)

Human poisoning from soil Cd was first observed in Japan in 1969. Much has been learned about this risk, and recent research has clearly shown that the most common Cd contamination (along with Zn in Zn/Pb ores, mining wastes, and smelter emissions) does not comprise risk except through rice (*Oryza sativa*) grown in flooded soils. In numerous locations in western countries where Cd+Zn contamination occurred and people grew crops and garden foods on the soils after adding enough limestone to prevent Zn-phytotoxicity, no disease has been found even in long term residents who grew home gardens. In some of these locations, soil Cd and Zn were as high as 100 ppm Cd and 10,000 ppm Zn. In Japan and China, soils with 2-10 ppm Cd caused rice to convey soil Cd and poison subsistence consumers. Malnutrition induced by subsistence rice consumption is now recognized to be fundamental to possible soil Cd risk. In rice grown on such soils with both Cd and Zn contamination, there is no increase in grain Zn even which grain Cd has risen 100-fold above background levels. No crop grown on aerobic soils with the normal ratio of Cd and Zn (100 g Zn per 1 g Cd) has been found to exclude Zn from edible plant tissues; usually Cd is excluded compared to Zn.

In rare situations of industrial contamination, soils become contaminated with Cd without the usual 100-fold higher Zn. In such cases, plants can appear healthy and have good yields but have unsafe levels of Cd accumulated from the soil. A few industries caused such contamination, those which use Cd pigments, or plastic stabilizer chemicals, or Cd-Ni battery manufacture. These industries are not known to have occurred on tribal lands.

If “Cd-only” soil contamination occurred, strongly acidic soils would favor Cd uptake. Sandy texture (lack of Fe and Mn oxides and clays) low in organic matter favors Cd uptake. Plant species differ strongly in Cd accumulation in such soils, with leafy and root vegetables most likely to accumulate the Cd. Extreme contamination and plant uptake would be necessary to cause disease if no staple food crops were grown on the contaminated soil.

One situation of Cd poisoning is especially relevant to tribal persons, the manufacture of jewelry using “silver solder”. This solder contains a high fraction of Cd to avoid using Pb in the solder. Workers can become contaminated, and they can contaminate their local environment from the fumes released, or from casual disposal of the dusts from the soldering. This was first reported for Arizona jewelry solders because one of the workers submitted her hair for diagnostic analysis and had remarkably high Cd contamination.

Cobalt (Co)

High soil Co may comprise food-chain risks to ruminant livestock and wildlife. Ruminants are much more sensitive to excessive Co than are monogastrics, perhaps because vitamin B12 is formed in the rumen and causes poisoning. Plants can accumulate up to 25 mg Co/kg dry leaves before phytotoxicity causes visible symptoms or stunting. But ruminant livestock may suffer chronic poisoning when forage Co exceeds about 10 mg/kg. This remains a theoretical risk because no case is known where it has occurred. Co usually occurs in serpentine soils where the Ni is at much higher concentrations and the Ni both causes phytotoxicity and inhibits Co uptake and translocation to shoots.

4) *Soil ingestion but not crop ingestion may comprise risk for Pb, As, F, etc.* The last category of elements are those which are not absorbed and translocated into plants, but comprise a risk through ingestion of soil, or of soil contamination of plants. Again, some elements in ingested soil do not comprise a risk even when relatively large amounts of soil are ingested chronically for a long period. These elements are so insoluble even in the stomach and intestine that animals are not harmed. Examples include Cr^{3+}, Al, Fe, Ti, Zr, and most of the periodic table. The elements where may cause risk to humans who ingest such soil include: Pb, F, As, and a few others. Although not found in nature, it seems very likely that ingestion of Hg rich soils would also comprise greater risk than consuming garden foods grown on the soil.

For these elements, soil ingestion comprises the important source of human risk. For high Pb and As soils from mining or smelting contamination, or geochemical sources, even if one were growing a home garden on the contaminated soil, plant uptake is so small that soil ingestion would be the first source of concern. If one will cease growing leafy and root vegetables, plants provide very little transfer of soil Pb and As to consumers.

5) *Rare hyperaccumulator plants*. Rare plants which evolved on mineralized soils have the ability to "hyperaccumulate" elements yet tolerate the element in leaves because of chelation or compartmentalization in vacuoles. Plants which accumulate and tolerate about 100-times higher foliar concentration of an element than commonly found for crop plants are considered hyperaccumulators. The trait gives protection against insects, bacterial and fungal disease, and even herbivores. The Se-accumulator species noted in "4" above are hyperaccumulators which can accumulate up to 1% Se even in the presence of high levels of soil sulfate, while sulfate strongly inhibits accumulation of Se by crop plants.

Several Zn+Cd and Ni+Co hyperaccumulator species also occur in the US, and an As accumulator has been found in FL which invaded from China. The Zn accumulator plants are a few species of *Thlaspi*; although many species are common in Europe, in the US there is little evidence that such Zn hyperaccumulator plants evolved. We have imported *Thlaspi caerulescens* for use in phytoextraction of soil Cd and Zn, and there is at least one report that this species occurs naturally in Rocky Mountain soils (called *Thlaspi alpestre*, an older name for this Zn,Cd accumulator species). Several other species and subspecies of *Thlaspi montanum* occur on serpentine soils in WA, OR, and CA which accumulate over 1% Ni in shoots. Another Ni-hyperaccumulator species was found in CA, *Streptanthus polygaloides*. It is conceivable that these species were unusual and selected for medicinal or ceremonial use, but I am not familiar with records of such use. Humans would have to ingest substantial amounts to suffer chronic poisoning.

Anecdotal evidence suggests that some people ingest at least some of these plants. Among persons who "rock garden", the unusual plants they try to grow as a challenge include the *Thlaspi* species known to accumulate Zn and Cd. Some individuals eat such plants while hiking in the mountains where such plants/soils occur.

Notes:

Davis, A.M. 1986. Selenium uptake in *Astragalus* and *Lupinus* species. Agron. J. 78:727-729.

"Forage quality potentials of plant introductions are unknown when introduced into the USA. Some native species of *Astragalus* are known to be Se accumulators and it is possible that some introductions could be accumulators also. This greenhouse study was undertaken to determine which, if any, of the untested 103 *Astragalus* and 68 *Lupinus* accessions were Se accumulators. Each accession was present in four replicates arranged in a randomized complete block design. Accessions were harvested at 24 weeks of age and the forage dried and analyzed. The AOAC extraction procedures were used and quantification was by colorimetric turbidimetric methods. Species of *Astragalus* accumulated Se in varying amounts from none to 61 mg/kg. None of the species of *Lupinus* accumulated Se at a potentially toxic level when grown in a soil mix (equal parts v/v; Shamel loam, quartz sand/commercial peat), that contained 18 mg Se/kg. These studies indicate that plants from 66 of the 103 *Astragalus* and none of the *Lupinus* accessions might accumulate toxic amounts of Se if grown on seleniferous soils. However, other unknown factors may make them unsuited for forage."

"Plants that accumulate Se do not form selenomethionine, and do not use it in the formation of proteins. While non-accumulators commonly put Se into protein amino acids.

Discuss the nature of Se poisoning of livestock from natural plants. The Se indicator plants, or Se toxic soil endemic tolerant plants, may accumulate high levels; from the field, Beath et al. collected *Astragalus bisulcatus* with 2590 ppm Se with only 20 ppm Se in the surface foot of soil. In normal plants, part of the Se is made into seleno-cysteine. Although toxicity is a concern where soil Se is high, at low soil Se, livestock suffer White Muscle Disease from Se deficiency. When they did this study, there were 131 species with 398 accessions of *Astragalus* in the USDA germplasm collection. He tested the part of the collection which had not been tested in 1972.

Used 20 cm pots; with a planting mix (1 kg) made from soil, sand, and peat. Se was not detected in this mix. He dusted the seeds with captan; used mechanically scarified seeds; thinned to 6 plants per pot. 25 mL of Hoagland solution were added to each pot at 6, 10, and 16 weeks of growth. Started adding Se when the plants were 8 weeks old; injected 5 mL with 0.002 g Se solution of Na selenate, and repeated the injection periodically until 18 mg Se were injected around inside the pot. The plants were harvested at 24 weeks; air dried; ground; wet ashed; and Se measured by colorimetric method.

The non-accumulator species of *Astragalus* contained 0-5 mg Se/kg dry wt., while the accumulator species contained from 2 to 61 ppm. Interestingly, when he compared different accessions of a species, found appreciable variation in Se accumulation. The following species were especially good Se accumulators: *A. adsurgens* var. *robustior*, 44 ppm; *A. asper*, 52 ppm; *A. bisculatus*, 43 ppm; *A. demetrii*, 47 ppm; *A. fraxinifolius*, 38 ppm; *A. galegiformis*, 41 ppm; *A. incanus*, 53 ppm; *A. scorpiurus*, 39 ppm; *A. tephroisioides*, 61 ppm; and *A. vulpinus*, 39 ppm. The last species was represented by 7 accessions, and shoot Se varied from 7, 17, 13, 15, 15, 39, 19 ppm. The *Lupinus* species did not have detectable Se by this method.

James, L.G., W.J. Hartley and K.R. Van Kampen. 1981. Syndromes of *Astragalus* poisoning in livestock. J. Am. Vet. Med. Assoc. 178:146-150.

Yang, G., S. Wang, R. Zhou and S. Sun. 1983. Endemic selenium intoxication of humans in China. Am. J. Clin. Nutr. 37:872-881.

"An endemic disease was discovered in 1961 in parts of the population of Enshi County, Hubei Province on the People's Republic of China. During the years of the highest prevalence, from 1961 to 1964, the morbidity was almost 50% in the 248 inhabitants of the five most heavily affected villages; its cause was determined to be selenium intoxication. The most common sign of the poisoning was loss of hair and nails. In areas of high incidence, lesions of the skin, nervous system, and possibly teeth may have been involved. A case is reported of a middle-aged female hemiplegic, whose illness and death apparently were related to selenosis. Daily dietary intakes of Se, estimated after the peak prevalence had subsided, averaged 4.99 (range 3.20 to 6.69) mg, and hair and blood Se averaged 32.2 and 3.2 Fg/mL, respectively. Up to 1000x differences occurred when selenium contents of vegetables, cereals, scalp hair, blood, and urine from the selenosis areas were compared with those from Keshan disease (Se deficiency) areas. The ultimate environmental source of Se was a stony coal of very high Se content (average more than 300 Fg/g; one sample exceeded 80,000 Fg/g). Se from the coal entered the soil by weathering and was available for uptake by crops because of the traditional use of lime as fertilizer in that region. This particular outbreak of human selenosis was due to a drought that caused failure of the rice crop, forcing the villagers to eat more high selenium vegetables and maize and fewer protein foods."

Turnip greens were very high in Se, 400 ppm, compared to 1/10 as high in other vegetables. Over 10000 times higher than in deficient village. Farm families consumed much turnip greens. In the endemic area, corn had 8.1, rice, 4.0, and soybean, 11.9 ppm Se! These levels are over 200 times higher than in normal crops. Cereals comprised 28-70% of total Se intake. Intakes were 3.2-6.69 mg/day, averaging 4.99 mg/day; high Se area without selenosis had 0.24-1.51, mean 0.75 mg Se/day; while normal area had 0.042-0.232 mg/day, mean 0.116. Deficient area had 0.011 mg/day. Drought stopped normal production of rice, and villagers relied more on corn and vegetables, which accumulated much higher levels of Se. Claim villagers often apply limestone, which would raise pH of area. Plant ash (from heating?) often returned to soils as well. Local pH not specifically reported. Corn had a pink coloration at the tip of the embryo, shown to be Se^0.

Soil from the Enshi, Hubei area contained 7.87 mg Se/kg, of which 0.35 ppm was water soluble. Non-endemic areas had soil Se=0.32 ppm with 0.011 to 0.04 ppm water soluble. Surface water contained significant amounts of Se, and may have contributed somewhat to the excessive Se intakes. Livestock had "alkali disease" or Se poisoning; pigs had often. Eggs were not hatchable, and if hatched, got beakless chicks! Exposures here were appreciably higher than in SD , NE, and Wyoming, and blood Se followed estimated exposures. They note information that selenium is much more poisonous than is organic-Se in foods, perhaps 5-fold.

"The present agricultural policy encourages the peasants to produce many more kinds of crops and exchange foods at the market."

