

SUMMARY REPORT

**HAIR ANALYSIS PANEL DISCUSSION:
EXPLORING THE STATE OF THE SCIENCE**

June 12–13, 2001

Prepared for:

The Agency for Toxic Substances and Disease Registry
Division of Health Assessment and Consultation
and
Division of Health Education and Promotion
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NOTE

This report was prepared by Eastern Research Group, Inc. (ERG), an ATSDR contractor, as a general record of discussion for the “ATSDR Hair Analysis Panel Discussion: Exploring the State of the Science.” As requested by ATSDR, this report captures the main points of scheduled presentations and highlights discussions among the panelists. This report is not a verbatim transcript of the meeting proceedings, nor does it embellish, interpret, or expand upon matters or agenda topics that were incomplete, unclear, or not addressed. Statements are the individual views of each panelist or meeting participant. Except as specifically noted, no statements in this report represent analyses or positions of ATSDR or ERG.

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FOREWORD

The Agency for Toxic Substances and Disease Registry (ATSDR) has found the expert panel process to be an effective tool for discussing and weighing scientific and public health issues. ATSDR convened one such expert panel to discuss the state of the science related to analyzing hair for environmental substances of concern found at hazardous waste sites. The panel consisted of individuals who represented state and federal government agencies, academia, and private practice and whose expertise, interests, and experience covered a wide range of technical disciplines that were critical to the issues being discussed. ATSDR convened the expert panel as part of an effort to begin formulating guidance on the use of hair analysis in exposure assessments. The panel met to discuss their opinions regarding hair analysis for 1½ days in June 2001 in Atlanta, Georgia. This document summarizes the panel discussions.

For ATSDR, the overarching objective of the panel discussion was to gain information on when to consider using hair analysis for exposure assessments. Exposure assessments are a necessary component of public health assessments and other related public health activities performed by the Agency for communities near hazardous waste sites. The Agency sought information about the overall utility, advantages, and limitations of hair analysis and how these factors would affect informed decisions on a site-specific basis.

The panel was asked to address a series of general questions about the science of hair analysis. These focused on exposure assessment and health interpretation of the results of hair analysis. The panel was strongly encouraged to avoid discussing the merits of hair analysis for drug testing or nutritional screening, unless such discussions involved a technical point that was directly applicable to environmental exposure assessment at hazardous waste sites. ATSDR did not seek consensus statements from the panel; rather, the panel was asked to discuss in detail specific issues related to methodology, factors influencing the interpretation of results, toxicologic considerations, data gaps, and research needs. The opinions expressed in the report are those of the individual panelists and may or may not represent those of ATSDR.

ATSDR views the panel discussions as a first step to sorting through the scientific issues regarding the advantages and disadvantages of hair analysis. ATSDR plans to weigh the information and data presented at the panel meeting and, over the next few months, develop interim guidance for its health assessors and other professionals who are asked by communities about the virtues of hair analysis as it relates to exposure and health evaluations at hazardous waste sites.

RADM Robert C. Williams, P.E., DEE
Assistant Surgeon General
U.S. Public Health Service
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Director, Division of Health Assessment and Consultation (DHAC)

Agency for Toxic Substances and Disease Registry

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LIST OF ABBREVIATIONS

AA	atomic absorption
ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Centers for Disease Control and Prevention
CLIA	Clinical Laboratory Improvement Act
DHAC	Division of Health Assessment and Consultation
DHEP	Division of Health Education and Promotion
EI	exposure investigation
EPA	U.S. Environmental Protection Agency
HIV	human immunodeficiency virus
IARC	International Agency for Research on Cancer
ICP-AES	Inductively coupled argon plasma atomic emission spectrometry
ICP-MS	Inductively coupled argon plasma mass spectrometry
ICP-OES	Inductively coupled argon plasma optical emission spectrometry
µg/L	micrograms per liter
NAA	Neutron activation analysis
NHANES	National Health and Nutrition Examination Survey
NRC	National Research Council
NTP	National Toxicology Program
PIXE	Proton induced x-ray emission
ppm	parts per million
PTH	parathyroid hormone
QA/QC	quality assurance/quality control

Abbreviations for Panelists' Names

RB	Dr. Robert Baratz
TC	Dr. Thomas Clarkson
MG	Dr. Michael Greenberg
MK	Dr. Michael Kosnett
DP	Dr. Dan Paschal
SS	Dr. Sharon Seidel
LW	Dr. LuAnn White

EXECUTIVE SUMMARY

The Agency for Toxic Substances and Disease Registry (ATSDR) convened a seven-member panel to review and discuss the current state of the science related to hair analysis, specifically its use in assessing environmental exposures. ATSDR invited a cross section of scientific experts in the fields of hair analysis, toxicology, and medicine to participate in 1½ days of discussions on a variety of topics, including analytical methods, factors affecting the interpretation of analytical results, toxicologic considerations, and data gaps/research needs. The meeting was held June 12 and 13, 2001, in Atlanta, Georgia.

Background

ATSDR convened this panel in response to (1) a growing number of inquiries from community members looking for assistance in interpreting hair analysis results and (2) agency interest in learning more about the utility of hair analysis in evaluating exposures and health effects at hazardous waste sites. The agency hopes to use the input received from this effort to develop guidance for agency health assessors on the use and interpretation of hair analysis data.

The general questions that ATSDR seeks to answer include:

- For what substances do reliable hair analysis methods exist?
- When is it appropriate/inappropriate to consider hair analysis in assessing human exposures to environmental contamination?
- What data gaps exist that limit the interpretation and use of hair analysis in the assessment of environmental contaminants?

This summary report presents the findings of the panel discussions. Central discussion points are highlighted below.

Overview of Discussions

Panelists engaged in a series of discussions to address ATSDR's questions, pointing to several limitations—having to do with the current state of the knowledge—on the usefulness of hair analysis in assessments of environmental exposures. Discussions focused primarily on metals and trace elements in scalp hair. Panelists considered the distinct differences between using hair analysis to identify exposures (Is the substance reaching people? Does a competing pathway exist?) and using it to predict, diagnose, or treat disease (What do hair concentrations tell us about the likelihood of harmful health effects?). Panelists noted that the latter is where the largest data gaps exist.

Although they were not required to reach consensus, the panelists did agree on the following summary statement related to the overall usefulness of hair analysis in evaluating environmental exposures:

For most substances, insufficient data currently exist that would allow the prediction of a health effect from the concentration of the substance in hair. The presence of a substance in hair may indicate exposure (both internal and external), but does not necessarily indicate the source of exposure.

For what substances do reliable hair analysis methods exist?

The group agreed that laboratory methods exist to measure the levels of some environmental contaminants in hair, but procedures need to be standardized to help ensure more accurate and reliable results (this includes ensuring that samples are collected by a trained person and establishing consistent sampling protocols, washing protocols, quality control/quality assurance procedures, etc.). Further, the panel agreed that testing should be targeted to the specific element of interest.

When is it appropriate/inappropriate to consider hair analysis in assessing human exposures to environmental contamination?

In general, panelists agreed that, before determining the appropriateness of hair analysis as an assessment tool, assessors should consider the following:

- (1) *The exposure type and period.* Take exposure histories to understand the likelihood that a particular substance will be in the hair at the time of testing and to identify other exposure sources (e.g., hair treatments).

Because the growth rate of hair is on average 12 centimeters per year, the panel concluded that hair analysis is not generally useful for evaluating very recent exposures or those longer ago than 1 year. Segmental analysis of hair (i.e., looking at concentration trends along the length of the hair) may have a role in documenting exposures over time (e.g., identification of a high-dose acute exposure). This would need to be considered on a subject-, substance-, and situation-specific basis.

- (2) *The type of substance and its behavior in the body.* Determine the biological plausibility that a particular substance will be present in hair and whether it is a marker of external contamination.

The group agreed that little is known about the transfer kinetics of substances into hair.

- (3) *The clinical relevance of a negative or positive finding.* Determine whether any dose-response relationship exists between chemical concentrations in hair and target organ effects/illness. Without an understanding of a dose-response relationship, useful interpretations will not be possible.

The panelists agreed that a relationship between contaminant concentrations in hair and any kind of measurable outcome have only been established for methyl mercury (e.g., the relation between maternal hair levels and observed developmental neurological abnormalities in offspring) and to a limited extent for arsenic (e.g., segmental analysis for forensic analysis), provided external contamination can be ruled out. There may be unique forensic settings for other substances.

The group also indicated the need to evaluate, on a substance- and exposure-specific basis, the extent to which hair analysis may be more advantageous than other biological sampling, such as blood or urine analysis.

What data gaps exist that limit the interpretation and use of hair analysis in the assessment of environmental contaminants?

The group identified several factors that limit the interpretation of even the most accurate, reliable, and reproducible laboratory results. These include:

- *The lack of reference (or background) ranges in which to frame the interpretation of results.* Assessors need a greater understanding of what is expected to be in hair in the absence of environmental exposures in order to determine whether detected levels are elevated as a result of environmental releases, including possible geographical or regional differences in background levels.
- *Difficulties in distinguishing endogenous (internal) from exogenous (external) contamination in hair.* Being able to make this distinction is important in evaluating internal doses of the substance of interest. The group voiced different views on the effectiveness of washing hair prior to analysis to eliminate external contamination. Some felt that the current literature suggests that there is no reliable washing method capable of separating external contamination from internal deposition of elements. It was suggested that identifying metabolites (or other unique markers of internal exposure) for substances of interest, where possible, is most helpful in distinguishing internal from external contamination.
- *A lack of understanding of how and to what extent environmental contaminants are incorporated into the hair.* Little scientific information is available on the uptake or incorporation of environmental contaminants into hair. Neither kinetic models nor metabolite data are known or fully understood for metals or environmentally relevant organic compounds.
- *The lack of correlation between levels in hair and blood and other target tissues, as well as the lack of epidemiologic data linking substance-specific hair levels with adverse health effects.* These correlations must be understood before hair analysis results can be used as a diagnostic tool or to predict health endpoints. The panel noted that hair analysis is not likely to play a role in evaluations of some of the more common health concerns associated with hazardous waste sites (e.g., cancer, birth defects).
- *Little information is available pertinent to the study of environmentally relevant organic compounds in hair.* The panel recommended taking advantage of what is known about hair analysis for testing drugs of abuse.

In moving forward, the panelists encouraged the standardization of sampling protocols and identified possible research areas. Before hair analysis can be considered a valid tool for any particular substance, research is needed to establish better reference ranges, gain a better understanding of hair biology and pharmacokinetics, further explore possible dose-response relationships, establish whether and when hair may serve as a better measure or predictor of disease than other biological samples (e.g., blood or urine), and learn more about organic compounds in hair.

Future ATSDR Activities

ATSDR plans to evaluate all the input received during the panel deliberations and generate a report on lessons learned from the panel discussions. In addition, the agency anticipates that the following activities will help all of ATSDR's divisions as well as professionals in the community.

- Providing education to physicians and other health professionals about hair analysis.
- Developing a generic fact sheet to help health assessors and communities communicate and understand hair analysis issues.
- Continuing to develop substance-specific toxicological profiles. The profiles are an excellent resource and contain information on biomarkers of exposure. In light of the panel discussions, additional language may be added regarding hair analysis (e.g., in terms of limitations, etc.).
- Developing guidance on hair analysis to support public health assessments and health studies conducted by the agency. That is, developing criteria for determining when to consider hair analysis as part of an ATSDR exposure investigation.

SECTION 1

INTRODUCTION

ATSDR convened a panel of seven experts to discuss the state of the science related to hair analysis, with specific focus on its utility in assessing environmental exposures. A 1½-day meeting held at the Radisson Executive Park in Atlanta, Georgia, on June 12 and 13, 2001, served as a forum for the panelists to discuss scientific issues related to the analysis and interpretation of hair data. The meeting, which was open to the public, also gave other interested parties the opportunity to observe the discussions, ask questions, and provide input.

This section details ATSDR's purpose for convening the panel (Section 1.1), how ATSDR selected panel members (Section 1.2), the charge to the panel (see Section 1.3), the meeting format (see Section 1.4), and the organization of this summary report (see Section 1.5).

1.1 Background

ATSDR conducts public health assessments to evaluate possible public health implications of contamination associated with hazardous waste sites and other environmental releases. An important step in ATSDR's assessment process is examining exposures to contaminants under site-specific conditions and determining whether people are being exposed to contaminants at harmful levels. In most of the agency's evaluations, the environmental concentration serves as a surrogate for "exposure."

Exposure concentrations, or estimated doses based on exposure concentrations, however, represent only one factor in a continuum of events that ultimately determine whether exposures will result in illness. Other factors include exposure conditions and various pharmacokinetic/pharmacodynamic events (e.g., absorption, distribution, metabolism, excretion),

as well as individual variability and susceptibility in the exposed population. To a large extent, ATSDR evaluates these factors qualitatively in its public health assessments.

To refine its assessments and/or to fill data gaps, ATSDR seeks ways to more precisely quantify exposures, such as measuring body burdens of a particular contaminant or its metabolites (e.g., lead in blood or arsenic and its metabolites in urine). On a site-by-site basis, ATSDR evaluates what additional exposure data it might be practical and useful to obtain to further support public health evaluations and ultimately to help determine the disease potential of a particular exposure.

In convening this panel, ATSDR's goal was to determine the overall utility of hair analysis as one such exposure assessment tool. Hearing various points of view will help ATSDR draw conclusions based on the best available science.

ATSDR plans to weigh the information and data presented at the panel meeting and, in the short term (i.e., over the next several months), independently develop some interim guidance for its health assessors and others at ATSDR who are asked by communities about the virtues of hair analysis in understanding exposures to, or the disease potential of, particular chemicals. For the purposes of the panel discussions, ATSDR was not seeking consensus of the panel on any particular issue, but rather scientific input (consistent or varied) for consideration by the agency. Also, the panel was *not* convened to discuss or evaluate the merits of hair analysis for other purposes (e.g., testing for drugs of abuse or nutritional screening). Again, the focus was on environmental exposures.

See the introductory remarks in Section 2 for additional background information.

1.2 Selection of Panelists

ATSDR identified candidates for the expert panel by reviewing the scientific literature in the field of hair analysis, researching professional organizations, and consulting with known experts within research institutes and other academic centers. The agency sought individuals who were experienced in the field of hair analysis and its interpretation for hazardous substances released to the environment.

To help ensure that a broad range of views was brought to the table, the agency sought individuals possessing a range of experience, interest, and expertise in the field of hair analysis. Potential candidates were ranked based on their level of technical expertise (i.e., either high, medium, or low) in each of the following categories:

- Hair analysis research
- Laboratory analysis
- Pediatric medicine
- Occupational medicine
- Forensic medicine
- Exposure assessment

Based on these criteria, ATSDR selected seven panelists, each of whom had expertise in one or more of the categories listed above. The collective expertise of the panel covered all categories, and individuals on the panel represented state and federal government, academia, and private practice.

Appendix A lists the names and affiliations of the panelists who participated in the meeting as well as a brief biographical sketch of each of the panelists.

1.3 Charge to the Panelists

ATSDR prepared a list of specific questions for the panel (referred to as the “charge”). Questions included a wide variety of topics designed to prompt discussions at the meeting. The main topics in the charge include:

- Analytical methodologies
- Factors influencing the interpretation of analytical results
- Toxicologic considerations
- Data gaps and research needs
- Identifying scenarios for which hair analysis may be appropriate

A copy of the charge to the panelists is included in this report as Appendix B.

Prior to the June 12 and 13, 2001, meeting, panelists were requested to review the charge and prepare initial responses to the charge questions (in the form of pre-meeting comments). To support their effort, panelists received six papers from the published literature and a bibliography of additional literature pertaining to hair analysis.¹ The purpose of this pre-meeting exercise was to stimulate panelists’ thoughts in relation to the charge questions and to serve as a stepping-off point for the 1½ days of panel discussions. Appendix C contains the panelists’ pre-meeting comments.

¹The papers provided to the panelists for their consideration included: Hopps 1977; Miekeley et al. 1998; Sky-Peck 1990; Seidel et al. 2001; Steindel and Howanitz 2001 (editorial); Yoshinaga et al. 1990; Wennig 2000.

Panelists provided additional relevant references with their pre-meeting comments and during panel discussions. An expanded bibliography of hair analysis literature is provided in Appendix D.

1.4 The Meeting Format

After some introductory remarks by ATSDR and an overview of hair physiology by one of the panelists, the panel engaged in open discussions related to individual charge questions.

Discussions generally followed the meeting agenda, as shown in Appendix E. However, as might be expected, some overlap occurred across topics due to the closely linked nature of the topics.

Dr. LuAnn White led the panel discussions. At the beginning of the meeting, she clearly stated the ground rules for the discussion:

- Focus on the scientific issues related to hair analysis.
- Focus on the specific charge topics. In the context of the charge questions, describe the advantages and disadvantages of using hair analysis.
- Limit discussions to topics directly or indirectly related to *environmental* exposures. Focus on the markers of environmental exposures or internal dose.
- Actively listen to one another and exchange ideas and different perspectives.

In addition to the panelists, approximately 50 observers attended one or both days of the meeting.

The observers included representatives from ATSDR, the Centers for Disease Control and Prevention (CDC), other federal and state agencies, commercial laboratories, and professional organizations. A list of the observers who attended the meeting is included in Appendix F.

Though the discussion at the meeting was largely among the panelists, observers were given three separate opportunities during the meeting to comment or ask questions (see the agenda) and

also were encouraged to provide written comments to ATSDR in response to the charge questions and panel discussions. Written comments received from observers after the meeting are included in Appendix G.

