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
Atlanta, Georgia

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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
and
Director, Division of Health Assessment and Consultation (DHAC)

Agency for Toxic Substances and Disease Registry

TABLE OF CONTENTS

Appendices

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
? g/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 competed 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

[1]The 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.

comments.

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:

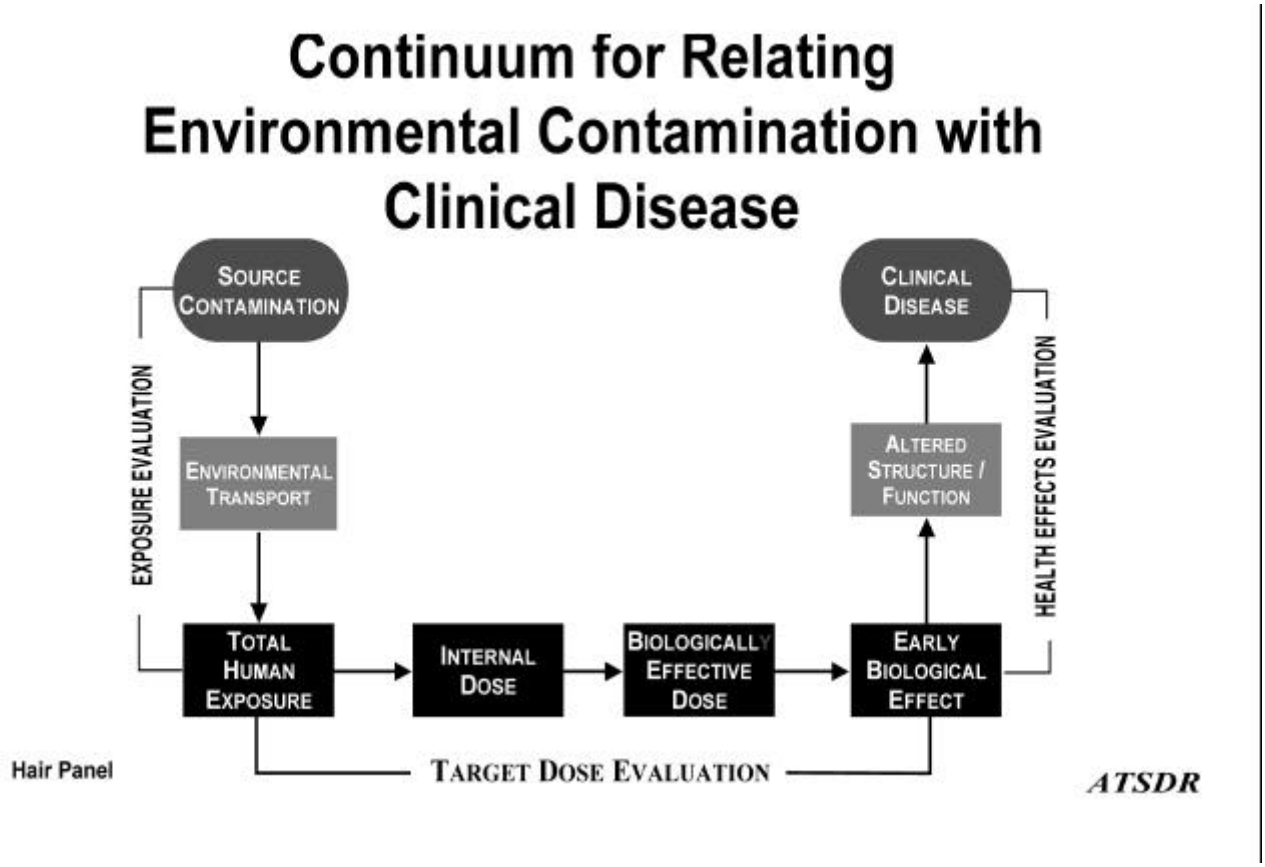

Figure 2-1. Continuum of events considered in the public health assessment process.

- Can an exposed population be identified?
- Does a data gap exists 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 (µg/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 µg/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" keratization, 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 rodents have regional variation in hair growth; the hair cycles, but in

waves across the body.

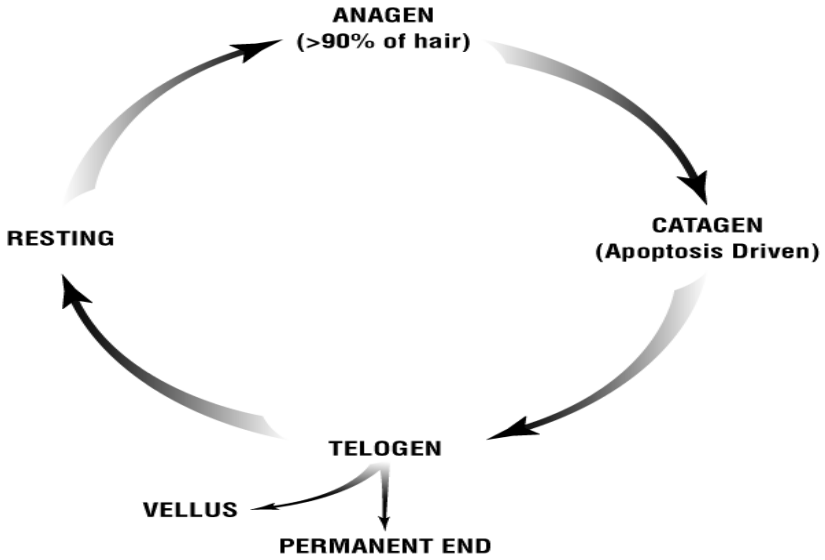

Figure 2-2. The hair growth cycle.

- *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, ?-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 sulphydral 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 (µg/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 µg/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 value 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 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 EPA[2] (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

[2]EPA 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 carcinogenic potential of environmental exposures (RB). Judging from 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 competed 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 for

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

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.

[4]One 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 Practice[1]

Model: Cause-Effect	?	Source	?	Exposure					?	Effect		
Indicators of exposure and effects		Substances known to be present at site		Potential dose	?	External dose	?	Internal dose		"Abnormality"	?	Disease or other health effect
Possible detectable or measurable parameters (examples)		Inventories of raw materials, byproducts, and final products		Concentra-tions in air, soil, water, and surface wipe samples		Personal air samples, wipe samples of skin, hair analysis; dosimetry		Concen-trations in blood, urine, and other body tissues and fluids; dosimetry		Bio-markers or other indica-tors		Bio-markers or other indicators
Type of assessment		Document review		Environmental				Biological		Biological and other		

ISSUES	Model	• 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
		  • 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	• 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	• Contamination • Stability during transport or storage • Preparation methods (e.g., cleaning, digestion)
	Laboratory methods	• 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
Doctor's Data

Day #1

Dr. Quig, from Doctor's Data (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 Cr^{6+} 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, Doctor's Data 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.

- Doctor's Data 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
Pyschemedics Corporation

Day #1

Dr. Schaffer, a trained industrial toxicologist with an interest in criminal justice and forensics, explained that Pyschemedics 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 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

[5]Dr. 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 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 Psychemedics hair analysis method, (2) information on hair testing and racial or color bias, and (3) information on the effectiveness of Psychemedics' 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

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