APPENDIX F

LIST OF COMMON PLANT USES BY NATIVE POPULATIONS

<u>Common Plant Uses</u>

Ingestion Exposure Pathway

Rita Blumenstein

	Common Name	Scientific Name *(If known)*	Portion of Plant Used	Use (*i.e., food, medicinal, or ceremonial*)	Potential for Metal Uptake *(H, M, L)*
1	Blackberry		Berries	Food	
2	Blueberry		Leaves, stems, berries	Medicinal	
3	Chamomile		Leaves	Medicinal	
4	Colt's foot			Medicinal	
5	Cranberry		Berries	Medicinal, food	
6	Currant, red and black		Berries	Medicinal, food	
7	Dandelion		Root, leaves	Medicinal, food	
8	Devil's club		Root, bulb, bark	Medicinal, ceremonial	
9	Fiddlehead		Shoot	Food	
10	Fireweed		Shoots, flowers, leaves	Medicinal, food	
11	Mint		Stem, leaves, flowers	Medicinal	
12	Raspberry		Flowers, leaves, berries	Medicinal, food	
13	Sour duck		Leaves	Medicinal, food	
14	Stinging nettles		Whole plant	Medicinal, food	
15	Twisted stock		Roots, berries	Food	
16	Valerian		Flowers, root	Medicinal	
17	Wild rhubarb		Shoots	Food	
18	Wild rose		Petals, hips	Food, medicinal	
19	Willow (Surra)		Leaves	Food, medicinal	
20	Wormwood (Stinkweed)		Leaves and stem	Ceremonial and medicinal	
21	Yarrow		Leaves, stems, flowers	Medicinal	

Common Plant Uses

Ingestion Exposure Pathway

Dolores Flores

	Common Name	**Scientific Name** ***(If known)***	**Portion of Plant Used**	**Use (*i.e., food, medicinal, or ceremonial*)***	**Potential for Metal Uptake *(H, M, L)***
1	Albaca-basil		Leaves	Leaves collected and dried (last 2 years used in herbal tea or spice for food)	
2	Canyon ragweed chicura		Root	Harvest root and dry, used fresh or in tea (dried root lasts 3-5 years)	
3	Chamomile		Leaves	Herbal tea or extract in oil	
4	Estafiete		Leaves	Brewed into tea, using fresh or dried leaves(dried leaves last 2-3 years)	
5	Cholla root		Root	Brewed into tea, used fresh or dried (dried leaves last 3-5 years)	
6	Yerba colorada		Leaves	Brewed into tea, applied in powder form (leaves last 3-5 years)	
7	Golondrina	*Euphorbia capitellata*	Leaves, stems	Brewed leaves used as eye or skin wash. Leaves and stems are used fresh or dry (dried leaves and stems last 1-2 years)	
8	Rue ruda			Oil or alcohol extract for skin (fresh or dry leaves last 2-3 years)	
9	Yerba manzo	*Anemopsis californica*	Leaves	Brewed into tea (fresh or dry leaves last 3-5 years)	

<u>Common Plant Uses</u>

Ingestion Exposure Pathway (continued)

Dolores Flores

	Common Name	**Scientific Name** ***(If known)***	**Portion of Plant Used**	**Use (*i.e., food, medicinal, or ceremonial*)***	**Potential for Metal Uptake *(H, M, L)***
10	Desert willow	*Chilopis linearis*	Leaves, bark	Bark and leaves brewed as antiseptic (bark and leaves last 3-5 years)	
11	Indian tea kanutio		Stem	Tea (dried leafless stem lasts 3-5 years)	
12	Yerba santa		Leaves	Tea (dried leaves last 2-3 years)	
13	Ocotillo bark		Blossoms, bark	Medicinal tea or applied fresh (dried bark lasts 2-3 years)	
14	Corn silk		Silk	Tea (dried or fresh)	
15	Elder flowers		Flowers	Tea	
16	Basil leaves		Leaves	Tea	
17	Sangrengado	*Jatropha cinerea, J. cardiophylla*	Stems	Stem juice used for eye infections	
18	Pods, sap, mesquite leaves		Leaves	Tea for infections	
19	Spearmint leaves		Leaves	Tea	
20	Aloe leaves		Leaves	Inner gel applied fresh or after roasting	

* Preparation practices are included for some uses.

<u>Common Plant Uses</u>

Dermal Exposure Pathway

Rita Blumenstein

	Common Name	**Scientific Name** ***(If known)***	**Portion of Plant Used**	**Use (*i.e., food, medicinal, or ceremonial*)**	**Potential for Metal Uptake *(H, M, L)***
1	Chamomile		Whole plant	Medicinal	
2	Colt's foot		Leaf	Medicinal	
3	Devil's club		Bark, root	Medicinal	
4	Stinging nettle		Whole plant	Medicinal	
5	Plantain		Leaves	Medicinal	
6	Wormwood		Leaves	Ceremonial, medicinal	
7	Yarrow		Leaves, stems	Medicinal	

Common Plant Uses

Dermal Exposure Pathway

Dolores Flores

	Common Name	**Scientific Name** ***(If known)***	**Portion of Plant Used**	**Use (*i.e., food, medicinal, or ceremonial*)**	**Potential for Metal Uptake *(H, M, L)***
1	Castor seed		Inner part of seed	Applied to scalp	
2	Rosemary		Leaves dried or fresh	Brewed and added to bath	
3	Jojoba seed		Seed is crushed	Hair is rinsed with solution	
4	Yucca root		Crushed, fibered	Used for washing hair	
5	Creosote		Leaves, stems	Antiseptic wash for ulcers	
6	Sagebrush		Leaves, stems	Antiseptic wash	
7	Aloe feria		Inner gel	For cuts, burns	
8	Comfrey root		Root and leaves	Brewed in large amount of water (used in bath water for rash)	
9	Mesquite trunk		Wood is burned	White ash applied to head	
10	Cholla stems		The stem or limb	Roasted until hot (applied for food pain)	
11	Prickly pear pad		Cleaned of spines	Sprinkled with powdered herbs and applied to infected wounds	
12	Thyme leaves		Leaves	Extracted in oil (alcohol applied to head)	
13	Judio yerba del indio	*Aristolochia watsonii*	Root or leaves	Root and leaves crushed (juice applied for sinus)	
14	Rue leaves		Leaves and stems	Oil extract applied to joints	
15	Pine leaves			Extracted in oil (used with other oils for congestion)	

<u>Common Plant Uses</u>

Inhalation Exposure Pathway

Rita Blumenstein

	Common Name	**Scientific Name** ***(If known)***	**Portion of Plant Used**	**Use (*i.e., food, medicinal, or ceremonial*)**	**Potential for Metal Uptake *(H, M, L)***
1	Yarrow		Whole plant	Medicinal	
2	Colt's foot		Whole plant	Medicinal	
3	Wormwood		Whole plant	Medicinal	
4	Sweet grass		Whole plant	Ceremonial	
5	Sage		Whole plant	Ceremonial	

Common Plant Uses

Inhalation Exposure Pathway

Dolores Flores

	Common Name	**Scientific Name** ***(If known)***	**Portion of Plant Used**	**Use (*i.e., food, medicinal, or ceremonial*)**	**Potential for Metal Uptake *(H, M, L)***
1	Cedar		Leaves, stems	Mesquite coals used to produce sacred smoke	
2	Copal white		Sap from elephant tree	Ceremonial aroma from sacred smoke	
3	Creosote	*Bursera fagaroids*	Flowers, leaves and stems	Medicinal ceremonial smoke	
4	Desert palm		Leaves	Ceremonial	
5	Mesquite		Bark trunk, root, leaves, pods, flowers	Ceremonial coals, medicinal, sacred smoke, food	
6	Osha root	*Ligusticum porteri*	Leaves, root	Medicinal, ceremonial	
7	Sage		Leaves	Medicinal, ceremonial	
8	Sweet grass		Grass	Ceremonial	
9	Tobacco		Leaves	Ceremonial	
10	Citrus peeling		Skin of fruit	Medicinal, aromatic	
11	Cinnamon cloves		Bark and seeds	Medicinal, aromatic	
12	Angelica		Root	Ceremonial, medicinal, aromatic	
13	Mints		Leaves	Medicinal, aromatic	
14	Rosemary		Leaves, stems	Medicinal, aromatic	
15	Rose buds		Bud	Medicinal, aromatic	
16	Peppers		Fruit, seeds	Medicinal, sacred smoke	
17	Jimson weed		Leaves dry or green	Smoke of herb used for asthma	

<u>Common Plant Uses</u>

Food Preparation/Preservation Methods

Rita Blumenstein

	Common Name	**Scientific Name** *(If known)*	**Type of Preparation/ Preserving Method** *(Provide brief description)*	**Potential for Metal Uptake** *(H, M, L)*
1	Nettles		Freeze whole young plant (blanch)	
2	Fiddle heads		Blanch and freeze	
3	Sourdocks		Blanch and freeze young shoots	
4	Lovages		Blanch and freeze young plants	
5	Lambs quarter		Blanch and freeze young plant	
6	Beach greens achack looks		Blanch and freeze young plant	
7	Goose tongue		Blanch and freeze young plant	
8	Massu Eskimo potato		Blanch and freeze root	

Common Plant Uses

Food Preparation/Preservation Methods

Dolores Flores

	Common Name	**Scientific Name** ***(If Known)***	**Type of Preparation/ Preserving Method** ***(Provide brief description)***	**Potential for Metal Uptake** ***(H, M, L)***
1	Tepari beans		Cooked by boiling	
2	Pinto beans		Cooked by boiling	
3	Lentils		Cooked by boiling	
4	Garbanzo		Cooked by boiling	
5	White corn		Roasted or boiled corn kernels	
6	Squash goard		Steamed or boiled	
7	Squash seeds		Dried or roasted	
8	Squash immature		Steamed or fried	
9	Squash flowers		Boiled or fried	
10	Wild rice or domestic rice		Boiled	
11	Wild oats or domestic oats		Boiled	
12	Cactus saguaro		Fruit eaten/ jam	
13	Prickly pear		Fruit and immature pads are cleaned, diced, and boiled	
14	Barrel cactus		Inner pulp is semi-boiled and packed in sugar	
15	Night blooming Cirrus cacti			
16	Chea seed pamitas		Seed crushed, set in water and simmered	
17	Field mustard		Fruit, root	
18	Verdolagas common purslane		Tender leaves are boiled	
19	White or red field clover		Leaves, flowers	
20	Chinita dandelion		Leaves	

Common Plant Uses

Food, Utilitarian, and Medicinal/Ceremonial Plants

Laurie Monti

	Common Name	**Scientific Name *(If known)***	**Portion of Plant Used**	**Geographic Area**	**Family**	**Habit**	**Perennial/Annual**
	Food Plants						
1	Agave	*Agave parryi*	Heart (base of stem)	Plains, high desert, dry slopes	Agavaceae	Succulent	Perennial
2	Amaranth	*Amaranthus spp. (palmeri, powellii)*	Leaves	Forests, high desert, washes, plains, disturbed areas	Amaranthaceae	Herb	Annual
3	Banana yucca	*Yucca baccata*	Fruit	High desert, plains, forests, riparian slopes	Agavaceae	Succulent	Perennial
4	Barberry	*Berberis haematocarpa*	Fruit	High desert, slopes	Berberidaceae	Shrub	Perennial
5	Blackberry	*Rubus procerus*	Fruit	Riversides, slopes, shady deciduous forest	Rosaceae	Shrub	Perennial
6	Cattail	*Typha latifolia*	Root	Wetlands, riversides, lakes	Typhaceae	Herb	Perennial
7	Currant	*Ribes spp. (cereum, aureum, pinetorum)*	Fruit	Forests, high desert, riparian	Grossulariaceae	Shrub	Perennial
8	Dropseed	*Sporobolus spp. (cryptandrus, contractus, airoides)*	Seed	Plains, high desert, washes	Poaceae	Grass	Perennial
9	Filaree	*Erodium cicutarium*	Leaves	Roadsides, forests, disturbed areas	Geraniaceae	Herb	Perennial
10	Lambs quarters	*Chenopodium album*	Leaves	Forests, high desert, washes, plains, disturbed areas	Chenopodiaceae	Herb	Annual
11	Juniper	*Juniperus spp. (osteosperma, monosperma)*	Leaves (ashes), fruit	Forests, cool desert canyons, roadsides	Cupressaceae	Shrub/tree	Perennial
12	Manzanita	*Arctostaphylos pungens*	Fruit	High desert, riparian slopes, forests	Ericaceae	Shrub	Perennial
13	Mesquite	*Prosopis velutina*	Fruit (legumes)	Disturbed areas, washes, rivers, plains, high desert, slopes	Fabaceae	Shrub/tree	Perennial

Common Plant Uses

Food, Utilitarian, and Medicinal/Ceremonial Plants (continued)

	Common Name	**Scientific Name *(If known)***	**Portion of Plant Used**	**Geographic Area**	**Family**	**Habit**	**Perennial/Annual**
14	Oak	*Quercus spp. (gambellii, emoryii, turbinella)*	Fruit (acorns)	Forests, riparian slopes, high desert	Fagaceae	Shrub/tree	Perennial
15	Pinon pine	*Pinus edulis*	Seed	Forests, cool desert canyons, roadsides	Pinaceae	Shrub/tree	Perennial
16	Prickly pear	*Opuntia spp.*	Fruit, leaves (pads)	High desert, forests, plains, dry slopes, disturbed areas	Cactaceae	Cactus	Perennial
17	Raspberry	*Rubus strigosus*	Fruit	Conifer forests, riversides, slopes, shady deciduous forest	Rosaceae	Shrub	Perennial
18	Rice grass	*Oryzopsis hymenoides*	Seed	Plains, high desert, washes	Poaceae	Grass	Perennial
19	Rocky Mt. beeplant	*Cleome serrulata*	Leaves	Roadsides, forest edges, disturbed areas	Capparidaceae	Herb	Perennial
20	Russian thistle	*Salsola iberica*	Leaves	Washes, roadsides, sandy plains, disturbed areas	Chenopodiaceae	Herb	Annual
21	Saltbush	*Atriplex canescens*	Leaves & fruit (ashes)	Plains, high desert, alkaline soils	Chenopodiaceae	Shrub	Perennial
22	Sego lily	*Calochortus spp. (nuttallii)*	Root (bulb)	Plains, high desert, desert, forest meadows	Liliaceae	Herb	Perennial
23	Skunkbush	*Rhus trilobata*	Fruit	Forests, cool desert canyons, high desert, plains, slopes	Anacardiaceae	Shrub	Perennial
24	Spring parsley	*Cymopterus spp. (purpurascens)*	Root	Plains, high desert, washes	Apiaceae	Herb	Perennial
25	Watercress	*Rorripa nasturium-aquaticum*	Leaves & flowers	Wetlands, riversides, lakes	Brassicaceae	Herb	Perennial
26	Wild oregano	*Monarda spp. (menthaefolia,*	Leaves & flowers	Riversides, wetlands, disturbed areas, washes	Lamiaceae	Herb	Perennial

Common Plant Uses

Food, Utilitarian, and Medicinal/Ceremonial Plants (continued)