1.5 The Report Organization

The organization of this report generally follows the list of topics outlined in the agenda and charge to the panelists. Section 2 includes a summary of opening remarks. Sections 3, 4, and 5 summarize the panelists' comments and discussions related to analytical methodologies, various factors influencing the interpretation of results, and toxicological considerations. Section 6 reports overall conclusions drawn by the panel, including data gaps and research needs.

Comments provided by observers throughout the meeting are presented in Section 7. Section 8 lists references cited in this summary report.

Note: In subsequent sections, the panelists' initials are used to attribute comments. They are as follows: Dr. Robert Baratz (RB), Dr. Thomas Clarkson (TC), Dr. Michael Greenberg (MG), Dr. Michael Kosnett (MK), Dr. Dan Paschal (DP), Dr. Sharon Seidel (SS), Dr. LuAnn White (LW).

SECTION 2

OPENING REMARKS AND PRESENTATIONS

Dr. Robert Amler, ATSDR's Chief Medical Officer, opened the meeting by welcoming panelists and observers and describing how the hair analysis panel discussions would help support the agency's public health mission. Dr. Allan Susten and Dr. Deanna Harkins, technical coordinators of the panel, reviewed the scientific issues related to hair analysis and the impetus for convening the hair analysis panel. They briefly described how hair analysis fits into the agency's public health assessment process, the goals and objectives of the panel discussions, and how the agency plans to use the scientific information obtained from panelists and observers.

To help ground subsequent discussions, panelist Dr. Robert Baratz provided an overview on the anatomy and physiology of hair. ATSDR's and Dr. Baratz's presentations are summarized below.

2.1 Welcome **Robert Amler, M.D.** **ATSDR Chief Medical Officer**

After welcoming all in attendance, Dr. Amler stated that ATSDR's overall mission is to protect people's health by identifying and preventing toxic exposures. Because recognizing problems and knowing how to evaluate them are key to the agency's ability to assess potential health threats, discussions such as those anticipated during the hair analysis meeting are of key importance. Dr. Amler noted that such discussions will help ATSDR sort through the advantages and disadvantages of using hair analysis in its exposure and health assessments.

Dr. Amler explained that the panel process has been shown to be a very effective means for discussing and weighing scientific issues. He further explained that these panel discussions will serve as a first step in developing agency guidance on the appropriateness of using hair analysis.

Because it is only a first step, additional areas of discussions may be necessary. Dr. Amler acknowledged that it would not be possible to obtain all the answers in this forum.

Dr. Amler thanked the individuals who were instrumental in initiating and organizing the panel discussions, including Mr. Robert Williams, Director of ATSDR's Division of Health Assessment and Consultation (DHAC); Dr. Gregory Christenson, Acting Director of ATSDR's Division of Health Education and Promotion (DHEP); Dr. Allan Susten, DHAC's Assistant Director for Science; and Dr. Deanna Harkins, Medical Officer, within DHEP. He also thanked Dr. LuAnn White for moderating the meeting. Lastly, he thanked all participants for their involvement in what promised to be fruitful discussions.

2.2 Purpose of the Meeting and Charge to the Panelists

**Allan Susten, Ph.D., D.A.B.T.
Assistant Director for Science
ATSDR/DHAC**

Dr. Susten described how the agency seeks to use the best available science in conducting its public health assessments. He indicated that the overarching goal for the panel discussions is to review the state of the science of the hair analysis field and help the agency evaluate the overall utility of hair analysis in its public health assessments. Specifically, the agency seeks to determine when it might be appropriate to use hair analysis in evaluating possible exposures and/or possible adverse health effects associated with environmental toxicants. Dr. Susten acknowledged that hair analysis is used for other purposes (e.g., drugs of abuse, forensics) but said that the focus of this forum was on the relevance of hair analysis to hazardous waste site evaluations.

To help illustrate the nature of the scientific input that helps the agency evaluate exposures and health effects, Dr. Susten displayed a “continuum” showing the components of ATSDR’s public health assessment process (see Figure 2-1). In doing so, he described the following components of the process:

- *Exposure evaluation*—Involves studying how hazardous substances can reach people, studying the means by which people can come in contact with hazardous materials, and determining the exposure concentration or dose at the point of contact.
- *Target dose evaluation*—Involves studying the distribution of a hazardous substance once it enters the human body and determining the internal and biologically effective doses.
- *Health effects evaluation*—Takes a closer look at the dose-response relationships of the substance(s) under evaluation and how the substance exerts its effect.

Dr. Susten explained that it is not enough to look at the estimated exposure concentration or exposure dose when evaluating the potential that a particular exposure will lead to clinical disease. To better understand the extent of exposures and the potential that a particular exposure will lead to disease, one needs to study the biology and the toxicology of the substance involved. Therefore, where possible, the agency seeks ways to estimate or measure internal dose and to assess whether such exposures might be associated with adverse health effects.

Through its work over the past decade or so, ATSDR has recognized that knowledge of environmental concentrations of hazardous substances alone is not enough to evaluate possible health effects. In response, the agency established a special “exposure investigation” (EI) section within DHAC to look specifically at biomonitoring and how it can be used to further inform the public health assessment process. Dr. Susten presented the criteria developed by ATSDR to determine whether biomonitoring should be considered to evaluate a site-specific exposure situation:

Continuum for Relating Environmental Contamination with Clinical Disease

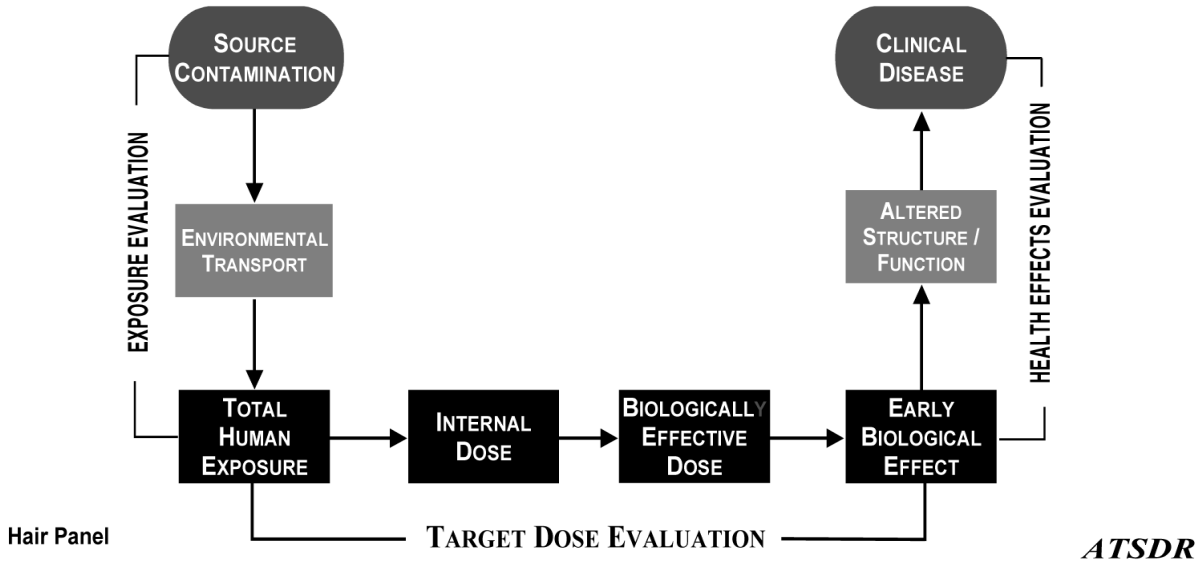


Figure 2-1. Continuum of events considered in the public health assessment process.

- Can an exposed population be identified?
- Does a data gap exist that affects ATSDR's ability to interpret whether a public health hazard exists?
- Can the data gap be filled with an EI?
- How would the EI results impact public health decision-making?

Dr. Susten stated that the panel's charge is to discuss scientific issues related to hair analysis that will help the agency determine the criteria for determining when hair analysis might be a useful tool in assessing public health exposures. He recognized that the science may not be available to support all analyses and that research may be needed.

In convening this panel, Dr. Susten emphasized, the agency's goal was to receive panelist and observer input on the following general questions:

- When is it appropriate to consider hair analysis in assessing human exposures to environmental contaminants?
- When is it inappropriate to consider hair analysis in assessing human exposures to environmental contaminants?
- What data gaps exist that limit the interpretation and use of hair analysis in the assessment of environmental exposures? What research is needed to fill these gaps?
- For what substances do reliable hair analysis methods exist (e.g., trace elements, organic compounds)?

ATSDR's primary interest in hair analysis, as was reiterated throughout the meeting, is using the best science when responding to an individual's request to interpret hair analysis results and determining when hair analysis at the population level may be helpful in demonstrating that an environmental exposure has occurred.

2.3 Impetus for Panel Discussions—A Case Example

Deanna Harkins, M.D., M.P.H.

Medical Officer

ATSDR/DHEP

Dr. Harkins described a recent site-specific scenario that served as a primary trigger for organizing the hair analysis panel. Specifically, hair analysis issues raised at a plating facility prompted ATSDR to look more closely at the criteria that should be considered when choosing hair analysis as an exposure assessment tool and the best way to interpret hair analysis results.

Dr. Harkins explained that the U.S. Environmental Protection Agency (EPA), ATSDR, and relevant state and local agencies have been working together to address community health concerns related to this particular facility. Dr. Harkins briefly reviewed how ATSDR evaluated potential exposures associated with releases from the facility (i.e., by examining the nature and extent of contamination and determining whether contaminants have moved from the source to a point where people might contact them), noting that the agency studies both past and current exposures. She re-emphasized Dr. Susten's point that evaluating exposures is only one step in evaluating possible public health hazards and that understanding the continuum of events between exposure and resultant disease is critical to determining the likelihood that a given exposure will have adverse effects.

Dr. Harkins provided the following summary of the issues reviewed during the assessment of the facility:

- Investigations at and around the facility revealed the presence of Chromium VI (Cr^{6+}) in groundwater. In response, affected residences were supplied with bottled water since 1977 and municipal water since 1997. Therefore no recent exposures have occurred.
- Chromium is found naturally in rock/soils and can be found in three valence states (0, 3+, and 6+). Also, Cr^{3+} is an essential nutrient: it is required for normal glucose metabolism and in the potentiation of the action of insulin, and it aids in the metabolism of fat and

cholesterol (Anderson 1997; Schroeder 1968; Mertz 1969; Hunter 1974). The National Academy of Sciences has established a safe and adequate daily intake for chromium in adults of 50 to 200 micrograms per day ($\mu\text{g}/\text{day}$) (NRC 1989). It has been reported that the daily dietary intake of chromium for a typical American is approximately half the minimum safe and adequate daily intake of 50 $\mu\text{g}/\text{day}$ (Anderson and Kozlovsky 1985). Chromium deficiencies have been shown to result in glucose intolerance, peripheral neuropathy, and decreased fertility (Anderson 1997). Because chromium is an essential nutrient and part of normal diets, it is difficult to measure body burdens from environmental sources.

- The primary health concerns expressed by site community members include birth defects, miscarriages, and cancer. Neither birth defects nor miscarriages are known to be associated with chromium exposures. Lung cancer and other respiratory effects have been associated with chromium exposures, but only in occupational settings where high doses of Cr^{6+} were received via inhalation. Cr^{3+} is not classified by EPA, the National Toxicology Program (NTP), or the International Agency for Research on Cancer (IARC) as a carcinogen.
- Site community members wanted to use chromium levels in hair as proof that they were exposed to chromium and clinically ill. In response, ATSDR, in cooperation with EPA, the state health department, and outside experts, held a series of meetings with the community, including the local medical community, to communicate *why hair analysis was not appropriate* for this site:
 - ATSDR determined that estimated chromium doses based on detected levels of chromium in groundwater were lower than those associated with any adverse health effects.
 - Because of the stomach's and gastric juices' high capacity for the reduction of Cr^{6+} , ingested Cr^{6+} is reduced to Cr^{3+} within minutes (Kerger et al. 1996). As a result, a person can tolerate ingestion of 50–100 milligrams of Cr^{6+} per day without risk of systemic effects (Donaldson and Barreras 1966; DeFlora and Wetterhan 1989).
 - Measuring chromium in hair would not demonstrate *past* environmental exposures.
 - The health effects of concern to the community are not known to be linked with chromium exposure.

Dr. Harkins stated that this case assessment led several ATSDR health assessors to inquire about

the overall utility of hair analysis. In turn, this has prompted the agency to look more closely at the scientific issues associated with hair analysis and to work toward developing guidance on when hair analysis might be useful in identifying environmental exposures and in evaluating disease potential.

2.4 General Physiology of Hair—An Overview

Robert Baratz, M.D., Ph.D., D.D.S.

To provide a foundation for subsequent discussions, Dr. Baratz described the general characteristics of hair and the underlying skin (e.g., structure, composition, growth patterns, growth cycles). Understanding the characteristics of hair, the temporal and spatial patterns of hair growth, and the factors that affect hair growth, for example, is important when collecting and interpreting hair analysis data. Dr. Baratz's presentation is summarized below.

- *Anatomy of hair.* Hair is encompassed in the follicle located below the skin surface in the dermis, the fiber-rich layer that makes up the bulk of the skin. The follicle has a connective tissue component (muscles) and glandular component (sebaceous glands). The muscles elevate the hair and the glands lubricate the hair.

The primary components of the hair follicle are the dermal papilla and the follicle cells. The dermal papilla is the “generative zone” of hair (it contains blood vessels, nerves, and pigment-forming cells). The follicle cells generate the hair shaft; the hair shaft is composed of essentially dead cells, which are the outermost layers of the epithelium and form a solid cylinder in the dermis. Mitotic activity at the base of the hair follicle generates different layers that will “keratinize” (see below).

- *Keratinization of hair.* Hair is composed of hard keratin (a family of proteins ranging in size from 20,000 to 70,000 Daltons) and is chemically denser than other forms of keratin (e.g., calluses, dander flakes). Keratinized cells contain more than 85% protein. Where the hair shaft separates from the follicle it undergoes “disjunctive” keratinization, which involves the splitting of layers and exposing surfaces not previously exposed.

Keratinized cells have a very distinctive appearance, and have tiny pores littering their surfaces. The cells are flattened and tightly bound to their neighbors in a very complex array. When they begin to split apart (by an unknown process), large “nooks and crannies” are formed. These types of anatomical features allow external environmental

agents to be easily trapped in the outer surface of the hair.

- *Elements found in hair.* Because so many elements are ubiquitous in the environment and therefore found in the human body, merely finding a particular element in the hair does not prove that it got there via a specific route/source, or that finding it has clinical significance.
- *Growth rates.* Hair growth varies depending on body region. For example, average eyelash/brow growth rates have been reported at 0.16 millimeters (mm) per day, scalp hair at 0.34 to 0.36 mm/day, and beard hair at 0.38 mm/day. Growth rates also are affected by age, gender, hair color, and ethnicity. For example, scalp hair in a prepubescent, adolescent, adult, and older adult have been reported at 0.41, 0.30, 0.34, and 0.32 mm/day, respectively (Myers and Hamilton 1951).

Interindividual variability also occurs. Scalp hair grows at an average rate of 1 centimeter (cm) per month, but can range from 0.6 to 3.36 cm/month (Harkey 1993). Thus, 12 cm can represent 3½ to 20 months of hair growth.

- *Growth cycles.* Hair grows in phases (see Figure 2-2). Usually, more than 90% of the hair is in the growing (or anagen) phase. The length of anagen varies from 2 to 6 years. The longer the hair, generally the longer the phases. For example, long hair tends to grow more slowly. Through apoptosis, the hair will begin to enter the relatively short catagen phase, during which the follicle will begin to regress and move toward the surface (the papilli will essentially disappear). During the next phase, telogen, the hair will actually fall out. If the cycle is complete, a resting phase will follow and then the follicle will resume the anagen phase. However, hair can “exit” the cycle and cease being a terminal hair. For example, it can become a vellus hair (non-pigmented “peach-fuzz” hair) or the hair follicle may permanently disappear, as is the case with male-pattern baldness.

Events known to affect the hair follicle and its cycle include local signaling events (e.g., cytokines, hormones, adhesion molecules). However, no firm theory of cycle control exists. Hypotheses include the presence of (1) a morphogenesis clock, (2) a cycling inducer, (3) a desynchronizer, and (4) an actual cycle clock, but none of these are specifically known.

- *Generation, cycling, and “patterning” of hair.* The hair growth cycle changes throughout life and varies based on species and body location. Patterning of hair is important to the generation and cycling of hair, and to how it relates to its neighbors (e.g., signaling goes on in various regions to space follicles in even arrays). Because of similarities in hair growth patterns, studying sheep hair growth has been useful in understanding human hair growth patterns. Rodent hair growth models, on the other hand, may not be applicable to

humans because regional variation growth; the hair waves across the

rodents have in hair cycles, but in body.

