Mercury in Ancient Mummy Hair from Peru, Chile, and Egypt –

Evidence of pre-industrial naturally occurring dietary exposure

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Scott Arnold*, Jennifer Parker, Nicolas Bloom, Arthur Aufderheide (deceased), and John Middaugh*

Authors Affiliations:

Drs Scott Arnold and John Middaugh are both former employees of the Alaska Section of Epidemiology and did most of the work for this report while working for the State of Alaska. Jennifer Parker worked for Nicholas Bloom, senior research scientist formerly with Frontier Geosciences Research and Consulting, who performed all of the analyses in 2001 when the specimens were made available. Dr Aufderheide provided the specimens for mercury analysis and provenance.

Scott Arnold, PhD*
The Dow Chemical Company
Toxicology and Environmental Research & Consulting
1803 Building
Midland, MI 48674
smarnold@dow.com

Jennifer Parker, Senior Associate
Windward Environmental, LLC
200 W Mercer Street, Suite 401
Seattle, WA 98119
jenniferp@windwardenv.com

Nicolas Bloom
Senior Research Scientist
Formerly with Frontier Geosciences Research and Consulting
414 Pontius Avenue North
Seattle, WA 98109

Arthur C. Aufderheide, MD (deceased)
Department of Pathology
University of Minnesota, Duluth School of Medicine
10 University Drive

Duluth, MN 55812

John P. Middaugh, MD*
Medical Epidemiologist
1711 W Lodge Drive
Phoenix, AZ 85041
jpmidd@cox.net

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Abstract

Increases in global atmospheric mercury emissions since the industrial era and concerns regarding human methylmercury exposure through fish consumption have increased interest in determining temporal mercury exposure trends. Using hair analysis, previous studies have documented mercury exposure in pre-industrial era mummies and hair bundles found at archeological sites from circumpolar regions. This study determined hair mercury concentrations (n=37) in three groups of mummies (5000 B.C. to 1300 A.D.) from the Atacama Desert near the Peruvian and Chilean border and Egypt's Western Desert. The magnitude of hair mercury concentrations corresponded to the archeological assessment of each group's dietary consumption of seafood. The Egyptian, Chilean, and Peruvian mummies had mercury exposures below the WHO guideline level of 1 ppm and were considerably lower than that of circumpolar pre-industrial populations.

Key words:

Mercury, mummy hair, Chinchorro, Chiribaya, Dakhleh Oasis

Introduction

Anthropogenic mercury emissions have increased global atmospheric mercury concentrations by approximately 3-fold since preindustrial times.[1] Due to recent concerns regarding human exposure to low-levels of methylmercury through fish consumption, there is interest in determining temporal exposure trends.[2-5] Mercury in hair is a well-established and stable biomarker of methylmercury exposure.[2,6-10]

Previous studies have documented hair mercury concentrations from 43 pre-industrial era mummies found in circumpolar regions.[11-15] These results provide insight regarding the extent of human exposure to methylmercury through fish and marine mammal consumption in pre-industrial times. Given the lack of information on prehistoric hair mercury levels from other regions of the world, we evaluated hair mercury and other trace metal concentrations of 37 mummies collected by Dr. Aufderheide from Peru, Chile, and Egypt.[16-19]

Methods

The hair of 40 well-preserved mummies from the Atacama Desert near the Peruvian/Chilean border and the Western Desert in Egypt were collected and analyzed for mercury (N=37) and other trace metals (N=3). These dry environments provide excellent preservation of ancient human remains (Aufderheide, personal communication). Three distinct mummy groups were studied representing northern Chile, southern Peru,

and Egypt. The age and sex of the mummies were determined by prior assessment in Aufderheide's lab (Table 1).

Northern Chile

The Chinchorro population lived in extreme northern Chile within the modern city of Arica at the mouth of the San Jose River.[16] Two sub-sites were excavated: Mo1 (c. 5000 B.C.) and Mo1-6 (c. 2000B.C.). Mo1 individuals (n=10) were anthropogenically mummified and their recovered hair was often derived from a wig fashioned from hair of others during that time period. Mo1-6 individuals (n=9) were naturally mummified with their hair attached. The pre-agricultural Chinchorro derived the majority of their nutrition from a marine environment (e.g., sea lions, fish, shellfish).[16,17]

Southern Peru

The Chiribaya population (n=9) lived near the modern seaport of Ilo in extreme southern Peru, approximately 100 kilometers north of Arica, Chile. The Chiribaya population (1150–1300 A.D.) were agropastoralists who supplemented their diet with small amounts of seafood.[16]