	Common Name	Scientific Name *(If known)*	Portion of Plant Used	Geographic Area	Family	Habit	Perennial/Annual
		pectinata)					
27	Wild potato	*Solanum spp.*	Root	High desert, PJ forests, plains	Solanaceae	Herb	Perennial
28	Wild rhubarb	*Rumex hymenosephalus*	Root	Wet areas, sandy plains, riversides, disturbed areas	Polygonaceae	Herb	Perennial
29	Wild rosemary	*Poliomintha incana*	Leaves & flowers	Rivers, sandy plains	Lamiaceae	Shrub	Perennial
30	Wild tea	*Thelesperma longipes*	Leaves & flowers	High desert plains, sandy areas, washes	Asteraceae	Herb	Perennial
31	Wolfberry	*Lycium pallidum*	Fruit	Disturbed areas, washes, rivers, plains	Solanaceae	Shrub	Perennial
	Utilitarian Plants						
32	Arrowweed	*Tessaria sericea*	Branches	Wetlands, riversides, lakes	Asteraceae	Shrub	Perennial
33	Beargrass	*Nolina microcarpa*	Leaves	High desert, plains, forests, riparian slopes	Agavaceae	Succulent	Perennial
34	Cliffrose	*Purshia spp. (stansberiana, mexicana)*	Branches	High desert, plains, forests, riparian slopes	Rosaceae	Shrub	Perennial
35	Cottonwood	*Populus spp. (fremontii, angustifolia)*	Root	Wetlands, riversides, lakes	Salicaceae	Shrub/tree	Perennial
36	Desert willow	*Chilopsis linearis*	Branches	Wetlands, riversides, lakes	Bignoniaceae	Shrub/tree	Perennial

Common Plant Uses

Food, Utilitarian, and Medicinal/Ceremonial Plants (continued)

	Common Name	Scientific Name *(If known)*	Portion of Plant Used	Geographic Area	Family	Habit	Perennial/Annual
37	Rabbitbrush	*Chrysothamnus nauseosus*	Branches	Forests, high desert, plains, slopes	Asteraceae	Shrub	Perennial
38	Seep willow	*Baccharis spp. (sarothroides, salicifolia)*	Branches	Wetlands, riversides, lakes	Asteraceae	Shrub	Perennial
39	Skunkbush	*Rhus trilobata*	Branches	Forests, cool desert canyons, high desert, plains, slopes	Anacardiaceae	Shrub	Perennial
40	Willow	*Salix spp. (lasiolepis, exigua)*	Branches	Wetlands, riversides, lakes	Salicaceae	Shrub	Perennial
41	Yucca	*Yucca spp. (angustifolia)*	Leaves	High desert, plains, forests, riparian slopes	Agavaceae	Succulent	Perennial
	Medicinal/Cermonial Plants						
42	Brickellbush	*Brickellia grandiflora*	Leaves & flowers	High desert, plains, forests, riparian slopes	Asteraceae	Shrub	Perennial
43	Ephedra	*Ephedra spp. (viridis, torreyana)*	Branches	High desert, plains, forests, riparian slopes	Ephedraceae	Shrub	Perennial
44	Globe mallow	*Sphaeralcea spp.*	Root	High desert, plains, forests, riparian slopes, disturbed areas	Malvaceae	Herb	Perennial
45	Gumweed	*Grindelia aphanactis*	Leaves & flowers	High desert, plains, forests, riparian slopes, disturbed areas	Asteraceae	Herb	Perennial
46	Horehound	*Marrubium vulgare*	Leaves & flowers	High desert, plains, forests, riparian slopes, disturbed areas	Lamiaceae	Herb	Perennial
47	Horsetail	*Equisetum spp. (hymenale, arvense)*	Whole plant	Wetlands, riversides, lakes, disturbed areas	Equisetaceae	Herb	Perennial
48	Mullein	*Verbascum thapsus*	Leaves, root	Disturbed areas, roadsides, forests	Scrophulariaceae	Herb	Perennial

Common Plant Uses

Food, Utilitarian, and Medicinal/Ceremonial Plants (continued)

	Common Name	Scientific Name *(If known)*	Portion of Plant Used	Geographic Area	Family	Habit	Perennial/Annual
49	Oregon grape	*Mahonia repens*	Root	Forests, riparian slopes, disturbed areas	Berberidaceae	Herb/shrub	Perennial
50	Osha	*Ligusticum porteri*	Root	Riparian slopes, forests	Apiaceae	Herb	Perennial
51	Red root	*Ceanothus fendleri*	Root	Forests, riparian slopes	Rhamnaceae	Shrub	Perennial
52	Snakeweed	*Gutierrezia sarothrae*	Leaves & flowers	Disturbed areas, forests, plains, high desert, washes	Asteraceae	Herb/shrub	Perennial
53	St. John's Wort	*Hypericum formosum*	Leaves & flowers	Wetlands, rivers, lakes	Clusiaceae	Herb	Perennial
54	Toadflax penstemon	*Penstemon linarioides*	Leaves & flowers	Forests, disturbed areas	Scrophulariaceae	Shrub	Perennial
55	Tobacco	*Nicotiana spp. (glauca, trigonophylla, attenuata)*	Leaves	Forests, disturbed areas, plains, washes, high desert	Solanaceae	Herb	Perennial
56	Valerian	*Valeriana spp. (edulis, arizonica)*	Root	Forests, wet areas, slopes, riparian areas	Valerianaceae	Herb	Perennial
57	Vervain	*Verbena spp. (macdougallii, bracteata)*	Leaves	Forests, disturbed areas, roadsides	Verbenaceae	Herb	Perennial
58	Yarrow	*Achillea millefolium var. occidentalis*	Leaves	Forests, disturbed areas, plains	Asteraceae	Herb	Perennial
59	Yellow dock	*Rumex crispus*	Root	Wetlands, rivers, lakes, disturbed areas	Polygonaceae	Herb	Perennial
60	Yerba santa	*Eriodictyon angustifolium*	Leaves	Forests, high desert, disturbed areas	Hydrophyllaceae	Shrub	Perennial

Potential sources of environmental toxins may include: mining, agricultural run-off, herbicides (especially along roadways), pesticides, illegal dumping, sewage contamination, hazardous waste spills, pollution, allelopathic chemicals from other plants, human waste, livestock waste, petroleum-based fertilizers and automobile exhaust.

APPENDIX G

MEETING AGENDA

ATSDR

Agency for Toxic Substances & Disease Registry
Division of Health Assessment & Consultation

Expert Panel Meeting: Tribal Exposures to Environmental Contaminants in Plants

Agenda

Meeting Chair: Donald Briskin
Meeting Facilitator: Candace Shelton

M O N D A Y , D E C E M B E R 4 , 2 0 0 0

8:00 AM	Registration	
8:30 AM	Welcome and opening prayer	*Austin Nuñez*
8:35 AM	Introductory statements	*Sharon Williams-Fleetwood*
8:45 AM	Purpose of meeting and introduction of panel participants	*Candace Shelton*
	Defining the problem and review of charge	*Donald Briskin*
	Exposure Scenarios and Traditional Plant Uses	
9:00 AM	Review lists of plants commonly used and activities that may result in exposure (via ingestion, dermal, and inhalation routes)	*Donald Briskin**
9:30 AM	Common types of plant uses by American Indians and Alaska Natives	*Donald Briskin**
10:15 AM	B R E A K	
10:30 AM	Use of non-vascular plants by American Indians and Alaska Natives	*Donald Briskin**
11:00 AM	Comparisons of the relative quantities of plant materials used	*Donald Briskin**
11:30 AM	Observer comments	
12:00 PM	L U N C H	
	Discussion of Factors that Influence the Uptake of Metal by Plants	
1:00 PM	Differences in uptake of metals across plant species	*Rufus Chaney**
1:45 PM	Important factors that affect plant uptake of metals	*Rufus Chaney**
2:30 PM	Estimating maximum concentrations in plants in the absence of sampling data	*Rufus Chaney**

*Discussion Leader

(over)

Discussion of Factors that Influence the Uptake of Metal by Plants (cont'd)

3:00 PM	Uniformity of distribution patterns of metals in plants across species . .	*Rufus Chaney**
3:45 PM	B R E A K	
4:00 PM	Discussion of available resources (e.g., published, unpublished, on-line) for referencing the various factors that impact plant uptake of metals	*Panelists*
4:30 PM	Important data gaps in the literature of plant uptake of metals	*Panelists*
5:00 PM	Observer comments	
5:30 PM	A D J O U R N	

T U E S D A Y , D E C E M B E R 5 , 2 0 0 0

Effects of Preparation and Preservation of Plant Materials on Exposure

8:00 AM	Review of lists of plant preparation methods and relative importance of preparation methods when evaluating exposure and dose	*Dolores Flores**
8:45 AM	Review of lists of food preserving methods and relative importance of food preservation methods when evaluating exposure dose	*Dolores Flores**
9:30 AM	Discussion of transformation of metals to more toxic forms during preparation and preservation of plant materials	*Donald Briskin**
10:15 AM	B R E A K	
10:30 AM	Use of plant materials for non-ingestion purposes and risk of exposure .	*Laurie Monti**
11:30 AM	Best available references and resources and identification of data gaps	*Panelists*
11:45 AM	Observer comments	
12:15 PM	L U N C H	

Additional Topics for Discussion

1:15 PM	Applicability of conclusions and recommendations presented in this meeting to non-metal contaminants in plants	*Laurie Monti**
2:15 PM	Guidance from panel members regarding additional resources for health assessors and issues of sensitivity regarding the traditional uses of plants	*Panelists*
3:00 PM	Observer comments	
3:30 PM	A D J O U R N	

* Discussion Leader

APPENDIX H

LIST OF REGISTERED OBSERVERS

Expert Panel Meeting: Tribal Exposures to Environmental Contaminants in Plants

List of Registered Observers

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APPENDIX I

"PARKING LOT" ISSUES

PARKING LOT ISSUES

1.*Sweat Lodges* - Interior temperatures up to 200 degrees. Potential dermal and inhalation exposures to contaminants in plant materials at these temperatures. Sage is burned in confined space for up to one and one-half hours. Also, sweetgrass and juniper is burned at the same time.

2.*Genetic Altering* - Recent visionary experiences by some traditional people reveal a time of aberrant life forms coming to earth, created by toxic wastes in our environment (Carol Locust).

3.*Regional Panels* - Include regional experts and elders and representatives from tribal colleges at future meetings (Brenda Brandon).

Examples:

Tribal Colleges - Native American Sciences by and for Native Americans.

Haskell Environmental Research Studies Center (HERS).

Plains Tribe (e.g., Oglala Lakota at Pine Ridge), issues include sage and selenium.

Montana (Salish Kootenai) and Stone Childs Fort Belknap), issues include sweet grass and organic pollutants.

California (Laytonville/Cahto), issues include angelica and metals.

Put mechanisms in place to work on this type of research ethicaly. Use HERS or other tribal college people.

- Community involvement is key to acceptance

- Clearly state purpose - to give alternatives, collection sites.

Cooperate with tribal colleges on culturally sensitive work - build tribal capacity.

Native American scientists are struggling to bridge the gaps between spiritual and science.

Our future includes Spiritual Advisors that are metals experts

Lets foster this and nurture it now.

This initiative includes intellectual recognition and financial support.

May need to include Indian Health Services expertise in certain study areas.

APPENDIX J

BIBLIOGRAPHY FOR HEAVY METALS IN/ON PLANTS
(Submitted by Craig McFarlane)

Bibliography for Heavy Metals in/on Plants

Chromium. Medical and Biologic Effects of Environmental Pollutants. National Academy of Sciences. Washington D.C. 1974

Arsenic. Medical and Biologic Effects of Environmental Pollutants. National Academy of Sciences. Washington D.C. 1977

Lead. Medical and Biologic Effects of Environmental Pollutants. National Academy of Sciences. Washington D.C. 1972

NUTRIENT DATA LABORATORY. USDA Food Composition Data. USDA Nutrient Database for Standard Reference, Release 13. http://www.nal.usda.gov/fnic/foodcomp/Data/index.html

Plants Database. Allows searching by common or scientific name. http://plants.usda.gov/

Brown, Dan 200. Animal Science 625 - Nutritional Toxicology. Spring 2000 Syllabus. The Nutritional Toxicology of Heavy Metals: Cadmium and Mercury. http://www.ansci.cornell.edu/courses/as625/1998term/Cadmium/cadmium.html

Office of Wastewater management

http://www.epa.gov/owm/

EPA rules for Biosolids (sewage sludge)

A Guide to the Biosolids Risk Assessments for the EPA Part 503 Rule

http://www.epa.gov/owm/bio/503rule/

A Plain English Guide to theEPA Part 503 Biosolids Rule

http://www.epa.gov/owm/bio/503pe/

Waste Management in Indian Country

http://www.epa.gov/tribalmsw/

Hazardous Waste Publications

http://www.epa.gov/tribalmsw/thirds/rehaz.htm

APPENDIX K

ANNOTATED BIBLIOGRAPHY OF SELENIUM PHYTOREMEDIATION

(Submitted by Rufus Chaney)

ANNOTATED BIBLIOGRAPHY OF Se-PHYTOREMEDIATION

Annotated Bibliography prepared by Rufus L. Chaney
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Last updated: February 25, 2001

The Primary accumulators commonly contain 1000-10,000 mg Se/kg (*Astragalus* (milkvetch), *Stanleya pinnata* (Prince's plume)(Cruciferae), *Oonopsis condensata* (Compositae); *Machaeranthera* (woody aster), *Brassica* (mustard). Secondary accumulators included *Atriplex* .

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60. Anderson, J.W. and A.R. Scarf. 1983. Selenium and plant metabolism. pp. 241-275. *In* D.A. Robb and W.S. Pierpoint (eds.) Metals and Micronutrients: Uptake and Utilization in Plants. Academic Press, New York.

61. Baker, D.C., L.F. James, W.J. Hartley and K.E. Panter. 1989. Toxicosis in pigs fed selenium-accumulating plant species or sodium selenate. Am. J. Vet. Res. 50:1396-1399.

