APPENDIX C

Pre-Meeting Comments

ATSDR
AGENCY FOR TOXIC SUBSTANCES
AND DISEASE REGISTRY

Hair Analysis Panel Discussion: Exploring the State of the Science

Pre-Meeting Comments

June 6, 2001

Notice

This booklet includes the panelists’ pre-meeting responses to the charge questions. It should be noted that the pre-meeting comments are preliminary in nature. The purpose of these comments is to stimulate meeting discussions. Some panelists’ technical findings might change based on discussions during the meeting; therefore, pre-meeting comments should not necessarily be considered the panelists' final opinions.

Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Note: Comments have been printed as received

LuAnn White, Chair

LuAnn White

Overview/Perspective from the Panel Chair

The overriding question for the use of hair analysis in environmental public health is the need to find reliable methods for assessing chemical exposure of people living in communities near hazardous waste sites. Hair sampling is tantalizing because it is a biological material that is readily available, noninvasive, and easy to collect. However, much controversy exists regarding the use of hair samples as an indicator for environmental exposure, health status, or disease state. The use and misuse of results from hair sampling has stimulated debate and at times, cast a shadow over the issue.

Complex questions linger regarding three overarching issues: 1) accuracy and reliability because of laboratory methods; 2) toxicokinetics of compounds and the biological variability among individuals; and 3) the relationship of the results to exposure and/or potential disease. Within each of these issues, multiple questions arise that include, but are not limited to, the reliability and reproducibility of the analytical methods; interlaboratory variability; types of compounds suitable for hair analysis; baseline of elements and compounds found in hair—for an individual and/or populations; influence of distribution, metabolism, storage and excretion on incorporation of compounds and elements into hair; and duration and level of exposure. Even if all of the methodological and toxicological questions can be answered, there are still great gaps in our knowledge as to the relationship between the concentration of a compound/element in hair and environmental exposure, and then between exposure and disease or reduced health status. Indeed, a lack of knowledge of these complex interrelationships exists with any biological sample and prevents full answers to many questions.

While there is much we do not know, there is a body of knowledge on hair analysis. The challenge is to define the parameters whereby hair analysis can be a valuable tool to assist in exposure investigations, but to guard against overinterpretations beyond our knowledge and experiences. Perhaps, identifying the issues will open the door to stimulate research to answer questions and fill our knowledge gaps.

Robert Baratz

Hair Analysis

By Robert S. Baratz, MD, PhD, DDS

Introduction

When physicians study a disease or process, they look for ways to evaluate that process in the body. Blood and urine are taken for testing because they are easily obtained and can be readily standardized. Normal values for populations can be set easily with such testing. Blood generally represents what is inside the body, and urine represents what is excreted from the body.

Hair testing has very limited usefulness in medical practice, because it does not represent either the tissues inside the body or what is excreted. Hair analysis is only useful for detecting exotic compounds that are not normally found in the body. Thus, for example, a medicine that someone is taking, might be detected in the hair. Poisons, such as arsenic, also show up in the hair. Elements normally found in the body -- such as copper, chromium, zinc, and even lead, mercury, and uranium-- will show up in the hair, but the levels are quite variable and have little or no practical or clinical significance.

Analysis of hair won't tell you about the source of an element found in the hair. Most minerals obtained by the body come from food or water. Foods are grown all over the country and thus, their constituents, more likely than not, have come from another region, in some cases, from another country. People are more commonly drinking bottled water and juices which also are coming from other regions. Thus, finding something in the hair or body in no way indicates the source of the material. This is especially problematic when dealing with elements that are somewhat ubiquitous in the environment. The most common source of lead, for example, can be from the solder joints of household plumbing. However, lead could also be introduced through any number of foods, and/or beverages. In some cases, even unglazed pottery used for serving food can be a source of lead contamination.

When hair analyses have been done rigorously in quantitative laboratory settings, it has been pointed out that great care must be taken to avoid possible sources of contamination. First, the hair itself must be processed in a uniform fashion to avoid introducing any exogenous material. Metal cutting instruments in sampling hair should be avoided. The hair sample must be standardized as to region of the scalp, length from the scalp and any washing done of the hair during processing. Even so, because hair grows at different rates in different people, there is still a great deal of uncertainty regarding even hair obtained close to the scalp. Many contend that such hair is of more recent vintage and thus more "representative" of the "body composition". There are no data, however, that confirm this idea. Hair seems to grow at an average rate of about 1 cm per month. However, a considerable portion of the hair shaft lies within the skin and thus hair that has been sampled that has already grown out represents hair that may be as many as several months old.

Even if hair analysis was a highly reproducible and valid test, done properly, there are virtually no data for correlation of findings with levels of elemental minerals found in other tissues or organs. Given the element of interest in the Colorado plateau region, it is important to point out that radioactive compounds from tailings are unlikely to go into hair. The agent of most interest is radon which ends up in the body as lead. Because radon exists primarily as a gas, the major organ that is affected is the lung. This reviewer is unaware of any studies that correlate amounts of material found in lungs with amounts found in hair.

As hair is handled in the laboratory, a number of possible contaminants can enter the hair from solutions used in processing. Rigorous care must be taken to check each and every reagent used in the laboratory. Even acid reagents can have significant amounts of trace elements within them when parts per billion are at issue. Water used in the laboratory for washing, hand washing or even wiping down counters may contaminate samples. Use of vaporization techniques such as atomic absorption spectroscopy can release agents into the laboratory air which would then end up contaminating other samples or solutions or both. Laboratory dust must be excluded since it too can act as a source of contamination. Even powder used to cover gloves of laboratory workers can result in significant contamination of the laboratory environment. Analyses are often done at the level of parts per billion and it takes very little contaminating material to change findings dramatically.

Many laboratories that handle hair fail to take into account that exogenous contaminants such as hair shampoos, swimming pools, shower water and the like can all add exogenous agents to hair. These include: selenium, bromine, zinc, copper and even arsenic. Some elements are removed by pre-washing before hair analysis. Acetone, a common washing agent, has been shown to remove sodium, bromine, and calcium. The same solution is known to add copper, iron, manganese, zinc, and mercury. Even the pH of washing solutions can effect the amounts of lead, mercury and cadmium found in hair samples.

Topic I – Analytic Methods

Types of Analytical Methods

The principal means for analysis of hair depends on the object of the analysis(es). Simple small molecules such as trace minerals, can be analyzed either using atomic absorption spectroscopy or mass spectroscopy. Analysis for organic compounds would depend on the specific compound being tested.

Some of these methods are exquisitely sensitive and small quantities of contaminants found in laboratory air from vaporization, dust, or coatings on lab-ware and/or contamination of test solutions can significantly affect results.

For trace minerals, results are commonly in the range of parts per billion, or smaller. Thus, exquisite attention to detail and lab cleanliness must be followed. Reliable analytical methods exist for detection of most trace minerals, however, quantification becomes an issue particularly when multiple overlapping peaks with spectroscopy occur. Moreover, sampling errors, and the nature of the starting material, often inhibit precise quantification. More commonly, qualitative results can be observed reliably.

For reasons to be discussed in later sections numeric quantitative evaluation of trace mineral substances is not clinically, forensically, or for matters of industrial hygiene, useful. This is largely due to the fact that normal ranges have not been established, cannot be established, or are irrelevant. A finding of an exotic substance that is never normally present is significant. Similarly, a change in order of magnitude of a trace substance that is normally present, and may, at high doses, be poisonous, often has clinical and other relevancies.

Hair analysis has been shown to be quantitatively useful for the detection of arsenic, and methyl mercury. Other validated uses of hair analysis are for the finding the presence of drugs of abuse or the presence of certain pharmacological agents.

In this reviewer's experience, commercial laboratories, as opposed to research laboratories, have been observed to have considerable variation in their performance. This variability is the result of inconsistent specimen preparation, source, and handling, inconsistent use of standards, and lack of multiple runs of the same material. Typically, only single samples are run and thus any variability within the laboratory and/or method are often unknown. Where multiple samples have been observed from the same laboratory on the same material, wide variations have been shown to exist. In the case of commercial laboratories, interpretation of results suggests that results are often misleading, inappropriate, and lack sufficient information to make them useful. Two reports by Barrett (1985) and Seidel and colleagues (2001) show that, at least in the case of commercial laboratories, reference ranges, results and interpretations vary considerably from laboratory to laboratory. This is not surprising considering the milieu in which this work is done, and the factors described above.

Topic II- Factors Influencing the Interpretation of Analytical Results

Regionally, there can be marked differences in elemental composition of hair even for the same element. For example, in 16 different regions of the scalp, antimony content was shown to vary considerably. Even with a person with a standardized diet and living conditions, the composition of hair at different distances out from the scalp itself can vary. This has been shown, in particular, for copper and zinc.

Additional problems in doing hair analysis show that there are difficulties in trying to measure more than two or three elements at the same time. In atomic absorption spectroscopy, one of the

more common methods for hair mineral content analysis, many elements give off multiple peaks which overlap. These absorption peaks obscure each other, negating the ability to do quantitative analyses accurately.

There is often a lack of precision and standardization in the amount of hair taken from any particular subject. Unless a uniform sample was taken from which all analyses were done, the validity of the analysis can be called to question.

Racial differences among subjects have also been found. There is considerable variability in calcium, iron, nickel, chromium, manganese, arsenic and lead levels between Caucasian subjects and blacks.

Similarly, age is a significant factor in metal composition of hair. Paschal and co-workers (1989) found marked differences in concentrations of 28 different metals in hair samples of 199 children compared with 322 adults. Age-dependent increases in calcium, barium, magnesium, zinc and strontium all occur up to about 12-14 years of age. Aluminum is shown to decrease with age. It has been hypothesized that metal composition of hair is related to skeletal and bony growth. Thus, adults undergoing osteoporosis would have differences in their hair composition related to those who did not have this problem. Similarly, anyone with any kind of bone abnormality would have findings that are non-standard.