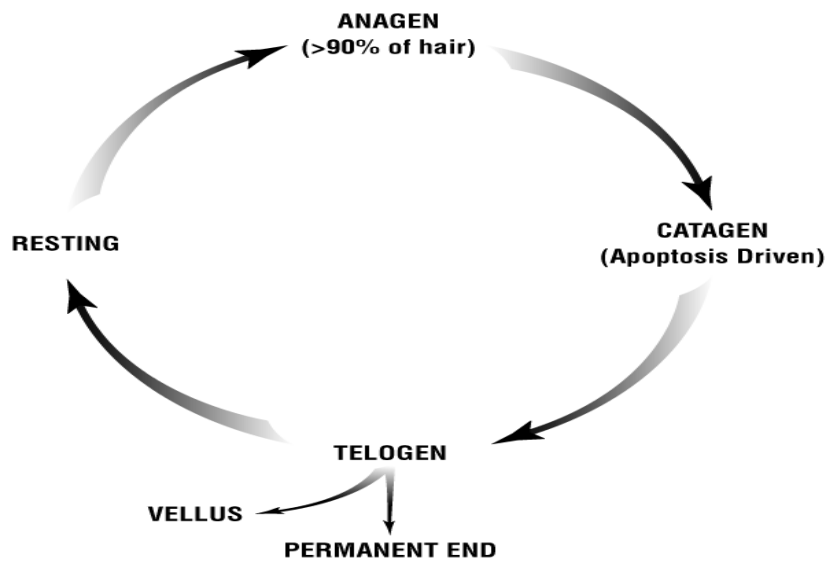


Figure 2-2. The hair growth cycle.

- Substances affecting hair growth.* A great number of substances can affect hair growth. For example, some drugs, such as alkalating agents, are cytotoxic and can make hair fall out (e.g., cancer chemotherapeutic agents). Other agents drive hair into telogen (e.g., heparin, Vitamin A, β -blockers, L-dopa, lithium, and some of the non-steroidals). Drugs that inhibit hair growth include parathyroid hormone (PTH) and PTH-related proteins. Variable agents also exist, such as Vitamin D. At low concentrations, Vitamin D may simulate hair growth, but at high concentrations hair growth is inhibited.

Substances such as testosterone, danazol, adrenocorticotropin hormone, metyrapone, anabolic steroids, glucocorticoids, retinoids, and insulin can lead to hirsutism (growth of hair where it does not normally occur). Cyclosporin, minoxidil, diazoxide, and

chromakalin increase the growth rate and size of hair (hypertrichosis). However, some regional variation may occur. For example, steroids will decrease the rate of growth of eyebrows, lashes, and hair on the extremities, but estrogen and testosterone will generally stimulate the growth of pubic and axillary hair.

Other factors can potentiate or inhibit hair growth by affecting the growth of the dermal papillae, hair, and follicle (see Table 2-1).

Table 2-1. Factor Effects

Factor	Effect on Hair Growth
Beta-fibroblast growth factor Platelet-derived growth factor	Potentiate growth of dermal papillae.
Transforming growth factor beta	Inhibits follicle proliferation, if induced by mitogens.
Interleukin-1 alpha	Inhibits growth of hair and follicle.
Epidermal growth factor	Stimulates growth.
Fibroblast growth factor-5	Inhibits growth.
Keratinocyte growth factor	Stimulates growth; induces keratinization.
Insulin-like growth factor-1	Accelerates growth of hair and follicle.
Skin damage (e.g., cut, scrape, burn, irritation)	Forces telogen to anagen (well-illustrated in rodent models).
Allergens (e.g., food)	Major changes in the skin, including hair loss.
Malnutrition	Protein/calorie deficiencies inhibit hair growth.
Fungal infection	Inhibits growth; hair may fall out.
Hypothyroidism	Diminution of eyebrows.
Viral agents (e.g, HIV virus)	Hair loss in patches.

Source: Jankovic and Jankovic 2001.

SECTION 3

SAMPLING AND ANALYTICAL METHODS

Panel discussions related to the sampling, handling, and laboratory methodologies used in hair analysis centered around the strengths and weaknesses of existing procedures and the lack of standardized methods for collecting and analyzing hair samples and reporting the results.

The group generally agreed that the technology exists to measure substances in hair, but variations in sample collection, preparation, and analytical methods can drive what will be measured in the final analysis. Therefore, the panelists encouraged the development of standard protocols for hair analysis to help ensure the generation of reliable and reproducible analytical results. In the interim, panelists encouraged laboratories to clearly document procedures used in their analyses, and encouraged users to be cognizant of these procedures when interpreting results. The group acknowledged that even if standard protocols were in place, the greatest challenge would still be interpreting the results from a practical and toxicologic perspective (see Sections 4 and 5).

Panelist Dr. Dan Paschal, research chemist at CDC, opened discussions on this topic with a brief overview of the advantages and limitations of existing analytical methods and approaches related to hair analysis. He emphasized that hair has real advantages in that (1) it can contain relatively high levels of hazardous substances of potential interest, including elements and some organic compounds, (2) it is easy to collect by relatively non-invasive methods, and (3) it is a stable specimen. He also commented on some of the limitations: lack of precision and accuracy of hair analysis results, external contamination, interindividual variations, lack of correlation with health effects, and lack of believable reference intervals.

In setting the stage for discussions on analytical methods, Dr. Paschal commented on published work related to reference intervals, detection limits, and hair concentrations of metals as a

function of age (DiPietro et al. 1989; Paschal et al. 1989). His specific comments are integrated in the sections that follow.

3.1 Sample Collection Methods

The panelists offered some varying opinions regarding the best way to collect samples. Topics discussed included preferred cutting tools, sampling location, and sample handling, as summarized below.

- *Selecting the appropriate cutting device.* Panelists offered differing views on what type of cutting tools should be used when collecting hair samples. One panelist noted that metals can be released from scissors and therefore recommended using *quartz* instruments (RB).

One panelist pointed out that if a stainless steel device is used, chromium and nickel results should be interpreted carefully, although he questioned whether use of stainless steel would really make a significant difference in the analytical results. This panelist questioned whether any data are available that document the extent to which chromium and nickel in stainless steel contribute to sample levels compared to quartz tools (MK).

In theory, said another panelist, labs that have used stainless steel scissors (for example) should run a careful blank for chromium. It is, however, difficult to do so: the variable concentration that is present in the specimen would be measured as well as the variable amount being introduced by the scissors. A chromium-free hair sample would be needed, which is not feasible (DP).

This same panelist stressed the importance of being sensitive to possible psychological and cultural issues when choosing a cutting tool. For example, children may be intimidated by certain types of shears or other cutting devices. Also, in certain cultures, hair is considered sacred. Touching, never mind cutting, is prohibited (DP).

One panelist suggested that if interferences due to the cutting instrument used are proven to be significant, a new instrument might need to be created that would be practical for field use (e.g., a relatively small tool with a quartz blade) (LW).

- *Collection location.* Because of differences in growth rates in different regions of the scalp, the location from which a sample is taken must be carefully considered to ensure consistency in measurements. For example, the anterior and the parietal regions grow

differently than the vertex (top), occipital (back), and temporal (side) regions (RB). In response to a question whether an optimal location exists, one panelist noted that defining an optimal sampling protocol is difficult (DP). At a minimum, it is important to choose a protocol that is practical in the field setting.

Another panelist noted the desire to identify a reproducible point on the skull. He suggested taking a sample from the nape of the neck (using a caliper to take the midpoint between the external auditory meatus), an area where hair is known to grow in a particular way (RB). Another panelist recommended sampling from the occipital region (SS).

In its 1989 study, CDC looked for a standard protocol but could not find one. Therefore, CDC defined its protocol as follows: Approximately 500 to 1,000 milligrams of occipital hair was collected using stainless steel scissors. Hair was pre-washed (using a non-ionic detergent). Samples were stored in pre-cleaned plastic bags that were rigorously tested. Therefore, within the context of the reference interval being generated, data were from specimens collected in a like fashion (DP).

- *Sample storage.* One panelist stated that plastic bags or other plasticware should not be used for storing hair samples unless the containers have been washed or cleaned. Zinc, he said, is used in plastic molding processes. Because detection limits are precise and relatively low, it is easy to record contamination from external sources; therefore, whatever container is used needs to be looked at with great scrutiny (RB).
- *Who should collect the sample.* One panelist stressed that people *not* be allowed to collect their own samples, put them in plastic bags, and ship them off to the laboratory (MG). Others agreed: only trained professionals should collect hair samples.

3.2 Sample Preparation Methods

Panel discussions on sample preparation focused on washing protocols. The group agreed that washing hair prior to analysis was an important consideration when external sources of the substance(s) being studied exist. Panelist-specific comments follow.

- One panelist stated that no washing method can distinguish between external contamination and internally deposited elements. She noted that a number of washing procedure “camps” exist, including the “no wash hypothesis” (Chittleborough 1980), use of a mild detergent, the washing procedure recommended by the International Atomic

Energy Agency that uses a solvent in water (adopted by many research groups), and more radical procedures that use chelating agents. Wide differences in results have been observed depending on the washing method (SS).

- External interferences can be especially significant with small children, so CDC uses a standard washing protocol (DP).
- The extent to which washing is necessary depends on the substance being studied and how the sample is being used. For example, washing is not necessary when one is testing for a substance for which no external source exists (e.g., methyl mercury). Other key questions to consider include: Are you looking at a spectrum or a specific agent/element at a hazardous waste site? Are you sampling for exposure information? Are you sampling to determine changes in exposures over time? (TC)

See Section 4 for additional discussions on hair washing, specifically as it relates to distinguishing between endogenous and exogenous sources of metals.

3.3 Analytical Methods

The group noted that reliable analytical methodologies do exist to measure and verify the presence of various substances in hair. Several panelists specified methods currently used for hair analysis in their pre-meeting comments; throughout the meeting, they mentioned some of the methods' strengths and weaknesses, as well as their applicability. This section highlights the points made during the meeting, but should not be considered an exhaustive discussion of existing methodologies. The methods discussed include:

- *Cold Vapor Atomic Absorption (AA)*. It was noted that this is the preferred methodology for measuring methyl mercury.
- *Inductively Coupled Argon Plasma Mass Spectrometry (ICP-MS)*. ICP-MS has widespread use in commercial laboratories. It can be used to measure methyl mercury, but it is difficult to get reproducible calibrations (DP). With certain types of mass spectrometry, stable isotope studies can help show the incorporation rates of certain elements in hair, which may help to answer some of the toxicology questions. For

example, a 20-day delay has been shown between the appearance of lead in blood and its appearance in hair (TC).

- *Inductively Coupled Argon Plasma Optical Emission Spectrometry (ICP-OES) or Inductively Coupled Argon Plasma Atomic Emission Spectrometry (ICP-AES)*. Of the commonly used methods, ICP-OES/AES is used the most (DP). This method makes it possible to generate a large amount of data on a large number of elements. It is a “quick and dirty” way of getting a global picture of the elemental composition of a hair sample.

It was noted that, historically, CDC used Jarrell Ash Model 1160 AtomComp (e.g., to generate the data cited in DiPietro et al. 1989). CDC presently uses a Jobin Yvon Ultima C (DP).

- *Neutron Activation Analysis (NAA)*. NAA has been used in forensics to measure trace elements in small quantities of hair. It can be used for segmental analysis of hair. Segmental analysis can reveal isolated elevations of contamination along the hair and provide information regarding the contamination of the length of the hair over time. Identifying patterns over time can help distinguish whether exposure is endogenous or exogenous (see also Section 4). These techniques are not widely commercially available, however (MK).
- *X-ray Fluorescence*. This technique is amenable, nondestructive, and multi-element. It also has the advantage of measuring the mass of hair as well as the amount of the element present in that segment of hair (TC). Another panelist noted that the distribution of mercury in segments along the length of a single strand of hair may be determined by x-ray fluorescence.
- *Proton Induced X-ray Emission (PIXE) Spectrometry*. This method was brought into play approximately 30 years ago. This method studies a cross section of hair, enabling identification of external versus internal contamination. This method has not been used very much because the instrument is expensive (TC). One panelist noted that single-strand analysis can be problematic if hair is in the non-growing phase (RB), although it was noted that this is not a problem if the sample is taken from the root (TC).

Another panelist commented on the variable success of the PIXE method. For example, differentiating internal and external arsenic may not always be that straightforward. In cases of internal uptake, peaks of arsenic on the external shaft of hair may be a consequence of appreciable cysteine residues and sulphhydryl groups. In cases of external contamination, washing procedures may lead to greater incorporation of external contamination into the shaft (MK).

Given the various methodologies that might be used, several panelists pointed out the importance of understanding the method and analytical equipment used when interpreting hair analysis results, noting that it is the laboratory's responsibility to clearly report the method used, quality assurance measures taken, any possible interferences, etc. Further, the data user should carefully consider this information when evaluating the results.

As stated by one panelist, it is easy to standardize measurements by using good standards and good laboratory practice (use of blanks, use of external verification) (DP). While the group recognized that valid methods exist, several panelists stressed that the challenge lies in the interpretation (see Section 4 of this report)

3.4 Other Methodological Considerations

The group discussed a number of other issues that influence the analytical results and should be considered when choosing methods and evaluating analytical data.

- *What amount of hair is needed for reliable analyses?* CDC has used between 500 and 1,000 milligrams of hair in its studies (DP). Another panelist commented that the amount selected depends on the analytical method used, but he is more accustomed to sample sizes in the 50 milligram range (TC). Down the road, there may be an interplay between the sensitivity of the method and the quantity of hair needed for analysis.
- *To what extent should multi-element analytical approaches be used?* The group agreed that a targeted (single-chemical) approach is preferable when analyzing hair for a particular environmental contaminant. The analytical method selected needs to be considered in the specific context of the substance and exposure situation under evaluation; both time and element need to be targeted (RB, MG, MK).

Serious interference problems can exist with instruments that test for a spectrum of metals (e.g., ICP instruments) (DP). According to one panelist's observations, laboratories do not always appear to account for these interferences: inconsistencies in approaches are seen across laboratories using ICP-MS and ICP-OES (SS). When performing OES, one must take a lot of care in choosing the emission wavelength used in

the measurement. Interferences from other elements can occur and must be considered. This is particularly true when one uses ICP-MS for elements with masses less than 80. Peaks can be the result of molecules made in the process of generating the ions. These can interfere with the peaks you are trying to measure (e.g., argon chloride and arsenic, both with nominal masses of 75 atomic mass units). A high-resolution MS, however, can resolve two such peaks (DP).

- *Other interferences.* Metals in acid solutions, as well as paint, dusts, gloves, etc. in the laboratory setting can be detected by the instruments used for hair analysis. Looking at low-level metals in a hair sample is therefore not a simple exercise (RB). These interferences might potentially overwhelm the amount that you may be seeking to measure in the hair sample (MK).
- *What about organic compounds?* A hair assay for benzene is being developed that is evaluating metabolic products in hair (data are proprietary). Such an assay may have a great impact on determining the feasibility of using hair analysis for organic chemicals (MG).
- *Quality assurance and quality control.* It is the responsibility of the laboratory to demonstrate its quality control procedures, such as standardizing procedures, running blank measurements, calibrating equipment, and verifying measurements externally through proficiency testing programs.

SECTION 4

FACTORS INFLUENCING THE INTERPRETATION OF ANALYTICAL RESULTS

Panelists identified several factors that influence, and more often than not can limit, the interpretation of hair analysis results. In light of these factors, the panelists generally concluded that hair analysis findings need to be used and interpreted very cautiously. Even if issues related to the reliability and reproducibility of the data are resolved, panelists stressed repeatedly, several factors limit the utility of hair analysis as an exposure and diagnostic tool. Scientists and clinicians currently know little about what such measurements mean in terms of predicting or treating clinical disease (see also Section 5, Toxicologic Considerations).

During the meeting, several factors influencing the interpretation of hair analysis data were discussed:

- Inconsistent sample collection and preparation methods (e.g., sample location, cutting method, sample storage). (Panelist discussions related to methodological issues are detailed in Section 3.)
- Difficulties in distinguishing metals deposited externally from those incorporated internally from the hair follicle.
- Exposure chronology and conditions (e.g., exposure period of interest, hair growth cycle, other exposures, etc.).
- The questionable reliability and variability of “reference” ranges. That is, what defines an “elevated” level?

4.1 Distinguishing Between Endogenous and Exogenous Sources of Metals

All of the panelists agreed that using hair analysis as an exposure or diagnostic tool for metal contamination is severely limited by difficulties in distinguishing between internal and external sources of metals. It is further complicated by the natural occurrence of many of the trace

elements (several of which are essential nutrients) within the body. The group recognized, however, that this distinction is not a limitation when a metabolite or a substance with no external source is being measured (e.g., organic compounds such as methyl mercury or many drugs of abuse).

Dr. Kosnett led discussions on the difficulties that exist in distinguishing endogenous and exogenous substances in hair. Other panelists expanded upon these issues. Individual points are summarized below.

- Hair analysis data do not necessarily enable you to determine where the measured contaminant came from and how it got there. High hair levels may provide “markers of potential exposure,” but that does not tell us how much is internally incorporated. If hair analysis is used in ATSDR’s evaluations of exposures to contaminants in air (e.g., in the form of particulates), water, or soil/dust, it must be realized that this distinction cannot necessarily be made (MK).
- An Alaskan study of arsenic levels in tap water, urine, and nails (Harrington et al. 1978), reveals some interesting trends. Individuals drinking bottled water, but bathing in tap water with arsenic averaging 345 micrograms per liter ($\mu\text{g/L}$), had higher average levels of arsenic in hair (5.7 parts per million, or ppm) compared to those drinking and bathing in tap water with arsenic containing 30 $\mu\text{g/L}$ (0.46 ppm arsenic in hair). Urine levels were similar, however. This example helps illustrate the difficulties in using hair concentrations alone to draw inferences regarding the magnitude of the internally absorbed dose of a metal (MK).
- Though they are not applicable to the example above (based on arsenic toxicokinetics), another reviewer noted that the following caveats could further confound the interpretation of such a scenario: (1) other exposures could be occurring (e.g., cooking, brushing teeth), (2) dermal absorption could be occurring, and/or (3) a pool of the contaminant could be sequestered in and later released from the bone (e.g., this can be true with tetracycline) (RB).
- *Effect of washing hair.* Dr. Kosnett described various studies that have looked at the role and/or effectiveness of washing hair in order to distinguish between endogenous and exogenous sources of arsenic. These studies suggest that no truly good washing method exists to remove arsenic: If hair is not washed aggressively, exogenous arsenic will remain. If hair is washed too aggressively, endogenous arsenic may be removed.

