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A study of the distribution of aluminum in human placental tissues based on alkaline solubilization with determination by electrothermal atomic absorption spectrometry

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Summary

Aluminum (Al) is a nonessential element known to induce neurotoxic effects, such as dialysis dementia, in patients on hemodialysis, with compromised kidney function. The role of Al in the progression of some neurodegenerative diseases, such as Alzheimer's disease (AD), is controversial, and remains unclear. The effects of Al on other vulnerable populations, such as fetuses and infants, have been infrequently studied. In the present study, Al has been measured in human placenta samples, comprising ~160 each of placenta bodies, placenta membranes, and umbilical cords, using electrothermal atomic absorption spectrometry (ETAAS) after atmospheric pressure digestion with tetramethylammonium hydroxide (TMAH) and ethylenediaminetetraacetic acid (EDTA). The sensitivity, or characteristic mass (m_0), for Al at the 309.3-nm line was found to be 30 ± 4 pg. The instrumental detection limit (*IDL*) ($3s$) for Al in solution was calculated as $0.72 \mu\text{g L}^{-1}$, while the method detection limit (*MDL*) ($3s$) was $0.25 \mu\text{g g}^{-1}$. Accuracy was assessed through analysis of quality control (QC) materials, including certified reference materials (CRMs), in-house reference materials (RMs), and spike recovery experiments, of varying matrices. Placental tissue analyses revealed geometric mean concentrations of approximately $0.5 \mu\text{g g}^{-1}$ Al in placenta bodies ($n=165$) and membranes ($n=155$), while Al concentrations in the umbilical cord ($n=154$) were about $0.3 \mu\text{g g}^{-1}$. Al was detected in 95% of placenta bodies, and 81% of placenta membranes, but only in 46% of umbilical cords.

Keywords

aluminum; placenta; electrothermal atomic absorption spectrometry (ETAAS); tetramethylammonium hydroxide (TMAH); ethylenediaminetetraacetic acid (EDTA)

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Introduction

Aluminum (Al), the third most abundant metal in the Earth's crust, is ubiquitous in nature. Long recognized as a nonessential trace element, Al is now known to be the causative agent in dialysis dementia.^{1, 2} In addition, accumulation of Al in bone has been shown to result in bone loss.³ Al has been implicated as a risk factor in some neurodegenerative diseases: in Alzheimer's disease (AD),^{4, 5} Al may exacerbate the formation of reactive oxygen species, leading to increased oxidative stress in the brain.^{6, 7} The metabolic actions of Al are similar to those of Fe in biological systems; and thus, accordingly, Fe levels in the body may modulate the toxicity of Al.⁸

Human exposures to Al are principally through ingestion of foods, drinking water, and pharmaceuticals;⁹ all of these routes can result in exposure to the fetus. However, little is known about the placenta's ability or inability to act as a barrier to Al. Animal studies suggest that excessive Al exposure during early developmental stages can result in pronounced developmental and neurological fetotoxicity.¹⁰⁻²¹ However, a review of some other animal studies concluded that exposure to Al *in utero* is unlikely to produce toxic fetal effects.²² Nonetheless, it is important to establish reference ranges for Al in human placental tissue, in order that we can recognize abnormally high exposures, and identify at-risk populations. Al accumulates to the greatest extent in bone, but can be found in many human tissues.^{23, 24} Typical Al concentrations in human placental tissue have not been established. Further, health effects associated with Al in placenta, as well as in the fetus itself, have not been studied. Fetuses can be extremely susceptible to adverse health effects resulting from exposure to nonessential trace metals such as Al, since the organs, excretory pathways, and blood-brain barrier are not yet fully developed.⁹

Electrothermal atomic absorption spectrometry (ETAAS) is a widely accepted technique suitable for the determination of Al in biological tissue.²⁴⁻²⁸ This technique offers detection limits in the ng L⁻¹ range, a sensitivity required for trace analyte determination in tissues, and a linear dynamic range sufficient for both normal tissue Al concentrations and elevated levels.²⁹ Tetramethylammonium hydroxide (TMAH) has been used as an alternative to conventional acid digestion for the determination of metals in biological samples. In particular, several groups have successfully measured Al in biological samples using ETAAS after digestion with TMAH.^{28, 30} In one study conducted by Zhou et al., ethylenediaminetetraacetic acid (EDTA) was also added during digestion, to enhance metal leaching from the sample, although Al was not one of the analytes measured.³¹

Here we report on the determination of Al in human placentas that were collected as part of a larger study conducted from 1993 to 1998 that focused on fetal exposure to Pb during pregnancy, i.e., the Albany Pregnancy Infancy Lead Study (APILS).^{32, 33} Al was measured in three human placental tissue components, using ETAAS after room-temperature digestion with TMAH and EDTA. Method validation was accomplished with the use of quality control (QC) materials of various matrices, as well as spike recovery experiments.

Experimental

Placenta collection and preanalytical procedures

Placentas were collected shortly after delivery, with informed consent, from 167 women participating in the APILS.^{32, 33} The collection of these tissues and their transfer to the Wadsworth Center were approved by the Institutional Review Boards (IRBs) of the New York State Department of Health (NYS DOH), Albany Medical Center Hospital (AMCH), and the University at Albany.^{32, 33} The protocol was subsequently approved for Al measurements (DOH IRB Protocol #06-007).

During the APILS, demographic data were collected from most of the women whose placentas were utilized in the present study ($n = 160$). The average age of women in the study was 24, and ranged from 15 to 38. The average parity was one, with a range of zero to seven. Forty-eight per cent of the women were African-American, 35% were Caucasian, and 13% were Hispanic. Fifty-seven per cent were characterized as inner-city residents of Albany, while 43% lived in other city or suburban areas. Forty-three per cent of women reported smoking at some point during pregnancy, at an average rate of $3 (\pm 5)$ cigarettes smoked per day. The sample population, and qualifications for participation in the study, have previously been described in greater detail.³²

Originally, these placentas were collected solely for the determination of Pb and Cd, and appropriate precautions were taken to avoid contamination. The standard operating protocol for collecting placenta at delivery required the entire tissue to be placed into a sealed plastic container provided by the Department of Pathology at AMCH in Albany, NY. The container was immediately transferred to the Dept. of Pathology where a small section ($<1 \text{ cm} \times 2 \text{ cm}$) of the myometrium membrane was removed for routine histological examination. While the standard pathology practice called for preserving placenta in formaldehyde solution, the protocol was altered for our study samples such that no preservative was added. The placentas were stored in a cold room until transferred intact to the Wadsworth Center's Clinical Trace Elements Laboratory at the NYS DOH, typically within 2-3 days. Samples of the plastic containers used by the AMCH were tested for Pb and Cd contamination *via* nitric acid leaching and determination by ETAAS, and none was detected.

Placenta samples were washed with doubled-deionized (DI) water, and divided into placenta body, placenta membrane, and umbilical cord with a tantalum knife. The placenta body was homogenized, due to its large size³⁴ and known heterogeneous distribution of some trace metals,³⁵⁻⁴¹ with a Stomacher 400 homogenizer (Seward, Worthing, UK). All three tissue components were freeze-dried (VirTis, Gardiner, NY; LABCONCO Corporation, Kansas City, MO, USA) using a slow, five-segment drying program that did not exceed room temperature. The majority of placentas were freeze-dried and stored long-term at -80°C , however, a small number ($n = 14$) were stored frozen for several years before being processed as described above.

The placentas were collected between 1993 and 1998, and analyzed from 2007 to 2009. Thus, placentas were stored at -80°C for approximately 10-15 years. The study was later extended to include analyses for additional trace elements, but no further contamination testing was deemed necessary since reasonable precautions had been taken for Pb and Cd.

When the samples were thawed for analysis, the placenta body was homogenized further, with an agate mortar and pestle (Laval Lab Inc., Quebec, Canada) inside a bench tent (Erlab, Inc., Rowley, MA, USA) to minimize contamination. This step produced a coarse powder suitable for digestion.

Digestion procedure

The digestion procedure used in this study was adapted from the method of Zhou et al.³¹ Accurately weighed samples of approximately 0.25 g were digested in 2 mL of 25% (w/w) TMAH and 2 mL of 0.1 mol L^{-1} EDTA in capped (finger-tightened) 50 mL polypropylene conical tubes (Sarstedt Inc., Newton, NC, USA). The samples were mixed overnight in an upright position on a rocker, in order to achieve adequate dissolution. Samples were vortexed (Barnstead Thermolyne Maxi-mix I Vortex Mixer, Dubuque, IA, USA) periodically throughout the digestion; in cases of incomplete dissolution at the end of the 24-hr digestion period, samples were sonicated (Branson Ultrasonics Corporation, Danbury, CT, USA) at 60°C for 60 min. Finally, the digestions were diluted with 1 mL DI H_2O and

vortexed. Resulting solutions were dark-colored, but translucent and homogeneous, characteristics that are typical of this type of digestion.⁴²⁻⁴⁴ Digestion blanks were periodically analyzed along with actual samples, and always contained Al concentrations that were below the method detection limit.

Although 167 placentas were obtained for analysis in this study, not all arrived at the Wadsworth Center from AMCH with body, membrane, and umbilical cord intact. Only 157 membranes and 154 umbilical cords accompanied the 167 placenta bodies received. Sample wet weights varied considerably from <9 g to >600 g (mean = 250 g) for placenta body, from <4 g to >70 g (mean = 49 g) for placenta membrane, and from <4 g to >120 g (mean = 26 g) for the umbilical cord. Freeze-dried sample weights varied from <2 g to >80 g for the placenta body, <0.05 g to 6.9 g for the placenta membrane, and from 0.25 g to 11 g for the umbilical cord. Further homogenization of the placenta body resulted in some additional sample loss. In a few cases, limited amounts of samples available made it difficult to perform repeated analyses for Al. Also, two placenta bodies were utilized specifically for method development purposes.

Instrumentation

Measurements were performed using an AAnalyst 800 atomic absorption spectrometer (PerkinElmer (PE), Shelton, CT, USA), equipped with a transversely heated graphite atomizer (THGA) and inverse longitudinal Zeeman-effect background correction. An AS-800 autosampler deposited 10 μL of placenta digest diluted 1 + 2 with $\text{Mg}(\text{NO}_3)_2$ matrix modifier onto a pyrolytically coated THGA tube containing a preinstalled L'vov platform (PE). Standard THGA tubes (not equipped with end caps) were used in this study. The AAnalyst 800 was operated with AA WinLab32 computer software (PE version 6.4.0.0191). Instrument parameters and temperature program settings used in this study are listed in Table 1.

Reagents and standards

Standard solutions ranging from 60 to 300 $\mu\text{g L}^{-1}$ Al were prepared from a 1,000 mg L^{-1} Pure Aluminum Spectroscopy Standard (PE) (99.99-99.9999% purity, traceable to NIST 3101a), and 1% (w/w) TMAH (25% (w/w) TMAH in aqueous solution, electronic grade, 99.9999% metals basis, Alfa Aesar, Ward Hill, MA, USA). Standards were prepared in acid-washed Nalgene polypropylene volumetric flasks (Thermo Scientific, Rochester, NY, USA), and stored in 15 mL conical polypropylene vials (Becton Dickinson and Company (BD), Franklin Lakes, NJ, USA) for easy access. Sample digestions contained 25% (w/w) TMAH in aqueous solution (Alfa Aesar) and 0.1 mol L^{-1} EDTA disodium salt ($\geq 99\%$ assay, Sigma-Aldrich Corp., St. Louis, MO, USA). A bovine liver powder was spiked with 5 $\mu\text{g g}^{-1}$ Al prepared from a 1,000 mg L^{-1} (0.100% w/v) Al atomic spectral standard solution, traceable to NIST (Baker Instra-analyzed, J.T. Baker, Phillipsburg, NJ, USA). This material was then digested, and used to assess analyte recovery. Calibration standards, digested placenta specimens, and QC materials were diluted 1 + 2 with a modifier solution containing 0.2% (v/v) HNO_3 (Veritas, double-distilled, ultra trace metals grade, GFS Chemicals, Powell, OH, USA), 0.1% (v/v) Triton X-100 (Laboratory Grade, Baker Analyzed, J.T. Baker), and 1.15 g L^{-1} (w/v) $\text{Mg}(\text{NO}_3)_2$ (SPEX Certiprep 99.999% purity, SPEX Industries, Inc., Metuchen, NJ, USA). The modifier solution was stored in a 250-mL amber, high-density polyethylene (HDPE) bottle, and prepared as necessary. Rinse solution was composed of approximately 1% HNO_3 and 0.0005% (v/v) Triton X-100 (Baker Analyzed, J.T. Baker). All solutions were prepared with DI water ($>18 \text{ M}\Omega\cdot\text{cm}$), produced using either a NANOpure Diamond UV/UF (Barnstead International, Dubuque, IA, USA) or a Milli-Q Plus UV (Millipore Corporation, Bedford, MA, USA) water system optimized for trace metals work. Autosampler wells (0.5 mL) were acid-washed before use.

Method validation

Method validation included analysis of a number of QC materials characterized for Al. Because placenta reference materials (RMs) are not available, a variety of other biological tissues and aqueous RMs certified for Al were analyzed, including NIST (National Institute of Standards and technology, Gaithersburg, MD, USA) Standard Reference Material (SRM) 1640 Trace Elements in Natural Water, NIST SRM 1547 Peach Leaves, NIST SRM 1515 Apple Leaves, and High-Purity Standards (HPS, Charleston, SC, USA) Bovine Liver Solution CRM. The RMs were chosen for method validation purposes because they are among the few that are certified for Al; two were of biological matrix, albeit plant tissue. Since NIST SRM 1640 and HPS Bovine Liver Solution CRM are aqueous solutions, they were not digested. Thus, measurements obtained for these RMs were only used to confirm the accuracy and precision of the method for undigested aqueous samples. However, the analysis of plant tissue includes the same sample preparation and digestion procedures as needed for the placenta samples. In addition to the analysis of the above RMs for Al, four caprine liver powder in-house RMs and one bovine liver powder were used to directly assess the precision of animal tissue Al measurements and recovery of spiked Al, respectively.⁴⁵ Endogenous levels of Al were repeatedly measured in the four caprine liver digests. A 5 $\mu\text{g g}^{-1}$ Al spike was added to bovine liver powder that was then subjected to TMAH/EDTA digestion, for determination of analyte recovery using the method. Placenta samples were analyzed in duplicate, with two replicates per aliquot. Aqueous calibrations were constructed for analysis.

Results and discussion

ETAAS performance with an alkaline medium

Graphite tube longevity—In this study, use of 25% (w/w) TMAH prolonged graphite tube longevity, as compared with use of concentrated HNO_3 . Tube lifetimes averaged 550 ± 150 firings ($n = 26$) in this study, compared with 160 ± 30 firings in a serum Al study performed in our laboratory with HNO_3 ($n = 19$).⁴⁶ Similarly, Skelly and DiStefano found that decreasing the concentration of concentrated HNO_3 in sample solutions from 20% to 6% increased the lifetime of the graphite tube, during an analysis of bone and brain tissue for Al.²⁷ Ribeiro et al. found tube lifetimes of >1000 firings for both HNO_3 and TMAH media, for Cd measurements.⁴⁷ However, when compared with Cd, Al measurements require higher pyrolysis and atomization temperatures, which result in faster graphite tube degradation. Martins et al. found extended longevity of the graphite tube for Cu when they used TMAH medium (over 800 firings per tube) as compared to acidic medium (400 firings per tube).⁴² Almeida et al. were able to perform over 900 Al measurements with the same graphite tube when they used TMAH.³⁰

Performance characteristics—Almeida et al. reported that the addition of TMAH to the graphite furnace at the time of sample introduction decreased the extent of carbonaceous buildup that is sometimes observed during Al measurements in serum samples by ETAAS.³⁰ Ribeiro et al. found lower RSD values for Cd, As, Ni, and Pb measurements in human hair samples when they used ETAAS with TMAH medium rather than HNO_3 .⁴⁷ Those authors pipetted calibration solutions, modifier, TMAH, and samples separately into the graphite tube so as to avoid analyte and modifier precipitation.⁴⁷ D'Haese et al. used a combination of TMAH and EDTA for the digestion of soft tissues, for the determination of Sr by ETAAS.⁴⁸ Those authors found that a digestion with TMAH yielded 'enhanced sensitivity' for Sr (note, however, that this observation was made on the basis of peak height measurements). D'Haese et al. also found that high concentrations of HNO_3 disrupt the Sr absorption signal during ETAAS analysis.⁴⁸

Atomization and pyrolysis curves—We performed pyrolysis and atomization studies using a placenta body digest with an Al concentration of 133 pg (Fig. 1). Method parameters and the optimized temperature program used in this study are shown in Table 1.

Characteristic mass (m_0)—The characteristic mass (m_0), i.e., the mass required to yield an integrated absorbance of 0.0044 s, calculated from daily calibration curves, averaged 32.8 ± 3.9 pg ($n = 77$ analyses). Experimental sensitivity was consistent with the manufacturer's expected m_0 , 31.0 pg.

Detection limits—Instrumental detection limits (*IDLs*) (3s of a reagent blank) and method detection limits (*MDLs*) (3s of a placenta digest) were calculated according to the IUPAC harmonized guidelines for method validation.⁴⁹ *IDLs* were calculated for a 2% TMAH reagent blank ($0.72 \mu\text{g L}^{-1}$), and *MDLs* were calculated for both a bovine liver powder digest ($0.7 \mu\text{g g}^{-1}$) and a placenta body digest ($0.25 \mu\text{g g}^{-1}$), to enable comparison between the two matrices ($n = 12$). The placenta body digest yielded the lower *MDL* of the two tissue digests, due to the higher endogenous Al concentration in the bovine liver digest. Thus, the *MDL* measured with the placenta body digest was used in this study.

Long-term QC studies and continuing validation—The accuracy and precision of the method was monitored over a 4 month period using CRMs, spike recoveries, and caprine liver secondary RMs (Table 2); the values listed demonstrate the capability of the method for measuring Al in human placental tissue. Al measurements in CRMs remained within $\pm 20\%$ of certified values throughout the study period, and RSDs found for caprine liver RMs were $\leq 20\%$, despite endogenous Al concentrations close to the *MDL*. Intermediate precision is reported for all QC materials. Similar performance was achieved for spike recovery experiments.

Outliers—Samples were analyzed in duplicate, i.e., two digestions, with two replicate injections each. Each replicate consisted of the mean of two injections. Grubbs' test was employed for the detection of outliers, and approximately 30 data points (either a single replicate data point or two averaged sample data points) were removed. In cases where data were deemed imprecise, some samples were re-analyzed.

Al in human placenta

Al profile—Al concentrations showed a skewed distribution in all three placenta components (Figure 2).

Comparison of mean values—Al concentrations found in each placenta component were statistically compared, using Dunn's Multiple Comparisons Test (Fig. 3). Concentrations found in the cord and placenta body were significantly different ($p < 0.001$), as were the concentrations in the cord and membrane ($p < 0.001$). There was no significant difference between Al concentrations in the body and membrane ($p > 0.05$). When individual placenta samples were source-matched (body, membrane, and cord of the same placenta) and re-analyzed using Repeated Measures ANOVA, followed by the Friedman statistic, the same relationship was found. That is, the cord and body, and cord and membrane, showed significant differences from one another ($p < 0.001$), while there was no difference between the body and membrane ($p > 0.05$). Thus, the data indicate that Al accumulates in placenta body and membrane tissue to a greater extent than in umbilical cord tissue.

The geometric mean, range of values, and 25th and 75th percentiles were calculated for Al concentrations in each sample component. In addition, the frequency of samples with detectable concentrations of Al was determined (Table 3). The umbilical cords had Al

concentrations that were about half as high as the concentrations found in the placenta bodies and membranes. Al was detected in 95% of placenta body samples, and 81% of membrane samples, but in only 46% of cord samples. The finding that Al is not as concentrated in the umbilical cord may be explained by poor transport across the placenta, or by the absence of suitable binding sites in cord tissue.

Al in placenta and other human specimens—Al concentrations in placental tissue were compared with those reported in the literature for other human tissues and biological fluids (Table 4). Placental tissue Al levels found in this study were similar to levels reported in other soft tissues by Bush et al.²³ Al concentrations found in placental tissues in this study (mean $0.5 \mu\text{g g}^{-1}$, range $<0.25 - 4.3 \mu\text{g g}^{-1}$ dry weight) are similar to those previously reported (mean $1.5 \mu\text{g g}^{-1}$ dry weight, range $0.68 - 5.44 \mu\text{g g}^{-1}$) in another study from the UK.⁵⁰ The method used in the previous study was neutron activation analysis, and the small difference in results between these two studies is remarkable given they were carried out using different analytical methods, on different human populations (i.e., of different geographical and dietary characteristics), and in different decades.

Conclusion

Aluminium was detected in human placental tissues at concentrations below $1 \mu\text{g g}^{-1}$ using an ETAAS method with Zeeman background correction following room temperature solubilization with TMAH/EDTA. In a population of 167 subjects, detectable levels of Al were found in 95% of placenta body samples and 81% of placenta membrane samples, but only in 46% of umbilical cord samples. Al concentrations in the latter were approximately half as much as those found in placenta bodies and membranes. Our results suggest that, while the placenta may serve as a partial barrier to Al exposure *in utero*, the developing fetus may still be vulnerable to cumulative exposure. Future research will focus on statistical comparisons between placental Al concentrations and epidemiologic variables.

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Figure 1. Pyrolysis (a) and atomization (b) curves for a placenta body digest containing approximately $13.3 \mu\text{g L}^{-1}$ Al (133 pg). The filled symbols denote optimal temperatures chosen for the determination of Al in placental tissue.

Figure 2. Al distribution in (a) placenta body, (b) placenta membrane, and (c) umbilical cord tissue samples.

Figure 3. