7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring copper, its metabolites, and other biomarkers of exposure to and affects of copper. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

Analytical methods and detection limits for copper in biological materials are given in Table 7-l. Copper in other biological materials such as hair and nails can be determined by using suitable procedures for dissolving the sample matrix and employing the same analytical techniques as with blood and tissue. These methods determine the total amount of copper in the sample. The methodology for analyzing biological material is similar to that used for environmental samples. The most commonly employed methods use atomic adsorption spectroscopy (AAS) or inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Araki et al. 1990; Lo and Araki 1989; Lopez-Artiguez et al. 1993). Differential-pulse anodic stripping voltammetry techniques have also been used to quantify copper in urine, yielding detection limits of 0.041 µg/L and an accuracy of 97% (Horng 1996).

7.2 ENVIRONMENTAL SAMPLES

Analytical methods and detection limits for copper in environmental media are given in Table 7-2. Analytical methods determine the total copper content of the samples. Determining specific copper compounds and complexes in samples is difficult. The most common methods used for environmental samples are AAS, either flame or graphite furnace, ICP-AES, and inductively coupled plasma-mass spectrometry (ICP-MS). Water and waste water samples can be analyzed for copper by EPA Test Method 200.1 (flame atomic absorption), 200.7 ICP-AES, or EPA Test Method 200.9 (temperature

Table 7-1. Analytical Methods for Determining Copper in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood or tissue	Acid digestion	Method 8005[a]; ICP-AES	1 µg/100 mL blood; 0.2 µg/g tissue	Not available	NIOSH 1987
Urine	Filter and polydithio-carbamate resin collection followed by low temperature plasma ashing or acid digestion	Method 8310[a]; ICP-AES	0.1 µg	Not available	NIOSH 1987
Tissue	HNO_3 digestion	AAS/graphite furnace	0.25 µg/g wet weight	103.1±7.7% mean recovery; 8.2±6.9% mean difference in duplicates[b]; 0.01% accuracy	Lowe et al. 1985
Toenails	HNO_3 digestion	AAS/graphite furnace	0.6 µg/g	<5% within run precision; 3.5% day-to-day precision	Wilhelm et al. 1991

[a]Simultaneous, multielemental analysis, not compound specific.
[b]Mean±1 standard deviation

AAS = atomic absorption spectrometry; ICP-AES = inductively coupled plasma-atomic emission spectroscopy

Table 7-2. Analytical Methods for Determining Copper in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Filter collection on 0.8 mμ membrane filter and acid digestion	Method 730, ICP-AES	1 μg	No bias identified	NIOSH 1987
Air	Filter collection on 0.8 mμ membrane filter and acid digestion	Method 7029, AAS	0.05 μg	No significant bias	NIOSH 1987
Water, waste water	Acidify with 1:1 HNO_3 to a pH<2	Method 220.1, AAS/direct aspiration	20 μg/L	0.9–29.7% bias between 7.5 and 332 μg/L	EPA 1983
Water, waste water	Sample solutions should contain 0.5% HNO_3	Method 220.2, AAS/furnace technique	1 μg/L	Not available	EPA 1983
Water, waste water	Filter and acidify sample	Method 200.7 CLP-M ICP-AES	6 μg/L	Not available	EMMI 1997
Water, waste water	Digestion with H_2SO_4 and HNO_3	Neocuproine, spectrometric	120 μg/L in 1 cm cell	Not available	Greenberg et al. 1985
Waste water	Adjust pH to 1.65–1.85, mix, filter	Method 200.1, flame atomic absorption	4 mg/L	Not available	EMMI 1997
Water, waste water	Filter and acidify	Method 200.7_M, ICP-AES	25 μg/L	Not available	EMMI 1997
Groundwater, surface water, and drinking water	Filter and acidify	Method 200.8, ICP-MS	20 μg/L	Not available	EMMI 1997
Marine waters	Digest in HNO_3, concentrate on iminodi-acetate chelating resin, elute with 1.25 M HNO_3	Method 200.10, ICP-MS	7 μg/L	Not available	EMMI 1997
Marine waters, estuarine waters, seawaters, and brines	Digest in HNO_3, concentrate on iminodi-acetate chelating resin, elute with 1.25 M HNO_3	Method 200.13, GFAA	5 μg/L	Not available	EMMI 1997
Soil, sediment, sludge, and solid waste	Digestion with HNO_3 and H_2O_2, reflux with dilute HCl	Method 7210, AAS	20 μg/L	As in Method 220.1	EPA 1986

