CHAPTER 6

Hair and Metal Toxicity

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6.1 Overview

Hair is most relevant to metals toxicology as a biological medium or biopsy material for analysis. Hair can be non-invasively obtained, easily stored and transported, and later analysed for the presence of certain metals.[1–3] Hair analysis is most informative when the metal of interest is a xenobiotic and the exposure route is ingestion. In these cases, it is most likely that hair analysis reflects an internal dose of the metal and not external contamination of the hair. Determination of hair mercury to estimate dietary methylmercury exposure is the best example.[4] A large body of epidemiological evidence correlates hair mercury concentrations to blood mercury levels and both of these to fish consumption in a dose-response fashion. In addition, hair is one of several biomarkers used in epidemiological studies of arsenicosis and arsenic-contaminated drinking water. Further, hair analysis can be used clinically and forensically to document thallium poisoning, an intoxication that also results in pathological changes in the hair.

Hair's utility as a biomarker is considered to be limited in most occupational environments where exposures are airborne. In these situations, hair is subject to exogenous contamination by the toxicant of interest. Therefore, distinguishing metal content internally distributed and excreted into the hair after absorption from metals that externally contaminate the hair surface is difficult, if not impossible.[2,4] Likewise, hair has limited usefulness when the metal of interest is both a potential occupational exposure and an essential, dietary trace element. In these cases, the metal may naturally be present in hair in varying amounts.

Timing is also important. Due to the hair shaft being non-vascular and to its growth rate, hair metal content is unlikely to accurately reflect very recent exposures (hours to several days). In addition, hair is also unlikely to document exposures that have occurred more than one year before the time of analysis.[4] In individuals with hair of sufficient length, however, segmental hair analysis may provide information regarding exposure over time.[4–6]

For each major metal of occupational and environmental interest, this chapter will:

a. briefly summarise the toxicology and kinetics in relation to hair
b. present the relative advantages and disadvantages of hair compared to other biomarkers of exposure
c. present the situations where hair analysis may be indicated to monitor and/or document human exposure.

Further, we will discuss the misuse of 'commercial' hair tests for panels of metals and minerals whose results are promoted as indicators of health, nutritional status and metal toxicity.

Finally, we will present an overview of methodological issues when using hair as a biomarker for metals.

6.2 Mercury

6.2.1 Toxicology

Mercury is a xenobiotic used in chloralkali production, some switches, fluorescent light bulbs and certain batteries. Elemental mercury (dental amalgams, thermometers and gas meters), methylmercury (fish) and ethylmercury (thiomerosal in vaccines) are the forms and potential exposures most relevant to the general population[7,8] (See Table 6.1). For most people, background mercury exposure and variability in blood mercury result primarily from the consumption of fish containing methylmercury, which bioaccumulates in seafood worldwide.[7–10]

Acute exposures to elemental mercury can produce acute lung injury, while chronic exposures may produce renal dysfunction and neuropathy. Frank poisoning results in additional neurological disturbances including tremor, behavioural changes ('erethism'), gingivitis[11,12] and progression to delirium/hallucinations.[13]

Clinical methylmercury intoxication is characterised by ataxia, tremor, constriction of visual fields, and cortical and cerebral atrophy[12,13] and is generally associated with hair levels greater than 50 parts per million (ppm) (>200 μg Hg L^{-1} in blood).[14] There is considerable debate regarding the 'safe' dose of

Table 6.1 *Major chemical forms of mercury associated with the general population exposure*

Form	*Absorption/ source of exposure*	*Major target organs*	*Major excretory pathway*	*Approximate whole body half-life*	*Usual biomarkers*
Hg^0	Inhalation of vapour	Kidney, CNS, PNS	Urine and faeces	60 days	Urine mercury
Methyl Hg	Ingestion of seafood	CNS	faeces	40–70 days	Blood and hair mercury
Ethyl Hg	Parenteral from vaccines	CNS, Kidney	faeces	7– 20 days	Blood mercury

Adapted from Clarkson *et al*. NEJM 2003[8]

methylmercury from fish in the diet, particularly for children and women of childbearing age.[10,15–17]

Thimerosal or thiomersal contains ethylmercury and has been used safely as a vaccine preservative since the 1930s. Ethylmercury has neurological effects similar to methylmercury, but is considerably less toxic, with higher blood concentrations required to cause poisoning.[18] Although adverse effects have not been documented, recently, thimerosal has been almost completely removed from US licensed vaccines to decrease childhood exposure as a precaution, but continues to be used in other countries.[8]

6.2.2 Kinetics and Relation to Hair

The different chemical forms of mercury influence the absorption, distribution, toxicological manifestations, excretion and useful methods of biological monitoring (Table 6.1). Metallic (elemental) mercury, methylmercury and ethylmercury all cross the blood-brain barrier. Elemental mercury also accumulates in the kidneys and renal excretion is important in its elimination from the body.[8,19]

Methylmercury penetrates into the central nervous system to a greater extent than ethylmercury due to the latter's larger size and faster decomposition, which may explain, in part, why ethylmercury is less neurotoxic than methylmercury.[18] In addition, ethylmercury is converted more rapidly to inorganic mercury and has a considerably shorter half-life than methylmercury.[8] A recent toxicokinetic study demonstrated that after immunisation with thiomerosal-containing vaccines, infants rapidly eliminated ethylmercury from the blood through the faeces (estimated half-life of seven days) with urine mercury undetectable in most samples.[20]

Methylmercury is distributed widely and concentrated in the blood. While methylmercury excretion is primarily faecal, it is also excreted into the hair where it accumulates and reaches concentrations ranging from 140 to 370 times that of blood.[21] The concentration in new hair is directly proportional to blood methylmercury concentration.[22]

6.2.3 Hair vs. Other Biomarkers

For elemental and inorganic mercury, toxicity thresholds and laboratory evaluation are based primarily on urinary excretion, its main route of elimination. A 24-hour urine collection is the best indicator of recent or chronic exposure to elemental or inorganic mercury.[19,23,24] Blood mercury can also detect intense, acute exposures to inorganic and elemental mercury.[23] Hair is not well-suited for monitoring exposure to elemental mercury vapour. First, it is limited by external contamination of the hair.[25] Second, most mercury in hair is in the form of methylmercury and reflects dietary exposure from seafood. Although one study found that average levels of mercury in scalp and pubic hair were significantly higher in dentists occupationally exposed to elemental mercury than controls,[26] the observed hair concentrations may still have reflected fish consumption.[27]

Blood mercury is the best test for current methylmercury exposure.[23] Because urinary excretion of methylmercury is low, an elevated blood mercury concentration

(with undetectable urine mercury) or a high ratio of blood to urine mercury supports exposure to an organic form of mercury. Investigators have used hair mercury measurements as a biomarker in populations with traditionally high fish or marine mammal consumption in attempts to elucidate benchmark doses for various effects.[10,28–30] In methylmercury exposure, the blood mercury concentration is directly proportional to the mercury concentration in new hair, and because methylmercury reaches much higher concentrations in hair, this tissue is more easily analysed. Methylmercury accounts for about 80% of total mercury in hair among those who eat fish,[10] but may account for less when methylmercury exposures are quite low.[31] The average ratio of hair mercury to blood mercury is about 250:1,[21] which means that a hair methylmercury value in ppm can be multiplied by four to find the corresponding estimated blood concentration in μg L^{-1}. Due to hair's growth rate, when measured from the scalp, each cm of hair reflects the average blood concentration for the last month.[22] Therefore, hair measurements provide the advantage of an integrated measure of exposure.[10] Blood concentrations, on the other hand, may fluctuate to a greater extent when fish consumption is intermittent and blood levels are not steady state.