Egypt

Hair samples obtained from nine individuals from the Egyptian Ismant el-Kharab (Kellis-1) site at the Dakhleh Oasis, approximately 400 km west of Luxor, were also analyzed.[17,18] These individuals lived during the Roman period (30 B.C. to A.D. 395) in

the northeastern portion of the Sahara Desert, and probably did not consume any marine foods.[16]

Laboratory Analysis

Assessment of External Contamination

Two different approaches were used to assess the extent of external contamination for the Peruvian/Chilean and Egyptian hair samples. For the Peruvian/Chilean mummy hair, one Peruvian and two Chilean hair samples were tested for external contamination. The hair samples were washed with 1% (v/v) Formula 409® detergent (which is low in mercury) in deionized water by shaking for one hour, followed by three rinses with deionized water. Water from the wash and rinses was analyzed for total mercury, methylmercury, and 13 additional trace elements (As, Cd, Cr, Cu, Fe, Pb, Mg, Mn, Mo, Ni, Sc, Se, Zn). Prior to mercury analysis, wash waters and rinsates were oxidized with 1% (v/v) of 0.2 M BrCl in 12 M HCl and hair samples were digested with concentrated HNO_3 (as described below). All samples were processed using clean sampling handling protocols in a low-mercury laboratory.

To aid in the evaluation of external contamination, the results of the three hair samples were compared to the concentration of trace elements in the certified hair reference material, International Atomic Energy Agency (IAEA-086), representing 20th century European hair.

A different approach was used for assessing external contamination for the Egyptian mummy hair samples because many were encrusted with a resin that was most likely

extracted from trees and diluted with bitumen-coal tar or petroleum derivative from the Dead Sea.[18] To determine if the resin contained mercury or if the weight of the resin encrusting the hair could bias the result of the mercury analysis, one Egyptian mummy hair sample and a certified hair reference material (IAEA-086) were split and extracted with either benzene or 1% (v/v) Formula 409® aqueous solutions and compared with the results of the unwashed hair. The benzene washes were performed using five consecutive 10 mL rinses of benzene. For the first two rinses, the samples were shaken six times for 10 minutes followed by centrifugation to separate the solids from the liquid. The final three benzene rinses were performed by shaking the sample for 1 minute in 10 mL of benzene followed by centrifugation and removal of the benzene. The washed hair was dried overnight at 65 °C. For comparison, approximately 0.3 grams of hair were also washed two times in a solution of 1% (v/v) Formula 409®. The samples were shaken at 10-minute intervals for 1 hour and centrifuged to separate the hair from the washing liquid. The samples were then rinsed twice with deionized water and dried overnight at 65°C.

Total Mercury

Prior to analysis of all hair samples for total mercury, approximately 0.1 gram of each sample was digested in 10 mL of hot, concentrated HNO_3 for approximately 3 hours, followed by dilution to 40 mL with a solution of 0.07N BrCl. Aliquots of each digest were analyzed for total mercury by $SnCl_2$ reduction, purge and trapping onto gold-coated sand, cold vapor atomic fluorescence spectrometric (CVAFS) detector for quantification.[20] The absolute detection limit for this instrument is approximately 0.1

picogram mercury, but all reported detection limits are determined by the variability of the measured blanks, according to the different extraction procedures. All reported data have been corrected for the mean method blank of a given extraction batch and for changes in instrumental drift on each analytical day.

Methylmercury

In order to prepare the hair samples for methylmercury analysis, approximately 0.1 gram of each sample was digested on a hot plate in 10 mL of a 25% (w/v) KOH/methanol solution for approximately 3 hours. Following completion of the hotplate digestion, the samples were diluted up to 40 mL with methanol (neat). From each KOH methanol digest, an aliquot of 0.5 mL was removed and aliquoted into a 60-mL Teflon distilling vial containing 45 mL of deionized water. Then 0.2 mL of 25% KCl, 0.1 mL of 9 M H_2SO_4, and 0.2 mL of 1% (v/v) ammonium pyrrolidinedithiocarbamate (APDC) solution were added to each vial, and the samples were distilled under nitrogen flow into a Teflon receiving vial until 40 mL of distillate was collected. Aliquots of the methylmercury in hair digests were analyzed using aqueous phase ethylation, purge and trap, isothermal GC separation, and CVAFS.[18,21] All reported data were corrected for the mean method blank of a given extraction batch, and for changes in instrumental drift on each analytical day. In addition, sample concentrations were corrected for the extraction efficiency (35%) as determined by matrix spike recovery results.