Most analyses on hair do not correlate positively with concentrations found in organs (Yoshinaga and co-workers 1990). It is intuitively obvious why this is likely so, since tissue concentrations involve both uptake and release which vary over time. Hair is essentially a one-way path out of the body. Likewise, some elements have significant diurnal variation. A good example is chromium (Sheard and co-workers 1980).

Many elements, when analyzed in the presence of other elements, can give false readings. The interaction of chromium with other anions and cations in hair may affect analytical results (Sheard and coworkers 1980). Merely painting the laboratory with particular types of paint, failure to use HEPA filters on the air intake and the presence of dust can easily affect sensitive analytical measurements.

A variety of hair treatments have been shown to alter hair trace element concentrations. (McKenzie, 1978). Other common issues are dyeing and permanent waving, shampooing, hair color, sex, seasonal variations, age, and growth rates. It is generally assumed that hair grows approximately 1 cm per month, however this must be verified in each individual tested. Hair growth is a function of individual factors as well as protein in the diet.

Many commercial laboratories claim to be able to detect and measure more than 20 elements in a single sample of hair, however, this is often accomplished without any specific knowledge of the patient’s medical history. What is more troubling, is that there is no definition of a normal range

(Hambridge 1982, Rivlin 1983; Manson and Zlotkin 1985, Barrett, 1985, Druyan and others 1998 and Seidel and others 2001;). Quality control in the laboratory is essential towards having useful data. Rigorous attention to detail, methodology, and sampling techniques must be followed. Even when known standards were used, because of the sensitivity of instrumentation, data varied commonly by up to 10%. (Nowak and Kozkowski 1998).

Variations in Sample Collection and Preparation Methods

A number of compounding variables limit interpretation of results from hair analysis. It is well known from the literature that the rate of hair growth varies from person to person, with nutritional and disease states, with the presence of particular drugs, with gender, age, ethnicity/race, with site on the scalp and/or other body parts. While some of the factors may be known in an individual case, others are unknown, or cannot be known. Thus, hair analysis from a particular individual is fraught with a series of uncontrolled variables and unknown data. It should be obvious that these belie making any precise quantitative diagnostic or forensic analysis. This becomes even more of a problem when dealing with trace minerals that are normally found ubiquitously in the environment and characteristically in foods, water, and air. Many trace minerals occur in human hair normally. Thus, finding them there is expected. Making interpretations based upon the quantitative analysis of these is fraught with uncertainty due to the unreliability of the data regarding exposure, timing, hair growth, treatment of the hair, diet, nutrition, and a host of other factors mentioned above.

Similarly, considerable variation exists from laboratory to laboratory in terms of sample preparation, whether a sample is washed, how it is digested, how long it is digested, and how it is handled after digestion.

Even more problematic is the development of “normal ranges” or “reference standards” (“reference ranges”). In most cases, population standards have not been developed. Thus, each laboratory has developed its own “reference range”. The major problem with this is that the source of the specimens used to create the “reference range” in a particular lab may be biased. Many commercial laboratories accept samples from a variety of practitioners, patients, and other sources. From reports this reviewer has seen, the precision of medical knowledge and facts regarding the source material is often poorly documented. Careful attention to uniform sample collection techniques is often also a problem.

So-called “normal” reference ranges do not exist for most trace minerals found in hair. The reasons for this are obvious. Considerable variation exists from person to person and the variety of unknown variables enter into the equation. Thus, there are no standardized “reference ranges” for most normal trace minerals. This has to do, in part, with the composition of hair. In essence, hair consists of keratinized or cornified cells packed into tight arrays in the hair shaft. These cells are fundamentally similar to the epidermis however contain proportionally more keratin fibrils and somewhat different materials in the thickened cell membrane that is left when the cells keratinize.

Hair, nails, horn, and some portions of the filiform papillae of some animal tongues are a so-called "hard" keratin compared with so-called "soft" keratins found in epidermis and oral and other, mucosae. All keratinized cells contain virtually no aqueous phase after keratinization. It is unclear if minerals are removed from these cells when they mature, or merely remain in the intracellular matrix. Along these lines, even if some minerals were found to be left "inside" such cells, each and every individual trace mineral would have to be studied in pre-keratinized cells and keratinized cells to see how it was handled.

Moreover, epithelium and its derivatives (hair) is an a vascular tissue with little intercellular space or material. What little extracellular material exists is primarily a lipid that forms a barrier to diffusion. The epithelium is neither a gland nor excretory organ but merely forms a protective layer. Thus, substances that would normally be excreted into various body fluids are normally not present in epidermal epithelium. Regionally, there is variability in the thickness of the epidermal epithelium and indeed there is some variability in the consistency of thickness of hair in different regions of the scalp. Hair in other body locations, axillary, pubic, limb, peri-anal, eyebrows, and eyelashes, all vary considerably in their structure, function, and growth rates.

Since hair is principally protein in nature, there is little need for trace minerals in the hair cells themselves. Trace minerals in the body are usually present as co-factors for enzymes. Keratinized cells are generally non-metabollic. After the filaments of keratine aggregate and are coated by other proteinatious material, the cell contents become essentially inert. Nuclear materials, enzymes, carbohydrates, and even lipids are essentially not present in the internal milieu of keratinized cells. Consequently, there would be no need for a regular array of minerals present from a functional point of view.

Some heavy metals may distribute into hair and become complexed with hair proteins. This would be due largely to interaction with free side chains on amino acids and/or forming crosslinks among protein chains as they may be denatured by heavy metals. Some heavy metals are well known as protein denaturants, e.g. mercuric chloride. They may become trapped in hair cells before they become completely keratinized. Whether or not this happens is largely unknown.

Finding trace minerals in hair is neither surprising nor a consistent finding. Because hair shafts consist of essentially of two portions, intra-epithelial and extra-epithelial, the possible absorption of extraneous material is possible in the extra-epithelial portion. The extra-epithelial portion is essentially free in the environment. Thus it is subject to washing, drying, chemical alteration, cosmetics, environmental pollutants present in the water or air, and a host of other chemical and physical insults. Not only may things be adsorbed and absorbed by the hair, but, substances may also be leached from the hair as well. Prolonged immersion and wetting of hair can cause some swelling of the cells of which hair is composed. This can diminish the barriers to diffusion of things both from the outside in, and from the inside out. Moreover, hair is being constantly exposed to scalp oils, and other glandular products excreted into the hair shaft space by sebaceous

and other glands present in skin. These provide an additional source of extraneous material to be adsorbed or absorbed onto or into the hair.

A common and highly variable factor in hair is hair growth. Hair growth can occur in several different ways. First, is the fact that hair undergoes a cycle in its normal growth. That is, hair is regularly shed from the scalp and other locations, and replaced by "new hairs". The stages of the growth (catagen, anagen, telogen) each have unknown times associated with them in particular subjects. Further complicating an understanding of growth is the fact that hair in humans is known to grow in a mosaic across the scalp. That is, any particular hair may be in a different state than its neighbors. A long list of drugs, hormones, and other factors can either accelerate or prolong the time a hair stays in a particular part of its growth cycle. Moreover, a number of other factors such as diet, nutrition, age, sex, hair color, and other factors are known to influence growth rates.

Growth can occur both longitudinally and in diameter. Hair in general varies from individual to individual in shape and not all individuals have a circular cross section of their hair. In particular, individuals with highly curled or "kinky" hair have hair that is somewhat flattened to a ribbon-like shape.

In general, hair growth in length is often described at approximately 1 cm per month. However, there is considerable variation in this from individual to individual and results can vary by a factor of 2 either in increase or decrease in rate of growth.

Topic #3: Toxicologic Considerations

As previously mentioned, among the mineral toxic agents studied only arsenic and methyl mercury have been shown to have reliable information on their presence and distribution in hair when viewed in comparison to their distribution in other organs.

To have predictive value, the values obtained from analysis of hair of a particular subject must be capable of yielding data that would be predictive for disease in general. This may prove to be considerably problematic in the case of heavy metals as the agents themselves may affect hair growth directly.

This reviewer is less well versed in arsenic and methyl mercury studies than others on the panel and wishes to defer to their knowledge and experience.

Topic #4: Data Gaps and Research Needs

Many of the data gaps in our knowledge of hair physiology and growth have been discussed earlier.

In one sense each trace mineral must be independently studied with regard to the best source for analytical material. In most cases it is likely that hair will prove to be a problematic source. While hair theoretically gives a longitudinal history of prior events, the speed of that history is largely unknown, and may even change over time. Whether this theory meets practice is also unknown. A better understanding of the physiology of hair growth is obviously an important area of research. This, of course, begs the question that hair may be useful at all for mineral analyses. This later point is yet unproven. A variety of data would suggest that hair is not useful for mineral analyses for most minerals, and that other body sources would be better—e.g. bone, teeth.

Knowledge of the dynamics of incorporation of a variety of environmental toxins, principally organic compounds, into hair would be desirable. Attendant to such a study would be studies of absorption, adsorption and leaching of such compounds.

Studies of the nature of differences in incorporation of materials into hair at different ages, by different sexes, different ethnic groups, and different hair colors would also be useful.

Topic #5: Identifying Scenarios for Which Hair Analysis May Be Appropriate

Hair analysis appears useful only for population studies where much of the individual variability can be eliminated. If a number of factors were known—duration of exposure, rates of incorporation into hair, effects on growth, amounts of leaching, sources of material that were found in hair, etc.—then useful data on exposure could be extracted. Correlating these with clinical findings is more problematic, since such are best done on the individual level, where hair analyses are likely more useful only for population studies.

Particularly for small molecules such as trace minerals, hair is unlikely to prove a reliable source of material for meaningful study.

Organic compounds that can be shown to incorporate into hair may be an area where hair analysis could be appropriate for following exposures to environmental toxins.

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Thomas Clarkson

Hopps (1977)

He provides background physiology and histology of human hair formation and growth

General questions

He gives no information

Topic 1 Analytical methods

No information

Topic 2 Factors influencing the interpretation of analytical results

He notes various pathways of metal into hair:
1) via the follicle into the hair matrix
2) secretion of metals in the sebum on to the hair surface
3) secretion of metal in exocrine sweat on to the surface of the hair
4) secretion of metals in apocrine sweat on to the surface of the hair.