Of major importance to studies of methylmercury's potential effects on the developing central nervous system, investigators have demonstrated that maternal hair and blood levels of mercury correlate well with the concentrations in umbilical cord blood, infant blood and infant brain tissue.[32–34] Grandjean *et al.*[33] found that the hair mercury in Faroese mothers who consumed a diet high in pilot whale was significantly correlated with umbilical cord blood taken immediately after delivery. Cernichiari *et al.*[34] examined the brains of 27 deceased Seychellois infants with matching infant blood samples, and matching maternal hair and blood. Maternal hair mercury was highly correlated (correlation coefficients 0.6–0.8) to concentrations in six infant brain regions and to maternal blood (correlation coefficient 0.82).

Table 6.2 summarises hair mercury values found in selected studies and advisory guidelines and relates them to blood mercury concentrations and exposure settings. Both background blood and hair mercury concentrations are determined by the frequency and type of fish consumption. The highest levels are seen in those consuming fish frequently, especially when predator fish high in mercury, such as swordfish and shark, are consumed regularly. In the US recent data demonstrate that average blood mercury concentrations are quite low,[35,36] which correspond to hair levels of 0.23 to 0.5 ppm.[31,35] Among frequent fish consumers in the US, however, it is not uncommon to observe higher blood mercury concentrations (10 to >50 μg L^{-1}),[7,49–51] corresponding to the hair levels of approximately 2–12 ppm, as observed by Airey[37] (See Table 6.2).

Neurotoxicity thresholds vary depending on the outcome in question. In addition, there are likely qualitative differences in exposure to methylmercury from fish, as opposed to methylmercury fungicides. Studies among populations that engage in high fish-consumption have found exposures that overlap with the lowest observed level for pre-natal effects of 10 ppm in hair determined during the Iraqi epidemic of poisoning due to fungicide-contaminated bread. Nonetheless, they have not observed cases of clinical congenital poisoning.[29,48]

Table 6.2 *Hair Mercury values in selected major studies and advisory guidelines; relation to blood mercury and exposure settings*

Exposure setting/location	*Hair mercury concentration in ppm*	*Blood mercury concentration in μg/L*	*Mercury concentration in fish in ppm*	*Comments*	*Reference*
Background					
Illinois, US	1.0+/−2.4 men 0.9+/−0.6 women	N/A	N/A	987 hospital employees and family members	Sky-Peck, 1990[3]
New Jersey, US	0.5 (87% < 1.0)	1.0(95% < 5)	N/A	189 pregnant women who averaged 1.6 fish meals a week.	Stern *et al.* 2001[35]
US	N/A	1.0(95% < 8)	N/A	1079 women from an NHANES sample, 73% reported < 3 fish meals in the 30 days prior to sampling	Schober *et al.* 2003[36]
US	0.23(0.05 to 2.3)	N/A	N/A	1276 samples from 356 women reporting no seafood consumption. Hair concentrations reflect methylmercury, not total mercury	Smith *et al.* 1997[31]
International: from 32 locations in 13 countries	2.3+/−2.8 (total)	N/A	N/A	559 samples	Airey, 1983[37]
	1.9+/−1.7			– Total mean excluding daily fish consumers	
	1.4+/−1.3			– Ate fish once a month or less	

Table 6.2 *(Continued)*

Exposure setting/location	*Hair mercury concentration in ppm*	*Blood mercury concentration in μg/L*	*Mercury concentration in fish in ppm*	*Comments*	*Reference*
	1.9+/−1.5			– Ate fish twice a month	
	2.5+/−2.2			– Ate fish every week	
	11.6+/−6.6			– Ate fish every day	
Health Advisory Guidelines					
US FDA	<50	<200	<1.0 (action limit)	Endpoint is development of overt neurological disease in adults based on review of 'Swedish expert group'.[38]	US FDA[14]
Canada	<10		<0.5 (guideline)	Endpoint is developmental toxicity. Safety factor of five was applied to adult threshold. Fish mercury guideline excludes large predator species, which should be limited to less than once per month	Health Canada[39]
WHO	<5	<20		Safety factor of ten applied to threshold for overt neurological disease in adults to derive limit	WHO, 1990[21]

US EPA	<1.2	<5.8		Endpoint is developmental neurotoxicity. Safety factor of ten applied to Faroe Islands benchmark dose level (58 ppb in blood/12 ppm in hair) for Boston naming test to derive limit	US EPA[10]
Studies of populations with high rates of fish-consumption					
Seychelles			0 to 1.6 (11 species)	36 mother-baby pairs and 40 fishermen. Estimated that Seychellois consume yearly 80–100 kg of fish per person	Matthews 1983[40]
			0.4 to 4.4 (dogtooth tuna)		
	12.0+/−6.6 (4.1 to 32)			-Mothers	
	15.2+/−11.5 (2.1 to 48)			-Babies	
	26.3+/−14.5 (5.5 to 68)	As high as 240		-Fishermen	
Seychelles		16	0.3(98% <0.7) (>20 species)	779 mother-infant pairs. Stated that the average Seychellois consumes 12 fish meals per week. Mean blood concentration based on correlation study of maternal hair and blood and infant brain tissue n = 27 (ref)	Cerniciari *et al.*, 1995[22]
					Cerniciari *et al.*, 1995[34]

Table 6.2 *(Continued)*

Exposure setting/location	*Hair mercury concentration in ppm*	*Blood mercury concentration in μg/L*	*Mercury concentration in fish in ppm*	*Comments*	*Reference*
					Myers *et al.*, 2003[41]
	6.9+/−4.5 (0.5 to 26.7)			-Mothers	
	6.3+/−3.3 (0.9 to 25.8)			-Children at 66 months of age	
Faroe Islands	4.3 (2.6 to 7.7 (25–75% range))	23 (13 to 41 (25–75% range))	3.3 (pilot whale)	Cohort of 1022 births where the diet periodically is high in pilot whale. About 50% of the mercury in pilot whale is methylmercury. A survey found that adults consume a daily average of 72 g of fish, 19 g of whale. Hair and blood data are for maternal hair at parturition and umbilical cord blood	Grandjean *et al.*, 1992[29] and 1997[33]
			0.07 (cod)		
	(15% of values > 10)				
New Zealand	8.3 (mean)	N/A	2.2 (shark consumed as 'fish and chips')	Values are for 73 'high' mercury mothers identified from among 935 heavy fish consumers from >10,000 mothers screened	Kjellstrom *et al.*, 1986[42]