Trace metals

Trace metals were determined for one Peruvian and two Chilean mummies in dilutions of HNO_3 digests via inductively coupled mass spectrometry (ICP/MS, Elan-6000) approximately following the protocols of EPA Method 1638. Hydride generation-atomic fluorescence (HG-AFS) was used to determine total selenium in hair.

Quality Assurance

Every analytical batch was accompanied by at least three identically processed method blanks, a sample extracted in duplicate, a matrix spike and matrix spike duplicate, and certified reference material (CRM) IAEA-086 (total and methylmercury in human hair). The CRMs used in the analytical runs as a calibration check standard were NIST-1641d (total mercury in water), a KOH/methanol digestion of NRCC DORM-2 (methylmercury in dogfish muscle digest), and NIST-1640 (trace metals in water).

Results

Assessment of External Contamination

One Peruvian and two Chilean hair samples were tested for external contamination. The maximum mercury concentration in the wash waters of each of the three hair samples was 0.017 mg/kg (ppm) dry basis, indicating minimal external contamination. The results of the trace element analysis were also used to assist in the determination of external contamination (Table 2). The concentrations of the trace elements (with the exception of manganese), were similar in magnitude to concentrations of the certified hair reference material, IAEA-086, representing 20th century European hair. The concentration of manganese in the Chinchorro (5000 B.C.) hair sample was 250- to 700-fold higher than

that detected in the other samples. Because these samples had very little external mercury contamination and the concentration of the majority of trace elements were consistent with values detected in modern hair, the remaining Peruvian/Chilean hair samples were not washed prior to mercury and methylmercury analysis.

For the Egyptian mummy hair sample, benzene efficiently removed the resin that encrusted the hair, and the benzene extraction did not affect the mercury concentration detected in the hair sample. There was no difference in the mercury concentration for the unwashed, aqueous-washed, or benzene-washed treatments for either the Egyptian mummy hair or the certified reference material hair samples, indicating this hair sample did not contain a significant amount of external mercury contamination. However, to remove the resin present on the Egyptian hair samples prior to mercury and methylmercury analysis, the remaining hair samples were extracted with benzene.

Total Mercury and Methylmercury

The results of the total mercury and methylmercury analysis for the groups of ancient mummy hair are presented in Table 3. The average concentration of total mercury detected in the Chilean and Peruvian hair samples, which ranged from 0.46 ± 0.51 mg/kg to 0.85 ± 0.81 mg/kg, was an order of magnitude higher than the average concentration detected in the Egyptian hair sample (0.049 ± 0.050 mg/kg). The average concentration of methylmercury detected in the two Chilean mummy groups (0.065 ± 0.12 mg/kg and 0.079 ± 0.095 mg/kg) was an order of magnitude higher than the average concentration of methylmercury detected in the Peruvian hair sample (0.0069 ± 0.012 mg/kg).

Methylmercury was detected at or slightly above the detection limit (0.0012 mg/kg) in two of the nine Egyptian hair samples (Table 3).

Discussion

This study extends the geographic range and nearly doubles the number of pre-historic hair mercury exposure assessments conducted to date. The magnitude of the average total mercury and methylmercury hair concentration for each group of mummies corresponded to the archeological assessments the seafood content of each group's diet. The mean mercury of the samples correspond to levels below the current WHO guidelines for mercury exposure assessed using hair samples (i.e., <1ppm).[22] The highest hair mercury and methylmercury concentrations were detected in the Chinchorro population, which derived the majority of their diet from a marine environment.[16,17] The Chiribaya consumed some seafood as part of their diet, and had an average methylmercury concentration that was 10 times lower than the Chinchorro. The hair of the Egyptian mummies contained essentially no detectable methymercury consistent with their terrestrial-based diet (Aufderheide, personal communication). The hair concentrations varied greatly within each group. Similar variation in hair mercury concentrations has been reported for mummies from Karluk, Alaska.[11]

For the Chinchorro population, the average concentration of total mercury and methylmercury was lower than the concentrations detected in the hair of the circumpolar region (Tables 3 and 4). In addition, the average percent of methylmercury to total mercury in the Chinchorro hair samples was 8 percent (Mo1, 5000 B.C.) and 11 percent

(Mo1-6, 2000 B.C.). The concentration of mercury in the hair of fish-eating populations is expected to be primarily methylmercury.[6] There are two possible explanations for the lower level of methylmercury in the Chinchorro hair samples: the concentration of methylmercury in the hair decreased overtime, or the Chinchorro were exposed to lower levels of methylmercury.