62. Banuelos, G.S., G. Cardon, B. Mackey, J. Ben-Asher, L. Wu, P. Beuselinck, S. Akohoue and S. Zambrzuski. 1993. Boron and selenium removal in boron-laden soils by four sprinkler irrigated plant species. J. Environ. Qual. 22:786-792.

"High concentrations of B and Se found in some arid environments are detrimental to sustainable agriculture. Vegetation management may be a remediation strategy designed to reduce soil B and Se concentrations to non-toxic levels. Two separate field experiments were conducted to study B and se uptake in four different plant species grown in soil containing high concentrations of B (water-extractable B ranging from 1-10 mg/kg soil) and Se (total Se ranging from 0.1-1.2 mg/kg soil). The four species were *Brassica juncea* L. Czern and Coss (Indian Mustard), *Festuca arundinacea* Schreb cv. Fawn (tall fescue), *Lotus corniculatus* L. (birdsfoot trefoil), and *Hibiscus cannibinus* L. (Kenaf). In the 1990 experiment, there were no differences in either tissue B or Se concentrations among the species. The mean tissue concentration was 105 mg B/kg DM and 0.75 mg Se/kg DM, respectively. In the 1991 experiment, mean shoot tissue concentrations of B ranged from a low of 96 mg/kg in tall fescue to a high of 684 mg B/kg DM in leaves of kenaf. Indian mustard accumulated the greatest amount of Se (>1 mg Se/kg DM), while the mean tissue concentration among the other three species was 0.36 mg Se/kg DM. For both experiments, soil samples were taken prior to planting and after harvest for each species to a depth of 0 to 30 and 30 to 60 cm,

and analyzed for water-extractable B and total Se. Summary data from all species indicated that extractable soil B and total Se concentrations were reduced between 0-60 cm soil depth by 52 and 48% in 1990, and by 24-13% in 1991, respectively. Planting any of the four species tested in B-laden soils may lead to a reduction in both B and Se concentrations in the soil."

Presently examining both Se removal and volatilization by the plants and the soil.

63. Banuelos, G.S., G. Cardon, T. Pflaum and S. Akohoue. 1992. Comparison of wet digestion and dry ashing of plant samples for boron analysis. Commun. Soil Sci. Plant Anal. 23:17-70.

64. Banuelos, G.S., R. Mead and S. Akohoue. 1991. Adding selenium-enriched plant tissue to soil causes the accumulation of selenium in alfalfa. J. Plant Nutr. 14:701-713.

"Greenhouse experiments were conducted to determine selenium uptake by alfalfa grown in soils amended with Se-laden mustard plant tissue. The experimental design was a completely randomized block with treatments consisting of 5, 10, 20, and 40 g of added dried Se-containing mustard tissue to the soil, which resulted in soil Se concentrations of 1.0, 1.6, 3.0, and 5.7 mg Se/kg, respectively. Four clippings of alfalfa were made and the vegetative portions analyzed for dry weight and total Se. Plant dry weight yields and heights of plants were significantly reduced only at the highest Se treatment rate. Mean tissue Se concn. increased from 1.8 mg Se/kg at the 5 g treatment rate to 6.0 mg Se/kg DM at the 40 g treatment rate. Based on this study, alfalfa can accumulate Se during establishment year when Se-laden mustard plant tissue is added to soil."

65. Mead and G.J. Hoffman. 1993. Accumulation of selenium in wild mustard irrigated with agricultural effluent. Agric. Ecosyst. Environ. 43:119-126.

"Three field plantings were conducted from April to October 1989 on the western San Joaquin Valley in central California to evaluate the uptake of selenium (Se) and other ions in wild mustard (*Brassica juncea* L. czern.) when irrigated with saline drainage water (EC_i of 14-18 dS/m, with an average concentration of 154 Fg Se/L) or non-saline water (EC_i of 0.8 dS/m and < 3 Fg Se/L). Dry weight yields were 25% lower and Se and other element concentrations higher in plants irrigated with drainage than with non-saline water. Irrigating wild mustard with agricultural effluent increases the Se content in plant tissue."

Because they had reported that Indian mustard accumulated Se in a greenhouse study, they took the next step and conducted a field study to see how effective this species might be in accumulating Se under field conditions. They make no mention of the genotype of *Brassica juncea* they used in this study. Did the experiment on a Twisselman silty clay loam, 120 km SW of Fresno, CA. Irrigated with drainage water with high salinity vs. high quality irrigation water normally used. The drainage water was quite high in some constituents: 5828 vs. 184 mg SO_4/L; 11 vs. 0.8 mg B/L; and o.o vs. 0.2 mg Se/L. The soil was initially high in sulfate as well. They started the plants in the greenhouse and transplanted to field; then grew 42 days before harvest.

The Se levels in tissues of the Indian mustard on non-saline vs. saline water were: Old leaves, 0.4/2.3; young leaves, 0.6/2.8; stalks, 0.5/1.9; and roots, 0.3/1.9 mg/kg DW. B reached nearly 300 mg/kg, and sulfate near 4% with the drainage water. Chloride was also high in the old leaves.

66. Banuelos, G.S., R. Mead, L. Wu, P. Beuselinck and S. Akohoue. 1992. Differential selenium accumulation among forage plant species grown in soils amended with selenium-enriched plant tissue. J. Soil Water Conserv. 47:338-347.

67. Banuelos, G.S. and D.W. Meek. 1989. Selenium accumulation in selected vegetables. J. Plant Nutr. 12:1255-1272.

"Greenhouse experiments were conducted to determine selenium uptake by sulfur-accumulating vegetables. Cabbage (*Brassica oleraceae* var. *capitata*), broccoli *Brassica oleraceae* var. *botyrtis*), Swiss chard (*Beta vulgaris* var. *cicla*), and collards (*Brassica oleraceae* var. *acephela*) were grown in a soil mix to which 4.5 mg of selenate or selenite had been added per kg of soil. Plants were grown to maturity, separated into plant organs, and the tissues analyzed for Se and sulfate. Vegetables grown in selenate laden soil significantly ($P < 0.05$) accumulated higher concentrations of Se than plants grown in selenite laden soil. The highest concentrations of Se and SO_4 were found in the broccoli floret and vegetable leaf tissues. A second greenhouse experiment examined the uptake of Se and SO_4 in broccoli grown hydroponically with increasing Se concentrations. Treatments consisted of three Se concentrations (2, 6, and 15 mg of selenate, added as Na_2SeO_4/L to a synthetic water solution including SO_4. Solution samples were taken weekly and analyzed for Se and SO_4. The removal or uptake of both Se and SO_4 by broccoli was positively related ($P < 0.05$) with tie at each Se concentration. After 6 weeks in Se treatments, uptake responses of Se and SO_4 were significantly different ($P < 0.05$) based upon analyses of covariance. Composite leaf samples were also taken from the broccoli plants and analyzed for Se and SO_4. Selenium concentrations were negatively correlated ($P < 0.08$) with SO_4 concentrations in the leaf tissue."

68. Banuelos, G.S. and D.W. Meek. 1990. Selenium uptake by different species in selenium enriched soils. J. Environ. Qual. 19:772-777.

69. Banuelos, G.S., D.W. Meek and G.J. Hoffman. 1990. The influence of selenium, salinity, and boron on selenium uptake in wild mustard. Plant Soil 127:201-206.

70. Banuelos, G.S. and T. Pflaum. 1990. Determining selenium in plant tissue with optimal digestion conditions. Commun. Soil Sci. Plant Anal. 21:1717-1726.

71. Banuelos, G.S. and G. Schrale. 1989. Plants that remove selenium from soils. Calif. Agric. 43(3):19-20.

"Initial results from greenhouse experiments suggest that some plants are able to lower selenium concentrations in soils by up to 50%. Use of these plant species to reduce concentrations to acceptable levels in problem soils on the west side of the San Joaquin Valley may be economically feasible."

They did a pot experiment to examine species differences in Se uptake into shoots, and checked the mass balance for the added Se to estimate volatilization. Started with a non-Se-contaminated soil, and added 3.5 mg/kg to 1 kg pots of soil; mixed thoroughly with the soil. Used species from the 3 categories 1)Primary Se accumulators, 2) secondary Se accumulators, and 3) normal. The Primary accumulators commonly contain 1000-10,000 mg Se/kg (*Astragalus* (milkvetch), *Machaeranthera* (woody aster), *Brassica* (mustard), and *Stanleya* (prince's plume). Secondary accumulators included *Atriplex* . For the milkvetch, shoot Se was 75/880 for selenite vs. selenate; and 25% was lost in the experiment. For the black mustard, 85/890; 15% lost. For wild mustard, 400/2500 ppm Se with 6% lost. For Australian saltbush, 85/1640 ppm Se, 6% lost. For saltbush, 70/875 ppm Se; 24% lost. In group 3, tested tall fescue: 60/580 ppm, 18% lost.

Did a separate comparison of several leafy vegetable species with 5 ppm added selenate or selenite: Swiss chard leaves (selenite/selenate), 12/750 ppm Se; collards, 38/235; cabbage, 65/450 ppm Se; and Broccoli, 50/370 ppm Se in leaves.

Difficulties with the experiment -- 1) added soluble Se salts rather than taking a mineralized or contaminated soil from the field; 2) watered with deionized water which minimizes sulfate competition with uptake of selenate; 3) did pot experiment rather than field.

Discussed the concept of phytoremediation to remove Se from the soil and use it as livestock feed supplement. But his model was 5 crops of young plants of mustard per growing season. This might be a way to treat the irrigation wastewater to remove Se. Notes the possible difficulties with salts, sulfate, and boron toxicity to plants in these salty wastewaters. The

plants clearly absorbed selenate more effectively than selenite, probably due to adsorption of selenite more strongly than selenate. Did not consider the "conversion of inorganic Se to organic forms which have higher phytoavailability than salts.

72. Beath, O.A. .... 1934. Certain poisonous plants of Wyoming activated by selenium and their association with respect to soil types. J. Am. Pharm. Assoc. 23:94-?

73. Beath, O.A., H.F. Eppsom and C.S. Gilbert. 1937. Selenium distribution in and seasonal variation of type vegetation occurring on seleniferous soils. J. Am. Pharm. Assoc. 26:394-405.

1. A quantitative selenium assignment for a seleniferous range plant requires not only a knowledge of range associations but specific information about the plant itself. 2. Selenium compounds in seleniferous range plants are dominantly organic. 3. Selenium in native seleniferous range plants is readily soluble in water at room temperatures. In this form it is available to and definitely absorbed by farm crops and forages. Even when applied in relatively large amounts such crops are non-chlorotic. 4. Sulfur and soluble sulfates in the presence of organic selenium derived from native range plants and illustrated by *A. bisulcatus* have been found not to inhibit selenium absorption by type cereals and other farm crops. 5. In several type seleniferous farm crops the combined selenium was fund to be partially soluble in water at room temperatures. In most cases more than 50% could thus be isolated in hay, cereals, straw, and vegetables. 6. Aqueous extracts from seleniferous hay were, when added to a non-seleniferous soil, capable of supplying growing wheat plants with available selenium. 7. Cattle and sheep excrete appreciable quantities of selenium in the feces when fed seleniferous hay. 8. Elemental selenium added to a non-seleniferous soil resulted in seedling *A. bisulcatus* and *A. pectinatus* plants becoming seleniferous (1150 ppm) in three months' time. 9. The solubility of an inorganic selenite salt was found to be greatly altered when applied to a soil. 10. Soil samples from a soil profile in a critical seleniferous poison area were studied in detail and data submitted relative to the form and distribution of selenium *in situ*. 11. The roots of certain native range plants were found in some instances to carry more selenium than the corresponding above-ground portion. 12. The roots of seleniferous cereals and vegetables, in the cases examined, were found to be distinctly seleniferous.

Report the characteristics of Se in these systems. For one test of volatilization of Se during air-drying, they used a composite sample of *Astragalus racemosus* collected at pre-blood stage and analyzed green gave the following loss upon air drying: green = 14,920 ppm; air dry = 13,900 ppm Se.

74. Beath, O.A., C.S. Gilbert and H.F. Eppson. 1939a. The use of indicator plants in locating seleniferous areas in the western United States. I. General. Am. J. Bot. 26:257-269.

75. Beath, O.A., C.S. Gilbert and H.F. Eppson. 1939b. The use of indicator plants in locating seleniferous areas in the western United States. II. Correlation studies by states. Am. J. Bot. 26:296-315.

76. Beath, O.A., C.S. Gilbert and H.F. Eppson. 1940. The use of indicator plants in locating seleniferous areas in the western United States. III. Further studies. Am. J. Bot. 27:564-573.

77. Beath, O.A., C.S. Gilbert and H.F. Eppson. 1941. The use of indicator plants in locating seleniferous areas in the western United States. IV. A progress report. Am. J. Bot. 28:887-900.

78. Bell, P.F., D.R. Parker and A.L. Page. 1992. Contrasting selenate-sulfate interactions in selenium-accumulating and nonaccumulating plant species. Soil Sci. Soc. Am. J. 56:1818-1824.