	6–10 (78% of sample)				Mitchell *et al.*, 1982[43]
	>10 (22% of sample)				
Quebec, Canada	>/= 6.0 (28% of sample)	N/A	N/A	3,599 persons from nine indigenous Cree communities tested in 1993–94. In 1988, 14% had hair mercury levels 15.0 ppm or greater	Dumont *et al.*, 1998[30]
	>/= 15.0 (3% of sample)				
Brazilian Amazon	11.8+/−8.0 (0.5 to 50)	N/A	0.53 (0.03 to 1.5)	Three riverside communities with mercury contamination from gold mining. N = 220, 327 & 321. Fish mercury concentrations shown are for carnivorous species	Santos *et al.*, 2000[44]
	19.9+/−12.0 (0.1 to 94)		0.49 (0.09 to 1.6)		
	4.3+/−1.9 (0.4 to 12)		0.12 (0.03 to 0.4)		
Brazilian Amazon	4.3+/−2.2 (0.4 to 12)	N/A	0.12 (0.03 to 0.4)	Four riverside communities not affected by gold mining. N = 321, 316, 499 & 214. Fish mercury concentrations shown are for carnivorous species	Santos *et al.*, 2002[45]
	4.0+/−2.1 (0.4 to 12)		0.3 (0.05 to 0.5)		
	5.4+/−3.1 (0.4 to 17)		0.04 (0.01 to 0.5)		

Table 6.2 *(Continued)*

Exposure setting/location	*Hair mercury concentration in ppm*	*Blood mercury concentration in μg/L*	*Mercury concentration in fish in ppm*	*Comments*	*Reference*
	8.6+/−6.3 (0.6 to 46)		0.3 (0.01 to 2.5)		
Poisoning Epidemics					
Minimata disease Victims (Minimata and Nigata, Japan)	>/= 50 (threshold for adult disease)	>/= 200 (threshold for adult disease)	9–24 (average), some samples up to 40 ppm, values are for fish	Industrial releases of methymercury into Minimata Bay and Agano River. Over 230 persons poisoned and over 50 deaths	FDA[14]
Iraqi epidemic 1971–1972	1 to >700	0 to 5000 (range)	9.1 (4.8 to 14.6) values are for methylmercury in ppm in wheat flour	Poisoning epidemic due to ingestion of bread baked with methylmercury treated grain. 6530 poisoning victims admitted to hospitals and 459 deaths	Bakir *et al.*, 1973[46]
	10–20 (threshold for fetal effects)	200 to 400 (threshold for adult disease)			Cox *et al.*, 1989[47]
	50 to 100 (threshold for adult disease)	>3000 (23% lethality)			Clarkson and Strain 2003[48]

N/A = not available

Only limited data are available regarding blood mercury concentrations after immunisation with thimerosal-containing vaccines[20,52] or after ethylmercury poisoning.[18] However, these studies do not provide information on the utility of hair mercury in ethylmercury exposures.

6.2.4 Indications for Hair Analysis

Properly handled hair samples are a recognised biomarker for methylmercury exposure in epidemiological studies using standardised protocols. This technique is well-established and provides several advantages over blood mercury as discussed above. In the clinical setting, however, problems may occur due to external contamination, use of hair samples far from the scalp, improper specimen handling and the use of 'commercial' hair tests.[53,54] Carefully collected samples analysed by experienced research laboratories may provide additional corroboration of exposure or longitudinal information in some cases.[23] Hair analysis is not indicated to assess potential toxicity due to elemental mercury from dental amalgams, and hair results should not be used to justify unproven interventions such as amalgam filling removal and chelation. (See below on commercial hair tests.)

6.3 Arsenic

6.3.1 Toxicology

The most common exposure to arsenic in the general population is from naturally occurring organoarsenates, primarily arsenobetaine and arsenocholine. These are concentrated in various types of seafood, including fish and shellfish, but are innocuous. Health effects from arsenic are largely from tri- and pentavalent inorganic arsenic species with occupational exposures from byproducts of mining, smelting and coal-burning and its use in various pesticides.[55] Worldwide, the most important exposures occur in discrete geographic areas with high levels of inorganic arsenic in groundwater.[56–59] Chronic arsenic-related health effects are endemic in these populations.

Large acute exposures generally result from ingestions and produce an inflammatory gastroenteritis with altered vascular permeability that can produce hypovolemia and shock. Chronic arsenic exposure causes a wide variety of skin lesions including melanosis, keratoses and palmar and solar hyperkeratoses, as well as non-melanoma skin cancers.[56,59] It is also associated with peripheral vascular or 'blackfoot' disease, neuropathy, hematopoietic disturbances and lung cancer.[55,56,58,59]

6.3.2 Kinetics and Relation to Hair

Arsenic is absorbed primarily through lungs and the gut.[19] It is distributed widely and rapidly cleared from the blood. Acutely, the highest tissue concentrations are found in the liver and kidney.[60,61] Chronically, arsenic accumulates in the skin, hair and nails due to their high content of sulfhydryl-rich keratin.[19,60,61] Other than

some accumulation in the lungs, arsenic is not significantly stored in internal organs. Inorganic arsenic species are detoxified in the body by methylation.[59] Excretion is predominantly renal, and 60–80% is excreted in methylated forms.[59,61] As the dose increases, the amount excreted as inorganic forms increases as well.[61]

6.3.3 Hair vs. Other Biomarkers

Because of its rapid clearance from the blood, blood testing is not utilised to assess occupational or chronic exposures to arsenic. A 24 hour urine collection is the method of choice for diagnosis of intoxication and monitoring occupational or environmental exposures. Urine concentrations can be deceptively elevated, however, by the consumption of seafood rich in organoarsenates within the last 24–72 hours.[19] For this reason, subjects should abstain from seafood for an adequate interval prior to collection and/or both total and inorganic arsenic species should be determined. If seafood consumption is responsible for an elevated total arsenic concentration, the resulting inorganic arsenic will be negligible.