- Smith (1964) showed that detected concentrations of arsenic in hair will vary depending on washing method, with no method shown to be capable of removing all arsenic. The results of applying different washing methods (to hair purposely externally contaminated with 12.08 ppm arsenic) are highlighted in Table 4-1. The arsenic concentration in hair before contamination was measured at 0.14 ppm.

Table 4-1. Effect of washing method and time on arsenic levels in hair

Washing Method	Washing Time (mins)	Arsenic (ppm)
Water	5	9.16
	15	5.78
	30	5.05
	60	5.03–6.21
Detergent (5%)	60	4.20–4.93
HCl (N)	60	4.92–6.26
NaOH (N)	60	0.40–0.70

Source: Smith 1964.

- Van den Berg et al. (1968) showed similar findings. Depending on the washing regime, this study revealed that even after 1,600 minutes of washing, externally deposited arsenic was still detected (MK).
- *Measuring total concentrations in hair.* Depending on the purpose of your testing, it may not be critical to distinguish between internal and external contamination. For example, in an industrial hygiene exposure investigation, identifying elevated levels of an element may be enough to suggest that the potential for exposure exists and protective measures are needed. While urine data may reveal that existing protective measures have prevented internal exposures, knowledge that employees have exposure potential may be important (e.g., contamination could be carried home) (MK, TC, MG). Several panelists reiterated, however, the limitations of using such data for clinical evaluation or interpretation.

4.2 Temporal Considerations and Exposure Conditions

Panelists agreed that, in determining whether to use hair analysis and in interpreting analytical results, one must carefully consider exposure chronology and conditions. Because hair growth is a factor in evaluating when a contaminant might become incorporated in the hair, one must consider it when deciding whether sampling hair will identify exposures over the period of interest. With regard to this, the panelists discussed these topics:

- *Window of exposure that hair levels may represent.* Growth rate is a key consideration. Assuming growth at approximately 1 centimeter a month, the hair on the average person's head generally represents a year or less of time. Therefore, hair analysis is not the best biological medium to serve as an indicator of very recent exposure or past exposures (greater than 1 year) (RB).
- *Using segmental analysis to study exposures over time.* If hair is looked at in a micro or segmental way, temporal patterns of exposure may be identified. Understanding *when* exposure might have occurred may be useful in documenting some historic exposures. As mentioned in Section 3, neutron activation analysis has been used to identify isolated elevations along small segments of a hair (e.g. millimeter[s] in length) (MK).

Segmental analysis has been shown to find isolated arsenic peaks at distal points along the hair shaft. For example, studies of past acute suicidal exposures to arsenic show distinct peaks migrating away from the scalp (Leslie and Smith 1978). Such analysis can reveal past exposures even when current urinary levels are normal. Curry and Pounds (1977) demonstrated peak concentrations of arsenic in hair migrating away from the scalp following the administration of medicinal arsenic (1 hour to 72 days after ingestion).

Segmental analysis may help ATSDR scientists identify *past* elevated exposures (e.g., acute high exposures from a spill event). Segmental analysis may also rule out exposures. Houtman et al. (1978), for example, studied hair in a population exposed to an accidental release of arsenic dust. Segmental hair analysis revealed that concentrations on the distal parts of hairs were elevated. However, it was determined that the higher levels were detected on portions of hair that would have been fully formed before the accident, thus establishing that the arsenic in hair was the result of external contamination. In some settings, a relatively uniform distribution of a metal such as arsenic along the length of sampled hair can reflect relatively stable, chronic ingestion, but even in those settings the contribution of external contamination cannot always be readily determined (MK).

The challenge of using segmental analysis to demonstrate exposure patterns is that it requires techniques that will enable the analysis of small quantities of hair (e.g., subcentimeter sections). It also requires collection of hair in a careful way, to preserve the orientation of the hair. Further, it has been shown that uptake of arsenic—even on deliberate external contamination—was not uniform. It has been hypothesized that the use of shampoos might account for the uneven distribution. This observation might limit the interpretation of segmental analysis for measuring patterns of endogenous levels (MK).

It was also noted that concentration increases towards the tip of the hair because it is exposed longer. This pattern is typical with external lead exposures. Increased concentration toward the tip is a useful clue regarding the extent of external contamination (MK, TC).

- *Understanding exposure conditions/histories.* Panelists suggested obtaining complete exposure histories as part of any hair analysis evaluation. A clinician or health assessor needs to understand the exposure situation and work within a framework of knowing when data may have a valid use. Using an *exposure questionnaire* as part of any hair analysis exercise will help the clinician/assessor identify sources of exposure, both site- and non-site related. Such information will ultimately help the assessor put available data into perspective (DP, TC, SS).
- *Age.* The age of the individual or population tested can affect the results and interpretation of hair analysis. Studies suggest, for example, that alkaline earths and zinc are not excreted as much in early years of life. The opposite is true with aluminum, of which children excrete higher levels than adults (Paschal 1989). When skeletal growth stops, the excretion of these substances into hair is relatively constant. As part of its National Health and Nutrition Examination Survey 99+ (NHANES), CDC studied mercury levels in the hair of children and women of childbearing age. Data suggest that children had lower mercury levels than adults (CDC 2001a; CDC 2001b) (DP).

4.3 Reference/Background Ranges

Discussions regarding reference ranges focused on uncertainties associated with levels of metals, etc., in “healthy” or “unexposed” individuals and the variability of reference ranges used by different laboratories. The panelists discussed how the uncertainties play out when one tries to interpret results of hair analysis.

Individual comments regarding currently available reference range data and inherent limitations are detailed below:

- The panelists discussed the importance of first clearly defining the term “reference range.” Two panelists expressed concern about using the term synonymously with “normal” because it implies that knowledge exists about associated health status, when in fact such information is largely unavailable. Reference ranges do not represent “background” or “controls,” nor do we know baseline levels for “normal” states of health (SS). We do not know what should be present in “healthy” hair (LW). Others suggested that it is *background* data that is ultimately being sought, noting that possible geographic and demographic differences need to be considered.
- A “normal” range does not exist for many elements. Unlike drugs, the presence of which would be considered “abnormal,” normal ranges need to be identified for metals. Building the database of normal levels would help assessors better understand and interpret hair analysis results. Considering hair data in the absence of reference data against which to compare them is therefore of limited utility (RB).
- The availability of a reference range does not mean we know the background or typical levels of *endogenous* incorporation. It does not necessarily represent what occurs naturally. It may represent external exposures to ubiquitous levels of contaminants (e.g., lead dust, etc.) (MK, TC). For ATSDR’s purposes, the key is distinguishing site exposures from non-site exposures (e.g., What are background levels where no known external exposure sources exist?). For example, will we be able to discern whether levels of a contaminant of interest are elevated in a potentially exposed population (LW)?
- DiPietro et al. (1989) reported analytical results for 271 adults, ages 20 to 73 years, for selected elements. In comparing the findings of this study with mean hair concentrations of the same elements reported by others, investigators concluded that results compare relatively well, given limitations and variability in hair analysis (DP).
- It might be useful to draw a distinction between *essential* trace elements and *non-essential* trace elements. One would expect a reference level of the essential trace elements in hair. The presence of non-essential elements, on the other hand, would suggest environmental exposure, deposited internally or externally (TC).
- According to two panelists, available reference ranges are often biased and based on small numbers. Some reference ranges are based on one or two old case reports (RB, MG).

- The validity of samples used to develop a reference range in the first place is unknown (RB).
- Available reference values may not relate to the population under study (RB).
- Reference ranges with an approximate 100-fold difference have been used by different commercial laboratories. What does this really mean from a biological perspective? (SS)
- One panelist emphasized that more important than understanding reference ranges is gaining an understanding of whether chemical-specific values have toxicologic or clinical significance. The availability of reference range information alone is inadequate to assess the clinical significance of a particular laboratory result; the fact that a reference range has been exceeded does not establish that the individual sustained a toxicologically significant dose (MK). Another panelist reminded the group that establishing reliable reference levels will inform assessors about the possible extent of *exposures* (LW).
- One panelist questioned whether CDC might consider additional hair analysis as part of NHANES efforts—providing an opportunity to collect data on a cross section of the population. It was speculated that if the science supported the need for such data collection, it could be proposed (funding aside) (LW, DP). NHANES 99+ did measure hair mercury of a selected subpopulation (children ages 1 to 5 and females 16 to 49 years) (CDC 2001a).

SECTION 5

TOXICOLOGIC CONSIDERATIONS

The panelists agreed that, in order to interpret hair analysis data in any meaningful way, scientists need a greater understanding of substance-specific relationships between levels in hair and other body compartments, including target tissues, and how those levels relate to adverse health outcomes. Much of the toxicology discussion, accordingly, centered around data gaps and research needs.

The panel chair stressed the importance of understanding to what extent a particular substance might enter the body, what could conceivably get into hair, and ultimately how such information can be used as an indicator of exposure and/or of possible clinical effects. Specific questions to consider included:

- What are the substance-specific pharmacokinetic factors (e.g., intake, absorption, distribution, excretion) that can influence the biologic uptake of specific substances and the concentration delivered and incorporated into the hair? How should half-life and possible storage pools within the body be considered?
- What substances are transported to the hair, and by what mechanism are they transported (e.g., how are environmental substances of interest incorporated into the hair)?
- What is the dose that causes effect at the target organ? If this is known, how does it relate to the concentration in the hair matrix?
- How do different patterns of exposure over time (e.g., as may be revealed by segmental analysis) help us understand possible acute versus long-term exposures, and how might these patterns correlate with potential health effects?

5.1 Pharmacokinetic Issues

The group acknowledged that little is known about the transfer kinetics of substances into hair (i.e., their “normal” percolation or rate of appearance in hair). Factors such as transit times, pools in the body, permeability of basal membranes, and co-factors that may be involved in transit are not known. Without this knowledge, interpretation of hair analysis results is greatly limited.

Individual panelist input focused on possible ways to fill data gaps. Specifically:

- Hair is a nonvascular tissue (separate from liquid phase transfer kinetics). Understanding the rate of uptake in the hair, if any, for substances of interest is of critical importance. Experimental models are needed (RB, MK).
- Implanting human hair on hairless mice, administering a radioactive isotope, and following its movement to hair may be an effective method for determining the incorporation of metals into hair (TC).
- Studying the uptake of arsenite used in the treatment of leukemia might be a possible human model to use to increase our understanding of pharmacokinetics and dose-response relationships, realizing that administered doses are much higher than they would ever be expected in an environmental setting (MK).
- Identifying the “transportable” form or metabolite(s) of substances of interest may provide the best biomarker. Methyl mercury may serve as a model. The key is understanding the transport mechanism. It may be worthwhile to pursue organo metals and their behavior (e.g, dimethyl arsenic acid, butyl tin); they may serve as more unique markers of exposure (TC, MK, SS, LW).
- When interpreting data, studying nutritional status should be considered because it may play a role in the uptake and distribution of metals. For example, iron and calcium can increase the uptake of lead into the hair. Zinc levels in hair may be high in “failure to thrive” cases because hair has stopped growing (LW, SS).
- Obtaining data to better correlate exposure, blood/urine, and hair levels would enable a better understanding of the relationship of elements in the various body compartments. It would help correlate external concentration with internal doses. Few such data exist, with the exception of NHANES data, which evaluate lead levels across hair, blood, and urine

(which correlated poorly). It was speculated that such substance-specific relationships could be studied further as part of the NHANES program (DP, LW).

5.2 Dose-Response and Clinical Relevance

The panelists concurred that relationships between hair and any kind of measurable outcome have only been established for methyl mercury and arsenic. The relationship between maternal hair and fetal brain levels of methyl mercury is the only well-documented hair/target tissue relationship; one panelist pointed to the benchmark dose of methyl mercury of 11 ppm in hair established by EPA² (RB, SS, TC, MK). Data for arsenic relate largely to forensic examinations; data do not exist for arsenic that offer disease-predictive value (e.g., long-term health outcomes). The group could not identify any other environmental substances for which any hard and fast clinical relationship has been established. Dose-response curves simply do not exist.

Panel discussions regarding current knowledge and the implications for using hair analysis are highlighted below:

- *Can hair analysis predict cancer and other common community health concerns?*
Common community health concerns relate to health outcomes such as cancer and birth defects (according to Dr. Harkins, ATSDR). Questions relate to what harm may have been done or what future risk may exist as a result of environmental exposures.

One panelist stated that it is not likely for hair analysis to be used to any large extent to address public health or individual concerns related to teratology or carcinogenicity (MG). This panelist did note, however, that current efforts to measure benzene in hair might in the future provide some predictive value for aplastic anemia, but only because of the known association between benzene and aplastic anemia. Another panelist re-emphasized that hair only provides an approximate 1-year time frame in terms of possible exposures, further supporting the conclusion that hair analysis has little predictive value in studies of the carcinogenic potential of environmental exposures (RB). Judging from

²EPA has established a methyl mercury benchmark dose (in maternal hair) of 11 ppm. This is equivalent to 46 to 49 micrograms of methyl mercury per liter of maternal blood; the critical effect is developmental neurological abnormalities in offspring (U.S. EPA 2001).

the current understanding of underlying science (particularly for carcinogens), another panelist commented, he would rather have exposure history instead of hair analysis data (DP).

- *Importance of establishing a clinical basis prior to testing.* A fair amount of discussion occurred regarding the criticality of establishing a clinical basis before pursuing hair analysis. Several panelists questioned the relevance of measured levels if they cannot be used to predict health endpoints. As in other discussions, the dichotomy of using hair analysis as an exposure tool versus a clinical tool was very evident.

The physicians on the panel strongly stated that a clinical basis must be established before hair analysis can be considered a useful tool. One panelist stressed that one should not collect data that one is not prepared to use (RB). In response to an acknowledgment that a community might press for hair analysis—for example, even in the absence of supportable scientific data—two panelists were emphatic that science must be the focus: politics, litigation, and any other underlying agendas must be put aside (RB, MG). In general, one must consider what doses, under what circumstances, are relevant (RB). Part of the challenge lies in communicating to the public what the current science enables us to do. No absolutes exist in toxicology and medicine. The exposure, the form, the presentation, and the distribution must be placed in the right context (RB).

Another panelist strongly stated that the predictive value of the test result must be weighed and communicated. He emphasized that should the science clearly show no plausible correlation for a particular substance or exposure situation, then hair analysis should not be considered (MK).

One panelist reiterated that in the absence of dose-response data, hair analysis may simply give us a better sense of exposure; it “raises some suspicion” of possible exposure and effects (TC). Measurements of particular substances in hair may be indicative of exposure, but not the risk of disease (LW).

- *Understanding the function of various elements in hair.* In order to ultimately understand dose-response relationships and the clinical significance of exposures, scientists need a better understanding of the role of various elements in the hair. Two panelists briefly commented on the basic lack of understanding of the function, if any, of metals, cations, etc., in hair. From a practical point of view, keratinized cells “are on their way out” with the purpose of protecting the skin and providing warmth. It is therefore difficult to determine the biological meaning of individual components in hair. Some elements maintain homeostasis (e.g. potassium). Other elements are co-factors in synthesis (e.g., chromium in collagen synthesis). Some elements, on the other hand, are ubiquitous and have no known purpose (e.g., lead, uranium) (RB, LW).

- *Substances for which hair analysis might prove useful.* Panelists provided a couple of examples of other elements for which hair analysis may hold some promise. The panelists agreed that if strong hypotheses exist, the scientific merit of these types of relationships may be worth pursuing (RB, DP, TC, SS).
 - *Thallium* might be useful in hair because it is an unusual toxicant. (Thallium was suggested based on a “classic picture” of thallium intoxication studied by CDC in Florida.) (DP)
 - The possible correlation between excessive *manganese* levels (as measured in hair) and violent and other antisocial behaviors has been studied in incarcerated populations. While study findings suggest some correlation and have some merit on the surface, many potentially confounding factors exist that need to be examined more closely, such as hair color, race, and social context (DP). Panelists questioned the overall scientific merit of the correlation, based on the possible lack of biological plausibility—that is, symptoms are not necessarily consistent with documented health effects associated with manganese (DP). One panelist noted that manganese exposures would more likely be expected to cause neurological effects that lead to more withdrawn or inactive behavior (e.g., Parkinson-like symptoms) (SS). Another panelist noted that, because manganese is an essential trace element, it is reasonable that it will get into hair (TC). Another study, by Bader et al. (1999), showed some correlation between axillary hair and airborne manganese (attributed to contamination by dust and water), but overall did not support the use of hair for manganese analysis (SS).

5.3 Choosing the Best Biological Marker

Panelists briefly discussed if and when hair may be more advantageous than other biological samples, such as blood or urine. From both an exposure and clinical perspective, panelists considered which approaches were most productive. Generally, based on current science, they concluded that hair may be used to provide historical exposure perspective within a fairly small window of time (i.e., 1 year). Panelists’ views are highlighted below:

- Two panelists emphasized that the following question needs to be answered in making such a determination: When might a substance be detected in hair, but not in urine (measure of excreted amount) or blood (measure of body compartment) (MG, LW)? Another panelist encouraged consideration of the following question: For what

substances do we have knowledge of the toxicologic implication of the measurement of the substance in hair compared to the measurement of the substance in other biological specimens (e.g., urine, blood, bone) (MK)?