Table 7-2. Analytical Methods for Determining Copper in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food	Closed-system digestion	AAS or ASV	0.32 µg/g (ASV), not reported (AAS)	94–100	Holak 1983
Biological tissues	HNO_3 digestion, reaction with H_2O_2	Method 200.3, ICP-MS	18 µg/L	Not available	EMMI 1997
Fish tissue (fresh edible tissue)	Dissociate tissue in tetraammonium hydroxide, acidify with HNO_3	Method 200.11, ICP-AES	18 µg/L	Not available	EMMI 1997

AAS = atomic absorption spectrometry; ASV = anodic stripping voltammetry; GFAA = graphite furnace atomic absorption; ICP-AES = inductively coupled plasma-atomic emission spectroscopy; ICP-MS = inductively coupled plasma-mass spectrometry

stabilized graphite furnace atomic absorption spectroscopy) (EMMI 1997). These methods are suitable for groundwater and surface water as well as domestic and industrial effluents. EPA Test Method 200.8 ICP-MS or EPA Test Method 200.15 ICP-AES are suitable for analysis of groundwater, surface water, and drinking water. EPA Test Method 200.8, EPA Test Method 200.10 (on-line chelation and ICP-MS), or EPA Test Method 200.13 (chelation and graphite furnace atomic absorption spectroscopy) are suitable for marine, estuary, and brine waters. If determination of dissolved and suspended copper is required, samples should be filtered using a 0.45 µm membrane filter. Suspended solids, as well as sludge and sediment, may be analyzed by EPA Methods 200.1 and 200.13 after an initial acid digestion with HNO_3. Interference by other elements is not a problem in the analysis. However, background correction may be required when using atomic absorption spectroscopy to correct for nonspecific absorption and scattering, which may be significant at the analytical wavelength, 324.7 nm (EPA 1986). In the determination of trace metals, major concerns are contamination and loss. Contamination can be introduced from impurities in reagents and containers as well as from laboratory dust. Losses may also occur due to adsorption onto containers.

Other analytical methods used for copper analysis include x-ray fluorescence, anodic stripping voltammetry, neutron activation analysis, photon-induced x-ray emission, as well as chemical derivatization, followed by gas chromatographic or liquid chromatographic analysis. Discussion of these methods is beyond the scope of this profile. However, methodology for the determination of copper has been reviewed by Gross et al. (1987) for food, by Fox (1987) for air, by MacCarthy and Klusman (1987) for water, and Lichte et al. (1987) for geological materials.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of copper is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of copper.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce certain uncertainties of human health assessment. This definition should not be interpreted to

mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Methods for determining background and elevated levels of copper in biological materials are well developed, sensitive, specific, and reliable. Standardized methods are available from NIOSH and other sources. The use of copper concentrations in toenails and hair has been investigated as surrogate markers of copper exposure, with validation studies currently underway.

Effect. No specific biomarkers of copper toxicity have been determined. Until such biomarkers are determined, the methodology needed to identify them cannot be established.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining background and elevated levels of copper in environmental media are well-developed, sensitive, and selective. Water is the medium of most concern, since the form of copper generally associated with health effects is soluble copper(II). Standardized methods of analysis for copper in air, water, soil, and food are available from EPA, NIOSH, and other sources. Analytical methods measure total copper. Therefore, the methods can not specifically analyze for a parent compound and a degradation product.

7.3.2 Ongoing Studies

Ongoing studies regarding new analytical methods for measuring copper in biological materials or environmental media were located in the literature. Dr. M. Longnecker at the National Institute of Environmental Health Sciences is working to validate toenail copper concentrations as a surrogate measure of exposure to copper. Development of high-performance liquid chromatography (HPLC) and derivatization techniques for identifying natural copper chelators in marine water is being conducted at Cornell University under the guidance of Drs. B.A. Ahner and J.W. Moffett. Dr. D.L. Sparks, at the University of Delaware, is developing x-ray absorption fine structure (XAFS) and atomic force microscopy (AFS) techniques for the study of metal/metalloid reactions in soil. Dr. J.F. Tyson and

colleagues at the University of Massachusetts at Amherst are developing liquid-liquid extraction pretreatment techniques that can be interfaced with HPLC-ICP-MS instrumentation.