Urine testing may also be of limited value more than 96 hours after exposure has ceased because most internal arsenic will have been eliminated.[61] Hair testing, therefore, has two distinct advantages. First, because arsenic accumulates in hair, hair analysis may identify arsenic exposure for longer periods. Second, hair binds inorganic arsenic, but seafood-derived organic forms of arsenic do not to accumulate in hair.[19,61–64] On the other hand, hair has the disadvantage of airborne contamination in occupational settings and can also bind exogenous arsenic from bathing in contaminated water.[61] Harrington *et al*.[65] found mean hair arsenic levels of 5.7 ppm in subjects bathing in arsenic-contaminated water, but who drank bottled water that was not contaminated. Unfortunately, no method is capable of removing all external arsenic contamination and none can distinguish externally deposited arsenic from that derived from ingestion.[19,61,66,67]

In general, background concentrations of arsenic in hair are usually well below 1ppm,[3,61,68] while health effects may be seen at concentrations exceeding 1ppm.[69] The dose-response relationship between hair arsenic concentrations and the degree of exposure or poisoning is approximate and may be misleading in individual cases.[61] There are several likely explanations, including the relative contribution of external contamination to total concentrations, individual differences in metabolism and hair deposition, and different analytical techniques especially with regard to washing. In settings where arsenic exposure is through inhalation or drinking contaminated water, hair arsenic concentrations reflect a combination of internal dose (personal exposure) and environmental contamination (airborne deposition or washing in contaminated water).[69] Table 6.3 compares Hindmarsh's[61] suggested guidelines for the 'approximate' interpretation of arsenic hair levels with data from selected studies of background concentrations, as well as various exposure settings. Toenails and fingernails can also be analysed for arsenic with similar advantages (chronic accumulation of inorganic arsenic) and disadvantages related to external contamination as observed with hair analysis. In epidemiological studies both hair and nail are often collected along with urine and environmental samples.[56–58,69]

Table 6.3 *Hair arsenic levels in selected studies in relation to urine arsenic and drinking water arsenic and Hindmarsh's[61] suggested guidelines for interpretation*

Exposure setting	*Hair arsenic concentration in ppm*	*Urine arsenic concentration in* μg/L^{-1} or μg g^{-1} *Cr*	*Drinking water arsenic concentration in* μg/L^{-1}	*Comments*	*Reference*
'Normal'	<1	N/A	N/A	Suggested guideline	Hindmarsh, 2002[61]
Background – generally unexposed persons	<1.1	N/A	N/A	271 adults in US without occupational exposure	DiPietro *et al.*, 1989[68]
	<1	N/A	N/A	987 Illinois hospital employees and family members	Sky-Peck, 1990[3]
	<1	N/A	N/A	20 unexposed adults	Bencko, 1995[70]
Background – polluted areas	3.1	N/A	N/A	40 adults in an 'exposed' area	Bencko, 1995[70]
	3.2+/−2.4	25+/−25	N/A	23 boys residing in a 'heavily polluted area'	Bencko, 1995[70]
	8.0+/−8.2	71+/−37	N/A	38 volunteers exposed to airborne arsenic through home coal burning, 29% prevalence of arsenic-related skin lesions	Shraim *et al.*, 2003[71]
Endemic areas with high concentrations of Arsenic in Groundwater	3.4+/−3.3	280+/−410	37% > 50, 11% >/= 300	4386 persons from arsenic-affected areas in Bangladesh	Rahman *et al.*, 2001[56]

Table 6.3 *(Continued)*

Exposure setting	*Hair arsenic concentration in ppm*	*Urine arsenic concentration in* $\mu g/L^{-1}$ or $\mu g\ g^{-1}$ *Cr*	*Drinking water arsenic concentration in* $\mu g/L^{-1}$	*Comments*	*Reference*
	1.5+/−1.6	180+/−2.7	25% > 50, 3% >/= 300	7135 persons from arsenic-affected areas in West Bengal, India	Rahman *et al.*, 2001[51]
	5.5+/−2.0	N/A	40	31 persons from rural areas of Victoria, Australia	Hinwood *et al.*, 2003[69]
	2.8 (0.25–12.4)	799 (24–3696)	57% > 50, 20% > 300	59 hair samples from Bihar, India, 63% prevalence of arsenic-typical neuropathy	Chakraboti *et al.*, 2003[72]
	5.6+/−0.4	140+/−9	212+/−15	59 persons with arsenic-related skin lesions from West Bengal, India	Mahata *et al.*, 2003[73]
	4.5+/−3.7	N/A	248+/−59	47 persons from West Bengal, India drinking contaminated water for 3–10 years	Mandal *et al.*, 2003[64]
Chronic poisoning	>/= 10	N/A	N/A	Suggested guideline, but may be less than ten	Hindmarsh, 2002[61]
	2.6 to 7.5	23 to 277	N/A	Three patients poisoned with arsenic-containing medications, all three with arsenic-related skin lesions and one with cytopenia and polyneuropathy	Chakraboti *et al.*, 2003[74]

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Lethal	Usually >45	N/A	N/A	Suggested guideline, can be lower, higher levels have also been reported in survivors	Hindmarsh, 2002[61]
	100	N/A	N/A	Patient died after ingesting 250 ml of copper acetoarsenite	Hindmarsh, 2002[61]
External contamination	Up to thousands	N/A	N/A	Suggested guideline	Hindmarsh, 2002[61]
	29 to 452	N/A	N/A	Five workers from a gold smelter	Hindmarsh *et al.*, 1999[75]
	36 (0.3 to 151)	N/A	N/A	39 antimony smelters	Bencko, 1995[70]

N/A = not available

6.3.4 Indications for Hair Analysis

Hair arsenic can be a useful indicator of chronic arsenic poisoning in clinical and forensic settings, but its presence should only be considered as circumstantial evidence in most cases, unless external contamination can be ruled out.[61] In individual patients, hair levels must be correlated with clinical findings of arsenic toxicity, urine measurements and possible exposure sources. Arsenic in hair has also been a valuable epidemiological tool in studies of individuals drinking arsenic-contaminated water when used along with urine, nail and water arsenic measurements.

6.4 Lead

6.4.1 Toxicology

Lead is a xenobiotic. Exposures may occur during mining, smelting and refining. It is the most widely utilised non-ferrous metal and used to make certain batteries, pigments, ammunition and solders.[76] Average population exposures in countries that have eliminated leaded gasoline have dropped sharply in recent years.[76,77] The presence of lead paint in older homes remains, however, an important source of exposure to children and construction workers.[76,78]

Lead is toxic to the haemal, renal, reproductive, cardiovascular and peripheral and central nervous systems.[76,78] Symptoms of lead toxicity in adults, if present, are varied and non-specific, including abdominal pain, fatigue, headache, arthralgias and myalgias. Central neurologic dysfunction depends on chronicity and severity and may range from subtle neurocognitive deficits to encephalopathy. Epidemiologically, increased lead body burdens otherwise not associated with disease in adults are associated with increases in blood pressure.[79–81]

6.4.2 Kinetics and Relation to Hair

Absorption is highest through inhalation, but can also occur through the gastrointestinal tract, and is increased by iron and calcium deficiencies.[19,76] Once absorbed, lead is distributed widely throughout the body, where it exists in three compartments or pools.[76] The half-life in blood and other rapid exchange tissues is on the order of weeks, while the in other soft tissues the half-life is measured in months.[19] Bone is the major endogenous storage site of lead, with a half-life of 5–15 years.[19,76] Excretion is primarily renal, but small amounts are also excreted through the bile, hair, nails and sweat.[19]