- How do we move toward establishing the “gold standard?” *Could* hair samples be a better way to non-invasively get a sample? Is it a valid measure and how does that relate back to blood or target organ levels (LW)?
- Hair samples may be considered preferable or less invasive under certain situations (e.g., pediatric exposures) (SS). Others commented that collecting blood or urine samples did not appear to be that much of an obstacle (MK, LW).
- Hair may be considered for retrospective purposes when blood and urine are no longer expected to contain a particular contaminant. Again, the distinction between the use of hair analysis as an exposure tool, rather than a diagnostic tool, was made (LW).
- From a clinical point of view, it is important to focus on what substances are of greatest interest, then ask what is the best way to analyze them. Is hair analysis the best way to measure body burden (instead of blood or urine)? For example, we may be able to analyze/identify many elements in hair, but it still may be more useful to look at blood levels. Blood may simply be the better body compartment to test from a scientific point of view regardless of whether we can test for a particular substance in hair. That is, what can potential levels in hair tell us that blood levels do not (RB)?
- An acute spike in hair might help document exposure, but generally will not help from a diagnostic perspective (MG, LW). Acute exposures are best measured through blood or urine (RB).
- Growth rate is a key consideration. Assuming growth at approximately 1 centimeter a month, the hair on the average person’s head generally represents a year or less of time. Hair analysis will therefore have limited usefulness in cases where exposures occurred more than a year prior to an exposure assessment (RB). While hair analysis may provide a snapshot of exposure conditions, it is not likely to predict long-term exposures (SS).

SECTION 6

CONCLUSIONS AND RECOMMENDATIONS

On the second day of the meeting, panelists reviewed earlier discussions and drew overall conclusions. During these final deliberations, panelists commented on the overall state of the science in hair analysis, the major limitations of hair analysis, topics for which a complete scientific understanding is not available, and research that might permit a better understanding of the science. The panel's general conclusions and recommendations are summarized below.

6.1 What Is the State of the Science of Hair Analysis?

Although consensus was not required, the panelists did agree on the following overall conclusion statement:

For most substances, insufficient data currently exist that would allow the prediction of a health effect from the concentration of the substance in hair.³ The presence of a substance in hair may indicate exposure (both internal and external), but does not necessarily indicate the source of exposure.

6.2 When Is It Appropriate To Consider Hair Analysis in Assessing Human Exposures to Environmental Contaminants?

The panelists recognized that hair analysis can serve two distinct purposes: (1) as a tool in identifying exposures (Is the substance reaching people? Does a competing pathway exist?) and (2) as a clinical tool (What is the threshold for adverse health effects?) The latter is where the largest data gaps exist. The panelists agreed that a body of literature describes specific conditions and uses of hair analysis for methyl mercury and arsenic. There may be a unique forensic setting

³This statement addresses only exposure to environmental contaminants and does not address substances of abuse.

for other metals. Segmental analysis with ultra-sensitive techniques may have a role in special cases—that is, subject-, substance-, and situation-specific cases (e.g., identification of high-dose acute exposure).

The group agreed on the general criteria that need to be fulfilled in order to consider hair analysis a valid assessment tool. Panelists encourage assessors to ask: What is the predictive value of a positive or negative test? Are data available to determine whether the measured level is of sufficient magnitude to be of pathological or public health importance? The following factors are key to that determination:

- (1) Defining the type of exposure that may have occurred and over what time period. (What do exposure histories tell us about the likelihood that a particular substance will be in hair at the time of testing?)
- (2) Understanding the type of substance and its behavior in the body. (Are data available that relate exposure to proportional uptake in hair? Is uptake in hair biologically plausible? Is it a marker of external exposure?)
- (3) Identifying the clinical relevance of a positive or negative finding. (Are any dose-response data available that will make useful interpretations possible?)

The panel provided this specific input on when hair analysis can be useful:

- From an exposure perspective, hair analysis can be useful for simply identifying or confirming exposures. Issues raised or reiterated included (1) the difficulty in distinguishing between internal and external contamination, (2) the qualitative nature of any such finding, (3) the inability to confirm the source of the substance under study, (4) the dilemma of not being able to “take it to the next step” (i.e., to use the results as a clinical tool). To overcome issues 1 through 3, it was noted, it may be more feasible for some substances to confirm the contamination source (e.g., based on the specific signature of the substance[s] of interest). Also, more sophisticated studies (e.g., looking at stable isotopes of certain metals) may now be possible (TC, MK, SS, LW).

- According to the current science, the primary utility of hair analysis is as a measure of historical exposure. The research focus needs to be on seeking data that establish dose-response relationships (SS).
- From a clinical perspective, the following conditions must be satisfied before hair analysis can be viewed as a reliable means to measure a particular substance: (1) hair contains a substance concentration that correlates with body organs, tissues, or fluids; (2) correlates exist and are predictive from a clinical and/or forensic perspective; and (3) hair can be used reliably to sample individuals, groups, and/or populations to measure the substance (RB).
- Theoretically, potential substances for which hair analysis may be useful include those for which the route of exposure would limit external contamination and those for which a metabolite might be measurable (MK).
- Because of general hair growth and cutting patterns, for exposures longer ago than a year or quite recent, hair analysis is not useful (RB, LW).
- Depending on the test or element under study, a negative test can help to rule out an exposure and any potential problem. Again, “negative” needs to be defined. That is, what is elevated (RB, MK, TC, LW)?
- Before considering hair analysis, a practical consideration is questioning whether there are any laboratories available that provide cost-effective services and reliable results (DP).

6.3 What Are the Limitations of Hair Analysis? What Data Gaps and Research Needs Exist?

Throughout the 1½-day meeting, the group identified various factors that currently limit the use of hair analysis in evaluations of environmental exposures. No specific research agenda was

proposed, but gaps in the scientific data were clearly identified.⁴ The limitations and data gaps were recapped by the panelists as follows:

- The lack of standard procedures for sample collection.
- The lack of standardization of methods and quality assurance/quality control (QA/QC) among laboratories.
- The possible over-interpretation of results far beyond the current body of scientific data and in light of limitations of techniques and procedures.
- External contamination from a variety of sources, which lowers sensitivity (e.g., environmental, hair treatments, personal hygiene, and others).
- The lack of a body of evidence to demonstrate the effect of washing hair on analytical results.
- The lack of reference ranges in which to frame the interpretation of results. Reliable reference ranges are needed—specifically, background or expected ranges in different geographical areas or regions. Reference ranges should be applicable to population of interest. The DiPietro (1989) data are a good start, but more data characterizing regional differences are needed.
- The lack of data related to uptake/incorporation of environmental contaminants into hair. For both metals and organic compounds, neither kinetic models nor metabolite data are known or fully understood. Identifying metabolites of substances of interest would be helpful, because they could serve as markers of internal exposure.
- The lack of correlation between levels in hair and blood and other target tissues.
- The lack of an epidemiologic database linking substance-specific hair levels and health end points.

⁴One panelist cited a pre-print of a paper by Jason Ditton, professor of criminology at Sheffield University, England, as a good overview of the potential problems associated with interpreting hair analysis results, which he felt were on par with panel discussions. The paper highlights uncertainties and intra-individual variability in hair growth rates and substance-specific incorporation rates. It also describes the challenges of external contamination issues, including variability in results depending on wash procedures. The paper concludes that hair analysis is not an “absolute dosimeter,” but rather a “chronometrically operating relativistic dosimeter” (RB).

It was re-emphasized that identifying measurable levels of particular substance in hair does not mean an adverse effect will occur or has occurred. From a medical perspective, many panelists felt strongly that there is little point in performing hair analysis for a substance if the findings cannot be used as a diagnostic aid. Justification needs to be provided for choosing hair analysis over blood or urine analysis, and a connection to a clinical endpoint is needed.

- A limited knowledge of the biological variations of hair growth with age, gender, race, and ethnicity.
- Insufficient data on environmentally relevant organic compounds in hair. However, information on testing for pharmaceuticals and drugs of abuse may have value for those looking at organic compounds.

Panelists repeated, throughout the discussions, the risk communication challenges that exist with any exposure or diagnostic tool. The limits of the state of knowledge need to be communicated as clearly as possible by laboratories, practitioners, ATSDR, etc. (RB, MG).

6.4 Recommendations

Panelists' recommendations focused on measures to standardize sampling protocols. The group agreed that such efforts would improve the overall usability and reliability of testing data. The group discussed sample collection, handling, and processing procedures. One panelist recommended considering hair analysis results *only if* the laboratory documents good practice in terms of handling and validation protocols (MK). It was also recommended that the governmental, commercial, and research laboratories pool their experience and help develop standard protocols (SS). Panelists offered the following specific recommendations:

- *Standardize sample collection procedures.* Samples should be ordered by a physician, taken for a defined reason, properly collected, and dealt with according to proper chain of custody procedures. A determination needs to be made regarding the best location on and distance from the scalp to test. No consensus was reached on the preferred cutting device. To avoid metal contamination, some panelists recommend using quartz or plastic or teflon-coated shears. Others questioned whether it really made that much of a difference.

Most important, everyone agreed, is for the laboratory to demonstrate the extent of contamination introduced, if any, during sample collection. Lastly, sample handling (chain of custody) procedures should be the same as those applied to other environmental samples.

- *Collect exposure histories.* Several panelists recommended obtaining exposure histories concurrent with collecting hair samples. Information should be collected for the year prior to the collection date, although one panelist pointed out that recall bias may likely be a limiting factor. Histories should consider environmental and treatment exposures. It was recommended that the questionnaire that has been used by CDC be used as a starting point or model. Lastly, any such questionnaire should be substance-specific.
- *Establish quality assurance protocols.* Use quality assurance methods for laboratory analyses recommended by the World Health Organization (1994). Specifically, (1) reference samples of the same matrix (hair) with known concentrations of the metal should be used as standards, (2) reference samples should contain the metal at approximately the same concentration as the sample, (3) if such reference materials are not available, analysis of quality-control samples at different laboratories by different analytical methods must be used, and (4) because results may vary over time and for different metals, results should be present for the corresponding time periods and metals (SS).
- *Require external validation.* Require performance evaluations of hair testing laboratories in the form of proficiency testing (e.g., running reference samples and evaluation of materials of unknown content). The Center for Toxicology in Quebec occasionally offers a hair analysis sample for ICP-MS (DP).
- *Require documentation.* Testing laboratories need to be challenged to make a deliberate day-to-day effort to demonstrate internal and external validation. Calibration and quality assurance methods need to be well-documented (DP, MK).
- *Encourage targeted analyses.* Target testing to the specific element of interest. Testing for multiple analytes increases uncertainty. Overlapping peaks may lead to the misinterpretation of results (MK).
- *Develop washing protocols.* Differing opinions were voiced regarding whether hair samples should be washed, but the panelists generally agreed that the effects of washing, when performed, need to be clearly documented by the laboratory. Individual panelist input is summarized below.

- The determination of whether or not to wash the sample is a substance-specific decision (SS).
- Insufficient data exist to measure the true effects of washing, so washing adds another layer of uncertainty when data are interpreted (MK).
- One panelist recommended examining the wash solution when washing (RB), but others questioned how to interpret the resulting data, fearing that it may add yet another layer of uncertainty (DP, MK).

6.5 Next Steps To Be Taken by ATSDR

Dr. Susten described ATSDR's anticipated next steps related to evaluating the utility of hair analysis. First, a summary report of this panel meeting will be generated and released (the report will be posted on ATSDR's Web site). Second, ATSDR will generate a report related to lessons learned from the panel discussions (and possibly publish it in the open literature). In addition, internally, the agency plans to do the following:

- Continue to provide education to physicians and other health professionals.
- Develop a generic fact sheet to help health assessors and communities communicate and understand hair analysis issues.
- Continue to develop substance-specific toxicological profiles. The profiles are an excellent resource and contain information on biomarkers of exposure. In light of the panel discussions, additional language may be added regarding hair analysis (e.g., in terms of limitations, etc.) on a substance-specific basis.
- Develop guidance on hair analysis to support public health assessments and health studies conducted by the agency. That is, develop criteria for determining when to consider hair analysis as part of an ATSDR exposure investigation.

These activities will help all of ATSDR's divisions as well as professionals in the community.

SECTION 7
OBSERVER COMMENTS

On both days of the panel discussions, observers were given the opportunity to provide input on issues related to the charge questions and panel deliberations. Observer comments received during the meeting are summarized below, alphabetized by observer's name. A full list of observers and their respective positions and affiliations is included in Appendix F. Observers were asked to provide appropriate references and data to support their statements where possible. Statements provided without reference are included, but have not been verified or validated by ATSDR or the panel. In some cases panelists responded to a particular observer comment or question; such responses are summarized in this section as well.

Observers were also encouraged to provide written comments after the June 12–13, 2001, panel discussions. Appendix G includes written comments from two individuals.

Erik Auf der Heide
ATSDR

Dr. Auf der Heide commented that considering sensitivity, specificity, and predictive value is as important as the reference range when interpreting laboratory data.

Sherlita Amler
ATSDR

Dr. Amler, a pediatrician, stressed her observations of over-interpretation and misinterpretation of hair analysis results.

She noted that a lack of knowledge exists among health care providers in terms of how to use hair analysis, citing two examples. She described a case of an autistic child with reportedly high

levels of mercury in his hair. The physician presumed that the elevations were due to his immunizations and ordered chelation in hopes of improving the autism. In another case, the interpretation of hair analysis results of a Down's Syndrome child as a dietary insufficiency led to the administration of high vitamin doses and an unusual diet. (Dr. Clarkson raised the point that misuse or misinterpretation of laboratory tests is not unique to hair analysis.)

Gary Campbell
ATSDR

Dr. Campbell emphasized the need to clearly define "normal" and "reference" ranges and to describe how these ranges are developed in the various laboratories. Understanding the meaning and derivation of such ranges is very important to individuals who need to interpret site-specific hair analysis results and understanding whether results may be elevated. Further, Dr. Campbell questioned what is known about possible geographical or regional differences in background concentrations of various substances in hair.

Robert Jones
CDC

Dr. Jones requested that the panel and ATSDR consider the following:

- Evaluate substances on a species-specific basis, not just on an element basis. Looking at the form in which elements such as arsenic, mercury, and selenium are present in hair may help to distinguish exposures due to the form released from a Superfund site from exposures to a form originating from another source.
- If ATSDR is considering hair analysis in its public health assessments, begin the process of generating substance- and species-specific quality control reference materials as soon as possible. Generation of such reference materials can take years.
- Include handling procedures and short- and long-term storage requirements (e.g., container and climatic conditions) in any standard protocol.

- Do not standardize hair analysis procedures too highly or you risk stifling innovation by laboratories. Strict standardization will not guarantee good quality control. Specific procedures or technologies should not be required as long as the laboratory can demonstrate the quality of its results. Proficiency systems (daily and longer-term), as recommended by the Clinical Laboratory Improvement Act (CLIA), are encouraged.

Melody Kawamoto
CDC/National Institute for Occupational Safety and Health

Dr. Kawamoto presented a schematic that integrated many of the concepts and issues being discussed by the panel (see Figure 7-1). She explained the interface between the many compartments within the body and how different testing methods help piece exposure information together. Specifically, Dr. Kawamoto discussed how different methods help assessors identify potential (environmental media sampling), external (wipes, breathing zone air samples, hair), and internal (hair, blood, urine) exposures to a particular substance and how that information may be integrated to evaluate potential health effects. She emphasized the importance of establishing a framework under which to conduct exposure and health effects evaluations, including clearly identifying the problem and the hypothesis under which you will proceed, identifying study design issues, and understanding sampling and analytical issues.

David Mellard
ATSDR

In reference to the arsenic conference held in San Diego in 2000, Dr. Mellard commented on a study in which a single volunteer showered in arsenic-contaminated water to help better understand internal versus external contamination. The study revealed that up to a certain level, no change in arsenic levels in hair were observed. Dr. Mellard suggested that perhaps further study is worthwhile to see if, for relatively low levels of arsenic in water, hair could be used as a measure of internal contamination, without worrying about external contamination.

Dr. Kosnett responded with a few words of caution: *In vitro* experiments have shown that external absorption is dependent on time. Therefore a single showering episode may not reflect a longer-term exposure or exposure through bathing. Having reviewed the literature, Dr. Kosnett indicated that he is not convinced yet that any cut-off point exists at which there is no element of external uptake of arsenic in hair from bathing.