"The shoots of primary Se-accumulating plant species can accrue Se to several thousand Fg/g dry weight, even when growing on gypsiferous soils, yet no detailed studies of the mutual antagonism between selenate and sulfate during plant uptake have been conducted with these species. In a comparative study, we grew a nonaccumulator (alfalfa, *Medicago sativa* L.) and a primary Se-accumulator (*Astragalus bisulcatus* [Hook.] Gray) in identical nutrient solutions with varied SeO_4 (2 to 80 FM) and SO_4 (0.5 to 15.5 mM) concentrations for 21 (alfalfa) or 32 to 35 d (*A. bisulcatus*). Shoot S concentrations in alfalfa were increased by increases in solution SeO_4, but only when shoot Se was above about 20 Fg/g, suggesting that SeO_4-induced stimulation of S uptake may be a result of incipient Se toxicity. Similar stimulations of S uptake were less apparent in *A. bisulcatus*. Alfalfa shoot Se concentrations ranged from 4 to 154 Fg/g, while the same treatments resulted in shoot levels of 175 to 1200 Fg/g in *A. bisulcatus*. Uptake of SeO_4 by alfalfa was profoundly inhibited by increases in solution SO_4, while Se uptake by *A. bisulcatus* was much less, although still significantly, affected. Comparison of molar Se/S ratios in plants and nutrient solutions indicated discrimination against Se by alfalfa, but preferential accumulation of Se by *A. bisulcatus*. A reevaluation of previously published results was in general agreement with our findings, and suggested that primary Se-accumulators have a unique ability to accumulate SeO_4 in the face of competition from SO_4. These species could thus prove useful in efforts to remediate Se-contaminated soils or sediments that are also enriched in So_4. Overall plant discrimination between SeO_4 and SO_4 may be related to (i) discrimination between the two analogues during initial absorption, and/or (ii) differential retranslocation of these elements to the root with subsequent efflux to the external solution."

Used chelator-buffered nutrient solutions. MES buffering. Had to add higher Fe to keep *A. bisulcatus* cultures; used EDTA-buffering. Interesting that this point had not been checked previously considering that the competition of selenate and sulfate had been carefully studied in normal plants.

79. Brown, T.A. and A. Shrift. 1981. Exclusion of selenium from proteins of selenium tolerant *Astragalus* species. Plant Physiol. 67:1051-1053.

80. Brown, T.A. and A. Shrift. 1982. Selenium: Toxicity and tolerance in higher plants. Biol. Rev. 57:59-84.

"1. Different plant species show considerable variation in their selenium content. Primary indicators, also termed selenium accumulators, many of which are members of the genus *Astragalus*, are highly tolerant of selenium; they are known to contain tissue levels of several thousand Fg selenium/g. Secondary indicators, tolerant to low concentrations of the element, may absorb up to 1000 Fg Se/g. Non-accumulators are poisoned by selenium. 2. The toxicity of selenate and selenite to most plants can be attributed to a combination of three factors. Firstly, selenate and selenite are readily absorbed from the soil by roots and translocated to other parts of the plant. Secondly, metabolic reactions convert these anions into organic forms of selenium. Thirdly, the organic selenium metabolites, which act as analogues of essential sulfur compounds, interfere with cellular biochemical reactions. 3. Incorporation into proteins of the amino acid analogues selenocysteine and selenomethionine, in place of the equivalent sulfur amino acids, is considered to be the underlying cause of selenium toxicity. The physical and chemical differences between selenium and sulfur will result in small, but significant, changes in the biological properties of a selenium-substituted protein. 4. Selenium-tolerant accumulator plants differ in at least two respects from sensitive species. Large quantities of Se-methyl-selenocysteine and selenocystathionine, two non-protein selenoamino acids rarely detected in non-accumulators, have been isolated from the tissues of selenium accumulators. In addition, selenium is kept from entering proteins so that the selenium levels in proteins of accumulator plants is significantly lower than the levels in selenium-sensitive plants. 5. Exclusion of selenium from the proteins of accumulators is thought to be the basis of selenium tolerance. Discrimination against selenocysteine during protein synthesis seems to prevent incorporation of this selenoamino acid into proteins of accumulators. Furthermore, synthesis of Se-methylselenocysteine and selenocystathionine, which results in diversion of selenium away from the synthesis of selenomethionine, will restrict the amount of this compound available for protein synthesis. 6. Selenium accumulation among unrelated plant genera is a striking example of convergent evolution. The possibility that accumulation of this element is associated with a nutritional requirement for selenium, although explored in the past, is still in need of further clarification."

81. Broyer, T.C., C.M. Johnson and R.P. Huston. 1972. Selenium and nutrition of *Astragalus*. II. Ionic sorption interactions among selenium, phosphate, and the macro- and micronutrient cations. Plant Soil. 36:651-669.

82. Cannon, H.L. 1952. The effect of uranium-vanadium deposits on the vegetation of the Colorado Plateau. Am. J. Sci. 250:735-7770.

83. Cannon, H.L. 1955. Description of indicator plants and methods of botanical prospecting for uranium deposits on the Colorado Plateau. *In*: Contributions to the geology of uranium. Bull. U.S. Goel. Surv. 1030:399-515.

"Two methods of botanical prospecting for uraniferous deposits on the Colorado Plateau are useful in semiarid country for prospecting for ore bearing beds at depths as much as 70 feet. By one method, tips of tree branches are sampled and analyzed for uranium content. Generally more than one part per million of uranium in ash indicates favourable ground for futher geologic exploration. The second method, that of mapping indicator plants, is used in senmi-arid parts of the Plateau at low altitude. The distribution of indicator plants is controlled by the availability of chemical constituents of the ore, such as selenium, sulfur and calcium. Plants of genus, Astragalus are most useful in prospecting for uranium deposits of high selenium content; plants of genera Allium and Eriogonum are most useful as indicators of deposits with a high sulfur content. Fifty indicator plants commonly associated with carnotite deposits and plants tolerant of mineralized ground are described and illustrated."

84. Cannon, H.L. 1960. The development of botanical methods of prospecting for uranium on the Colorado Plateau. Bull. U.S. Goel. Surv. 1085-A..

85. Cannon, H.L. 1960. Botanical prospecting for ore deposits. Science 132:591-598.

REVIEW. This paper is a long review in Science of the concept of using bioindicator plants, or plants with good soil:plant relationship for biogeochemical prospecting. Includes data on *Arabidopsis thalianum* found growing on bags of ZnO at Friedenville, PA. This plant is a "cress closely related to *Thlaspi* of Europe" which contained 9000 ppm Zn DW, or 60000 ppm Zn in the ash (leaf:ash=15%, or 6.7-fold higher). Paper otherwise contains little information about metal concentrations in plants, except for average metals in ash of plants growing on uncontaminated soils.

86. Cannon, H.L. 1971. The use of plant indicators in ground water surveys, geological mapping, and mineral prospecting. Taxon 20:227-256.

87. Cannon, H.L. and F.J. Kleinhampl. 1956. Botanical methods of prospecting for uranium. pp. 801-? *In* Proc. Int. Conf. Peaceful Uses Atomic Energy. United Nations, Geneva. Vol. 6. Paper A/CONF. 8/P/509.

88. Davis, A.M. 1972. Selenium accumulation in *Astragalus* species. Agron. J. 64:751-754.

"Some *Astragalus* species are known to be Se accumulators and are toxic to grazing animals. The Western Regional Plant Introduction Station, Pullman, WA, had 108 accessions representing 49 species of *Astragalus*, mostly of non-North American origin, available for this study. The Se accumulation characteristics of these had not been determined and therefore their

potential as forage in areas of seleniferous soil was open to question. Plants were greenhouse grown in soil containing 18 ppm Se. The forage was dried and analyzed for total Se by AOAC methods. Selenium content varied from a high of 213 ppm in *Astragalus tephrosoides* Boiss. to a low of no detectable Se in 45 accessions. Sixty accessions, representing 26 species, accumulated 5 ppm Se or more and could be poisonous to grazing animals."

The original separation of phenotype was done on the basis of germination in presence of Se on filter papers. The accumulators were tolerant, non-accumulators were not tolerant (Trelease). According to Beath, of the 29 groups (tribes) of *Astragalus*, only 6 tribes contain hyperaccumulators (*Bisulcati, Galegiformis, Lonchocarpa, Ocreati, Podoschiericarpi, and Preussii*).

The nature of Se accumulation by the introduced species of *Astragalus* was unknown, so he tested these accessions. 10 cm clay pots, with 500 g of soil. Used soil; dusted seeds with captan; allowed plants to become established before started adding Se to the soil. Added only selenate in this study; added total of 3 ppm Se, in 10 day increments until 18 ppm had been added. Shoot Se varied from 0 to 213 ppm. Three accession accumulated more than 100 ppm Se: *A. tephrosoides* (213 ppm), *A. icanus* (121 ppm), and *A. siliquosus* (123 ppm). Where multiple accessions of a species were studied, variation was considerable.

There were 3 North American species represented. *A. bisulcatus* grew poorly without added Se, but grew better and accumulated lots of Se when Se was added.

When he analyzed the soil for Se at the end of the growth period, found only 2 ppm Se when they were supposed to contain 18 ppm. Is that the first evidence of phytoremediation? Beath had noted that when they air dried the *A. bisulcatus*, compared to analyzing fresh leaves, a significant fraction was lost.

89. Davis, A.M. 1986. Selenium uptake in *Astragalus* and *Lupinus* species. Agron. J. 78:727-729.

"Forage quality potentials of plant introductions are unknown when introduced into the USA. Some native species of *Astragalus* are known to be Se accumulators and it is possible that some introductions could be accumulators also. This greenhouse study was undertaken to determine which, if any, of the untested 103 *Astragalus* and 68 *Lupinus* accessions were Se accumulators. Each accession was present in four replicates arranged in a randomized complete block design. Accessions were harvested at 24 weeks of age and the forage dried and analyzed. The AOAC extraction procedures were used and quantification was by colorimetric turbidimetric methods. Species of *Astragalus* accumulated Se in varying amounts from none to 61 mg/kg. None of the species of *Lupinus* accumulated Se at a potentially toxic level when grown in a soil mix (equal parts v/v; Shamel loam, quartz sand/commercial peat), that contained 18 mg Se/kg. These studies indicate that plants from 66 of the 103 *Astragalus* and none of the *Lupinus* accessions might accumulate toxic amounts of Se if grown on seleniferous soils. However, other unknown factors may make them unsuited for forage."

"Plants that accumulate Se do not form selenomethionine, and do not use it in the formation of proteins. While non-accumulators commonly put Se into protein amino acids.

Discuss the nature of Se poisoning of livestock from natural plants. The Se indicator plants, or Se toxic soil endemic tolerant plants, may accumulate high levels; from the field, Beath et al. collected *Astragalus bisulcatus* with 2590 ppm Se with only 20 ppm Se in the surface foot of soil. In normal plants, part of the Se is made into seleno-cysteine. Although toxicity is a concern where soil Se is high, at low soil Se, livestock suffer White Muscle Disease from Se deficiency. When they did this study, there were 131 species with 398 accessions of *Astragalus* in the USDA germplasm collection. He tested the part of the collection which had not been tested in 1972.

Used 20 cm pots; with a planting mix (1 kg) made from soil, sand, and peat. Se was not detected in this mix. He dusted the seeds with captan; used mechanically scarified seeds; thinned to 6 plants per pot. 25 mL of Hoagland solution were added to each pot at 6, 10, and 16 weeks of growth. Started adding Se when the plants were 8 weeks old; injected 5 mL with 0.002 g Se solution of Na selenate, and repeated the injection periodically until 18 mg Se were injected around inside the pot. The plants were harvested at 24 weeks; air dried; ground; wet ashed; and Se measured by colorimetric method.

The non-accumulator species of *Astragalus* contained 0-5 mg Se/kg dry wt., while the accumulator species contained from 2 to 61 ppm. Interestingly, when he compared different accessions of a species, found appreciable variation in Se accumulation. The following species were especially good Se accumulators: *A. adsurgens* var. *robustior*, 44 ppm; *A. asper*, 52 ppm; *A. bisculatus*, 43 ppm; *A. demetrii*, 47 ppm; *A. fraxinifolius*, 38 ppm; *A. galegiformis*, 41 ppm; *A. incanus*, 53 ppm; *A.*

scorpiurus, 39 ppm; *A. tephroisioides*, 61 ppm; and *A. vulpinus*, 39 ppm. The last species was represented by 7 accessions, and shoot Se varied from 7, 17, 13, 15, 15, 39, 19 ppm. The *Lupinus* species did not have detectable Se by this method.

90. Eustice, D.C., F.J. Kull and A. Shrift. 1981. In vitro incorporation of selenomethionine into protein by *Astragalus* polysomes. Plant Physiol. 67:1059-1060.

91. Evans, C.S., C.J. Asher and C.M. Johnson. 1968. Isolation of dimethyl diselenide and other volatile selenium compounds from *Astragalus racemosus* (Pursh.). Aust. J. Biol. Sci. 21:13-20.

"Volatile selenium compounds from intact *Astragalus racemosus* plants and from oven-drying tops or roots of the same species collected on activated charcoal and fractionated according to solubility in water or diethyl ether. The ether-soluble fraction contained two compounds which could be separated by gas-liquid chromatography. One of these compounds was shown to be dimethyl diselenide. The other compound has not been positively identified. The water-soluble fraction contained two as yet unidentified selenium compounds separable by anion-exchange column chromatography. The compounds appeared to be similar to, or identical with, the compounds isolated from lucerne in a previous study. Intact plants released the same four volatile selenium compounds as oven-drying tops or roots, but the yield of these compounds from oven-drying tops or roots was much greater than from intact plants."

Apparently this Se accumulator produced dimethyldiselenide rather than dimethylselenide which is produced by non-accumulator species such as cabbage. Used activated charcoal (reported by Lewis et al. to collect volatile Se compounds from plants), and gas chromatography. Showed the dimethyldiselenide quite readily.

92. Francis, A.J., J.M. Duxbury and M. Alexander. 1974. Evolution of dimethylselenide from soils. Appl. Microbiol. 28:248-250.

93. Frankenberger, W.T., Jr. and U. Karlson. 1989. Environmental factors affecting microbial production of dimethylselenide in a selenium-containing sediment. Soil Sci. Soc. Am. J. 53:1435-1442.