6.4.3 Hair vs. Other Biomarkers

Measurement of lead in whole blood is the best marker of current and recent exposures and correlates well with acute and sub-chronic toxicities.[76] Bone lead, on the other hand, provides the best estimate of body burden and can be determined non-invasively by X-ray fluorimetry (K-XRF).[82–84]

Background lead concentrations in hair have been described as high as 12+/−15 ppm,[3] but others have found much lower levels with the 90th percentile <10 ppm[68] and a geometric mean of 2.4 ppm.[85] Among highly exposed workers, hair lead content is significantly correlated with blood lead, but may represent a mixture of internal dose and external contamination.[19,86] As a screening test for lead poisoning in childhood, hair lead measurement had poor sensitivity for identifying children with blood lead $\geqslant 10$ μg dL^{-1}.[87] Niculescu *et al.*[88] found a strong correlation ($r = 0.72$, $p < 0.001$) between blood lead and hair lead in a group where 84% of blood lead values were $\geqslant 40$ μg dL^{-1}, but a much weaker correlation ($r = 0.03$, $p < 0.05$) where 84% of blood lead values were <40 μg dL^{-1}. Foo *et al.*[89] found a significant correlation between hair lead and blood lead ($r = 0.85$, $p < 0.0001$) among occupationally exposed persons with a geometric mean blood lead of 34 μg dL^{-1} and range of 3–77 μg dL^{-1}.

6.4.4 Indications for Hair Analysis

Hair analysis for lead has limited epidemiological utility due to external contamination and inadequate sensitivity and specificity at lower levels of exposure, now known to be health concerns. It is not indicated for clinical diagnosis and not indicated for childhood or workplace screening programs. It may have limited applications in ecological and exposure assessment studies as a proxy marker of environmental contamination.[90] Anecdotally, segmental hair analysis was useful in corroborating the timeline of lead ingestion in a case of criminal poisoning.[5]

6.5 Cadmium

6.5.1 Toxicology

Cadmium is a xenobiotic that occurs naturally with zinc and lead. Occupational uses include electroplating, cadmium alloy and battery production, welding solders and cadmium pigments.[91] Tobacco smoking is the most important source of non-occupational exposure to cadmium.[19,91,92] Large acute inhalational exposures can produce acute lung injury, while chronic exposures are nephrotoxic and epidemiologically linked to emphysema and bone demineralization.[91]

6.5.2 Kinetics and Relation to Hair

Similar to lead, cadmium is absorbed well *via* inhalation and to a lesser extent through the gut.[19,91] Absorption is increased by calcium, iron and zinc deficiencies. Cadmium is distributed throughout body tissues, with roughly 50% of the body burden found in kidneys and about 15% in liver. Significant accumulation occurs in both anatomic sites with a biological half-life of 10–30 years in kidney and 5–10 years in liver.[19,91] Smokers have on average twice the body burden of non-smokers.[19,93] Excretion is primarily renal, with lesser amounts excreted in the faeces, saliva, hair and nails.[19]

6.5.3 Hair vs. Other Biomarkers

A number of parameters are useful for assessing different aspects of cadmium exposure and toxicity. Both blood and urine cadmium are considered to reflect a mixture of recent exposure and body burden, which depend on the intensity, duration and continuity of the exposure.[19] Thresholds for nephrotoxicity are best established for urine cadmium.[94–96] In addition, β-2-microglobulin can be quantified in urine and is a marker of early renal toxicity. The concentration of β-2-microglobulin may correlate well with the degree of nephrotoxicity.[97] Finally, XRF can be used to estimate the cadmium body burden by directly measuring the kidney or liver cadmium content.[19]

Background cadmium concentrations in hair are usually less than 0.5–0.8 ppm.[68,85,92,98] Experience with the use of hair cadmium as a biomarker is limited and its utility is severely hampered by the inability to distinguish external contamination from endogenous deposition.[19] It has been used in some parts of Europe as one of several indicators of environmental exposure/contamination.[92,93,98–100] In general, higher cadmium levels in hair are observed in more polluted areas. In addition, men and boys accumulate more cadmium in hair than women and girls, presumably due to spending a greater time outdoors on average.

Several lines of evidence suggest that hair cadmium does not reflect internal dose or body burden. First, cigarette smoking, the major exposure for most adults, has only a minimal effect on hair cadmium concentrations, but is a major determinant of blood and urine cadmium.[92] Second, Erzen and Kragelj[100] found the correlation between blood and hair cadmium concentrations in military recruits to be non-significant. Third, Hac *et al.*[93] compared the cadmium concentrations in renal cortex and hair in 65 paired samples from autopsies and found no correlation between renal and hair cadmium levels. They concluded that hair is not a good indicator of personal exposure to cadmium. Several other animal and human studies have also failed to find significant correlations between cadmium concentrations in hair and liver, kidney or lung.[2]

6.5.4 Indications for Hair Analysis

At present, there are no clinical indications for the measurement of cadmium in hair or as a biological marker of internal cadmium dose or body burden. It may have a role as a proxy marker of human interaction with environmental contamination.

6.6 Manganese

6.6.1 Toxicology

Manganese is an essential trace element that serves as a cofactor for several enzymes.[101,102] It also has a number of industrial uses including manganese alloys, dry cell batteries, paints, fertilisers and in the gasoline additive, methylcyclopentadienyl manganese tricarbonyl (MMT).[101,102] Occupational exposures also occur in mining and smelting. Manganese compounds are respiratory tract irritants.[103]

Chronic inhalation exposure is associated with neuropsychiatric disturbances, with advanced neurological disease closely resembling Parkinsonism.[101,102]

6.6.2 Kinetics and Relation to Hair

Occupational exposure occurs primarily from inhalation, but some gastrointestinal absorption also occurs. Manganese may share certain absorptive and metabolic pathways with iron.[102,104] The major body storage site is the liver, but accumulation also occurs in the brain and kidney.[19] Excretion is primarily faecal through the bile,[102] but small amounts are also excreted in the urine, hair, sweat and nails.[19]

6.6.3 Hair vs. Other Biomarkers

In general, bio-monitoring has proven difficult for manganese. While average manganese concentrations in biological media among exposed groups are higher than in non-exposed groups, significant overlap may occur, and correlation with individual exposures and the severity of toxicity is poor.[19,101] There is also little dose-response consistency among manganese concentrations in blood, urine and hair, especially for individuals.[101] Blood manganese may reflect recent exposure, but due to rapid elimination, there is little correlation with past exposure.[102] On an aggregate basis, urine manganese has shown some correlation with air exposures,[19] but did not discriminate between exposed workers and non-exposed referents in one recent study.[105]

Table 6.4 summarises hair manganese levels across various studies and their relation to concentrations in other biological media and exposure setting. Background hair levels are usually reported as less than 0.3 ppm, however, differences in analytical technique or unaccounted for external contamination may yield higher results. Sky-Peck[3] found mean manganese hair concentrations in healthy volunteers to be slightly above 1 ppm using energy dispersion XRF, while Dipietro *et al.*[68] and Paschal *et al.*[85] used inductively coupled argon plasma emission spectroscopy (ICAP). Higher background levels in hair were also seen among the 'unexposed' referents in the Bader *et al.*[105] and Boojar and Goodarzi[103] studies, perhaps due to a small degree of unaccounted for external contamination.