Figure 7-1. Evaluation and Solution of Environmental and Occupational Health Problems: Critical Analysis in Practice¹

Model: Cause-Effect		Source	Exposure				Effect	
Indicators of exposure and effects	Possible detectable or measurable parameters (examples)	Type of assessment	Potential dose	External dose	Internal dose	"Abnormality"	Disease or other health effect	
			Concentrations in air, soil, water, and surface wipe samples	Personal air samples, wipe samples of skin, hair analysis; dosimetry	Concentrations in blood, urine, and other body tissues and fluids; dosimetry	Bio-markers or other indicators	Bio-markers or other indicators	
			Environmental		Biological	Biological and other		
			Document review					
I S S U E S	Model	<ul style="list-style-type: none"> Hypothesis or problem statement clearly defined Scientific plausibility (e.g., toxicity, biokinetics, relationship between time of exposure and time of assessment) Motives and desired results <ul style="list-style-type: none"> application of scientific methods to research theoretical questions application of available scientific knowledge to provide answers to questions from the public 						
	Public health criteria and study design	<ul style="list-style-type: none"> Selected parameters to be measured are valid with respect to the model Interpretation possible (e.g., dose-response relationship, population norms, intra- and interindividual variability) Relevance of interpretation to the problem statement (e.g., prevention possible) or to questions from the public (e.g., predictive value, risk communication) Feasibility (technical feasibility and cost feasibility) Timeliness Ethics 						
	Collection and handling	<ul style="list-style-type: none"> Contamination Stability during transport or storage Preparation methods (e.g., cleaning, digestion) 						
	Laboratory methods	<ul style="list-style-type: none"> Validity, reliability, accuracy, precision, sensitivity, specificity Quality control (proficiency tests, coefficient of variation) 						

¹ Dr. Kawamoto provided this schematic following the meeting, as a work in progress, as a visual display of the various concepts presented as part of the panel discussions. It is an expanded version of a hand-drawn figure presented at the meeting.

David Quig

Day #1

Dr. Quig, from . . . (a commercial laboratory), expressed extreme gratitude for being invited to this meeting and offered his opinion on a variety of topics related to analytical methods and factors affecting the interpretation of laboratory results:

- As a screening tool, no one laboratory test exists that is absolutely definitive. It is critical that hair analysis results be looked at in careful consideration of patient symptoms and exposures. Hair analysis is not a test to end all tests.
- A targeted approach is necessary for certain elements. There is no question, for example, that chromium is extremely difficult to measure. One laboratory using high-resolution mass spectrometry is getting closer to being able to measure Cr⁶⁺ in blood. However, interference problems do not exist for *all* the elements.
- Hair treatment is an important issue and clearly affects hair analysis results. Dr. Quig has worked on a study of 150 hair products (pre-published status); the most common contaminants identified include tin, aluminum, silicone, and phosphorous. Only two products have been found to contain mercury and arsenic (Denorex and Aquanet), which could confound hair analysis for these elements.
- Ethnicity/race needs to be factored in when evaluating hair analysis results. For example, the reference ranges for Caucasians should not be used for African Americans. The basic profile is very different between the two.
- With respect to growth rates, the difference between the very young and the very old is significant.
- Distinguishing internal versus external levels is impossible. Some laboratories claim they have an algorithm for making such distinctions. Any such claim should be seriously questioned.
- In Dr. Quig's experience, laboratories do take into account the type of container in which samples are stored.

- Using hair analysis for an individual can be acceptable and useful—for example, when tracking occupational exposures of a particular person over time (e.g., a worker exposed to lead).
- Washing procedures are a critical part of the hair analysis protocol (with the possible exception of methyl mercury testing). It would not be desirable, for example, to test unwashed dreadlocks.
- The only time Dr. Quig has seen significantly elevated mercury in hair levels in non-fish-eating individuals is with dentists exposed occupationally to mercury vapor. In questioning whether this was internal or external contamination, a comparison of scalp and pubic hair confirmed equally high levels; this suggested internal exposure. Again, it is critical to look at hair analysis screening in context of other measurements (e.g., blood).
- As indicated by the panel, it is important to realize that the presence of organic toxins (e.g., DDT) is not “normal.” It is equally important to recognize that we are all subjected to exposure to a variety of organic compounds and toxic metals. It is therefore important to consider multiple exposures.
- Standardization of laboratories is a necessity. The same methods and sensitivities should be required. It is not surprising that Seidel et al. (2001) found different reference ranges across the laboratories studied, because the laboratories used different analytical methods (i.e., ICP-MS versus OES) that have a 1,000-fold difference in the detection limits. This discrepancy should not be used as a reason for not using hair analysis, but as the impetus for advocating standard protocols.

Day #2

Dr. Quig provided more comments toward the end of the second day of the meeting. His stated opinions are summarized below:

- If done correctly, hair analysis can be a useful tool.
- No question exists that gross ineptness has been observed at some commercial laboratories. The issue of interlaboratory differences is not sufficient reason, however, to conclude that hair analysis is not of value. It is simply a question of tightening up sampling/analytical protocols and QA/QC procedures.

- Regarding quality control issues, [his laboratory] has been pressing for the establishment of standardized procedures for hair analysis under CLIA and the Health Care Financing Administration. The fact that procedures are not yet in place is not a reason not to do hair analysis; it is a matter of the organizations catching up with the needs of the time.
- Regarding washing protocols: A laboratory should produce a reasonable report describing its washing protocol. The user of the data should look for this information before interpreting the data.
- A standardized procedure can and should be set for sample collection.
- Statements by panelists regarding the over-interpretation and misuse of hair analysis were not relevant to the specific charge of this meeting and should not be of concern to ATSDR.
- [His laboratory] only accepts hair samples from licensed physicians or for research purposes. Dr. Quig agreed that hair samples should only be submitted by trained practitioners.
- Dr. Quig suggested looking at research conducted by Needleman (University of Pittsburgh) and Masters (Dartmouth) before dismissing the utility of hair analysis for evaluating lead exposures.
- Sound literature does exist on manganese and aberrant behavior, although the literature is criticized by the panel. Dr. Quig referenced a follow-up study comparing manganese levels in prisoners committing violent versus nonviolent crimes. With regards to the symptoms and the neurotoxicity of manganese, psychological effects range from apathy progressing to violent reactions and loss of tolerance. The physiology of manganese toxicity is well-established in the literature. Manganese has a high propensity to bind to myelin pigmented dopaminergic neurons in the brain.
- Reference ranges are not based exclusively on small data pools (e.g., “n=2”), as suggested during some of the panel discussions. Available reference ranges are based on 28 years of doing hair analysis. As methods improve, so will reference ranges. Data sets are expanding to include documentation of variations in levels of elements between Caucasians and African Americans, as well as transcontinental differences.

Barry Sample
Quest Diagnostics

Dr. Sample speculated on the possible value of measuring wash solutions as well as washed hair in attempts to further distinguish between internal and external exposures. Wash solution may provide a better sense of external levels and the hair may provide a better indication of the total internal burden. At a minimum, Dr. Sample suggested incorporating wash evaluation into any standard protocol.

Based on his experience looking at drugs, Dr. Sample acknowledged that data may not exist to set the “normal range.” In order to do so, one needs to understand the different rates and methods of incorporation into the hair. He suggested that there may be some value, in an occupational setting, in developing an individual reference range.

In response, Dr. Kosnett commented that workers may not be the best population to study for normal ranges because of the potential for external exposures in various work places. Dr. Seidel noted that further research is needed into the utility of studying wash water. Studies suggesting that easily removed fractions represent exogenous sources and the not so easily removed fraction represents endogenous sources have been disproved.

Michael Schaffer
Psychemedics Corporation

Day #1

Dr. Schaffer, a trained industrial toxicologist with an interest in criminal justice and forensics, explained that Psychemedics performs hair analysis as part of workplace drug testing. He asked participants to keep an open mind and consider the science of hair analysis very carefully. Knowledge gained from the last 10 years of testing hair for drugs of abuse can, he said, be used

to enhance the knowledge base for using hair analysis for environmental/public health evaluations. He stressed that his experience in the drug testing arena has revealed that hair analysis is not totally unreliable. Good science and good analyses have supported legal cases. If the proper analytical tools and washing procedures are used, valid interpretations can be made.

Dr. Schaffer recognizes that drugs of abuse are different than trace metals. Working with mass spectrometry, metabolite profiling has helped identify uniquely internal measures of the substance of concern. It has taken 10 years, but such tools are now available.

Dr. Schaffer stressed that hair offers a unique matrix, recognizing that there is much that is not known or understood. In time, he feels, hair analysis will likely provide a lot of useful information.

Day #2

Dr. Schaffer expressed concern that some of the statements made during the panel discussions could be misinterpreted or used inappropriately. Specifically, he wanted to make certain that caveats were provided with panel conclusion statements so that it is clear that hair analysis for substances of abuse is appropriate and based on good science; the conclusions drawn by the panel should apply to environmental contaminants only.

Dr. Schaffer also responded directly to Dr. Baratz's overview of the Ditton paper.⁵ He took exception to the implication that hair analysis may not be suitable for testing drugs of abuse. He stated that conducting hair testing with the proper safeguards is defensible and has been upheld by the courts. He noted that no hair color or ethnicity bias exists. *In vitro* studies have shown

⁵Dr. Baratz clarified that his purpose in presenting the Ditton paper was to summarize some of the key aspects and possible pitfalls of hair analysis. Dr. Baratz noted that the author, a chemist, has done studies on drugs of abuse and has shown the validity of hair analysis for testing drugs of abuse.

incorporation of drugs in different types of hair, but those drugs can be removed by washing as quickly as they are bound to hair. The Department of Health and Human Services (Substance Abuse/Mental Health Services Administration) is currently writing draft guidelines for the incorporation of hair analysis into the federal workplace drug testing program. A pilot proficiency survey is also available to help address quality control issues; the model is urine drug testing.

Subsequent to the June 12–13, 2001, panel discussions, Dr. Schaffer submitted additional comments and supporting literature. He provided (1) a partial listing of those cases demonstrating judicial acceptance of the Psychomedics hair analysis method, (2) information on hair testing and racial or color bias, and (3) information on the effectiveness of Psychomedics' washing procedures for ruling out external contamination. (See Appendix G.)

Margaret Schonbeck
Colorado Department of Public Health and Environment

Ms. Schonbeck questioned whether hair analysis would be a valid consideration at an arsenic exposure site (soil pica/soil ingestion) where urine sampling is already planned.

Dr. Kosnett commented that a hair assay could reveal the potential for exposure, but that environmental and urine data will have already provided that information. It is not likely that hair analysis would provide additional insight. Dr. Baratz re-emphasized that one must examine the clinical utility before considering hair analysis. Does it have any predictive value? Without symptom or disease history, or unless you have a quantifiable dose-response relationship, hair analysis data will not help. Dr. Baratz expressed concern that collecting hair samples as another means of documenting exposure will only muddy the waters. Dr. Seidel suggested collecting, analyzing, and archiving the data, but being clear with the community up front what the data can and cannot be used for. Dr. White emphasized the distinction between medicine and public

health, which can sometimes cause confusion and tension in the community. That is, medicine is looking at the individual and treatment options, while public health is looking at populations and possible risk factors.

Anthony Suruda

Association of Occupational Environmental Clinics

Rocky Mountain Center for Occupational and Environmental Health

Dr. Suruda questioned whether nails are more susceptible to external contamination by metals than hair. In response, Dr. Kosnett noted that, in some forensic investigations, the distal portions of nails have shown correlation with poisoning. Some studies have investigated whether the inner surface of the nail may be less likely to contain elevated levels of arsenic as a result of external contamination. Study findings suggest that external contamination of nails is an issue as it is in hair. For example, a study that measured arsenic in nails over time following arsenic ingestion revealed the following: (1) elevated levels of arsenic were measured in distal segments of unscraped nails (believed to be deposited by sweat); (2) scraped nails during the same period did not reveal elevated levels; and (3) samples of scraped nails taken later in time showed elevated arsenic levels (as a result of the ingestion episode). As with hair, it is questionable whether methods exist to clearly distinguish between externally and internally deposited contamination.

Dr. Suruda indicated that he was requested to evaluate an individual with peripheral neuropathy 9 months after possible exposures to lead and arsenic. Total arsenic urinalysis had been performed closer to the time of exposure, but not a fractionated analysis. To evaluate past exposures, a toenail sample was taken down to the growth plate, which was negative. These results were used to conclude that the individual *had not* been exposed to arsenic within the past year.

Dr. Suruda noted that the charge to the panel was to examine aspects of hair analysis related to public health assessments. Dr. Suruda commented that he is more often faced with questions from *individuals* (practitioners, community members) looking for assistance in interpreting hair analysis results. He expressed hope that the panel and ATSDR will consider the utility of hair analysis in the assessment of public health as well as for individual assessment. Dr. Suruda noted that ATSDR's toxicological profiles and other agency documents have great credibility within the scientific community and that he looks forward to further guidance (e.g., biological

monitoring guidelines) to assist in his evaluations. Even if all the answers are not available, Dr. Suruda said, hair analysis should be ranked with other methods of monitoring (e.g, blood, urine).

Regarding research needs, Dr. Suruda indicated the need for a population-based study on how hair analysis is used and what impact it has had. Questions to consider include: Can it be used to identify poisoned individuals? How many people are unnecessarily alarmed or mistreated on the basis of hair analysis? What type of reports do practitioners receive on hair analysis? Dr. Suruda expressed concern regarding what he referred to as “junk science.” For example, he pointed to a laboratory report that indicated “lead is slightly above detection limit” and that the “zinc to mercury ratio is extremely high.” The report indicated that these ratios do not indicate disease; however, it also indicated that research has shown that this “will eventually lead to other disturbances in metabolic function.” Physicians and other practitioners need to recognize that they do not often know what results mean and should be cautious in what they report.

SECTION 8
REFERENCES

The following papers were reviewed by the panelists prior to the meeting for their consideration when reviewing the charge questions.

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See also the bibliography of hair analysis references provided in Appendix D.

APPENDIX A

List of Panelists

Panelist Biographical Sketches



Hair Analysis: Exploring the State of the Science

Panel Participants

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Hair Analysis: Exploring the State of the Science

The Panel

Robert Baratz

Dr. Baratz is the founder of two corporations in the medical device area. He was also the associate medical director for Harbor Health Services in Boston. He currently provides consulting services for clients such as the U.S. Food and Drug Administration, ABC News, CBS News, NBC News, American Dental Association, and the states of Iowa, California, Colorado, and Minnesota. His area of research interest is the development and formation of the skin and its appendages, including hair. He is currently working with the state of Wisconsin regarding chelation therapy and use of hair analyses. Dr. Baratz is the national spokesperson for the American Dental Association regarding alleged mercury toxicity. He received his A.B. in biology and his M.D. from Boston University. Dr. Baratz received his Ph.D. in cell biology and anatomy and his D.D.S. from Northwestern University. He has published more than 150 papers.

Thomas Clarkson

Dr. Clarkson has been a professor at the University of Rochester for over 20 years, where much of his research has involved conducting epidemiologic-toxicologic studies on populations exposed to mercury and its compounds and studying the pharmacokinetics of mercury in animals and humans. He has studied the toxicology of hair analysis, including coauthoring two studies on the subject, entitled “The biological monitoring of mercury in the Seychelles study” and “Monitoring methyl mercury during pregnancy: maternal hair predicts fetal brain exposure.” Dr. Clarkson served as a panelist at an NIEHS Workshop on Assessment of Mercury Exposure. He has also co-edited various books, including *Biological Monitoring of Toxic Metals* and *Advances in Mercury Toxicology*. Dr. Clarkson is a member of the Society of Toxicology and the Society for Trace Element Research in Humans. He serves as a reviewer for numerous national and international scientific journals, such as *Journal of Applied Toxicology*, *British Journal of Industrial Medicine*, and *New England Journal of Medicine*. Dr. Clarkson received his B.S. in chemistry and his Ph.D. in biochemistry from the University of Manchester.

Michael Greenberg

Dr. Greenberg is a professor and toxicology fellowship program director at the Medical College of Pennsylvania–Hahnemann School of Medicine. He also serves as chief of the Division of Occupational, Environmental and Hyperbaric Emergency Medicine at the Allegheny University of the Health Services School of Medicine (Department of Emergency Medicine). In addition, Dr. Greenberg is a senior consultant for the Philadelphia Poison Control Center. He has performed more than 1,000 exposure assessments and has extensive experience both interpreting and clinically utilizing hair testing in forensic, occupational, and environmental toxicology venues. Dr. Greenberg has published more than 100 articles and abstracts in occupational and environmental toxicology. He also is the editor in chief of the book *Occupational, Industrial and Environmental Toxicology*, published by Mosby, St Louis, Missouri. Dr. Greenberg received his B.A. and M.A. in biology from Hofstra University, his M.D. from Temple University School of Medicine, and his M.P.H. in occupational medicine from the Medical College of Wisconsin. He is board certified in Occupational/Environmental Medicine, Medical Toxicology, and Emergency Medicine.

Michael Kosnett

Dr. Kosnett is an associate clinical professor at the University of Colorado Health Sciences Center. His main specialty area is occupational and environmental toxicology. One of his research areas is the application of laboratory data to clinical epidemiology and clinical assessment in humans exposed to heavy metals, particularly arsenic. He has been regularly involved in reviewing and utilizing quantitative information on human exposures to toxic substances and has designed and implemented biological monitoring programs. He has served as a consultant to ATSDR at the Bunker Hill Superfund site in Idaho, and acts as a toxicology consultant to the California Department of Health Services, Occupational Lead Poisoning Prevention Program. He also has experience in assessing the impact of toxic substances on children. Dr. Kosnett was a member of the National Research Council's (NRC's) Subcommittee on Arsenic in Drinking Water and testified on the subcommittee's behalf to a U.S. Senate Committee on Environment and Public Health. He contributed to sections of the 1999 NRC report *Arsenic in Drinking Water* which discussed the use of hair analysis in assessing exposures to arsenic in drinking water. He also has served on the Committee on Toxicology of the NRC, and as vice president of the American College of Medical Toxicology. Dr. Kosnett recently gave the keynote address at the 2001 ATSDR Partners in Public Health Meeting, entitled "Elemental Mercury Exposure and Human Health: Controversial Issues Regarding Low Level Exposure." Dr. Kosnett received his B.S. in molecular biophysics and biochemistry from Yale University, his M.D. from the University of California San Francisco, and his M.P.H. in environmental health sciences from the University of California at Berkeley.