94. Gutenmann, W.H., C.A. Bache, W.D. Youngs and D.J. Lisk. 1975. Selenium in fly ash. Science 191:966-967.

"Selenium, at concentrations exceeding 200 ppm dry weight, has been found in white sweet clover voluntarily growing on beds of fly ash in central New York State. Guinea pigs fed such clover concentrated selenium in their tissues. The contents of the honey stomachs of bees foraging on this seleniferous clover contained negligible selenium. Mature vegetables cultured on 10% (by weight) fly ash amended soil absorbed up to 1 ppm of selenium. fly ashes from 21 states contained total selenium contents ranging from 1.2 to 16.5 ppm. Cabbage grown on soil containing 10% (by weight) of these fly ashes absorbed selenium (up to 3.7 ppm) in direct proportion (correlation coefficient r=0.89) to the selenium concentration in the respective fly ash. Water, aquatic weeds, algae, dragonfly nymphs, polliwogs, and tissues of bullheads and muskrats from a fly ash-contaminated pond contained concentrations of selenium markedly elevated over those of controls."

First collected yellow sweet clover, then later white sweet clover growing of fly ash landfills in NY State. The yellow sweet clover had 5.3 ppm Se in whole shoots, while the white sweet clover had 14 and 69 ppm Se; the ash had Se levels of 22.9 (Lansing) and 21 (Endwell) ppm, and were very deep, 4.6 and 23 m. The young growing shoot tips had much higher Se, up to 200 ppm. A farm pond had been built adjacent to the Lansing landfill in 1972, and was stocked with bullheads. Windblown fly ash from trucks contaminated the pond

compared to a control farm pond 16 km away. These data were an early demonstration of the bioaccumulation of Se in aquatic food chains.

95. Gutenmann, W.H. and D.J. Lisk. 1979. Absorption of selenium from coal fly ash-amended soil by *Astragalus racemosus*. Bull. Environ. Contam. Toxicol. 23:104-.

96. Gutenmann, W.H., I.S. Pakkala, D.J. Churey, W.C. Kelly and D.J. Lisk. 1979. Arsenic, boron, molybdenum, and selenium in successive cuttings of forage crops grown on fly ash amended soil. J. Agr. Food Chem. 27:1393-1395.

"Alfalfa, birdsfoot trefoil, brome, orchard grass, and timothy were grown on soil amended with 112.5 metric tons per hectare of coal fly ash and untreated soil. Five successive cuttings of each crop were analyzed for arsenic, boron, molybdenum, and selenium. Of the fly ash treated crops boron showed a consistent increase mainly in the legumes, while selenium increased mainly in the grasses. Molybdenum showed a consistent increase in all of the cuttings of all crops grown on fly ash. Arsenic increased mostly in the first cutting of the crops. Crop yields on the two treatments appeared comparable."

Because fly ash could supply B and Se, commonly deficient in soils, they followed the phytoavailability of the these elements in ash treated soils for 2 years (5 cuttings). At Ithaca, NY. Arkport fine sandy loam, pH 6.0 when started. Obtained fresh fly ash from Milliken Station, a coal-burning power plant in Lansing, NY, on Cayuga Lake. The pH of the fly ash was 5.0; the plant burns 2300 t coal/day, and produces 460 t fly ash/day. They added 5% fly ash to the soil (50 T/A or 112.5 t/ha). Soil/Ash contained: As, 8.8/23; B, 10/14; Mo, 2.2/2.9; and Se, 1.5/5.1 ppm. Un-replicated plots. After amendment, the soil pH fell to 5.9. Each plot got equal NPK fertilizer, 2.7 kg of 15-6.5-12.5% N-P-K. The plots were rotovated to obtain good mixing. The crops grown were 'Honeyoye' alfalfa, 'Empire' birdsfoot trefoil, 'Saratoga' brome, 'Penn Mead' orchardgrass, and 'Climax' timothy. The crops were planted in area of 1-x-3 m on each plot using a mechanical seeder in the spring of 1977. Each plot got 35 g of soluble 20-8.7-16.6% N-P-K fertilizer after each cutting. Irrigated in summer of 1978. They report the total concentration of each element in each cutting of each crop, for each plot. No statistical analysis except over all cuttings, for either legumes or grasses. As was increased significantly in first cuttings, but hardly different in subsequent cuttings. Boron was somewhat increased in the legumes, but not really after the first cutting of the grasses. Mo was increased a little in all crops, while Se was increased in the first cutting of the legumes, but much more in all cuttings of the forage grasses -- 2-5 fold. They advised that if the fly ash was to be used as a fertilizer for Mo, B, and Se, the composition of the fly ash and the crops would need to be monitored to make sure that other elements did not have large changes which required regulatory attention. Somewhat supportive, a remarkable position for Lisk et al.

97. Hamilton, J.W. and O.A. Beath. 1963. Selenium uptake and conversion by certain crop plants. Agron. J. 55:528-531.

"All plants studied possess the ability to absorb selenium from the soil. This Se is metabolized and stored in the plant tissue in sufficient quantities to render the plant material capable of producing toxic effects when eaten by animals. All plants and, in some instances, their seeds or grains contained both organic and inorganic Se. Flaxseed, safflower seed, and the root portion of sugar beets contained relatively low levels. Sunflower plants possessed the highest Se-absorbing ability."

Did a greenhouse study of Se accumulation by crop plant species [barley; buckwheat; dent corn; flax; flint corn; ladino clover; millet; oats; rape; red clover; rye; safflower; sorgo; sudangrass; sugar beets; sunflower; wheat]. Used fairly low soil Se as selenate to avoid phytotoxicity (< 5 ppm); had previously seen that even 20 ppm Se as organic Se species (incorporated powdered shoots of *Astragalus*) was not phytotoxic to higher plants. Interestingly, sunflower accumulated the highest Se levels among the species examined. No accumulator plants in this study. In this pot study, wheat grain reached over 100 ppm Se, dangerously high!

98. Hamilton, J.W. and O.A. Beath. 1963. Uptake of available selenium by certain range plants. J. Range Manage. 16:261-265.

99. Hamilton, J.W. and O.A. Beath. 1964. Amount and chemical form of selenium in vegetable plants. J. Agr. Food Chem. 12:371-374.

100. James, L.G., W.J. Hartley and K.R. Van Kampen. 1981. Syndromes of *Astragalus* poisoning in livestock. J. Am. Vet. Med. Assoc. 178:146-150.

101. Karlson, U. and W.T. Frankenberger, Jr. 1988. Determination of gaseous selenium-75 evolved from soil. Soil Sci. Soc. Am. J. 52:678-684.

102. Karlson, U. and W.T. Frankenberger, Jr. 1989. Accelerated rates of selenium volatilization from California soils. Soil Sci. Soc. Am. J. 53:749-753.

103. Karlson, U. and W.T. Frankenberger, Jr. 1990. Volatilization of selenium from agricultural evaporation pond sediments. Sci. Total Environ. 92:41-54.

104. Kleinhampl, F.J. 1962. Botanical prospecting for uranium on South Elk ridge, San Juan County, Utah. *In:* Botanical prospecting for uranium on the Colorado Plateau.Bull U.S. Geol. Surv. 1085-D:105-188.

105. Kleinhampl, F.J. and C. Koteff. 1962. Botanical prospecting for uranium in the Circle Cliffs area, Garfield County, Utah. *In:* Botanical prospecting for uranium on the Colorado Plateau.Bull U.S. Geol. Surv. 1085-D:85-104.

106. Kleinhampl, F.J. 1962. Botanical prospecting fro uranium on South Elk ridge, San Juan County, Utah. *In:* Botanical prospecting for uranium on the Colorado Plateau.Bull U.S. Geol. Surv. 1085-D:105-188.

107. Lewis, B.G. 1976. Selenium in biological systems, and pathways for its volatilization in higher plants. pp. 389-409. *In:* J.O. Nriagu (ed.) Environmental Biogeochemistry. Vol. 1: Carbon, Nitrogen, Phosphorus, Sulfur and Selenium Cycles. Ann Arbor Sci., Ann Arbor, MI.

108. Lewis, B.G., C.M. Johnson and T.C. Broyer. 1974. Volatile selenium in higher plants: The production of dimethyl selenide in cabbage leaves by enzymatic cleavage of Se-methyl selenomethionine selenonium salt. Plant Soil 40:107-118.

"The volatile selenium compound produced by cabbage (*Brassica oleracea* var. *capitata*) when cultured on media containing either selenite or selenate is dimethyl selenide, $(CH_3)_2Se$. The dimethyl selenide arises from enzymatic cleavage of a Se-methyl selenomethionine selenonium compound."

109. Mayland, H.F., L.J. James, K.E. Panter and J.L. Sonderegger. 1989. Selenium in seleniferous environments. pp. 15-50. *In:* L.W. Jacobs (ed.) Selenium in Agriculture and the Environment. SSSA Spec. Publ. 23. ASA, CSSA, SSSA, Madison, Wi.

110. Mikkelsen, R.L., F.T. Bingham and A.L. Page. 1989. Factors affecting selenium accumulation in agricultural crops. pp. 65-93. *In:* L.W. Jacobs (ed.) Selenium in Agriculture and the Environment. SSSA Spec. Publ. 23. ASA, CSSA, SSSA, Madison, Wi.

111. Mikkelsen, R.L., A.L. Page and F.T. Bingham. 1986. Geochemistry and health in California: Recent experiences with selenium. pp. 413-423. *In:* D.D. Hemphill (ed.) Trace Substances in Environmental Health, Vol. 20. Univ. Missouri, Columbia.

112. Mikkelsen, R.L., A.L. Page and G.H. Haghnia. 1988. Effect of salinity and its composition on the accumulation of selenium by alfalfa. Plant Soil 107:63-67.

SULFATE. ALFALFA. "Alfalfa (*Medicago sativa* L.) was grown in greenhouse sand culture to examine the effect of salinity composition and concentration on Se accumulation by plants. In a 2 x 2 x 4 factorial experiment, salinity was added as either chloride or sulfate salts to the irrigating solution to achieve an EC of 0.5, 1.5, 3.0, and 6.0 dS/m. Selenium was added to the nutrient solution at a concentration of 0.25 or 1.0 mg Se(VI)/L. Following the third cutting, the roots were washed and all plant material analyzed for dry weight and Se. Plant biomass production decreased with additions of Se or salinity, regardless of composition. In the presence of Se, the yield reduction was greater with Cl^-) salinity than with sulfate salinity. Plant Se accumulation was reduced from 948 ppm Se to 6 mg Se/kg in the presence of sulfate salts (0.5 mmol sulfate/L vs. 40 mmol sulfate/L) due to an apparent Se(VI)-SO_4 antagonism. This Se-SO_4 antagonism prevented accumulation of Se and reduced Se-induced toxicity. A lesser antagonistic effect on Se accumulation was observed between chloride and sulfate. A synergetic interaction between sulfate and selenate increased plant S concentrations in the presence of the relatively low basal sulfate concentrations but not at the higher solution sulfate concentrations. In many areas, soil and water containing high Se concentrations also contain large amounts of sulfate. The occurrence of sulfate with Se reduces plant accumulation of Se(VI) and may lower the risk of Se overexposure to animals feeding on forage material grown on the high selenate-sulfate regions."

11 L plastic pots for sand culture; in greenhouse. 100 L reservoir for bulk nutrient solutions with pH adjustment. Used 0.5 mM P, 1.25 mM Ca, 0.2 mM $MgSO_4$, 0.5 mM $Mg(NO_3)_2$; included 5 mg Fe/L as FeEDDHA. Kept between 6.5 and 7.

113. National Research Council. 1983. Selenium in nutrition. National Academy of Sciences, Washington, DC.

114. Ng, B.H. and J.W. Anderson. 1978. Synthesis of selenocysteine synthesases from selenium accumulator and non-accumulator plants. Phytochem. 17:2069-2074.

115. Nigam, S.N. and W.B. McConnell. 1976. Metabolism of $Na_2{}^{75}SeO_4$ in *Astragalus bisulcatus*, lima bean, and wheat: A comparative study. J. Exp. Bot. 27:565-571.

"A comparative study of the metabolism of $Na_2{}^{75}SeO_4$ in *Astragalus bisulcatus*, lima bean, and wheat has been carried out. The results indicate that all three plants metabolize selenium extensively. Important differences were observed in the distribution of radioactivity between the various fractions isolated from the plants. Compared to the protein fraction, the free amino acid fraction from *A. bisulcatus* contained a higher percentage of radioactivity. The converse was true for wheat and lima bean. As *A. bisculatus* proteins contained a significant percentage of radioactivity, it is suggested, that the differences in the toxicity of selenium towards wheat, lima bean and *A. bisulcatus* are difficult to explain in terms of the differences in its incorporation into the protein of the three species."

An early species comparison with radioisotope Se. But used the "cut stem" method to get label into the plants, so this is at best a comparison of metabolism under conditions where high uptake had occurred. Found that a higher fraction of Se went into proteins in wheat and lima bean, while more stayed in the soluble amino acid pool in the accumulator. Estimated volatilization by difference, and found nearly the same level of volatilization from all three species.

116. Nigam, S.N., J.-I. Tu and W.B. McConnell. 1969. Distribution of selenomethylselenocysteine and some other amino acids in species of *Astragalus*, with special reference to their distribution during the growth of *A. bisulcatus*. Phytochem. 8:1161-1165.

117. Ohlendorf, H.M. 1989. Bioaccumulation and effects of selenium in wildlife. pp. 133-177. *In:* L.W. Jacobs (ed.) Selenium in Agriculture and the Environment. SSSA Spec. Publ. 23. Soil Sci. Soc. Am., Madison, WI.

118. Ohlendorf, H.M., J.E. Oldfield, M.K. Sarka and T.W. Aldrich. 1986. Embryonic mortality and abnormalities of aquatic birds: Apparent impacts by selenium from irrigation drain water. Sci. Total Environ. 52:49-63.

119. Olson, O.E., E.J. Novacek, E.I. Whitehead and I.S. Palmer. 1970. Investigations on selenium in wheat. Phytochem. 9:1181-1188.

120. Olson, O.E. and A.L. Moxon. 1939. The availability, to crop plants, of different forms of selenium in the soil. Soil Sci. 47:305-311.