Hair monitoring generally demonstrates higher values among exposed groups than unexposed groups, but it is unclear to what extent this reflects internal dose vs. external contamination.[103,105,106] Loranger and Zayed[106] compared garage workers exposed to higher ambient levels of manganese through the gasoline additive MMT to other workers with lower ambient exposure. The garage workers had a significantly higher mean hair manganese, 660 ppb, compared to the controls, 390 ppb, but their estimated total manganese intakes were similar when dietary sources were accounted for. When one contrasts the blood and hair levels of the poisoning cases reported by Woolf *et al.*[104] and Ono *et al.*[107] with the heavily exposed workers in the Bader *et al.*[105] and Boojar and Goodarzi[103] studies, clearly, a significant portion of the hair manganese observed in the battery workers and miners results from external contamination. Further, the hair results shown in Table 6.4

Table 6.4 *Hair manganese levels in selected reports in relation to urine and blood manganese concentrations and exposure settings*

Exposure setting	*Hair manganese concentration in ppm*	*Urine manganese concentration in* $\mu g/L^{-1}$ *or* $\mu g/g^{-1}$ *Cr*	*Blood manganese concentration in* $\mu g/L^{-1}$	*Comments*	*Reference*
Background	0.22 (90% <1.1)	N/A	N/A	271 adults in US without occupational exposure	DiPietro *et al.*, 1989[68]
	0.23 (mean)	N/A	N/A	322 adults in US (extension of DiPietro *et al.* study)	Paschal *et al.*, 1989[85]
	1.1+/−0.6 men	N/A	N/A	987 Illinois hospital employees and family members	Sky-Peck, 1990[3]
	1.2+/−1.3 women				
MMT exposure	0.66 (0.12–2.47)	N/A	7.6 (2.8–14.5)	37 garage mechanic ('exposed'). Garage workers had greater exposures to ambient manganese through MMT in gasoline exhaust, but total estimated manganese exposure including diet similar to control workers.	Loranger and Zayed, 1995[106]
	0.39 (0.06–1.19)	N/A	6.7 (0.2–13.1)	27 other blue collar workers ('less exposed'), controls	
Dry cell battery manufacture	8.2 (0.9–27.7)	0.49 (0.1–2.2)	13.8 (6.1–23.3)	39 highly exposed workers, selected from the area with the highest ambient manganese concentrations	Bader *et al.*, 1999[105]
	5.2 (0.5–17.2)	0.33 (0.1–1.3)	11.7 (3.2–23.0)	22 moderately exposed workers, selected from the area with intermediate ambient manganese concentrations	

	4.6 (0.4–29.8)	0.26 (0.1–1.8)	10.7 (3.9–25.8)	39 low exposure workers, selected from the area with the lowest ambient manganese concentrations	
	2.2 (0.4–6.2)	0.39 (0.1–1.2)	7.5 (2.6–15.1)	17 unexposed referents	
Manganese mining				145 manganese miners tested after 4 to 7 years of exposure	Boojar and Goodarzi, 2002[103]
	23+/−4	14.8+/−3.3	18.6+/−3.1	Smokers	
	20+/−4	13.2+/−3.2	16.7+/−3.5	non-smokers	
	1.4+/−0.4	1.4+/−0.3	1.5+/−0.4	65 matched controls smokers	
	1.6+/−0.5	1.5+/−0.4	1.6+/−0.3	non-smokers	
Poisoning cases	3.1 (nl <0.26)	8.5 (nl <1.1)	38 (nl <14)	10-year-old boy exposed to drinking water with elevated manganese, psychometric testing suggested impaired memory	Woolf *et al.*, 2002[104]
	1.4 (nl <0.1)	<1.0	43 (nl <25)	17-year-old male welder with involuntary myoclonic movements	Ono *et al.*, 2002[107]

N/A = not available; MMT = methycyclopentadienyl manganese tricarbonyl

for Bader *et al.*[105] are for the most proximal, first centimeter of axillary hair. Further analyses of the second and third centimeters of axillary hair demonstrated progressively higher manganese content, suggesting increasing contamination over time.

6.6.4 Indications for Hair Analysis

No single biological medium definitively and reliably reflects individual manganese exposure or toxicity. Therefore, hair manganese analyses should be considered as an additional source of potential exposure information in both individual cases and group studies, along with blood and urine manganese, and when possible environmental manganese determinations. The potential for external contamination must also be considered. Given the wide variation in reported background hair concentrations, control specimens should be obtained and analysed using the same methods. Hair manganese is more likely to be informative regarding biological dose when the exposure is through ingestion rather than ambient air. In the latter case, the hair may be a surrogate measure of environmental manganese contamination.

6.7 Thallium

6.7.1 Toxicology

Thallium salts are toxic, and thallium sulfate has been used as a rodenticide. While a 1970s ban on its use in the US has reduced accidental poisonings, it continues to be used in some countries as a rodenticide, as well as being implicated in homicide attempts.[108–110] Anecdotal occupational exposure to thallium has also been described in the manufacture of a special glass.[111]

Characteristically, the ingestion of thallium salts produces gastrointestinal symptoms, followed by the onset of a painful ascending neuropathy.[108,109] The neuropathy can be progressive with associated weakness. Acute alopecia begins after about a week, and can progress to complete baldness over several weeks.[109] Characteristically, the alopecia is painless, hair can be pulled out in clumps with little effort, and the inner one-third of eyebrows are spared.[108,112–114] In addition to diffuse alopecia, thallium intoxication can cause blackening of the hair roots, which is seen when these are examined microscopically.[108,112,114] Hoffman believes, however, that untrained observers may have difficulty in noting this diagnostic feature.[108,109]

6.7.2 Kinetics and Relation to Hair

Thallium is readily absorbed by inhalation, gastrointestinal absorption and dermally.[109,110] Subsequently, thallium is widely distributed throughout the body and concentrated in the kidneys.[109,110] Excretion is predominantly renal and faecal, but thallium is also excreted into the saliva, nails and hair.[19,115] The elimination half-life for thallium has been reported to be anywhere from 2–30 days.[19,108,115]

Thallium does not have an internal anatomic reservoir, but binds sulfhydryl groups with high affinity like those in keratin, which likely explains its concentration in hair and nails.[108,115]

6.7.3 Hair vs. Other Biomarkers

Thallium can be determined in blood, urine, hair and nails. Because most information relating to thallium concentrations in biological media comes from poisoning cases, little is known about background concentrations or the quantitative relationship between exposures, internal doses and the relative concentrations among different biological media.[19] Acutely, the measurement of thallium from a 24-hour urine collection is the diagnostic test of choice.[108,109] Both urine and blood thallium concentrations are usually elevated in poisoning cases and are commonly measured in these cases.