Dan Paschal

Dr. Paschal has more than 20 years experience as a research chemist in CDC's Nutritional Biochemistry Branch, Division of Environmental Health Laboratory Sciences, within the National Center for Environmental Health. He is responsible for establishing and maintaining "state of the art" analytical methods for toxic elements in biological samples, participating in transfer of appropriate technology to state or other environmental health laboratories, and supervision of technical research involving method development and specimen collection protocols. Dr. Paschal has studied various aspects of hair analysis and published papers related to the "age-dependence of metals in hair" and "reference intervals for 28 elements in nonoccupationally exposed adults in the United States and effects of hair treatment." Dr. Paschal also consults with a wide variety of state and local health professionals to evaluate analytical methods, quality control procedures, and exposure assessment approaches. Dr. Paschal received his B.S. in chemistry and his Ph.D. in analytical chemistry from the Georgia Institute of Technology.

Sharon Seidel

Dr. Seidel is a toxicologist for Impact Assessment, Inc., a contractor for the California Department of Health Services. Prior to that she served as a toxicologist for the U.S. Environmental Protection Agency Superfund Program in Region 9. She has studied the health implications of a number of environmental exposures, including children's exposures to pesticides. Her primary involvement with hair analysis is a recent comprehensive assessment of practices of commercial laboratories performing a broad suite of mineral analyses being used as a clinical assessment tool and to identify toxic exposures. Her findings were published in the January 2001 *Journal of the American Medical Association*. Dr. Seidel received her B.A. in chemistry from the University of California, San Diego, and her Ph.D. in pharmacology from the University of Iowa.

LuAnn White

Dr. White is an associate professor and director of the Center for Applied Environmental Public Health at the Tulane School of Public Health and Tropical Medicine. She has designed and implemented the key elements of the Tulane/HAMMER project for the U.S. Department of Energy to improve environmental restoration and waste management worker health and safety through education and training. She is an innovator in the use of distant learning technologies. Dr. White is a diplomate, American Board of Toxicology, and has promoted the use of Geographic Information Systems for toxicologic research. She developed a model for curriculum development in environmental health which provides a skills-based approach to environmental education. Dr. White also serves on ATSDR's Board of Scientific Counselors. Dr. White received a B.S. in chemistry from St. Mary's Dominican College and a Ph.D. in pharmacology/toxicology from Tulane University.

APPENDIX B

Charge to the Panel

Charge to the Panel

Hair Analysis: Exploring the State of the Science

The Agency for Toxic Substances and Disease Registry (ATSDR) is holding a panel discussion to review and discuss the current state of the science related to hair analysis. ATSDR has invited a cross section of scientific experts in the fields of hair analysis, toxicology, and medicine to participate in 1½ days of discussions on a variety of topics, including analytical methods, factors affecting the interpretation of analytical results, toxicologic considerations, and data gaps/research needs. The panel will discuss whether hair analysis is a useful tool in evaluating exposures to hazardous substances present in the environment. The agency will use the input received during the discussions to develop a framework for determining when measuring contaminant levels in hair can help support scientifically defensible public health evaluations.

Background

ATSDR conducts public health assessments to evaluate possible public health implications of contaminants associated with hazardous waste sites and other environmental releases. An important step in ATSDR's assessment process is examining exposures to contaminants under site-specific conditions and determining whether people are being exposed to contaminants at harmful levels. In most of the agency's evaluations, the environmental concentration serves as a surrogate for "exposure."

Exposure concentrations, or estimated doses based on exposure concentrations, however, represent only one factor in a continuum of events that ultimately determine whether exposures will result in illness. Other factors include exposure conditions and various pharmacokinetic/pharmacodynamic events (e.g., absorption, metabolism, excretion), as well as individual variability and susceptibility in the exposed population. To a large extent, ATSDR evaluates these factors qualitatively in its public health assessments.

To refine its assessments and/or to fill data gaps, ATSDR sometimes identifies ways to more precisely quantify exposures, such as measuring body burdens of a particular contaminant or its metabolites (e.g., lead in blood). On a site-by-site basis, ATSDR evaluates what additional exposure data might be practical and useful to obtain to further support public health evaluations and ultimately to help determine the disease potential of a particular exposure. *ATSDR seeks to determine the overall utility of hair analysis as one such exposure assessment tool.*

Charge to Panel Members

General Questions

ATSDR's overall goal is to receive expert opinion on the following four general questions related to hair analysis. Panelists should keep these questions in mind when answering the specific charge questions that follow.

- When is it appropriate to consider hair analysis in assessing human exposures to environmental contaminants?
- When is it inappropriate to consider hair analysis in assessing human exposures to environmental contaminants?
- What data gaps exist that limit the interpretation and use of hair analysis in the assessment of environmental exposures? What research is needed to fill these data gaps?
- For what substances do reliable hair analysis methods exist (e.g., trace elements, organic compounds)?

Specific Charge Questions

Discussions on the first day of the meeting will focus on answering questions that pertain to Topics #1, #2, and #3 below. In asking these questions, ATSDR seeks a critical review and assessment of the *state of the science pertaining to hair analysis*. The second day of the meeting will be devoted to identifying critical data gaps and research needs, and also identifying scenarios for which hair analysis should/should not be considered in light of limitations in analyzing and interpreting hair data (Topics #4 and #5).

Topic #1: Analytical Methods. Discuss/review basic sampling and laboratory methodology used in hair analysis.

- What analytical methods currently exist?
- For what substances do reliable analytical methods exist?
- For what purposes are these methods typically used (e.g., diagnostics, forensics, industrial hygiene)?
- What amount of hair is needed? Is it dependent on the substance being tested? If so, specify substance-specific requirements.

- To what extent are multi-element analytical approaches used? Concern: Accuracy and/or sensitivity for a specific element may be sacrificed.
- Intralaboratory variability: How variable are results and interpretations?
- Interlaboratory variability: How variable are reference ranges, results, and interpretations?

Topic #2: Factors Influencing the Interpretation of Analytical Results. Discuss to what extent (qualitatively and/or quantitatively) the following factors influence the interpretation of hair analysis data. Provide substance-specific examples. Note any additional factors not highlighted below that are critical to or limit the interpretation of hair analysis data.

- Variations in sample collection and preparation methods, including:
 - ▶ Hair sample scalp location and homogenization.
 - ▶ Laboratory sample preparation and washing methods.
 - ▶ Laboratory calibration standards and proficiency testing programs (QA/QC procedures).
 - ▶ “Normal” reference ranges.
 - ▶ “Abnormal” or “toxic” concentration ranges.
- Exposure of hair sample to the external environment (e.g., shampoos, bleaches, dyes, permanent waving, relaxers, styling products, hair sprays, hot dryers and curlers, tobacco smoke).
- Distinguishing between endogenous and exogenous sources of metals in hair.
- Distinguishing between exposures associated with site contamination versus exposures from typical background and other sources.
- Hair color, location of hair on the scalp, and hair diameter.
- Gender, ethnicity/race, diet, age, geographical location, and season.
- Rate of hair growth.

Topic #3: Toxicologic Considerations. Discuss to what extent hair analysis data can be used to predict adverse health outcomes. Cite specific examples/substances for which information is available.

- What is known about biological uptake of specific substances and the concentration delivered to and incorporated into hair?
- What is the relationship between chemical concentrations in the hair and blood compartment and target organs? For what chemicals does a correlation exist between specific chemical concentrations in other body tissues, organs, fluids, subcellular fractions, or metabolic pools?
- What is the dose-response relationship between chemical concentration in hair and target organ effects?
- Ultimately, what is the relationship between chemical concentrations in hair and disease? What is the disease-predictive value?
- Is information available defining “normal” ranges of chemical concentrations in hair that have physiological and health-related significance?

Topic #4: Data Gaps and Research Needs. In light of Topics #1–#3, provide recommendations to fill data gaps and overcome/clarify limitations in hair analysis and interpretation.

- Specify data gaps and limitations that most significantly limit the use of hair analysis in public health evaluations, both in terms of analytical methodologies and toxicologic interpretations.
- Identify hair analysis research needs for ATSDR’s research agenda; identify specific recommendations for future studies.

Topic #5: Identifying Scenarios for Which Hair Analysis May Be Appropriate. Considering the factors identified in the matrix below, and in light of issues identified under Topics #1–#4, discuss when hair analysis *may or may not be* appropriate for evaluating exposures to environmental contaminants. This matrix will assist ATSDR in developing a framework or decision logic for determining when to conduct hair analysis.

Exposure Scenario	Chemical/ Exposure Pathway	Exposure Chronology	Exposure Duration	Measurable Health Effects (Y/N)
- Individual - Community - Population (List both appropriate and inappropriate scenarios)	E.g., ingesting lead in soils	- Past - Present	- Acute - Chronic	- Specify

Reference Materials

When addressing the charge questions, please provide citations for references that ATSDR should consider when evaluating hair analysis issues. ATSDR has developed a bibliography for your review (primarily post-1985). Please identify additional key studies or papers.

The following journal articles and papers have been provided for panelist review and consideration. ATSDR selected these papers to represent the breadth of the issues to be discussed by the panel. Their selection does not indicate ATSDR’s position on any particular issue. ATSDR recognizes that these only represent a sampling of the many peer-reviewed papers on the subject of hair analysis. The purpose of reviewing these papers is to help stimulate thought and discussion related to the charge questions.

- 1) Hopps H. The biologic bases for using hair and nail analyses for trace elements. *Sci Total Environ.* 1977; 7:71-89.
- 2) Miekeley N, Dias Cameiro MTW, and Porto da Silveira CL. How reliable are human hair reference intervals for trace elements? *Sci Total Environ.* 1998; 218:9-17.
- 3) Seidel S, Kreutzer R, Smith D, McNeel S, Gilliss D. Assessment of commercial laboratories performing hair mineral analysis. *JAMA.* 2001 Jan 3; 285(1):67-72.

- 4) Sky-Peck H. Distribution of trace elements in human hair. *Clin Physiol Biochem.* 1990; 8:70-80.
- 5) Steindel S, Howanitz P. The uncertainty of hair analysis for trace metals. *JAMA.* 2001 Jan 3; 285(1):83-85. Editorial.
- 6) Wennig R. Potential problems with the interpretation of hair analysis results. *Forensic Sci Int.* 2000 Jan 10; 107(1-3):5-12. Review.
- 7) Yoshinaga J, Imai H, Nakazawa M, and Suzuki T. Lack of significantly positive correlations between elemental concentrations in hair and in organs. *Sci Total Environ.* 1990; 99:125-135.

APPENDIX C

Pre-Meeting Comments



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.

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LuAnn White, Chair

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 proteiniatious 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 within 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 chemicals. 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 quantitative 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 cationic 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

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 $\mu\text{g/g}$ (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 (≈ 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 μg (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 adsorption 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 an 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 an 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 µg/L (range 1.0 to 2450 µg/L). A subset of subjects whose wells contained arsenic averaging 345 µg/L consumed only bottled water. Although they had relatively low arsenic levels in urine (average 43 µg/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 µg/L in water) had hair arsenic concentrations averaging 0.46 ppm, and urine arsenic levels averaging 38 µg/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

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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Sharon Seidel

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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APPENDIX D

Hair Analysis Bibliography

Hair Analysis Bibliography

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APPENDIX E
Meeting Agenda



Hair Analysis: Exploring the State of the Science

Agenda

Day 1	Tuesday, June 12, 2001 Discussing the State of the Science in Hair Analysis
8:00 AM	Registration
8:30 AM	Introductory remarks <i>Robert Amler, MD</i> <i>ATSDR Chief Medical Officer</i>
8:45 AM	Purpose of meeting and review of the charge <i>Allan Susten, PhD, DABT</i> <i>Assistant Director for Science, DHAC</i>
9:00 AM	Impetus for panel discussions—a case example <i>Deanna K. Harkins, MD, MPH</i> <i>Medical Officer, DHEP</i>
9:10 AM	Introduction of panelists <i>LuAnn White, PhD, DABT</i> <i>Panel Chair</i>
9:20 AM	General physiology of hair—an overview <i>Robert Baratz, MD, PhD, DDS</i>
9:40 AM	<i>Topic #1: Analytical methods Panelists</i>
10:30 AM	Break
10:45 AM	<i>Topic #1: Analytical methods (continued) Panelists</i>
11:30 AM	Observer comments
12:00 PM	Lunch
1:00 PM	<i>Topic #2: Factors influencing the interpretation of analytical results Panelists</i>
3:15 PM	Break
3:30 PM	<i>Topic #3: Toxicologic considerations Panelists</i>
4:45 PM	Observer comments
5:15 PM	Adjourn

(over)

Day 2

**Wednesday, June 13, 2001
Developing Recommendations**

- 8:00 AM Review of day 1 issues *Panel chair*
- 8:15 AM *Topic #4: Data gaps and research needs* *Panelists*
- 9:00 AM *Topic #5: Identifying scenarios for which hair analysis may be appropriate* . *Panelists*
- 10:15 AM Break
- 10:30 AM Observer comments
- 11:00 AM Conclusions/recommendations *Panelists*
- 12:30 PM Adjourn

APPENDIX F
List of Observers



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Hair Analysis: Exploring the State of the Science

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APPENDIX G

Post-Meeting Observer Comments

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Michael Schaffer

Buck Grissom, Ph.D.
Health Science Administrator (Biomedical Sciences)
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Comments Concerning the Interpretation of Hair Analysis

I have used only data from hair analysis to help determine when an exposure occurred (see Item 1). There are too many variables to use hair data for any other purpose (see Item 2). I have commented on several instances in which hair data had been misused/misinterpreted by citizens and health care providers. Example: I received a call from a concerned parent. His son's symptoms were as follows: dizziness, poor skin color, poor mental acuity, and blackouts (petit mal seizure like symptoms). A physician had analyzed his son's hair for metals and recommended chelation therapy at a cost of \$6,000. His son's hair levels were within levels typically reported for control groups in hair analysis studies. Neither a source nor a pathway had been identified. No one else was in the area was having similar health problems. Their water was not contaminated. I told the parent to get a second opinion. Additional testing was needed to determine his son's health problems; hair analysis was inadequate. Moreover, chelation therapy is not risk free. I suggested the closest Association of Occupational Environmental Clinics clinic or a pediatrician trained to diagnose neurological symptoms before proceeding with chelation therapy.

General Questions (page 2)

1. When is it appropriate to consider hair analysis in assessing human exposures to environmental contaminants?

COMMENT: If a source and pathway have been identified, hair analysis may provide information concerning episodic exposures (i.e., frequency and duration of exposure).

2. When is it inappropriate to consider hair analysis in assessing human exposures to environmental contaminants?

COMMENT: Information concerning sources, pathways, etc. (i.e., is exposure plausible), of interest to ATSDR is required before attempting to interpret hair data. Interpretation of data from hair analysis in the absence of environmental data is conjectural.

3. What data gaps exist that limit the interpretation and use of hair analysis in the assessment of environmental exposures? What research is needed to fill these data gaps?

COMMENT: Internal sources of metals detected in hair need to be distinguished from external sources.

COMMENT: External sources of arsenic need to be distinguished (e.g., air, food, water, medicinals, and hair dyes). Is there a hazardous waste site involved?

COMMENT: Analytical methods that result in elimination of intra- and inter-laboratory data variability are needed.

Specific Questions

4. Topic #2 (page 3)

- Factors Influencing the Interpretation of Analytical Results.

COMMENT: This section lists many of the factors that confound interpretation of hair data. I agree with Dr. Baratz's comment: I do not want information that I cannot interpret. Even under the best circumstances, hair data are exceedingly difficult to interpret. In cases where hair levels exceed levels expected in a population, additional information is needed.

5. Topic #3, Toxicologic Considerations (page 4)

- Is information available defining "normal" ranges of chemical concentrations in hair that have physiological and health significance?

COMMENT: Terms such as standard or normal hair levels of metals or reference ranges for metal levels in hair need to be carefully defined—i.e., what constitutes normal? All reference values for hair need to be representative of the population being evaluated. For example, groundwater levels of arsenic (e.g., 100 ppb) have been reported to be elevated in some areas of Utah, Michigan, and Maine. Hair levels of arsenic in these areas are likely to be greater than hair levels in areas with low levels of arsenic in groundwater (e.g., 2 ppb).

COMMENT: What do hair levels above a reference value mean? How will hair data be interpreted? Reference values are frequently used for purposes for which they were not intended. A law firm sent a letter to the U.S. EPA citing the CDC lead guidance as a basis for not conducting an environmental investigation requested by EPA. The letter stated that the blood lead levels in the community were not above 20 micrograms per deciliter ($\mu\text{g}/\text{dL}$) and did not consistently exceed 15 $\mu\text{g}/\text{dL}$; therefore, an environmental investigation was not needed.

**Michael Schaffer
Psychomedics Corporation**

- Partial listing of cases demonstrating judicial acceptance of the Psychomedics hair analysis method.
- Information on hair testing and racial or color bias.
- Information on the effectiveness of Psychomedics washing procedures for ruling out external contamination

PARTIAL LISTING OF THOSE CASES DEMONSTRATING JUDICIAL ACCEPTANCE OF THE PSYCHEMEDICS HAIR ANALYSIS METHOD

A. Employment Cases

Scott v. The City of New York. et al., Civil Action No. 98-C V-1902 (ERK), (U.S.D.C., Eastern Dist. NY, March 21, 2001), a case involving a claim of constructive discharge based on race and gender, was dismissed via summary judgment. In making its decision, the court relied on the plaintiff's hair test result, which was positive for marijuana, as well as plaintiff's prior admission of use.