"Six soils from seleniferous farms were analyzed for various important constituents including total and water-soluble sulfur and total, water-soluble, acid-soluble, and organic selenium. Ten plantings were made on the soils in the greenhouse, and the plants were analyzed for selenium to determine the availability of the selenium in the six soils to various plants. The availability of the selenium in soils appears to be dependent upon the amount of water-soluble selenium, which in turn seems to be dependent upon or correlated with the amount of organic selenium in the soil. The total sulfur content and the water-soluble S content of a soil appear to be of little or no significance in determining the availability of selenium to plants in a naturally seleniferous soil. The selenium cycle and the forms of selenium in soils are discussed briefly."

Beath had suggested the model of the "Converter" plants. When the Se accumulator plants have produced organic Se forms, and die, these increase the organic Se in the soil organic matter pool. This Se is adsorbed quite differently from the inorganic Se species. Crop plants take up much lower levels of Se than the accumulator plants, but usually incorporate the Se into protein; while the accumulator plants put little of their Se into protein, thereby protecting themselves from the Se. To do this experiment, they collected soils from 10 farms reported to be seleniferous. They analyzed the soils for different Se

compounds and pools, and grew corn, wheat, oats and barley; were grown twice; then one planting of sorghum and one of mustard. When the plants got about 12 inches tall, they harvested the vegetative plants and started the next crop cycle. All the soils were calcareous, although not extremely so. Most were heavy textured. For the 6 soils used, the average shoot Se level was 16.4, 12.1, 0.15, 0.65, 3.2, and 2.5 ppm DW. For the first soil, the crop responses were: Wheat-1, 35.0; Wheat-2, 28.6; corn-1, 16.3; corn-2, 9.4; barley-1, 28.0; barley-2, 13.8; oats-1, 23.0; oats-2, 29.8; sorghum, 4.8; and mustard, 85.0. The vegetative tissues were analyzed.

Crop uptake of Se was related to water soluble Se, which in turn was related to organic Se. The data are not very firm, and no statistics were conducted at that time.

121. Panter, K.E., W.J. Hartley, L.F. James, H.F. Mayland, B.L. Stegelmeier and P.O. Kechele. 1996. Comparative toxicity of selenium from seleno-*DL*-methionine, sodium selenate, and *Astragalus bisculcatus* in pigs. Fund. Appl. Toxicol. 32:217-223.

"Selenium is an essential micronutrient, although ingestion in excess in pigs can cause disease conditions including neurological dysfunction and chronic skin and hoof lesions. Controlled feeding trials in growing swine, using the same Se content in feed sources, resulted in higher concentrations (*p* #0.05) of Se in blood and organs of pigs fed seleno-*DL*-methionine compared to those receiving *Astragalus bisculcatus* or sodium selenate. Clinical signs of Se toxicity including neurological signs of paralysis were more severe and occurred sooner in the *A. bisculcatus* group than in the sodium selenate or seleno-*DL*-methionine groups. All five pigs fed *A. bisculcatus* developed neurological signs of paralysis, and in four the signs occurred within 5 days of the start of treatment. Four of five pigs fed sodium selenate also developed paralysis, but this occurred 4 to 21 days after treatment began. The firth pig in the group developed signs of chronic selenosis. Two of five pigs fed seleno-*DL*-methionine developed paralysis on 9 and 24 days, respectively, and the remaining three developed chronic selenosis. Selenium fed to pigs in three forms [plant (*A. bisculcatus*), sodium selenate, or seleno-DL-methionine] resulted in neurological dysfunction and lesions of symmetrical poliomyelomalacia. These were most severe in the *A. bisculcatus* group, which also had polioencaphalomalacia. Although seleno-DL-methionine caused the greater increase in tissue and blood Se concentrations, this did not correlate with severity of pathological changes, since animals fed *A. bisculcatus* developed more severe and disseminated lesions."

Different *Astragalus* species have different toxic factors, including nitro-containing *A. miser*, swainsonine-containing *A. lentiginosus*, locoweeds) and the Se-containing *A. bisculcatus*. The control diet contained 0.4 mg Se/kg; 25 mg Se/kg was added to this basal diet in the form of the three test materials, and fed up to 6 weeks. The *Astragalus bisculcatus* contained 300 mg Se/kg DW. This species contains water soluble (non-protein) forms of Se rather than selenocysteine found in crop plants proteins when high Se is provided to crop plants.

122. Parker, D.R., A.L. Page and D.N. Thomason. 1991. Salinity and boron tolerances of candidate plants for the removal of selenium from soils. J. Environ. Qual. 20:157-164.

"Agricultural drainage water from the west side of the San Joaquin Valley, CA is highly salinized, and is often contaminated with an assortment of metals and metalloids, including Se. Among proposed disposal options, vegetation management may be a critical component of remediation strategies designed to reduce soil or sediment concentrations of Se to safe levels. Soil salinity (mostly sodium sulfate) and B pose serious limitations to the use of many plant species. We screened a number of cultivars or lines of species from the genera *Astragalus, Leucaena, Medicago, Trifolium, Elymus, Elytrigia, Festuca, Leymus, Oryzopis, Psathyrostachys, Puccinellia, and Sporobolus* for tolerance to salinity and B using solution culture methods. Considerable variation in tolerance to salinity, both within and across species, was observed during seed germination. electrical conductivities required to produce a 50% reduction in germination (EC_{50}) ranged from 5 to 30 dS/m. Boron levels up to 4.0 mM had only minimal effects on germination. The most promising genotypes, representing some 15 species, were then tested for salinity and B tolerance during the seedling growth stage. Lines of five species (*Astragalus bisculatus, A. racemosus, Elytrigia pontica, Puccinellia distans, and Sporobolus airoides*) appeared most promising; all exhibited EC_{50} values > 20 dS/m and were unaffected by B concentrations up to 4.0 mM during seedling growth. *Astragalus bisculatus* and *A. racemosus* are considered primary accumulators of Se; their tolerance of high salinity and B during seedling growth make them particularly good candidates for remediation of Se-enriched soils and sediments."

123. Pate, J.S. 1983. Patterns of nitrogen metabolism in higher plants and their ecological significance. pp. 225-255. *In:* J.A. Lee, S. McNeill and I.H. Rorison (eds.) Nitrogen as an Ecological Factor. Blackwell Scientific Publ., Oxford.

Supposed to have a comment that the high Se in Se accumulating legumes may limit their predation by chewing insects. From Marschner, 1995.

124. Peterson, P.J. and G.W. Butler. 1962. The uptake and assimilation of selenite by higher plants. Aust. J. Biol. Sci. 15:126-146.

125. Presser, T.S. and H.M. Ohlendorf. 1987. Biogeochemical cycling of selenium in the San Joaquin Valley, California, USA. Environ. Manage. 11:805-821.

126. Reamer, D.C. and W.H. Zoller. 1980. Selenium biomethylation products from soil and sewage sludge. Science 208:500-502.

"Inorganic Se compounds are converted to volatile methylated species (dimethyl selenide, dimethyl diselenide, and dimethyl selenone or methyl methylselenite) by microorganisms in sewage sludge and soil. In the absence of added Se, no volatile Se compounds were detected. All samples were evaluated without the addition of nutrients and in the presence of air or nitrogen. The methylation process may be an important step in the detoxification process for microorganisms exposed to high concentrations of Se."

Only got volatile Se when added selenite or Se^0 to soil or sludge. Sludge contained 3.2 Fg Se/g DW. Addition of 1 ppm to 1000 ppm supported release of much volatile Se. At low addition, only Me_2Se was formed, but with higher addition (10-1000 ppm), got increasing amounts of Me_2Se_2 and the selenone. In most systems, the Me_2Se would predominate. Se^0 was a much poorer substrate than selenite. Used resin to collect the volatile Se compounds during culture in a respirometer flask. Some selenite was reduced to Se^0 in the media, as shown by accumulation of red-brown deposits. Aerobic environment produced more volatile Se than anaerobic environment.

127. Rosenfeld, I. and O.A. Beath. 1964. Selenium: Geobotany, Biochemistry, Toxicity and Nutrition. Academic Press, New York.

128. Shaw, W.H. and J.W. Anderson. 1974. Comparative enzymology of the adenosine triphosphate sulfurlases from leaf tissue of selenium-accumulator and non-accumulator plants. Biochem. J. 139:37-42.

129. Shrift, A. 1969. Aspects of selenium metabolism in higher plants. Annu. Rev. Plant Physiol. 20:475-494.

130. Shrift, A. and J.M. Ulrich. 1969. Transport of selenate and selenite into *Astragalus* roots. Plant Physiol. 44:893-896.

"After incubation for 1 hr with ^{75}Se-selenate, excised roots of *Astragalus crotalariae*, a selenium-accumulating species and *A. lentiginosus*, a nonaccumulator, had absorbed radioactivity to levels well over the external concentrations. About 98% of the radioactivity was ethanol-soluble, and when analyzed by column and paper chromatography and by electrophoresis proved to selenate. This and previous evidence shows an active transport for selenate. Considerably less radioactivity was absorbed when ^{75}Se-selenite was supplied to the excised roots, and the levels of the ethanol-soluble radioactivity did not exceed the external concentration. A good deal of the radioactivity was ethanol-insoluble. Analysis of the soluble radioactivity from both species showed appreciable conversion of selenite to other forms."

131. Shrift, A. and T.K. Virupaksha. 1963. Biosynthesis of Se-methylselenocysteine from selenite in selenium-accumulating plants. Biochim. Biophys. Acta 71:483-485.

"No abstract." Research had shown that the accumulators converted Se to soluble organic forms, while the others put amino acids with Se into proteins. Trelease et al. (1960) isolated Se-methylselenocysteine from an extract of dried leaves of *A. bisulcatus*. Shrift studied Se species formed when $^{75}SeO_3$ was supplied to *A. crotalariae* and *Oonopsis condensata*, both well known Se accumulators. Examined the plant extracts for Se compounds using chromatography and electrophoresis. Had authentic Se-methyl-selenocysteine for comparison. The radioactive compound of se in both these species was the Se-methylselenocysteine. Used a number of different separation systems with same result. Tested other compounds to see whether they would co-chromatograph or co-electrophorese. When looked at methyl-cysteine, found that the Se analog would co-crystallize with the sulfur analog! Thus, 3 accumulators all formed the Se-methylselenocysteine. Looked at one non-accumulator species of *Astragalus*, *A. canadensis*, and found quite different Se compounds, in particular cysteine and methionine.

132. Shrift, A. and T.K. Virupaksha. 1965. Seleno-amino acids in selenium-accumulating plants. Biochim. Biophys. Acta 100:65-75.

"The biosynthesis of organic selenium compounds has been investigated in *Stanleya pinnata* (Cruciferae), *Oonopsis condensata* (Compositae) and six species of *Astragalus* (Leguminosae), plants that are known to accumulate selenium. Excised leaves were supplied with $^{75}SeO_4$, $^{75}SeO_3$, or $^{35}SO_4$. Analysis of the trichloroacetic acid soluble components revealed that selenate was less efficiently utilized than was selenite. The major soluble organic selenium compound in all plants was Se-methylselenocysteine (CH_3-Se-CH_2-$CHNH_2$-COOH). *Stanleya pinnata* also synthesized appreciable amounts of selenocystathionine (HOOC-$CHNH_2$-Ch_2-CH_2Se-CH_2-CH_2CHNH_2COOH). Small amounts of a compound tentatively identified as Se-methylselenomethionine () and of a selenium-containing peptide were found in several of the accumulators. Plants supplied with $^{35}SO_4$ made approximately equal mounts of S-methylcysteine and glutathione. The absence of selenoglutathione in several of the plants which biosynthesized glutathione was noted, suggesting that sulfur and selenium may not entirely follow the same pathway in these particular plants."

133. Soltanpour, P.N. and A.M. Workman. 1984. Use of NH_4HCO_3-DTPA soil test to assess availability and toxicity of selenium to alfalfa plants. Commun. Soil Sci. Plant Anal. 11:1147-1156.

134. Terry, N., C. Carlson, T.K.Raab and A.M. Zayed. 1992. Rates of selenium volatilization among crop species. J. Environ. Qual. 21:341-344.

"The rate of volatilization per plant was measured for 15 crop species grown hydroponically (for 18-48 days) depending on the species) in growth chambers. Selenium was supplied as 20 FM Na selenate in 0.25 Hoagland's solutions. Selenium volatilization was determined by enclosing plants in a Plexiglas plant chamber and trapping the volatile Se emissions in alkaline peroxide traps. The results show that rice, broccoli, and cabbage volatilized Se at the fastest rates, i.e. 200 to 350 Fg Se/m^2 leaf area per day (150-2500 Fg Se/kg plant dry matter/day). Carrot, barley, alfalfa, tomato, cucumber, cotton, eggplant, and maize had intermediate rates of 30-100 Fg Se/m^2/day (300-750 Fg Se/kg DM/day). Sugarbeet, bean, lettuce and onion had the lowest rates, i.e., less than 15 Fg Se/m^2/day (<250 Fg Se/kg DM/day). Comparing all plant species, Se volatilization rate was found to be highly correlated with Se concentration in plant tissues; we suggest that the ability of plants to absorb Se may be an important factor contributing to high rates of Se volatilization."

Note the potential importance of sulfate competition with selenate uptake by a particular species may be controlling in the actual rate of emission in the field. Because the source of contamination they are interested in is the drainage water, high sulfate accompanies the selenate.

Among the highest phytovolatilization of Se rates was by broccoli at 1.3 mg Se/d/m^2 = 13 g/day/ha = 4.8 kg/hayr.

135. Trelease, S.F. 1942. Identification of selenium indicator species of *Astragalus* by germination tests. Science 95:656-657.

136. Trelease, S.F. 1945. Selenium in soils, plants, and animals. Soil Sci. 60:125-131.

137. Trelease, S.F., A.A. DiSomma and A.L. Jacobs. 1960. Seleno-amino acid found in *Astragalus bisulcatus*. Science 132:618.

"Ion-exchange and filter-paper columns were used in a separation of amino acids from an extract of *Astragalus bisulcatus*. Two amino acids were identified, S-methylcysteine and Se-methylselenocysteine."

Used available separation techniques, trying to separate Se and S amino acids. Were successful. First found that the Se in accumulator plants was Se-methylselenocysteine, a soluble but non-protein amino acid. The common name for *A. bisulcatus* is two-grooved milk vetch.

138. Trelease, S.F. and H.M. Trelease. 1938. Selenium as a stimulating and possibly essential element for indicator plants. Am. J. Bot. 25:372-380.

139. Trelease, S.F. and H.M. Trelease. 1939. Physiological differentiation in *Astragalus* with reference to selenium. Am. J. Bot. 26:530-535.

REF-VER/Copy [Se in Soil/Plants: Trelease et al.] "*Astragalus racemosus*, growing in solution and sand cultures, was greatly stimulated by selenium (as selenite) in concentrations from 0.33 to 9 ppm; these tests confirm earlier experiments in suggesting that selenium may be an essential microtrophic element for this species of *Astragalus*. In contrast, *Astragalus crassicarpus* was not stimulated; it was instead poisoned by selenium, being severely injured by a concentration as low as 0.33 ppm (as selenite). *Astragalus racemosus*, having a higher tolerance than *Astragalus crassicarpus* to selenium, was able to accumulate correspondingly higher concentrations of this element from solutions containing selenite. The greenhouse tests of growth in artificial media confirm field observations in showing a physiological differentiation of *Astragalus* species into two groups, those which seem to require selenium for their development, and so serve as indicators of seleniferous soil areas, and those which do not utilize selenium."

Compared two species they had studied before. Grew them in nutrient solution and in sand culture; contained 0.72 mM P, Fe-tartrate, Mn, B, Cu, Zn, Si, Al, I, Ni, Na, Li, As, and Co. Used Na-selenite. As selenite concentration increased, yield of accumulator species increased, while yield of the non-accumulator species declined to near 0. In the solution culture experiment, the Se concentration in the plant shoots were not all that different. In the sand culture, Yield was increased for *A. racemosus*, from 9.4 to 26.4 g/pot, and contained 12 vs. 1090 ppm Se for 0 and 9 mg Se/L; for *A. crassicarpus*, used 0 vs. 9 ppm first 12 days, and then 0 vs. 1 ppm; yields were 8.44 and 1.13 g/pot, and the Se in shoots was 4 vs. 141 ppm. Considering differences in concentrations, not very different responses! May not have been the same for selenate as found for selenite; and selenate is the normal form mobile in soils and absorbed by roots.

In the field, *Astragalus racemosus* shows no toxic symptoms of Se even when it contains very high Se levels. Beath, Gilbert and Eppsom (1937) collected a plant with 15,000 mg Se/kg. Maybe these species can accumulate higher Se from the organic Se normally found in the really Se-toxic soils. In a previous test by Trelease and Trelease, the plants had somewhat reduced yield when the shoot Se contained over 2000 ppm Se, again from a selenite source.

They then reviewed information about the unusual response of *A. racemosus*; plants have been collected in the field with from < 10 to 15,000 ppm Se; median level about 100 ppm, but a bunch of samples at > 1000 ppm Se. The low Se levels may occur in old plants or leached old leaves, while young plant tissues from the same area always had Se. They did not make a annual cycling study of Se in these plants in the field.

Because the Se accumulators convert inorganic Se into organic Se compounds and build up phytoavailable Se in soils, they convert a non-toxic soil into a toxic soil because all plant species can absorb the Se from the residues of the Se accumulator plants.

140. Ulrich, J.M. and A. Shrift. 1968. Selenium absorption by excised *Astragalus* roots. Plant Physiol. 43:14-20.

"Absorption of selenate and selenite by excised roots of *Astragalus crotalariae*, and selenium accumulator, and *A. lentiginosus*, a non-accumulator, was favored by $CaCl_2$ and a pH of 4.0. The uptake of selenate and possibly selenite, is metabolically linked. Roots of a number of *Astragalus* species were examined, and in all cases selenate entered the roots much faster than selenite. In these short-term experiments there was no relation between uptake of the two ions and classification of a species as selenium-accumulator or non-accumulator."

141. Virupaksha, T.K. and A. Shrift. 1963. Biosynthesis of selenocystathionine from selenate in *Stanleya pinnata*. Biochim. Biophys. Acta 64:791-793.

142. Virupaksha, I.K. and A. Shrift. 1965. Biochemical differences between selenium accumulator and non-accumulator species. Biochim. Biophys. Acta 107:69-80.

"The predominant soluble organic selenium compounds synthesized from $^{75}SeO_3$ and $^{75}SeO_4$ by excised leaves of four species of *Astragalus* (Leguminosea), chosen because of their inability to accumulate selenium, was Se-methyl[^{75}Se]selenomethionine. Three of the species also synthesized smaller amounts of Se-methyl[^{75}Se]selenocysteine, which could not be detected, however, in a fourth species. Appreciable amounts of a peptide which contained selenium were found in each of these species. The results are compared with previous findings which showed that the predominant seleno-amino acid in selenium accumulator species of *Astragalus* was Se-methyl[^{75}Se-selenocysteine], whereas Se-methyl-[^{75}Se]-selenomethionine either could not be detected or occurred only in traces. The ability to synthesize Se-methyl-selenomethionine from selenite or selenate is, therefore, suggested as a biochemical basis for distinguishing non-accumulator from accumulator species of *Astragalus*. Comparable studies on the assimilation of $^{35}SO_4$ by two of the non-accumulator species showed that most of the ^{35}S occurred in glutathione and S-methyl-methionine. S-methyl-[^{35}S]-cysteine could not be detected in either species, one of which had, however, synthesized Se-methyl-[^{75}]-selenocysteine from $^{75}SeO_3$. Differences in the metabolism of sulfur and selenium had also been observed previously in accumulator species of *Astragalus*."

Used excised leaves fed isotope through the cut stem! Otherwise used best techniques at the time. If one had to identify the key difference between these accumulator/tolerant and non-accumulator/sensitive species, it would be the very low formation of Se-methyl-selenomethionine in the accumulator species.

143. Yang, G., S. Wang, R. Zhou and S. Sun. 1983. Endemic selenium intoxication of humans in China. Am. J. Clin. Nutr. 37:872-881.

"An endemic disease was discovered in 1961 in parts of the population of Enshi County, Hubei Province on the People's Republic of China. During the years of the highest prevalence, from 1961 to 1964, the morbidity was almost 50% in the 248 inhabitants of the five most heavily affected villages; its cause was determined to be selenium intoxication. The most common sign of the poisoning was loss of hair and nails. In areas of high incidence, lesions of the skin, nervous system, and possibly teeth may have been involved. A case is reported of a middle-aged female hemiplegic, whose illness and death apparently were related to selenosis. Daily dietary intakes of Se, estimated after the peak prevalence had subsided, averaged 4.99 (range 3.20 to 6.69) mg, and hair and blood Se averaged 32.2 and 3.2 Fg/mL, respectively. Up to 1000x differences occurred when selenium contents of vegetables, cereals, scalp hair, blood, and urine from the selenosis areas were compared with those from Keshan disease (Se deficiency) areas. The ultimate environmental source of Se was a stony coal of very high Se content (average more than 300 Fg/g; one sample exceeded 80,000 Fg/g). Se from the coal entered the soil by weathering and was available for uptake by crops because of the traditional use of lime as fertilizer in that region. This particular outbreak of human selenosis was due to a drought that caused failure of the rice crop, forcing the villagers to eat more high selenium vegetables and maize and fewer protein foods."

Turnip greens were very high in Se, 400 ppm, compared to 1/10 as high in other vegetables. Over 10000 times higher than in deficient village. Farm families consumed much turnip greens. In the endemic area, corn had 8.1, rice, 4.0, and soybean, 11.9 ppm Se! These levels are over 200 times higher than in normal crops. Cereals comprised 28-70% of total Se intake. Intakes were 3.2-6.69 mg/day, averaging 4.99 mg/day; high Se area without selenosis had 0.24-1.51, mean 0.75 mg Se/day; while normal area had 0.042-0.232 mg/day, mean 0.116. Deficient area had 0.011 mg/day. Drought stopped normal production of rice, and villagers relied more on corn and vegetables, which accumulated much higher levels of Se. Claim villagers often apply limestone, which would raise pH of area. Plant ash (from heating?) often returned to soils as well. Local pH not specifically reported. Corn had a pink coloration at the tip of the embryo, shown to be Se^0.

Soil from the Enshi, Hubei area contained 7.87, of which 0.35 ppm was water soluble. Non-endemic areas had soil Se=0.32 ppm with 0.011 to 0.04 ppm water soluble. Surface water contained significant amounts of Se, and may have contributed somewhat to the excessive Se intakes. Livestock had "alkali disease" or Se poisoning; pigs had often. Eggs were not hatchable, and if hatched, got beakless chicks! Exposures here were appreciably higher than in SD , NE, and Wyoming, and blood Se followed estimated exposures. They note information that selenium is much more poisonous than is organic-Se in foods, perhaps 5-fold.

"The present agricultural policy encourages the peasants to produce many more kinds of crops and exchange foods at the market."

144. Zayed, A.M. and N. Terry. 1992. Selenium volatilization in broccoli as influenced by sulfate supply. J. Plant Physiol. 140:646-652.

"The influence of sulfate supply on the rate of selenium volatilization is broccoli (*Brassica oleraceae* var. *botrytis* cv. Green Valiant) was investigated. Plants were cultured hydroponically in growth chambers. Sulfur was supplied at five different concentrations: 0.25, 0.5, 1.0, 5.0, and 10.0 mM in half-Hoagland's solution. All treatments received 20 FM Na_2SeO_4. Measurements were made of the rate of Se volatilization per plant, Se and S concentrations in root, stem, and leaf blades, and of plant dry weight and leaf area. Each increase in sulfate level from 0.25 to 5 mM caused a progressive decrease in the daily rate of Se volatilization which decreased from 96.7 (at 0.25 mM) to 13.8 (at 10 mM) Fg Se/square meter of leaf surface. The concentrations of Se in plant tissues (stem, leaf, and root) responded differently to increased sulfate level than did Se volatilization rate: tissue Se concentration did not change with increase in sulfate from 0.25 to 1 mM and decreased only at the higher sulfate levels, 5 and 10 mM. The step-wise decrease in Se volatilization with each increase in sulfate was however, correlated strongly with a step-wise decrease in the ratio of Se:S in plant tissues. These results suggest, that with increasing sulfate supply, sulfate in plant tissues increasingly competed with selenate for the enzymes of the S-assimilation pathway; this internal competition most likely led to a decreased production of selenoamino acids, especially selenomethionine (required for the production of the volatile form of Se, dimethylselenide), thereby reducing S volatilization rate."

145. Zayed, A.M. and N. Terry. 1994. Selenium volatilization in roots and shoots: Effects of shoot removal and sulfate level. J. Plant Physiol. 143:8-14.

"Broccoli plants were grown hydroponically in growth chambers with 20 FM Se supplied as selenate. The separate contributions of root and shoot to the volatilization of Se by plants supplied with six different levels of sulfate (ranging from 0 to 10 mM) in half-Hoagland's nutrient solution were determined. Most of the Se volatilized by broccoli plants was from the roots which volatilized about 26 times faster than the rate of shoots. The removal of the shoot markedly increased the amount of Se volatilized over the following 72 hr, the detopped rot attaining rates that were 20 to 30 times the rate of the intact root. Comparable results were also obtained for five additional species, rice, cabbage, cauliflower, chinese mustard, and wild brown mustard (*Brassica juncea*). Part of the volatilization of Se by plants may involve microbes, i.e., bacteria. This is indicated by the fact that when prokaryotic antibiotics were added to the nutrient solution, the total rate of Se volatilization by root (broccoli) and nutrient solution was significantly decreased, much more than could be accounted for by the loss of microbial volatilization from the nutrient solution alone."

Previous work had indicated that shoots emitted more of the Se than roots, but may have suffered experimental errors. They grew 'Green Valiant' broccoli at 25E; 2.5 mM Ca nitrate; 1.0 mM KH_2PO_4, 3 mM KNO_3; 1 mM $MgSO_4$, 0.5 mM NaCl; 23.1 uM B, 4.6 Mn, 0.38 Zn, 0.16 Cu, 0.052 Mo, and 44.8 FM FeEDTA. $MgSO_4$ varied to vary sulfate. All treatments got 10 FM Na_2SeO_4. Volatile Se was collected with a scrubber solution.

146. Zieve, R. and P.J. Peterson. 1981. Factors influencing the volatilization of selenium from soil. Sci. Total Environ. 19:277-284.

147. Zieve, R. and P.J. Peterson. 1984. The accumulation and assimilation of dimethylselenide by four plant species. Planta 160:180-184.

"Plants of *Agrostis tenuis* Sibth., *Hordeum vulgare* L., *Lycopersicon esculentum* Mill. and *Raphanus sativus* L. were grown hydroponically in sealed systems and fumigated with 8 Fg/m^3 [^{75}Se]-dimethylselenide. The accumulation of ^{75}Se was measured and the shoot tissues were extracted to examine the products of ^{75}Se assimilation. Characteristic differences were observed between species in the accumulation of ^{75}Se and the transport from shoots to roots. High-voltage electrophoresis and chromatography of extracts made with 80% aqueous ethanol revealed the presence of inorganic selenite as an assimilation product as well as the selenium analogues of glutathione and methionine. Extensive incorporation of ^{75}Se into protein-bound selenomethionine was observed in all plant species."

Very straight forward study now understood to be the way the world works. They got to it sooner than others.