Because thallium concentrates in the hair, elevated hair thallium concentrations can be used to help confirm the diagnosis of poisoning.[108,109,114,116] Table 6.5 summarises the results of hair analysis in selected reports from the literature. While hair levels in this table are presented in ppm to maintain consistency with the remainder of the chapter, thallium content in hair is usually described in units 1000-fold lower (ppb or ng g^{-1}). Limited data describe background concentrations of thallium in biological media. Available information suggests that these levels should be very low and less than 20 ppb in hair.[111,114,118] Among poisoning victims, hair concentrations range from 48 to 35,000 ppb with most between 150 to 1500 ppb. In Rusyniak *et al.*'s[114] case series, there was a good correlation between 24-hour urine thallium excretion and the thallium concentrations in the hair taken simultaneously (Figure 6.1). With regard to timing, elevated thallium concentrations in hair have been found as early as 2–3 weeks after ingestion in poisonings[6,114,116] and as late as 13 months after the cessation of their occupational exposures.[111] Thallium also concentrates in nails and they have been analysed in poisoning cases,[108,116] but there are insufficient data available to determine the relative advantages of hair vs. nails.

6.7.4 Indications for Hair Analysis

Analysis of thallium content in hair should be considered as complementary to urine and blood testing in confirming the diagnosis of acute thallium poisoning, possibly as early as two weeks after the onset of symptoms. Unlike urine samples, hair is unlikely to demonstrate elevated concentrations immediately after a single ingestion, however, given the relatively short elimination half-life of thallium, hair analysis may be especially useful in cases where several months have elapsed since the cessation of exposure. Analytic methods must be capable of quantifying concentrations in the ppb range.

6.8 Commercial Hair Tests and Their Potential Misuses

Commercial hair analyses are promoted as a means of determining a patient's nutritional status and exposure to toxic heavy metals. Although hair analysis for

Table 6.5 *Hair thallium levels in selected published reports in relation to urine and blood thallium concentrations and exposure settings*

Exposure setting	*Hair thallium Concentration in ppm*	*Urine thallium concentration in μg/L^{-1} or μg per 24 hours*	*Blood thallium concentration in μg/L^{-1}*	*Comments*	*Reference*
Background	<0.020	<10	N/A	Source of background values not specified	Rusyniak *et al.*, 2002[114]
	N/A	<5	<2	Source cited as Mulkey and Oehme, 1993[117]	Mercurio and Hoffman, 2002[108]
	<0.012	N/A	N/A	Unexposed control subject's hair analysed. Thallium content below detection limit of 0.012 ppm	Hirata *et al.*, 1998[111]
	<0.016	N/A	N/A	Source cited as Schoer, 1984	Yoshinaga *et al.*, 1993[6]
Poisoning cases	0.252 (0 to 1.324)	1127 (0 to 5885)	N/A	Mean values for 12 victims of a combined thallium and arsenic poisoning taken after about 16 days of symptoms. Suspected to be a criminal act	Rusyniak *et al.*, 2002[114]
				73% incidence of neuropathy overall and present in all six patients with hair thallium >0.100 ppm.	
				5/6 patients with hair thallium >0.100 ppm had alopecia; 0/6 patients with hair thallium <0.100 ppm had alopecia	
	0.53	532	275	21-year-old with alopecia, severe ascending neuropathy and coma. Biological media tested for thallium about one month after she was presumably poisoned for a second time	Mercurio and Hoffman 2002[108]

	35	N/A	N/A	38-year-old who eventually died of thallium poisoning. Hair sampled three weeks after hospitalisation	Yoshinaga *et al.* 1993[6]
	1.46	1022	400	Three victims of a thallium poisoning presumably criminal. Timing of hair sampling about three weeks after onset of symptoms	McCormick and McKinney, 1983[114]
	10.69	3838	240		
	12.69	N/A	N/A		
Manufacture of a special glass containing thallium carbonate	0.020	N/A	N/A	Worker 1: handled thallium-containing materials for four years and presented with alopecia and polyneuropathy. He was tested 32 months after exposure cessation	Hirata *et al.*, 1998[111]
	0.576			Worker 2: replaced worker 1 in previous job handling thallium-containing materials. He was tested 13 months after exposure cessation and also complained of alopecia	

N/A = not available

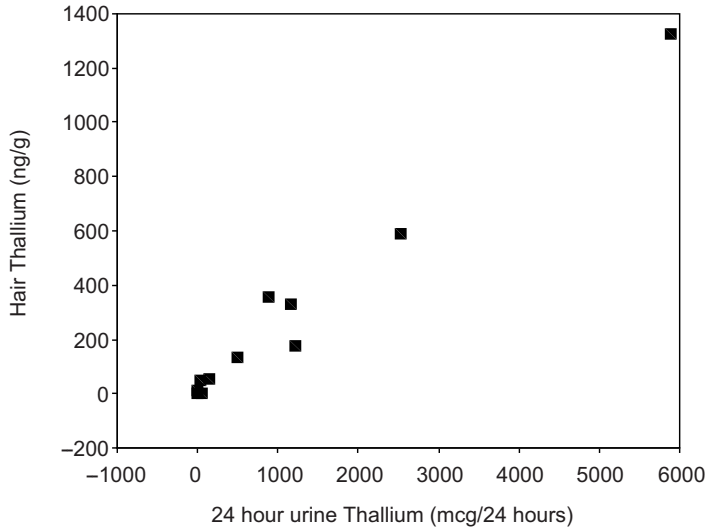

Figure 6.1 *Hair thallium values for 12 victims of a poisoning plotted against 24-hour urine thallium. Both hair and urine were collected after about 16 days of symptoms. Data are from Rusyniak et al.*[114]

heavy metals has a number of valid applications, as described above, commercial analyses that simultaneously determine large numbers of metals and minerals have long been misused. In 1974, Lazar[119] wrote: 'Trace-metal hair analysis, as advertised by several commercial laboratories, is done often on hair samples supplied by clients, often by mail, usually of uncertain origin and without adequate history of previous environmental or personal exposures... Such analysis does provide numerical results, but these results are of very little true value and unfortunately are frequently used to support questionable diagnoses or recommendations'.

Subsequent to Lazar's editorial, commercial hair analyses of this type have been studied, and criticised and discouraged by various investigators and committees.[2,4,7,53,54,120–124] Unfortunately, such unconventional practices continue today, with patients sending in hair samples to commercial laboratories directly or through a variety of 'alternative' practitioners. Seidel *et al.*[53] calculated that nine US laboratories were performing an annual average of 225,000 hair analyses at a cost of almost $10 million.