He notes that apocrine sweat may not be important for scalp hair.

He discusses the relative merits of head versus pubic hair and concludes that scalp hair is to be preferred

He discusses some reports where lead and arsenic have been measured in scalp hair. The metal level depend on the distance from the scalp/ Lead tends to increase towards the tip of the hair strand.

Arsenic appears to be accumulated in hair and may present a historical record of tissue levels. However hair can accumulate external arsenic in the form of arsenite. Animal experiments indicate arsenic is excreted in sweat.

Variable data have been obtained with cadmium

He gives a table of normal levels of metals in hair.

He notes that attempts to distinguish external versus internal uptake of metals have usually been unsuccessful

Topic 4 Toxicological consideration

No information

Topic 5 Data gaps and research needs

No information

Miekelay et al (1998)

Compared two methods of measuring metal in samples of scalp hair taken from 1,091 adults living in Rio de Janeiro. They also sent a test sample to commercial laboratories for comparison.

General questions

The article indicates the need to revise reference interval for normal levels of metals in hair

Topic 1 Analytical methods

The article claims that ICP-AES (inductively coupled plasma atomic emission spectrometry is out of date with poor detection limits but is still used by most commercial laboratories. The article claims that ICP-MS (inductively coupled mass spectrometry) is the method of choice.
Tables are presented comparing reference limits published by five commercial laboratories indicating wide differences between laboratories for certain metals. Tables are also presented indicating wide differences in results for certain metals on two hair samples circulated blind to the same five commercial laboratories. However, results for some metals yielded reasonable agreement. These metals included Na, Ca, Mg, Mn, Cu, Fe, Zn. The following metals gave reasonable agreement if results from one of the laboratories were excluded: Pb, Cd, Ba, Ni, Li, P, B, Cr, Mo.

Topic 2 Factors influencing the interpretation of analytical results

No information

Topic 3 Toxicological consideration

No information

Topic 4 Data gaps and research needs

The study indicates the need to revise reference limits for some metals.

Sky-Peck (1990)

He performed X-ray fluorescence analysis in six carefully aligned samples of hair from 987 employees and their families at a major medical center in Cook County, Illinois. The purpose was to elucidate factors that might affect concentrations of trace elements in human scalp hair

General questions

He concludes that hair analysis should only be used as a screening method along with other measures of the nutritional status of the patient.

More data are needed on factors affecting trace elements in hair before hair can be used as a quantitative tool to assess the nutritional status of any trace element.

Topic 1 Analytical methods

He used only X-ray fluorescence analysis. He did not describe how the weight of hair was obtained. Usually Compton scattering is used to measure the hair mass. This does not appear to be the method used in this report

Topic 2 Factors influencing the interpretation of analytical results

The method of washing the hair sample can influence the levels of certain trace elements. The mild washing procedure used in the report did not affect levels of 14 selected trace elements. Treatment with peroxide produced a statistically significant reduction in S, Ca, Fe, and Zn. The reduction in Ca was almost complete and Zn was reduced substantially. Hg levels were not affected.

Permanent waving produced a statistically significant increase in levels of 6 trace elements. Levels of Ca, Ni and As were more than doubled. Mercury was unaffected.

Brunettes and blondes differed significantly in only three trace elements, F (slightly lower in blondes); Mn (slightly lower in blondes); and Pb (almost double in blondes). Compared to brunettes and redheads differed statistically in 5 trace elements. Iron was almost doubled in red heads. Mercury was slightly reduced.

Blacks differed from Caucasians in 10 trace elements. Ni, As, and Pb in blacks were more than twice as high as in Caucasians. Orientals differed from Caucasians in 9 trace elements. Ca and Pb in Orientals were a factor of 2 below corresponding levels in Caucasians. Mercury was the same.

Note: Elements differed according to age. Ca in the older group was less than 50% of the younger group. Br was five times higher. Hg was unaffected.

The longitudinal profiles differ according to the trace element. The levels of As, Hg, Cu, Fe, Zn, S and Se were steady and unaffected by distance from the root end. On the other hand, the levels of Pb, Ni and Mn rose sharply towards the tip of the hair strands suggestion external contamination. Ca and Sr showed less pronounced changes.

The results indicate that the levels of certain trace elements are influenced by a number of factors. It would appear that Pb, Ni and Mn are affected by external contamination.

On the other hand, levels of Hg appear to be robust and unaffected by all but one of the factors tested in this report. For one factor, natural hair color of redheads versus brunettes, there was a statistically significant difference in mercury levels, but this difference was quantitatively small.

The most stable trace elements were S, Cu, Zn, Se, Cr, and Rb because these were not changed by more than a factor of 2 by any of the factors tested in this study.

The most unstable elements were Ca, which was affected by more than a factor of 2 by five of the six factors tested. Pb was affected by four factors, and Ni. Br, and Sr by three factors.

Topic 3 Toxicological consideration

No information

Topic 4 Data gaps and research needs

The paper stresses the need for more data on factors affecting levels of trace elements in hair

Seidel et al. (2001)

The authors sent a common hair sample to six commercial laboratories for trace element analysis Different levels were obtained However, it is difficult to evaluate the data without knowing the correct level. These levels can be compared to the normal ranges for each laboratory.

The authors also checked on the accreditation of the labs and on the dietary advice given on the basis on the findings

General questions

The authors argue that there are few if any trace elements that have been validated as indicators of dietary sufficiency or of toxicity. Methyl mercury may be the only substance for which toxic dose response relationships have been established.

Topic 1 Analytical methods

The labs tests used atomic fluorescence or mass spectrometry detection methods. The authors note that the mass spec. method is much lower detection limits

Topic 2 Factors influencing the interpretation of analytical results

The labs can be compared in terms of identifying with elements are outside their normal range. All six labs agreed that the following elements were with their normal range: Ba, Be, B, Cd, S and Ti. All labs agreed that Mn and Mo were outside their normal range. For the following elements all

labs except one agreed on classifying according to their normal range: Al, As, Pb, Mg, Hg, Ni, and Zn.

Thus, for approximately half the elements tested, there was reasonable agreement between the commercial labs.

Topic 3 Toxicological consideration

There is lack of toxicological information of the value of hair element concentration as a biomarker for tissue levels, especially levels in the target tissue. This information is available only for methyl mercury.

Topic 4 Data gaps and research needs

As mentioned above, the data gaps are in dose response information and in relating hair levels to levels in the target tissue

Steindel & Howanitz (2001)

The authors provide editorial comment on the paper by Seidel and provide a discussion of proficiency testing in clinical chemistry laboratories.

General questions

They point out that the current lack of normal ranges for trace elements in hair make interpretation of results impossible. They comment of the difficulty of making nutritional conclusions from hair data

Topic 1 Analytical methods

No information

Topic 2 Factors influencing the interpretation of analytical results

The authors listed many problems in interpretation of hair data including external contamination and the absence of reliable reference standards and uniform methods for processing the hair samples.

Topic 3 Toxicological considerations

No information

Topic 4 Data gaps and research needs

More data are needed on inter-laboratory comparisons

Wennig (2000)

This is a review article on the incorporation of drugs into hair. It presents a useful review of hair physiology and biochemistry. It gives recommendations for collection and storage of hair samples.

It gives no information on trace elements in hair.

Yoshinaga et al (1990)

The paper compares the concentration of a number of trace elements in hair with corresponding concentrations in several organs and tissues obtained at autopsy.

Unfortunately, little detail was given on how the hair samples were collected or on the length of the hair samples.

General questions

Topic 1 Analytical methods

A commonly used analytical method was used (ICP-AES). Quality control tests were made.

Topic 2 Factors influencing the interpretation of analytical results

The varying length of the hair samples may have influenced the result and accounted for the poor correlations.

Topic 3 Toxicological consideration

The main finding was that levels of Ca, Mg, P, and Zn in hair did not correlate with tissue levels or body burden

They were not able to draw any conclusions about Fe, Cu or Se as the appropriate tissues were not available for analysis

Topic 4 Data gaps and research needs

More information is needed on hair versus levels in autopsy tissues. The hair length should be restricted to a short segment close to the scalp.

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Michael Greenberg

Topic #1: Analytical Methods

Comments:

The laboratory analytical methods available are capable of defining the qualitative existence of a variety of pharmaceuticals, drugs of abuse, and occupational/environmental toxicants. The operative word here is "qualitative". Quantitation of specific levels are not, in my opinion and experience, either generally reliably reproducible and/or clinically useful. Specific analyte levels are essentially of little or no value in the determination of so-called cut-off levels (e.g., PELs, TLVs, "safe levels"), "normal levels," or other designators which rely on reference levels. In addition, the analytical techniques currently in use are capable of providing "segmental analysis" of hair, which in turn can provide a historical picture of various qualitative (not quantitative) exposures over time. In addition, hair analysis may help to derive an essential time frame which may indicate, based on the average rate of hair growth, the time of inception for various exposures.

The amount of hair needed for analysis may be dependent on the specific analyte sought as well as the temporal relationship between exposure and hair harvest.

One of the most important shortcomings for hair analysis, as it currently exists, is the fact that reference ranges may often be unreliable. Laboratories frequently base their reference ranges for specific analytes on limited case reports in the medical literature or exclusively on data derived from animals, which has limited applicability to humans. These facts contribute to substantial limitations with regard to interpretation of results. Variability undoubtedly exists from one laboratory to another. Certainly these facts limit the clinician's ability to interpret and utilize hair-derived values beyond the potential qualitative information that might come from hair testing of any individual.

Thus, at this time, it may be prudent to recommend that hair testing for all substances (drugs of abuse, occupational toxicants, environmental toxicants) be limited to qualitative determinations as opposed to quantitative determinations. The goal of quantitation for any laboratory analyte is to derive clinical algorithms that translate into levels that indicate disease, dysfunction, or specific risks for disease or dysfunction. With regard to hair testing in its current state, there is little evidence that there is sufficient reliability to use quantitation for these purposes.