It is possible that environmental conditions contributed to a decrease in methylmercury contained in the Chinchorro hair. Similarly, low levels of methylmercury compared with total mercury have been detected in the hair of mummies from the Karluk Archeological site in Kodiak, Alaska (Table 4). This population also relied heavily on a marine-based diet. Egeland et.al. hypothesized that poor environmental conditions (e.g., moist soil containing high amount of tannic acids) could have affected the hair strands.[11] However, the excavation sites for the Chinchorro are located in one of the driest places on earth.[16] Thus, the conditions are conducive to excellent preservation of mummies.

It is possible that methylmercury in the Chinchorro hair samples spontaneously degraded over the centuries. To our knowledge, the Chinchorro mummy specimens (2000–5000 B.C.) are the oldest to be evaluated for methylmercury exposure to date. Previous specimens collected from circumpolar mummies were dated 400–1150 A.D. (Table 2). While it is presumed that methylmercury in hair is stable overtime, hair samples of this age have not been previously analyzed for methylmercury. The hair from the two Chinchorro groups (who were presumed to have comparable diets) contained similar methylmercury concentrations even though they differed in age by 3000 years (2,000 –

5,000 B.C.), suggesting that methylmercury concentrations may not have decreased overtime.

The Chinchorro may have been exposed to lower concentrations of methylmercury because they consumed fish/marine mammals lower on the food chain. However, sea lion bones and hides have been uncovered in the graves of the Chinchorro, indicating they did consume carnivorous marine mammals.[16]

Methylmercury concentrations could have been lower at the time the Chinchorro lived. The concentration of methylmercury in hair of the Chinchorro was approximately 19-fold lower than methylmercury concentrations detected in the hair of mummies recovered from the circumpolar north (Tables 3 and 4). Additionally, the methylmercury concentrations in the ancient South Pacific may have been lower than those in the more recent circumpolar north.

The range of selenium concentrations detected in the one Peruvian and two Chilean hair samples (0.86 mg/kg to 1.28 mg/kg) was similar to the average concentration reported for mummy hair from Greenland, Karluk and the Aleutians, which ranged from 0.15 mg/kg to 2.9 mg/kg,[11,14,15] and selenium in the certified hair reference material representing 20th century European hair (1.1 mg/kg). The range of cadmium concentrations detected in the one Peruvian and two Chilean hair samples (0.44 mg/kg to 1.0 mg/kg) was somewhat lower than the concentration detected in mummy hair from Greenland, Karluk, and the Aleutians, which ranged from 1.1 mg/kg to 5.2 mg/kg (Table 5).[11,14,15] The high

manganese concentration (7,709 mg/kg) detected in the Chinchoro hair sample (5,000 B.C.) reflects manganese in paint that the Chinchorro (5000 –3000 B.C.) commonly applied to the bodies during mummification (Table 2).[16]

The age and presumed diet of these well-preserved mummies presented a unique opportunity to determine ancient exposure to methylmercury. The results presented here document methylmercury exposure presumed?? from marine fish or mammal consumption in individuals from antiquity. These data provide additional evidence of the extent of and variability in pre-industrial human exposure to methylmercury. The available data suggest that prehistoric methylmercury exposures were higher in the circumpolar regions.

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Table 1. Age and Sex of Mummies from Chile, Peru, and Egypt

Mol Group 1: 1984 Arica, Northern Chile Chinchorro c 5000 BC				**Ilo Group: Southern Peru Chiribaya c 1150–1300 AD**		
Mummy No.	**Age (yrs)**	**Sex**		**Mummy No.**	**Age**	**Sex**
2-C2	3	I		21	30	F
7-C1	15	M		44	45	M
7-C2	I	I		70	45	F
11	I	I		94	27	M
17-C3	3	I		111	18	F
21-C1	13	F		114	21	F
23-C1	30	F		121	33	M
23-C4	40	F		123	38	M
23-C12	22	F		125	6	I
24	1	F				
Mo1-6 Group: 1987 Arica, Northern Chile Chinchorro c 2000 BC				**Egypt, Dakhleh Oasis c 30 BC to AD 395**		
Mummy No.	**Age**	**Sex**		**Mummy No.**	**Age**	**Sex**
9	60	M		EG-98, T-106	15	F
16	12	I		EG-98, T-111	23	M
18	40	M		EG-98, T-118	35	M
22	35	M		EG-98, T-122	37	M
32	45	F		EG-98, T-123	50+	M
38	I	I		EG-98, T-124	50+	M
39	13	F		EG-98, T-125	40	M
40	60	F		EG-98, T-130	7	M
41	58	M		EG-98, T-131	8	M
50	50	M		EG-98, T-132	4	I
U4	I	I				