In Jones et al. v. City of Chicago, Civil Action No. 99 C 8201, (U.S.D.C., Northern Dist. IL, November 28, 2000), a case involving claims of race bias in hair testing, the United States District Court granted summary judgment in favor of the City of Chicago and dismissed the case. The Court found that not only was some of the evidence inadmissible, but also that "the remaining admissible evidence would be insufficient for a trier of fact to find that the [Psychomedics] hair test is more likely to result in false positive results for African-American applicants than for white applicants..."

In Cruse v. Whirlpool Corp., Civil Action No. 99-2129, (U.S.D.C., Dist. AR, June 23, 2000), the United States District Court found no merit to plaintiff's allegations that the Psychomedics hair test ("RIAH") was racially biased against African Americans and, as such, granted the defendant's motion for summary judgment. "Summary judgment is not appropriate unless all the evidence points toward one conclusion . . ." (citing Hardin v. Hussman Corp., 45 F. 3d 262 (8th Cir. 1995)). The defendant's expert offered through written testimony that "there is absolutely no scientific support for the notion that plaintiff's test result could be positive because of her race." The court considered the plaintiff's failure to offer any statistical evidence in support of her claim of racial bias in granting the defendant's motion.

In Gregory Hicks et al. v. City of New York et al., Index No. 119154 (1999), the Supreme Court of the State of New York upheld the termination of three officers through the use of Psychomedics' hair analysis drug testing.

In Brinson v. Howard Safir, et al, 680 N.Y.S. 2d 500, 255 A.D. 2d 247, (N.Y.A.D. 1 Dept. 1998), the New York Supreme Court Appellate Division upheld the lower court's determination of the accuracy of Psychomedics hair testing performed on an NYPD officer. Subsequent to this decision, the plaintiff filed suit in federal court, (E.D.N.Y. Civil Action No. 98-CV-2784 (ERK)(JMA)), claiming, in part, that he had not been afforded procedural due process before he was terminated from his position. The court, in granting the defendants' motion for summary judgment, found that the plaintiff was afforded, and took advantage of, every opportunity to appeal his dismissal. The court also referenced the Appellate Division's holding that "there was reasonable suspicion to order the testing...and there was no reason to doubt the accuracy of the test results."

In Matter of Brown v. City of New York, 250 AD2d 546, 673 NYS2d 643, (1998), the New York Supreme Court Appellate Division affirmed the New York Police Department's discharge of a New York City police officer for failure to pass a Psychomedics hair analysis drug test. Claims of contamination and inadequacies of testing were determined to be devoid of merit.

In Nevada Employment Security Department et al. v. Cynthia Holmes, 914 P.2d 611 (Nev.1996), the Nevada Supreme Court held the following with regard to a stand alone Psychomedics hair test utilized to deny unemployment benefits:

We acknowledge that there are, arguably, no certainties in science. See Daubert v. Merrell Dow Pharmaceuticals, Inc., U.S. ___, 113 S.Ct. 2786, 2795 (1993). Nonetheless, we conclude that RIA [hair] testing especially when coupled with a confirmatory GC/MS test, is now an accepted and reliable scientific methodology for detecting illicit drug use.

...we conclude that Holmes' ingestion of cocaine, subsequently proven by the RIA screening and confirmatory GC/MS test constitutes misconduct within the definition of NRS. 6 12.385.

In Bass v. Florida Department of Law Enforcement, Criminal Justice Standards and Training Commission, 627 So.2d 1321 (Fla. Dist. Ct. of Appeals, 1993), the plaintiff, a corrections officer, appealed from the decision of a hearing officer that her criminal justice certification should be revoked based on a positive urinalysis. The Court in Bass held that evidence of a negative Psychomedics hair analysis was erroneously excluded and that "the radioimmunoassay analysis of human hair to determine cocaine use is generally accepted in the scientific community." On remand, the hearing officer disregarded the hair analysis results as well as a subsequent negative urinalysis result and again recommended the revocation of the plaintiff's certification. The plaintiff appealed a second time in Bass v. Fla. Dept. of Law Enforcement, 712 So. 2d 1171 (Ct. App. Fla 1998), in which case the Court affirmed the ruling of the lower court holding that hair analysis should be admitted as it is "precisely the tool which is used when there is a claim of error in a urinalysis for cocaine."

B. Parole Revocation

In United States v. Medina, 749 F. Supp. 59 (E.D. N.Y. 1990), the court ordered a hair test to determine if a probationer, in a parole revocation hearing, had violated his parole by utilizing drugs in the preceding months. In revoking parole, after a positive Psychomedics hair test, the court found that:

Extensive scientific writings on RIAH hair analysis establishes both its reliability and its acceptance in the field of forensic toxicology when used to determine cocaine use.

In his decision, Judge Weinstein, the author of a treatise on evidence, analyzed the admissibility of hair analysis in the Medina case under the Federal Rules of Evidence as well as the older Frye evidence standard and concluded hair analysis was admissible under both. In addition, Judge Weinstein took judicial notice of extensive writings which support the acceptance of the reliability of RIAH.

C. Unemployment Insurance Appeal Board/Administrative Law Judge Decisions

The decisions of the Department of Labor to deny benefits to claimants who are terminated after receiving positive hair test results for drugs of abuse are routinely upheld by Administrative Law Judges and the State's respective appeal or review boards. The decisions are upheld based on the established reliability of Psychomedics' hair analysis, which is demonstrated in numerous peer reviewed scientific publications.

In In the Matter of Patrick Forte, New York Appeal Board No. 477610 (4/7/00), the Unemployment Insurance Appeal Board upheld the determination of the Administrative Law Judge, (A.L.J. Case No. 097-0852 1), in affirming the decision of the Department of Labor to disqualify a probationary police officer, ("claimant"), from receiving benefits. The claimant was disqualified after his termination due to willful misconduct. The claimant submitted to a hair test, which results were positive for cocaine use. The claimant argued that either the hair sample was contaminated due to his exposure to crack cocaine vapors, or that he "passively ingested" small amounts of cocaine. The Appeal Board found that due to the fact that the claimant's results showed a cocaine level 4-8 times the cutoff level and that benzoylecgonine, a cocaine metabolite, was also detected, it was unlikely that the claimant "passively ingested" cocaine. The Appeals Board recognized that it had previously been demonstrated to the Board successfully that Psychomedics' laboratory's washing techniques eliminated the issue of external contamination.

In In re Claim of Delbert Otto, B 95-02542-000 (1996), the State of Ohio Unemployment Compensation Board of Review, ("Board of Review"), overturned the Hearing Officer's ruling that the claimant was discharged without just cause and was entitled to benefits. The Board of Review found that expert testimony demonstrated the reliability of the Psychomedics hair test which detected quantities of marijuana in the claimant's hair.

See also In the Matter of Otis K. McBride, State of New York, A.L.J. Case No. 099-17766 (1999); In the Matter of James Rawls, State of New York, S.S.A. No.120-42-0562 (1998); In the Matter of Claimant, State of Indiana, Case No. 93-1BA-1 IOB (1994); and In the Matter of Brian J. Berrigan, State of New York, Index No. 121899 (1998).

D. Arbitrations

Hair analysis has been upheld in arbitrations between Anheuser-Busch, Inc. and its unions:

- In an October 1999 decision, the collection of body hair for analysis was upheld.
- In a July 1999 decision, union claims of improper specimen collection, and age, race and gender bias related to slow hair growth were found to have no merit and the issues were resolved in favor of the Company.
- In an August 2000 decision, it was determined that random hair testing of employees in safety sensitive positions did not violate their state constitutional rights to privacy. The Psychomedics hair

test was deemed “a reliable method for detecting employee drug use, [which] therefore served to further the Employer’s legitimate safety interest.”

In *United States Steel, A Division of USX Corp. and United Steelworkers of America, Local 1557*, Case No. USS-38, 287 (1999), the Arbitrator ruled:

We find that hair testing for drugs is legitimate under the LCA and scientifically valid. Psychomedics’ wash procedures are effective in removing environmental contamination. The 5.0ng/10mg cutoff level for cocaine is appropriate in light of field studies. There was no bias here on the basis of race or hair color. The chain of custody was unbroken. The Company has satisfied us that Grievant ingested cocaine during the period covered by the Last Chance Agreement. That material violation of the LCA was proper cause for discharge.

Hair analysis was also upheld in *US Steelworkers Local 4134 & Lone Star Steel Co.*, Case No. D22-96 (1997); *Battle Mountain Gold Co. & Operating Engineers Local 3* (1998); *Cooper Tools and United Automobile, Aerospace, Agricultural Implement Workers of America, AFL-CIO, Local 1774*, Grievance No. 005 (2000); and *United States Steel, A Division of USX Corp. and United States Steelworkers of America, Local 1014*, Case No. USS-41, 820 (2001).

Hair Testing and Racial or Color Bias

Every large scale population study dealing with race and or color bias has concluded that hair color or race as factors do not lead to any statistically significant variations that would create a “bias.” Several studies utilizing Psychomedics’ methodology, (extensive washing of the sample, complete digestion and removal of melanin, the color component of hair), have established that there is no systematic bias occurring with this specific technology.

A large study on the issue of possible racial bias and drug testing was originally reported in *Forensic Science International* in 1993. The study involving 1200 real world cases showed that with all three methods of reporting utilized, (self-reports, urine testing and hair analysis) the same positive percentage ratio between Caucasians and African-Americans was achieved.

An even larger study, published in the July 1999 *Journal of Occupational and Environmental Medicine* by Dr. Benjamin Hoffman, compared the 1997 results of hair and urine tests on over 1800 black and white candidates for a large municipal police force. Again, no racial bias was found comparing hair testing to urine testing.

In a 1999 study, published in Drug Testing Technology – An Assessment of Field Applications, “An Analysis of the Racial Bias Controversy in the Use of Hair Assays” concluded from the analysis of numerous data sets that any effect of hair color or race would be negligible as a factor in the outcome of a hair test. The authors of the study reported that in side-by-side comparison with hair, urine and self-reports, the racial differential in positive rates compared to self-reports was actually greater in urine than in hair analysis.

In January 2000, Dr. Mieczkowski’s meta-analysis of all available published studies that included data on drug test results matched to race or hair color was published in *Forensic Science International*. These studies included European research where participants were dosed with known quantities of drugs. In no instance, in any study, was a statistical bias shown to exist.

Most recently, in the *Bulletin of the International Association of Forensic Toxicologists*, an analysis of over 56,000 cases showed no significant relationship between hair color and a likelihood to test positive for cocaine.

The “potential” to create bias issues exists with any specimen, including urine– as any element that affects the matrix could arguably lead to a “biased” result.

- A) Diet has a significant impact on urine excretion. Some ethnic diets may greatly influence a urine result.
- B) Patterns of water retention/urine excretion in women are influenced by menstrual cycles that may create longer detection times in women.

- C) Body weight and size influence the amount of drugs that would be found in urine.
- D) Certain medications influence urine output and drug excretion rates.
- E) The ability of the body to effectively process drugs is influenced by age which increases retention times.
- F) Water intake and activity dramatically influence drug excretion rates in urine. A sedentary person in a wheelchair could retain drugs in urine significantly longer than an athletically active person who hydrates his or her system.

None of these “potential bias” issues have presented much of a problem in workplace testing. This is largely due to the fact that normal biovariability between individuals overwhelms any single element and, of course, a person claiming any sort of bias would first have to admit drug use.

[Note: Dr. Schaffer provided copies of the following supporting journal articles]

Hoffman B. Analysis of race effects on drug-test results. *Journal of Occupational and Environmental Medicine*. 41(1999) 612-614.

Mieczkowski T and Newel R. An evaluation of patterns of racial bias in hair assays for cocaine: black and white arrestees compared. *Forensic Science International*. 63 (1993) 85-98.

Mieczkowski T and Newel R. An analysis of the racial bias controversy in the use of hair assays. In: *Drug Testing Technology: Assessment of Field Applications* (ed: Mieczkowski, T.), CRC Press (1999), Boca Raton, pp. 313-348.

Mieczkowski, T and Kruger, Michael. Assessing the effect of hair color on cocaine positive outcomes in a large sample: a logistic regression on 56,445 cases using hair analysis. *Bulletin of the International Association of Forensic Toxicologists*. (2001), 9-11.

Environmental Contamination

Psychemedics employs several independent approaches which in combination, rule out the possibility of a positive result from external sources.

- a) The rigorous chemical washing of hair for extended periods of time.
- b) The analysis of the contents of these washes followed by a comparison of the drugs remaining in the hair.
- c) Measurement of metabolites, the unique compounds created by the body's processing of the drugs. These metabolites are normally not present in the environment or in smoke. For example, marijuana smoke does not contain carboxy THC - the metabolite that Psychemedics identifies in marijuana positives.
- d) Use of cut-off levels with hair, as with urine, to prevent any passive internal exposure from producing a positive result. Because of the constancy of drug concentrations in hair, these cut-off levels more accurately reflect use, and are therefore safer than those used by urinalysis.

Several studies by Dr. Thomas Mieczkowski of the University of South Florida¹ dealt with the real world issue of external contamination and its removal by appropriate wash procedures. The studies concerned the passive contamination of undercover narcotic officers who, in the course of their duties, had continuing and extensive contact with cocaine, operated in cocaine rich environments and interacted frequently with cocaine users and cocaine dealers. The officers handled cocaine in the process of buying and selling and when they made arrests or seized contraband.

These undercover officers effectively mimicked drug users in all respects, except usage. In his studies, Dr. Mieczkowski found that the officers had some amount of detectable cocaine on the outside of their hair as a contaminant. However, even in this extreme contamination scenario the hair was easily cleansed. Dr. Mieczkowski concluded that the commercial wash procedures utilized (Psychemedics) were effective methods for removing external contamination from hair and that external contamination did not present a difficult problem with properly performed hair analysis.

In a contamination study utilizing an early Psychemedics wash procedure researchers exposed volunteers to crack smoke in a small, unventilated room (2.5 x 3 x 2.5 m) and exposed cut hair to the equivalent of smoke vapors from 5000 lines of cocaine in closed beakers. In all cases, after washing, the exposed

¹Passive Contamination of Undercover Narcotics Officers by Cocaine: An Assessment of Their Exposure Using Hair Analysis. Microgram, 1995.

Distinguishing Passive Contamination from Active Cocaine Consumption: Assessing the Occupational Exposure of Narcotics Officers to Cocaine. Forensic Science International (84) 1997.

contaminated hair tested negative. The authors concluded that deposition of cocaine from even these extreme contamination scenarios was washable.² Also in the study, hair from admitted cocaine users tested positive, hair from non-users tested negative and hair from non-users who admitted being present in crack environments also tested negative. It is not likely that any employee would claim an exposure scenario greater than being in an enclosed room while 5000 lines of cocaine were vaporized or handling cocaine more frequently than an undercover narcotics officer or evidence technician.

Most recently, in a contamination study presented at the Society for Forensic Toxicologists this past October, Psychomedics' extensive wash procedures were compared to the short wash results obtained in an earlier cocaine contamination study and were shown to be effective at distinguishing contaminated hair from user hair.

Due to the hyper-sensitivity of urine tests, it is well recognized by the scientific community that false positives due to passive internal exposure to drugs are far more likely for urinalysis than for hair analysis (e.g., the opiate false positive problem of urinalysis due to poppy seed ingestion). The Department of Health & Human Services found that over 87% of urine opiate confirmed positives were overturned by medical review officers because ingestion of poppy seeds as well as some medications could cause urine opiate cutoff levels to be exceeded. The studies of Dr. Hans Sachs and those of others have shown that even the massive ingestion of poppy seeds is incapable of producing interpretive false positive hair analysis results. Additionally, the hair of heroin users contains stable amounts of the heroin metabolite, 6 MAM (an absolute marker of heroin). Testing for the 6 MAM metabolite in urine is required under the amended NIDA urine guidelines, (the amended guidelines also increase the cut off levels from 300 ng/mL to 2000 ng/mL). Unfortunately, while 6 MAM is identifiable in hair for months, it has an extremely short half-life in urine and for all practical purposes will be detectable at best only in persons who use heroin on the day of their urine test. This makes the confirmation of heroin use extremely problematic for urine testing creating false negatives.

NIDA scientist, Dr. Cone, experimentally demonstrated that as little as one-hundredth of a line of cocaine (i.e., 1 or 2 mg) can produce interpretive false positive urinalysis results.³ These small quantities can be inadvertently ingested by a non-drug user (e.g., a spouse) who may be in the constant presence of a drug abuser. In contrast to the resistance of hair to drug penetration, the lungs and gastrointestinal tract have absolutely zero resistance. In actual fact, drugs are transported by active transport mechanisms into the interior milieu, i.e., by breathing or by active membrane processes. Such active internalization can cause interpretive false positive urine results by minute amounts of cocaine if the timing of the test is in close proximity to the passive ingestion.

Unlike hair, there is no method to remove this contamination from urine or to differentiate between active drug use and unknowing exposure to a drug that may rise above cut off levels, e.g., spiked food or drink. Unlike urine, hair can be segmented to substantiate or refute these claims. Additionally, a completely new hair sample can be obtained that will replicate the same time frame of the original sample eliminating concerns or claims of sample mix-up. New samples replicating the same time frame cannot be obtained with urine as most drugs are completely flushed from the system in a couple of days.

²Hair Analysis of Cocaine: Differentiation Between Systemic Exposure and External Contamination. *Journal of Clinical Pharmacology*, 1992.