Laboratory washing procedures prior to digestion may significantly alter the hair content of various analytes. For example, when hair is tested for THC, if it is washed with methanol, THC concentrations may be reduced by as much as 85% (Forensic Drug Abuse Advisor, 1996) by virtue of this process. It is reasonable to expect that similar degradations in analyte concentration occur when other analytes are involved.

Hair pigmentation is a critical factor in the interpretation of the concentration of certain compounds and their metabolites incorporated into hair. Melanin is responsible for the pigmentation. The color and the melanin content of human hair samples differs over a wide range. Once deposited into hair, chemicals may remain detectable for a period of months to years. However, if disposition into hair is influenced by those properties attributed to hair color, then certain persons may test positive more frequently than other persons. Removal of the melanin from hair digests prior to hair analysis may reduce the effect of melanin on the total chemical concentration by excluding the drug bound to the pigment. In one study (Hold KM, et al), the effect of melanin removal by centrifugation of hair digests on cocaine concentrations was investigated. Two sets of hair samples from five cocaine users were analyzed for cocaine and metabolites. A solution consisting of 10 mL of 0.5M Tris buffer (pH 6.4) to which is added 60 mg D,L-dithiothreitol, 200 mg SDS, and 200 U Proteinase K, was used to digest the hair. Two milliliters of this solution was added to 20 mg of hair and incubated at 37 degrees in a shaking water bath (90 oscillations/min) overnight. The samples were removed from the water bath and mixed. One set was centrifuged at 2000 rpm and divided into supernatant and melanin pellet. The

other set was not centrifuged. Internal standards were added to all tubes. The samples were further extracted, derivatized, and analyzed by gas chromatography-mass spectrometry. A mean of 8.8% (standard deviation [SD] 7.0%) of the total cocaine concentration (supernatant and pellet) was left behind in the pellet. The same experiment was repeated—except that the melanin pellet was redigested with 0.1 N HCl. After redigestion of the melanin pellet, the mean cocaine concentration in the pellet was 3.8% +/- 4.0% (mean +/- SD) of the total cocaine concentration in hair. These investigators felt that their data demonstrate that removal of melanin from hair digests by centrifugation does not eliminate hair color bias when interpreting cocaine concentrations.

TOPIC #2: FACTORS INFLUENCING THE INTERPRETATION OF ANALYTICAL RESULTS

Comments:

Exposure of hair sample to the external environment could be an important factor in confounding results on both a quantitative and qualitative basis. By way of example, many over the counter hair coloring preparations contain lead acetate (e.g., Grecian formula). This may persist for long periods on hair shafts and thus confound hair testing results for lead. It is also unclear if the use of coloring agents containing lead acetate alters or enhances the hairs ability to bind other analytes or potential toxicants.

Based on the medical literature that describes the use of hair testing for substances of abuse there are differences in hair uptake of various substances based on ethnicity. For example, negroid hair has been suggested to bind cocaine residues with greater affinity than caucasoid hair.

There are also reports in the literature that the ability to bind various chemicals and drugs may depend on endogenous hair color as well as if hair has undergone bleaching. For example, bleached hair radically lowers the drugs [of abuse] content of hair. This may explain the

observation that many competitors on the professional biking circuit sport bleach blond hair (Kintz). Blond hair has been shown to not bind cocaine or its metabolites as well as pigmented hair (Hubbard). In addition, there was no evidence of a dose-related incorporation of these drugs and metabolites into non-pigmented hair. The concern is that similar circumstances may occur with regard to specific occupational or environmental toxicants and chemicals.

Based on a study presented by Reid et al. indicating that gray hair takes up less cocaine than non-gray hair, it is possible that gray hair may also alter the utility of hair analysis in other settings. The Reid study evaluated cocaine levels in the same individuals by comparing the levels in gray and non gray hairs from the same person. In a similar study (Rothe et al.), hair samples from 15 patients receiving medical treatment with amitriptyline, carbamazepine, chlorprothixene, diclofenac, doxepine, indomethacine, maprotiline, or metoclopramide, or with a chronic heroin and cocaine abuse, were separated into white and pigmented fibers and both fractions were independently investigated by GC-MS. The drugs were found in pigmented fibers as well as in white fibers, but the concentrations in the white fibers were smaller than in the pigmented ones for most of the samples investigated. The concentration ratio of the drugs or their metabolites in both hair fractions (white/pigmented) was found to be between 0.09 and 1.57 (mean 0.70, 30 concentration pairs). There are large differences in this ratio between different subjects with the same drug; whereas for different drugs in the same subject—in many cases—similar ratios were measured. As reasons, a different grade of pigmentation of the hair and the influence of the drug structure are discussed. From these results it follows that the natural hair color is an important parameter in the evaluation of drug concentration in hair. Again, similar effects may be seen when dealing with occupational and environmental toxicants.

The rate of hair growth may be an important factor in the ability to identify the presence of various materials based on time of exposure. Sources usually indicate that head hair grows at the rate of 1-2 cm per month. That in itself represents a range encompassing up to a 100% difference

in hair growth rate. Obviously, comparisons of individuals whose hair growth rates differ by a factor of 100% is problematic.

TOPIC #3: TOXICOLOGIC CONSIDERATIONS

Relatively little is known about the biological uptake of specific substances with regard to the concentration delivered to and incorporated into hair. There is essentially no data that reliably establishes the relationship between chemical concentrations in the hair and blood or other target organs for most chemcials. More specifically, and more importantly, no dose-response data currently exists with regard to chemical concentrations in the hair and blood or other target organs. In addition, no disease predictive value exists for any quantatative data that has been derived to date with regard to the hair concentration of drugs or chemicals.

Rollins et al have suggested that the ionization state of any given chemical is what determines whether or not it will bind with hair melanin. These investigators reported that catiomnic drugs are more likely to bind with melanin when compared with anionic drugs. This study may provide some guidance with regard to the binding ability of other toxicants of concern.

TOPIC # 4: DATA GAPS AND RESEARCH NEEDS

Comments:

The data gaps that most significantly limit the use of hair testing in public health evaluations are 1) the lack of accurate and reliable reference range data and 2) the lack of specific information about dose response relationships with regard to the relationship between chemical concentrations in the hair and blood or other target organs. In my estimation, these two items constitute the most pressing research needs with regard to hair testing.

Future studies must address these basic data gaps in order to even begin to decide if hair testing has clinical screening or other clinical usefulness.

TOPIC # 5: IDENTIFYING SCENARIOS FOR WHICH HAIR ANALYSIS MAY BE APPROPRIATE

Comments:

Hair testing for acute exposures is clearly not the best alternative for determination of either dose or exposure with regard to any potential toxicant. If acute exposure is defined as the pre-distribution time frame, then blood or urine testing would be far superior to hair testing in any scenario. However, in the event of a single exposure (as opposed to an ongoing exposure) the use of hair testing after the completion of the pre-distribution phase of kinetics may be helpful in qualitatively identifying the fact that exposure has indeed occurred and/or generally timing that exposure. The use of hair analysis in this setting may have forensic as well as public health value.

In the setting of chronic exposures, hair analysis may have value in identifying and documenting a given exposure. This, again, may have forensic, civil-legal, and risk assessment value for individuals as well as communities and populations. Obviously, the length of any given individual's hair may limit the use of hair analysis, as well as how frequently the hair is cut.

In any scenario, however, the state of the art is such that specific and measurable health effects will generally not be uncovered by hair analysis. In addition, public health and/or individual risk assessment determinations will be limited by whatever conclusions may be drawn by what is essentially a qualitative and not quantitative toxicological evaluation.

ADDITIONAL CONSIDERATIONS:

Comments:

One interesting study (Al-Delaimy, et al) used hair analysis to measure the relation between workplace smoking policies and exposures to environmental tobacco smoke (ETS) of workers in bars and restaurants. In this study, 114 workers were questioned about sources of exposure to ETS and smoking habits, and details of the smoke-free policy in their work place were recorded. A hair sample was collected from each participant and tested for nicotine. Among non-smoking workers, hair nicotine levels varied strongly according to the smoke-free policy at their place of work. Those working in 100% smoke-free restaurants had much lower levels than staff working in bars with no restrictions on smoking, and levels were intermediate for staff working in places with a partial smoking ban. Hair nicotine levels among nonsmokers working in places with no restriction on smoking were similar to hair nicotine levels of active smokers. The findings from this study highlight the substantial levels of exposure of bar and restaurant staff from patrons' smoking.

The potential sources for confounding variables in the hair testing arena are truly legion. This fact is demonstrated in one instance by a paper from Japan wherein investigators sought to draw a relationship between head hair mercury and health. However, in the end, these investigators discovered that “some subjects who showed a high total mercury level made habitual use of toilet soap containing much mercury.” Thus, the confounding effect of an unusual source for a heavy metal can interfere with effective hair analysis.

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Michael Kosnett

Michael J. Kosnett, MD, MPH

June 4, 2001

The following are preliminary comments regarding some topics that constitute the charge to the panel. However, I am still in the process of reviewing some relevant studies and therefore may revise or amend this material in a subsequent submission.

Topic #1: Analytical methods

The key analytical methods currently used by clinical laboratories to measure trace elements in hair appear to be inductively coupled plasma atomic emission spectrometry, and inductively coupled plasma mass spectrometry (Miekeley et al, 1998; Seidel et al, 2001). Graphite furnace atomic absorption spectrometry has been used to measure arsenic in hair, with reported limits of detection of 0.005 to 0.01 ? g/g (Rebel et al, 1998; Hewlett et al, 1995). Total and inorganic mercury in hair has been determined by cold vapor atomic absorption (Boischio and Cernichiari, 1998; NRC, 2000), and the difference between total and inorganic Hg yielded by this method has been used as a surrogate for the methyl mercury hair content. Methyl mercury in hair has also been determined directly by gas chromatography using a tritium foil electron capture detector (Smith et al, 1997). Selenium in hair has been measured fluorometrically after complexation with 2,3-diaminonaphthalene (Yoshinaga et al, 1990). The preceding methods appear to have generally required a hair specimen size on the order of 50 mg or more. Although commercial laboratories commonly measure the submitted hair sample in bulk, the methodology is sufficiently sensitive to allow investigators to yield segmental analysis (^{3} 1 cm) on bundles of hair for which information on the alignment and distance from the root has been preserved. Segmental analysis may potentially offer information on the temporal pattern of exposure to the element in question that is of value in epidemiological and forensic investigations.