(*I=indeterminate)*

Table 2. Trace Elements Detected in Ancient Hair Collected from the Atacama Desert of Chile (N=2) and Peru (N=1)

Group/Location	Estimated Age of Hair Sample	Trace Metal Concentration, mg/kg (ppm), Dry Weight														
		Mg	Sc	Cr	Fe	Mn	Ni	Cu	Zn	As	Se	Mo	Cd	Hg	Me Hg	Pb
Chiribaya/Peru	1150–1300 AD	377	2.39	0.69	199	10.5	1.48	8.73	75.2	0.82	1.2	0.26	0.44	0.091	0.06	0.41
Chinchorro (Mo1-6)/Chile	2,000 BC	313	0.94	0.86	161	29.4	0.36	8.28	108	0.37	1.3	0.50	0.57	0.186	0.01	0.29
Chinchorro (Mo1)/Chile	5,000 BC	155	0.88	0.70	446	7,709	2.16	14.0	180	31.6	0.86	0.76	1.0	0.275	0.03	2.27
20th Century European (IAEA-086)		150	0.01	--	110	10	--	18	160	--	1.1	--	--	0.50	0.20	--

IAEA: International Atomic Energy Agency

Table 3. Average Detected Total and Methylmercury Concentrations in Ancient Hair from Peru, Chile, and Egypt

Group/Location	Estimated Age of Hair Sample	Number of Samples	Total mercury		Methylmercury	
			mg/kg (ppm), Dry Weight			
			Mean	Standard Deviation	Mean	Standard Deviation
Dakhleh Oasis/ Egypt	30 BC - 395 AD	9	0.049	0.050	0.0014*	0.0002*
Chiribaya/Peru	1150 - 1300 AD	9	0.46	0.51	0.0069	0.012
Chinchorro (Mo1-6)/Chile	2,000 BC	9	0.47	0.30	0.065	0.12
Chinchorro (Mo1)/Chile	5,000 BC	10	0.85	0.81	0.079	0.095

**Methylmercury was detected in 2 of 9 samples (0.0012 and 0.0015 mg/kg) at or above the estimated detection level of 0.0012 mg/kg*

Table 4. Average Total Mercury and Methylmercury in Ancient Human Hair from Circumpolar Regions

Location	Estimated Age of Hair Sample (AD)	Number of Samples	Total Mercury	Methylmercury	Reference
			mg/kg (ppm), Dry Weight		
Karluk One, Kodiak, Alaska	1160-1660	16	1.3	0.03	Egeland et al. 1999
Barrow, Alaska	1460	2	3	N/A	Toribara and Muhs 1984
N. Baffin Island, Canada	1400–1150	8	N/A	1.7	Wheatley and Wheatley 1988
Umanak, Greenland	1400	6*	3.1	N/A	Hansen et al. 1989
		2†	10	N/A	
Kagamil Island, Aleutian Islands, Alaska	1445	4*	5.8	1.2	Egeland et al. 2009
		5†	--±	1.4	

N/A: not analyzed

* *Adults*

† *Children*

± *Not reported because of external mercury contamination*

Table 5. Cadmium and Selenium Concentrations in the Hair of Ancient Remains

Group/location	Estimated Age of Hair Sample	Number of Samples	Selenium		Cadmium	
			Concentration ppm Dry Weight			
			Mean	SD	Mean	SD
Chiribaya (Ilo)/Peru	1150–1300 AD	1	1.2	--	0.44	--
Chinchorro (Mo1-6)/Chile	2000 BC	1	1.3	--	0.57	--
Chinchorro (Mo1-T)/Chile	5000 BC	1	0.86	--	1.0	--
Kagamil Island, Alaska	1445 AD	5	2.9	2.1	1.1	1.6
Karluk, Alaska	1160–1660 AD	16	0.15	0.14	5.2	5.7
Umanak, Greenland	1400 AD	Unknown	0.5	--	2.8	--