In our experience, such commercial testing is typically targeted at patients with unexplained somatic symptoms, as well as others with idiopathic diagnoses such as autism and amyotrophic lateral sclerosis. Characteristically, these analyses are not element specific, nor based on exposure history, but test from 15–39 different minerals and metals.[7,53,122] Statistically, this multiple testing increases the probability of 'abnormal' results based on chance alone.[7,123] The results are often used to justify questionable therapies such as extensive vitamin and other supplementation regimens, mercury amalgam filling removal and chelation.[7,53,120–122]

Reliability studies of commercial hair analyses using duplicate hair samples have demonstrated discordant reference range intervals, poor result reproducibility and divergent nutritional recommendations from different laboratories analysing identical samples.[53,120] In 1985, Barrett[120] sent duplicate samples from two healthy volunteers to thirteen different commercial laboratories. He found considerable divergence in results for identical samples sent to different laboratories as well as those sent to the same laboratory. Seidel *et al.*[53] recently updated the Barrett investigation by sending a split sample of scalp hair to six laboratories that perform 90% of the commercial hair analyses in the US. Reported values for 12 minerals varied more than 10-fold on the split sample. Also, statistically significant extreme values for the same element compared to other laboratories' results were found for 14 of 31 minerals analysed by more than three laboratories.

Additionally, case reports of patients labelled as having heavy metal toxicity on the basis of commercial hair tests, which subsequently received second opinions, describe inaccurate diagnoses based on hair testing.[2,7,122,125] Such patients often experienced needless anxiety based on the inaccurate diagnoses. They also usually lacked significant exposure histories, and when conventional blood and urine tests were performed had normal concentrations of the metals in question.

A summary report of the US Agency for Toxic Substances and Disease Registry (ATSDR) panel on hair analysis encapsulated the experts' view on commercial hair analyses with the following statement: 'Universally, the panelists expressed concern about the misuse of hair analysis to justify and support unnecessary and unethical medical therapy'.[4] We agree that in clinical situations, hair testing for heavy metals should be targeted to specific elements based on the patient's occupational and environmental history and/or exposure assessments, should be used only as an adjunct to more reliable biomarkers and should utilise experienced reference or research laboratories.

6.9 Important Methodological Issues in Hair Metal Analysis

There are a number of unresolved methodological issues that limit the clinical use of hair analysis and must be taken into account when hair is used for forensic and/or epidemiological purposes. The ATSDR-convened panel,[4] has discussed and reviewed these in depth and the reader is directed to the full meeting report for additional information and bibliography.[126] While the panel recommended the standardisation of hair sampling protocols, a definitive consensus was not reached. Logically, the ideal methodology may differ depending on the metal of interest.

6.9.1 Sampling

Hair from various scalp locations may grow at variable rates, while hair segments closer to or more distant from the scalp will reflect different chronological periods of exposure. In addition, although hair growth rate is often assumed to be 1.1 cm per month for the purpose of timing exposures, the average growth rate may range from 0.6 to 1.5 cm per month with additional variation among individual

subjects.[10] Therefore, one must decide where on the scalp to sample from and how far from the scalp to include in the analysis sample. While a universally agreed upon anatomical location does not exist, consideration should be given to sampling from a reproducible point on the skull.[126] The amount of hair collected should exceed the minimum required by the laboratory for a valid analysis, while not being cosmetically unacceptable to the subject. To avoid sample contamination, cutting tools and sample storage vessels should not be made of or treated with the metals of interest for the analysis to be performed.[126]

6.9.2 Cosmetic/Hygiene Products

Personal care products may contain metals that can be adsorbed onto hair. For example, Grecian formula®, a hair dye, has been reported to contain lead.[3] Other hair treatments such as permanents and hair waving may alter the content of copper, arsenic and mercury.[3,127] Dandruff treatments containing selenium sulfide could potentially alter hair mercury concentrations.[85] Bleaching of the hair alters melanin content and therefore, potentially affects metal binding.[128] The effects of different shampoos and the frequency of hair washing on hair metal content have not been thoroughly studied,[126] but because different sample washing procedures have been noted to affect results (see below), it follows that shampooing habits could potentially affect metal recovery for certain elements.

6.9.3 Sample Washing

Decisions regarding the necessity of washing and the washing method should be substance specific.[126] When the metal of interest can only reach the hair internally (*e.g.* dietary MeHg) and is not an external contaminant, washing is not necessary.[126,129] Washing protocols become important when the metal of interest has an external source, but there is no universal agreement as to an ideal method.[2,70,130–132] Many groups use the acetone washing procedure recommended by the International Atomic Energy Agency.[133] One should recognise several facts about washing hair samples prior to analysis. First, different washing methods utilising different solutions and/or different durations of washing may produce significant variation in results.[67,68,132] Second, washing only incompletely removes external contamination, but may also remove some internally deposited metals.[2,126]

6.9.4 Sample Preparation

Hair proteins bind metals. Therefore, in order to be analysed for metal content, hair must be digested into a form in which metals can be ionised for measurement.[54] Digestion methods differ among laboratories and may be an additional source of variation.[134]

6.9.5 Analytical Methods

No less than six analytical methods exist for measuring metal content in hair.[126] Atomic absorption spectrophotometry (AAS) is applicable to several

metals including lead,[5,88–90] mercury,[44,45] arsenic,[58,69,72] cadmium[92,93,98] and manganese.[103,105] Cold vapour atomic absorption (CVAA) is preferred for MeHg analysis.[126] CVAA has been widely applied in epidemiological investigations.[22,29,34,41] Using the 'Magos' reagents, it can distinguish between total and inorganic mercury concentrations (allowing methylmercury to be determined as the difference).[22] Inductively coupled argon plasma spectrophotometry techniques (ICP-OES, ICP-MS, ICP-AES) can be used to simultaneously determine multiple elements and are commonly used.[6,68,71,111,126]

6.9.6 Targeted Analyses

The ATSDR panel did reach general consensus that analytic approaches should be targeted to preferably a single metal and a specific exposure context.[126]

6.9.7 Interpretation

Sampling location, hair growth rate, sample washing and preparation, the potential confounding of cosmetic and/or hygiene products, as well as, for most metals, the lack of universally accepted reference ranges (or adverse effect levels) greatly affect the ability to make accurate estimations of individual patients' metal exposures based on hair analysis. In epidemiological investigations, these limitations may be surmountable by stringently standardising all procedures for all participants and samples. In this way, investigators will be able to mitigate individual variation and compare relative exposures within groups of study participants. Ideally, internal validation studies that correlate hair metal content to appropriate blood and/or urine biomarkers of the same metal should also be done on a subset of the participants. This technique makes results comparable in a quantitative fashion with other studies and levels of metal exposure associated with documented biological effects.

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