Neutron activation analysis (NAA) has been used in forensic investigations and occasionally in epidemiological or clinical studies for the sensitive determination of certain trace elements in minute quantities of hair. For example, neutron activation analysis has been used to measure arsenic in 2 mm segments of an individual hair, each segment weighing approximately 3 ? g (Smith, 1964; Curry and Pounds, 1977). NAA has also been used to measure the hair content of Zn, Au, Cu, Mn, Hg, Sb, and Th (Jervis, 1968; Cornelius, 1973). The distribution of mercury in 2 mm segments along the length of a single strand of hair may be determined by nondestructive x-ray fluorescence (Cox et al, 1989, cited by NRC, 2000). Proton induced x-ray emission has been used to measure the spatial distribution of multiple elements in 10 micron increments across axial cross section of a single shaft of hair (Cookson and Pilling, 1975; Hindmarsh et al, 1999).

A multitude of factors influence the quality control of laboratory hair analysis. These include the finite limitations of the assay method (ideal method recovery and precision), and the variability associated with within-run and day to day operation of the assay (actual method recovery and precision). Although not necessarily reflective of a systematic review of the literature, a few references may be cited as offering examples of operational precision in research investigations.

Using NAA to measure 7 elements in a single specimen of hair, the coefficient of variation ranged from 5.92% in the case of Mn (mean concentration 1.65 ppm) to 15.7% in the case of Sb (mean concentration 0.18 ppm) (Cornelius, 1973). Wilhelm et al (1989) reported a day to day coefficient of variation of approximately 6% for atomic absorption measurement of Zn, Pb, Cu and Cd in hair. The issue of inter-laboratory variability of multi-element hair analysis for trace elements provided by commercial laboratories using ICP-AES and ICP-MS has recently been addressed by Miekeley et al (1998) and Seidel et al (2001), both of whom obtained widely discrepant results from split samples sent to 4 to 6 different commercial laboratories.

Topic #2: Factors Influencing the Interpretation of Analytical Results

One of the most fundamental factors impacting the potential utility of hair analysis as an *exposure assessment tool* in public health evaluations is the limited capacity of such measurements to distinguish external contamination from internal incorporation. In particular, multiple studies have noted that toxic metals may become incorporated into hair following external contact with metal containing dust, soil, water or hair care products. There is no reliable analytical approach that can distinguish this external contamination from elevations in hair metal content that result from metal ingestion or inhalation (Chittleborough, 1980). Although pre-analysis washing or rinsing methods are often used in an attempt to selectively remove external contamination, there is no standardized approach that has been shown to achieve the desired result.

The experience with arsenic, a toxic metalloid that is often encountered through environmental exposures, is a case in point. *In vitro* studies have demonstrated that hair incorporates appreciable amounts of arsenate and arsenite from aqueous solutions, and that the extent of absorption increases with duration of contact time and moderate decrements in pH (e.g. pH 3 to 5) (Atalla et al, 1965; Bate, 1966; Van den Berg et al, 1967; Fergusson et al, 1983). Adsorption of arsenic to hair may also be substantial following contact with arsenic containing dust (Atalla et al, 1965). The extent of adsorption may vary significantly along the length of a single hair (Maes and Pate, 1977). Adsorption-desorption experiments demonstrate that externally deposited arsenic cannot be completely removed from hair by a variety of washing and rinsing techniques (Smith, 1964; Atalla et al, 1965; Van den Berg et al, 1968). Moreover, washing may complicate interpretation further by partially removing arsenic present in hair as a result of internal incorporation (Atalla et al, 1965; Van den Berg et al, 1968; Young and Rice, 1944). Studies with other metals have reported similar findings with respect to adsorption onto hair from external contamination, and variable removal of both internal and externally derived traces by washing regimens (Chittleborough, 1980; Fergusson et al, 1983; Wilhelm et al, 1989).

The problems posed by this inability to distinguish external adsorption from internal incorporation places substantial constraints on what can be learned from the results of hair analysis for an environmental toxin where the suspected route of human exposure is via contact with contaminated dust, soil, airborne particulate, or tap water. Although these routes of exposure might result in ingestion or inhalation of an environmental toxin and its subsequent appearance in

hair through incorporation at the hair follicle, they also create ample opportunity for the agent to become externally adsorbed onto hair via airborne deposition, hand to hair contact, or bathing. In such settings (which are probably characteristic of the majority of sites subject to ATSDR health assessments), the finding of elevated levels of a environmental toxin in the hair of a given subject or a study population is *limited at best to establishing the potential* for that subject or population to have come into contact with the agent in a manner that may have resulted in ingestion or inhalation. In addition to being a test of low specificity, the information on potential exposure gleaned from an elevated hair level in such settings is likely to be qualitative in nature. That is because with the notable exception of methyl mercury, quantitative information on the relationship between ingestion or inhalation of a environmental toxin and its concentration in hair is limited, and appears to be subject to considerable inter-subject and inter-population variability.

Again, an example derived from the measurement of arsenic in hair is instructive. Although several epidemiological studies have noted a correlation between levels of arsenic in hair and arsenic in dust, soil, or water, (e.g. Bencko and Symon, 1977; Hartwell et al, 1983; Valentine et al, 1979), the hair arsenic levels may not correlate with levels of arsenic in urine (Harrington et al, 1978; Hewlett et al, 1995). For example, Harrington et al (1978) studied hair and urine arsenic levels in a community near Fairbanks, Alaska, where the arsenic concentration of water obtained from domestic wells averaged 224 ? g/L (range 1.0 to 2450 ? g/L). A subset of subjects whose wells contained arsenic averaging 345 ? g/L consumed only bottled water. Although they had relatively low arsenic levels in urine (average 43 ? g/L), the arsenic concentration of their hair was high, averaging 5.74 ppm. Subjects consuming water from domestic wells with the lowest levels of arsenic (less than 50 ? g/L in water) had hair arsenic concentrations averaging 0.46 ppm, and urine arsenic levels averaging 38 ? g/L. Thus, the arsenic level in hair varied by 14-fold, despite similar levels of arsenic in urine. The authors noted the likely implication that the elevated hair arsenic levels were probably due to external contamination derived from bathing in, but not drinking, the high arsenic well water.

Topic #3 To what extent may hair analysis be used to predict adverse health outcomes? *and* Topic #5, Under what scenarios may hair analysis be appropriate for evaluating exposures to environmental contaminants?

From a medical standpoint, there appears to be no disease or illness caused by an environmental toxin for which there is a general medical consensus that the results of hair analysis would form the basis for specific medical treatment.

In the case of methyl mercury, segmental maternal hair analysis may have diagnostic value as a biomarker of fetal exposure to levels of this neurotoxin that are associated with a postnatal risk of adverse neurobehavioral development (NRC, 2000). Some data suggest that the level of hair methylmercury in children and adults may also be a biomarker of exposure associated with adverse effects on neurological function and other health endpoints (NRC, 2000). Because most contemporary exposure to methylmercury is confined to ingestion via seafood, there is little

potential for high hair levels of methylmercury to be a result of external contamination. In most populations whose level of seafood ingestion is of a sufficient magnitude to pose a potential health risk from methylmercury, measurement of total mercury in hair may be an acceptable surrogate for measurement of methylmercury in hair.

In certain settings, segmental hair measurement of arsenic (and potentially other toxins such as thallium) may be of diagnostic and/or forensic value in identifying or confirming a high dose toxic exposure or poisoning that terminated months (but not years) in the past. For example, segmental analysis of a sufficiently long hair might help to confirm a suspicion that an episode or outbreak of severe gastroenteritis followed by peripheral neuropathy that occurred 8 to 10 months in the past was likely to have been the consequence of acute arsenic or thallium poisoning. Months after the exposure ended, levels of arsenic or thallium in the urine may have fallen to normal values, and high peak levels in the hair (or nails) may offer the only remaining confirmatory forensic evidence. It should be noted that although the hair measurements in such scenarios might conceivably be of value in confirming past poisoning, the epidemiological database on hair analysis is insufficient to use these measurements to predict the risk of latent diseases such as cancer.

Supplemental comments from Michael J. Kosnett, MD, MPH (submitted June 21, 2001)

1. A key factor to be addressed prior to ATSDR's use or interpretation of hair testing is the predictive value of a positive or negative test with respect to detecting an exposure and/or internally absorbed dose of a toxic substance of sufficient magnitude to be of pathological or public health significance.

2. One of the inherent limitations of hair analysis arises from the fact that hair represents a matrix that is in direct contact with the external environment and as such may be subject to greater contamination than other analytes traditionally used in biological monitoring, such as blood, urine, or even expired air.

Supplemental references submitted by Michael J. Kosnett, MD, MPH (June 21, 2001)

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Dan Paschal

Daniel C. Paschal–CDC

ATSDR Hair Analysis Workshop

June 12–13, 2001
Atlanta, GA

Charge Questions for Panelists:

Analytical Methods

1) **What analytical methods currently exist?**

Analytical methods for hair analysis include cold vapor atomic absorption analysis (1); graphite furnace atomic absorption (2); inductively coupled argon plasma optical emission spectrometry (3,4); inductively coupled argon plasma mass spectrometry (5); proton induced X-Ray emission (PIXE) spectrometry (6) ; X-Ray analysis (7); and neutron activation analysis (8).

2) **Substances/elements for which reliable analyses exist** include:

a) mercury- methyl and inorganic (1);

b) arsenic (2,8);

c) aluminum (3,4);

d) gold (3,4);

e) boron (3,4);

f) barium (3,4);

g) beryllium (2,3,4);

h) calcium (3,4);

i) cadmium (2,3,4);

j) cobalt (3,4);

k) chromium (2,3,4);

l) copper (2,3,4);

m) iron (3,4);

n) lithium (2,3,4);

o) magnesium (2,3,4);

p) manganese (2,3,4);

q) molybdenum (3,4,5);

r) sodium (3,4);

s) nickel (2,3,4);

t) phosphorous (3,4);

u) lead (2,3,4,5);

v) antimony (3,4);

w) selenium (2,3,4,5);

x) strontium (3,4);

y) titanium (3,4);

z) thallium (2,3,4,5);

aa) vanadium (2,3,4);

bb) zinc (2,3,4);

cc) drugs of abuse -cocaine, PCP, opiates (9,10)

3) **For what purposes are these methods typically used**?

Forensics- As

Exposure evaluation- As, Cd, Cr, Hg, Mn, Pb, Se, Al

Diet/Nutrition Status- Ca, Mg, Na, Se, Sr, V, Zn, Cu, Co

4) **What amount (g) of hair is needed**?

0.1-0.5g (4,5)- Amount depends on type (occipital or other) and detection limit (4,5,9,10).

5) **Intralaboratory variability** (within-lab/run precision and accuracy)- MUST be evaluated with a stable, homogeneous, well-characterized pooled material.

6) **Interlaboratory variability**-(among laboratories accuracy and precision)-
evaluation can be by regulation (CLIA or state/county/city licenses) or voluntary participation in Quality Assurance/Quality Control programs- e.g. Center for Toxicology of Quebec (http://www.ctq.qc.ca/ctqintre.html http://www.ctq.qc.ca/icpms.html).

Factors Influencing the Interpretation of Analytical Results

Variations in sample collection

A variety of sample preparations have been suggested to sort exogenous (presumable contamination from exposure to the external environment) and endogenous metals and drugs from collected hair specimens. These vary from no treatment, washing with deionized/distilled/ultrapure water only to washing with ionic or non-ionic detergents, either alone or in concert with organic solvent washes. For details and references, see (2).

Sampling methods

CDC has standardized the specimen collection and washing for hair, based on studies conducted internally and reported (4,5) in the literature. We obtain about 0.5 grams of occipital hair, and wash with a non-ionic detergent. Quality control is preformed by analysis of reference materials from NIST (SRM 1643d-Trace Elements in Water; SRM 1641d Mercury in Water), and a digested hair sample characterized by our operational method(s). Normal or "reference" ranges for 28 elements were published (4). "Abnormal" ranges would be those outside (generally higher than) the 95% upper limits for these analytes- toxic levels vary considerable depending on the adverse health outcome for each individual toxicant.

Exposure of hair to external environment

includes copper from certain chlorinated swimming pools, lead from lead acetate "Grecian Formula", selenium from dandruff shampoo ("Selsun"); zinc from "herbal" shampoos (Herbal

Essence; Head and Shoulders), lead , cadmium, mercury and arsenic from dust, dirt, smoke, etc (4,5,11).

Exogenous and endogenous

hair levels are difficult to distinguish, due to the high porosity of hair, and ineffective and non-standard "washing" procedures. The ideal washing/cleaning procedure would remove ONLY exogenous metals or other analytes- unfortunately, none have been reported (4,5,12,13,14).

Hair color

pigmentation (melanin?) (15) and location (4,5,11) have been demonstrated to affect hair concentrations of several analytes.

Gender, ethnicity

affect hair metals concentrations due to presence or absence of gender-linked hair treatment activities (e.g. coloring, permanent) and pigmentation (4,5,11).

Rate of Growth

of hair has been assumed by many investigators to be relatively "constant" at about 1 cm/month (4,5,11) but is known to vary somewhat with age/gender/season (4,5,11).

Toxicologic Considerations

Biological uptake of metals (4,5,11,16,17) and drugs of abuse have been extensively studied and described.

Relationship between hair and other tissue concentration levels, including urine (18) , whole blood (1,19) and serum (20) as well as other tissues (21) has been studied and described to some degree. The most complete and compelling evidence exist for hair mercury/blood mercury

(methylmercury) and for arsenic in hair/urine/fingernail/tissue (1,21,23,24). Other metals and drugs of abuse are less well characterized (17).

Dose response relationships have been demonstrated in very few recognized studies—only hair mercury and arsenic have been clearly associated with body burden and health (adverse) effects (25,26). Other evidence, e.g. correlation between the concentration of manganese in hair and behavioral disorder or violence, is less compelling (27).

Data Gaps

Methodological- Quality control/quality assurance- although some laboratories are licensed for trace metals determinations, there are very few (28) proficiency testing programs or reference materials available (29,30) for evaluation and documentation of precision and accuracy of laboratory analytical systems.

Toxicological- Serious disagreement exists as to "reference" (normal or expected) values for a large number of elements. Drugs of abuse can often be detected at low concentrations; there is some disagreement as to the correlation between results of hair testing for abused drugs and more conventional determinations of drugs in urine, exhaled breath, or other (29).

Research Needs- Simply stated, carefully designed studies of exposure, body burden, and hair concentrations are needed to move beyond "anecdotal" levels of documentation. These studies, will, unfortunately, be limited by available funds and other resources.

Scenarios Where Hair Analysis May Be Appropriate

Exposure	**Pathway**	**Chronology**	**Exposure Duration**	**Measurable Health Effects (Y/N)**
Individual	Ingestion (MeHg)	Past	Chronic	Yes (if high)

Individual	Ingestion Inhalation (As 3/5)	Past	Chronic	Yes (if high)
Individual	Ingestion Lead	Past	Chronic	Yes (if elevated)

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ATSDR Hair Analysis Workshop - Charge questions:
Topic #1: Analytical Methods.

Atomic absorption spectroscopy (AAS) is commonly used for individual elements, and can now do more than one element at a time. Lead, for example, is commonly measured by graphite furnace AAS. A well-established conventional laboratory with forensic services typically measures individual elements or a small panel of elements in hair for chronic exposure (e.g., first panel - mercury by cold vapor AAS; lead, arsenic, chromium and cadmium by graphite furnace (GF)-AAS; second panel - cadmium, manganese, nickel and thallium, all by GF-AAS). The AAS methods are considered well-established methods. The amount of hair required for either AAS panel (above) is 0.5 gram. Other analytical methods have the potential to measure a number of elements simultaneously, including inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and mass spectroscopy (ICP-MS). Newer ICP-AES instruments can attain a sensitivity equivalent to single element AAS. ICP-MS is a more sensitive method than AES.

In a carefully conducted study, a major research laboratory at the Centers for Disease Control and Prevention (CDC) reported the determination of 28 elements in hair from non-occupationally exposed U.S. populations.[1] These investigators used ICP-AES for all elements except mercury, which was measured with an LDC mercury monitor. The required minimum hair sample weight was 0.5 gram. Miekeley et al. more recently reported results for ICP-MS analysis of a suite of elements from hair in a Brazilian population, with improved sensitivity compared to ICP-AES.[2] The amount of hair required was approximately 0.3 gram.

Of the 9 commercial “nutritional hair analysis” laboratories currently operating in the United States, 3 indicate that they primarily use ICP-MS, 4 primarily use ICP-AES, and 1 reports use of directly coupled plasma (DCP)-AES. DCP-AES is an older technique that is potentially less stable than ICP-AES. On average, these laboratories measure 26 elements per hair sample. Nutritional hair analysis laboratories require between 0.3 and 1 gram for the AES methods, and

0.25-1 gram for ICP-MS. Puchyr et al. also discuss preparation of hair for elemental analysis by ICP-MS from a nutritional hair analysis laboratory perspective. [3]

Other investigative techniques for measuring elements in hair are reported in the scientific literature. A general discussion of common methods is provided by Jacobs and by Haraguchi et al. [4,5] Various other methods and example references, e.g: Differential pulse voltametric (DPV); [6] Instrumental Neutron Activation Analysis (INAA); [7,8] Microwave-Induced Plasma Mass Spectroscopy (MIP-MS); [9] Capillary electrophoresis (CE) and High Performance Liquid Chromatography (HPLC); [10] and Particle Induced X-ray Emission (PIXE).[11]

Laboratory variability has been investigated for the commercial "nutritional hair analysis" laboratories on several occasions.[2,12-14] Inter-laboratory variability was high for reference ranges, results, interpretations and health advice. For example, for one hair sample that was split and sent to six of the laboratories, there was a difference of an order of magnitude or more between laboratories in reported results for over 10 elements, including arsenic, lead, and mercury.[13] In the same split hair sample, no two laboratories flagged the same element as high, and laboratories had conflicting health interpretations and dietary recommendations based on their analysis of the sample. When intra-laboratory variability was investigated for nutritional hair analysis laboratories, results were similarly discrepant.[12]

Topic #2: Factors Influencing the Interpretation of Analytical Results.

A.) Sample collection and analysis:

Sample collection and preparation methods can have a significant impact on the data collected. Hopps notes that scalp hair has about 90% of follicles in the growth phase at any given time, growing at about 0.45 mm/day.[15] Scalp hair grows in a mosaic pattern over the scalp, with similar growth activity in the various regions of the scalp. However, sampling near the face is usually avoided due to increased likelihood of contamination from sebaceous secretions and facial hygiene products/cosmetics. Miekeley et al. note that larger samples of scalp hair (50 g.), cut into

<1cm pieces and manually homogenized, showed homogeneity in repeated analyses of aliquots of the samples.[2]

Commercial nutritional hair analysis laboratories frequently offer the option of collecting samples of axillary or pubic hair. Hair from these regions of the body grows more slowly, with a much greater proportion in the resting phase, and is likely to be subject to external contamination from apocrine gland secretions, in addition to use of personal hygiene products, clothing, etc. There are no published reference ranges for elements from non-scalp hair. A lack of correlation has been shown between scalp and pubic hair for Ca, Cu, Fe, Mg and Zn.[16]

Homogenization can be a concern, particularly if long lengths of hair are collected. Concentrations in hair of a number of environmentally-important elements have been shown to increase from the proximal to distal end of hair, e.g. Pb, Cu, Fe, Mn, and Zn.[17,18] Contamination is also a concern if the laboratory uses a grinding tool that introduces contaminants, as occurred in the preparation of one hair reference material, where Al, Fe, Ti, Mn, and Mg contamination were introduced through use of an agate ball grinding mill.[19]

Sample preparation and washing methods vary greatly and can cause different analytical results. Chittleborough provides a detailed review of these issues.[20] Various washing recommendations include: no-wash;[20] use of a standardized washing procedure recommended by the International Atomic Energy Agency (IAEA) which uses a nonpolar solvent-acetone and deionized water;[21] a mild ionic detergent-sodium lauryl sulfate emulating a detergent shampoo;[1] and more extreme methods including a chelating agent, EDTA;[22] and others (see review by Chittleborough).[20] There is no washing method presently available which is capable of reliably removing external contaminants without also affecting endogenously-deposited elements.[20,23-25] While a no-wash approach offers the least disturbance to endogenous elements, the demonstration by scanning electron microscopy of dust, dead skin, etc., adhering to much of the length of unwashed hair samples discourages use of this approach.[26] Other aspects of laboratory sample

preparation that may be critical include procedures which minimize loss of more volatile elements, such as mercury, during sample dissolution.

A major stumbling block in interpreting metals data for hair is laboratory analytical error. The World Health Organization recommends the following quality assurance methods for laboratory analyses. 1) Reference samples of the same matrix (hair) with known concentrations of the metal (element) should be used as standards. 2) Reference samples should contain the metal (element) at about the same concentration as the samples. 3) If such reference materials are not available, analysis of quality-control samples at different laboratories by different analytical methods must be used (i.e., split samples). 4) Since results may vary over time and for different metals (elements), results should be presented for the corresponding time periods and elements.[27]

There are various certified reference materials (CRM), for one (mercury) or multiple elements in hair, which meet certification requirements including certified values with a stated level of confidence in each value.[19,28-30] There is no certified hair reference material for all elements currently analyzed by commercial "nutritional hair analysis" laboratories. The Chinese hair CRM, reportedly used by four of these laboratories, certifies 17 elements: Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Na, Ni, Pb, Se, Sr, and Zn - about half the elements tested by these laboratories. A common practice among these and other laboratories is to use aqueous element standards, or other non-hair standards such as bovine liver. The difficulty with this is the possibility of complex matrix interferences in the hair sample that are not accounted for by the calibration standard. Reference ranges cited by commercial U.S. nutritional hair analysis laboratories show some rather broad inter-laboratory variations, e.g. arsenic (<0.06 vs. <5 ppm), lead (<0.8 vs. 2-20 ppm), and lithium (0.0035-0.025 vs. 1.25-3 ppm).[13]

Investigations of "nutritional hair analysis" laboratory practices using split samples have shown wide discrepancies.[2,12,13] An approved proficiency testing program for hair element analysis is not available under the Clinical Laboratory Improvement Act (CLIA). This type of analysis is

classified as a high-complexity test, with method and accuracy verification left up to the individual laboratory.

"Normal" reference ranges are largely undefined, due to the wide variation in elemental hair concentrations in presumed healthy populations. Contributing factors include geography, age, sex, ethnicity, hair type, hair treatments and other exogenous exposures. Examples of U.S. studies follow. DiPietro et al. published reference intervals for 28 elements in a non-occupationally exposed U.S. adult population.[1] These investigators used extensive questionnaire data to control for many of these factors. A partial list of geometric means for healthy adults in this study includes: arsenic (0.15 ppm); cadmium (<0.15 ppm); nickel (0.39 ppm); and lead (2.43 ppm). A number of population studies have been conducted for mercury in hair. For methylmercury, the geometric mean hair concentration for U.S. women reporting some seafood consumption was 0.36 ppm, and 0.24 ppm for no seafood consumption.[31] Published clinical references for biomonitoring for metals/elements in hair are sparse. These include arsenic (<1 ppm) and thallium (?5-10 ppb)[32] and mercury (<1ppm) and nickel (0.01-1.8 ppm).[33] These are secondary to the established blood and/or urine reference levels, and the problem of external contamination is noted as a major stumbling block which limits the use of the hair references.

Generally speaking the use of the term "normal" is misleading. What is being estimated is a background or baseline level for a population, typically by geographic region, rather than a state of health. Methylmercury data are an exception. Methylmercury exposures commonly occur through consumption of fish and seafood. Clear dose-response relationships have been demonstrated between dietary consumption of mercury-contaminated fish and concentrations in human hair. Methylmercury is the only metal (compound) which has a health benchmark based on hair concentrations. The U.S. EPA has a reference dose (RfD) for methylmercury of 0.0001 mg/kg body wt/day. This is based on a benchmark dose of 11 ppm in maternal hair, equivalent to a maternal blood level of 44 micrograms/L, for developmental neurological abnormalities in infants.[34] Several reference range studies for methylmercury are available.[31,35,36]

B.) Other factors influencing analytical results:

Hopps notes the sources of elements in hair as: 1) papilla (contacted by blood and lymph) during hair formation; 2) sebaceous glands, sweat glands, desquamating skin cells (endogenous exposures not necessarily related to blood/organ concentrations); 3) and exogenous materials.[2] Salts of sodium, potassium, and calcium predominate in sweat, but minor amounts of other elements are also found, e.g., zinc.[15] There is evidence for an extra input route from the root sheaths into the hair shaft, other than longitudinal growth, complicating the picture of a simple blood compartment:hair relationship.[11] Finally, the lipids and waxes in sebum and skin may contribute to sealing exogenous contaminants into the hair shaft.

Exogenous contaminants can range from personal care products to elements present in air, water, soil, occupational environments, etc. As mentioned above, there is currently no washing method capable of removing exogenous elemental contaminants while leaving endogenous elements undisturbed. Chemicals such as methylmercury, which are generally from dietary sources, suffer less from this drawback, provided unusual sources of inorganic mercury do not complicate the picture, e.g., mercury vapor in occupational settings. Practically speaking, public health concerns are often related to exposure, and hair can serve as an index of overall exposure, if not of biological uptake.

Examples of external contaminants of hair include both personal care products and environmental sources. Hair is a porous material (witness the rapid uptake of water and increase in weight during washing) and may bind through weak ion-exchange sites (e.g., Na, K, Ca, Mg), and through stronger bonds, particularly with sulfur, (e.g, arsenic). Arsenic binds avidly to hair, due to the sulfur content of keratin. Exogenous arsenic is readily taken up by hair and cannot be differentiated from endogenous arsenic.[15] It has been shown that adsorption of other metals such as Al, Cd, Cu, Pb and Zn into scalp hair from aqueous solutions cannot be reversed even by extreme washing methods.[25] Hair treatments such as permanents can alter such binding.[37] Dandruff shampoos containing selenium can contaminate hair.[38] DiPietro et al. noted significant

difference between dandruff shampoo vs. regular shampoo for Na, Se, and Ti for men, and between permanents/color and any shampoo for Ba, Ca, Cu, Mg, Na, and Sr for women.[1] Hair dyes may contain metals, e.g., lead in "Grecian Formula."[39] Sky-Peck also found that peroxide bleaches and permanents altered S, Ca, Fe, and Ni in hair, peroxide altered Zn, and permanents increased Cu and As concentrations.[39]

Soil, house dust, and water may contribute contaminants.[40] Air serves as a contamination source.[41] This is a major concern in occupational settings. Cadmium is an example of a metal where environmental sources contribute to concentrations in hair, e.g., drinking water and dust levels and seasonal influences.[42]

As noted, gender, ethnicity, diet, age, geographic location, and season are capable of influencing hair reference ranges in populations. Sky-Peck found the following for a healthy midwestern U.S. population: 1) gender – females had higher Ca and Ni and lower Pb, Br and Se compared to males; 2) hair color - blondes had less Fe than brunettes, red-heads had more Fe and Cu; 3) ethnicity/race - Blacks had increased Ca, Fe, Ni, Cr, Mn, As, and Pb, and decreased Hg, compared to Caucasians; Orientals had decreased Ca, Fe, Cu, Mn and Pb; 4) age – a decrease in S, Ca and Sr, and an increase in Pb with age; 5) geography – increased hair strontium in areas with elevated strontium in drinking water, and increased hair lead in industrial/older residential areas.[39] Sky-Peck notes that some of the differences in gender and ethnicity may be due to differences in hair treatment and/or environmental exposure. While Sky-Peck found no differences between gray hair and natural hair, other investigators have noted pigmentation effects,[43] and it is known that various chemicals, including metals, will bind to melanin.[44]

Other investigators have studied age-related differences in hair elements. Paschal et al. observed age-dependent increases in Ca, Ba, Mg and Sr (Group 2A alkali elements) and Zn up to 12-14 yrs in U.S. residents.[45] In comparison, an Italian study showed increases in Cu, Zn, Cr and Br, and decreases in Fe, Mn and Sr up to 8 yrs.[46] In Japanese children, Zn decreased up to 12-14

yrs, and Cu showed a similar trend.[47] The reason for differences between laboratories and/or populations is not presently known.

Baseline reference values for elements in clinical specimens, including hair, have been referenced by international location.[48,49] International differences are identified for hair Zn, Cd, Cu, Mn and Pb. Some of this geographical difference may be due to differences in environmental metal concentrations, industrialization, etc. Seasonal differences in hair element concentrations, e.g. cadmium, may be due to time spent outdoors and contact with soil, dust, etc.

Topic #3: Toxicological Considerations.

As discussed above, methylmercury is the only element (compound) for which sufficient data exist to define the relationship between concentrations in blood, concentrations in hair, and effects on the target (the developing fetus). It is also the only element (compound) with a health benchmark, the U.S. EPA reference dose, based on a threshold concentration in human hair. It should be noted that this threshold was identified based on massive poisoning incidents in human populations and not on typical (dietary) exposures.[34,50] Forensic medicine has used hair to assess poisoning by other elements, e.g, arsenic and lead. However, these document overwhelming poisoning exposures, rather than a threshold for earliest/most subtle adverse health effects. Nor is there a need in these instances to differentiate between a “normal” background and subtle increases in exposures. Such a distinction is difficult due to the wide variations in background reference ranges. This has caused a number of investigators to conclude that results for an individual are not likely to be meaningful with respect to less drastic environmental/dietary exposures, and that statistical analyses of group data must be employed.[13,42,51,52] Finally, if the goal is also to provide an index of body burden, rather than simply document exposure to environmental contaminants, the lack of a washing technique capable of reliably separating exogenous contaminants from biologically-deposited elements is a substantial concern and must be addressed.

Of the trace elements that have been tested in hair, only a few have research data relating hair concentrations to blood levels and/or tissue concentrations. Aside from mercury, the focus has largely been on aluminum, arsenic, cadmium, chromium, copper, lead, selenium, and zinc. Data highlights are summarized below.

Aluminum (Al) – Aluminum is elevated in hair only in extreme exposures (and even then is inconsistent), and is unrelated to serum or bone aluminum.[53-56] Aluminum dietary intake is unrelated to aluminum in hair, even with controlled dietary intake.[57] Aluminum in hair is not a useful biological indicator of exposure.

Arsenic (As) – Arsenic is well taken up in hair. Animals show a dose-related increase in hair arsenic.[42] Forensic hair tests can determine the time-course of chronic arsenic poisoning.[58] Increased arsenic in soil (<20 to 370 ppm soil As) show a slight correlation with slightly elevated hair arsenic using group statistics (0.02 ppm to 0.06 ppm hair As).[59] Consumption of drinking water with elevated arsenic concentrations showed a correlation with hair arsenic, using group comparisons.[60-62] This correlation was not seen in a study where drinking water exposure was only modestly above a legal threshold.[63] Group statistics show elevated hair As in patients with Blackfoot disease.[64]

Cadmium (Cd) – Animal studies show conflicting results with respect to any correlation between cadmium in hair and the target organ, the kidney.[42,52] The most significant non-occupational exposure to cadmium occurs through tobacco smoke. Smokers have elevated blood cadmium levels compared to nonsmokers. Studies show conflicting results with respect to hair cadmium concentrations in smokers versus non-smokers.[65-67] A nationwide German environmental survey found little correlation with cadmium in hair and active cigarette smoking, although it was the major predictor for blood and urine cadmium concentrations. In contrast, outdoor activities, seasonality, and cadmium in tap water were more important predictors in hair cadmium concentrations, emphasizing the role of exogenous deposition of cadmium into hair.[67]

Copper (Cu) – Taylor's review notes that animal studies showed a proportional relationship between copper in hair and liver.[52] Yoshinaga et al. found no significant correlation between hair copper and various internal organs, including the liver, in autopsy samples.[68]

Literature studies of human populations show conflicting results with respect to hair versus serum copper.[52,69,70] Serum copper is generally higher in women than in men.[33,49] However, hair copper is inconsistent with respect to sex. Contiera and Folin found no effect of sex on hair copper.[71] Sky-Peck found a modest correlation ($p<0.025$) for higher hair copper in women compared to men (24 vs. 20 ppm).[39] In human patients with biliary cirrhosis, or Wilson's disease (systemic copper intoxication), with increased liver copper, hair copper was typically not increased.[52] Further studies of Wilson's disease confirmed these findings, with no increase in hair copper in patients with this disease.[72] In copper deficiencies (malnutrition or Menkes syndrome), hair copper was not significantly reduced.[52]

Chromium (Cr) – Studies of hair chromium are somewhat limited. A large study (40,872 patients) in England found age-related decreases in hair chromium for males and females [0.98 ppm (mean at age 1-4 yrs) to 0.5 ppm (mean at age 70 plus yrs)], slightly lower hair chromium in males ages 25-49 years, and a correlation between hair and serum chromium, all statistically significant.[73] In comparison, a U.S. study found no difference in hair chromium by sex or age in 987 individuals.[39] Hair chromium has been hypothesized to increase in gestational diabetes (in early pregnancy), compared to non-diabetic pregnant women.[74] Hair chromium measurements have been used in monitoring occupational exposures, although blood and urine chromium are the standard biological indices.[75]

Lead (Pb) – There are a number of studies relating lead exposure to tissue concentrations, including hair. Animal studies show a dose-dependent correlated increase in lead in bone and hair during the exposure period.[76] Isotopic tracer studies have shown the deposition of lead into human facial hair, interpreted as the integral of a blood lead pool over approximately 3 months.[77] In humans, hair analysis can be used to demonstrate lead poisoning.[72] Occupational exposures show a correlation between blood and hair lead.[52,78] Lower-level exposures have more variable results,[52] but larger studies appear to support a relationship between hair and blood lead.[42] Exogenous deposition of lead onto scalp hair may be influential, e.g., season, dust exposure, and hair treatment.[42] Centers for Disease Control (CDC) investigators compared hair and blood samples from 189 children to gauge the accuracy of using hair to screen for lead poisoning (mean

blood lead 9.8 ug/dl; mean hair lead 7.2 ppm).[79] Hair lead as a screening method had a 57% sensitivity and 18% false-negative rate. The investigators concluded that hair lead measurements are NOT an adequate method of screening for childhood lead poisoning. The reliable measure of individual lead exposure is a blood lead test.

Selenium (Se) - Animal studies show that: 1) hair selenium is strongly influenced by the chemical form of selenium and the level in the diet, with a greater increase for L-selenomethionine than sodium selenate 2) sodium selenate increases hair selenium but not muscle selenium (the largest body Se pool); and 3) dietary methionine deficiency increases selenium deposition in hair.[80] These observations suggest caution when evaluating environmental selenium exposures. Population measurements have shown a correlation between low hair selenium and selenium-deficient soils.[81] The hair-to-blood selenium ratio is calculated to be ?3 in dietary selenium deficiency, increasing to 10 as toxic levels are approached. Hair selenium will continue to rise far beyond the plasma saturation concentration, indicating contribution from another body pool.[82] A hair concentration of >5 ppm Se is reported to be associated with elevated exposure, while a concentration <0.12 ppm Se is reported to be associated with chronic selenium deficiency.[83] However, most population studies have preferred blood or urine to indicate selenium exposure.[84] Exogenous contamination with selenium-containing dandruff shampoos is a serious confounding factor in developed countries.[1] Yoshinaga et al. found no significant correlation between selenium concentrations in hair and in internal organs.[68]

Zinc (Zn) – Zinc in hair has been reviewed by several authors.[52,85-88] These reviewers note that hair is a difficult medium for interpretation of zinc status. The interpretation of zinc concentrations in hair can be obscured by confounders such as sex, body composition, and hair treatment.[89] In severe zinc deficiency, hair growth slows, producing normal or even elevated hair zinc concentrations.[87] Yoshinaga et al. found no significant correlation between concentrations of zinc in hair and in various internal organs.[68] Administration of zinc in the diet did not increase zinc in beard hair.[90] Serum zinc is typically decreased in dialysis patients. Hair zinc in these patients is not consistent with serum findings.[91]

In conclusion, with the exception of methylmercury, there is no good indication that hair analysis offers any improvement over currently available clinical tests to determine individual biological exposure to metals/metalloids of concern.[92] Occupational texts note that hair analysis is unproven to detect toxic chemicals in the body to account for symptoms and inappropriate in the diagnosis of "environmental" illness.[93] Group statistics on hair data, preferably geometric means, may be useful in population screening for exposure to some of these metals (e.g., arsenic). Confounding factors, such as hair treatments, must be controlled for in these studies. Analysis of hair minerals to predict nutritional status is a practice not supported by the state of the science.

Topic #4. Data Gaps and Research Needs.

Generally speaking, further information is needed on concentrations of elements in the hair of individuals with known exposures to trace elements, particularly where environmental exposures are of concern. Laboratory studies of elemental concentrations in blood and target tissues compared to hair concentrations are needed. Such data are important if one is to hypothesize that there is a relationship between hair element concentrations and critical/target organ effects. Clinical studies correlating hair concentrations with clinical conditions (deficiencies or elevations) may also be helpful. Further work is needed on sample washing methods. Standardization on one washing method is important for comparison of studies.

Specific recommendations:

? Do not use hair analysis for individual nutritional assessment. The state of the science does not support this application.

? If hair analysis is undertaken for comparison of groups, choose element(s) for which the literature supports such an approach, e.g., methylmercury, e.g., NOT aluminum.

? When studying control versus exposed groups, chose a group size of sufficient statistical power to determine differences between group means, based on current literature findings.

? Use geometric means in analyzing group data.

? Collect blood and/or urine samples for comparison with the hair results in the analysis of group data. If this is not feasible for the entire study population, choose a subset of sufficient size to provide statistically meaningful comparison data.

? A questionnaire should be administered to each individual in the study, determining: age, sex, ethnicity, hair wash and hair treatment history including products used on hair, swimming habits, time spent outdoors, occupation, smoking history, etc. (e.g, DiPietro et al., 1989).

Topic #5: Identifying scenarios for which hair analysis may be appropriate.

Exposure Scenario	Chemical/ Exposure Pathway	Exposure Chronology	Exposure Duration	Measurable Health Effects (Y/N)
Individual – severe poisoning/forensic	Mercury, Arsenic, Lead	Past / present	Acute (1-2 months min.); chronic	Possible with very high exposure
Group/population:	Methylmercury-diet (fish, seafood)	Past / present	Acute (1-2 months min.); chronic	Unlikely unless very high exposure
	Arsenic, Cadmium, Lead	Past / present	Acute (1-2 months min.); chronic	Unlikely unless very high exposure

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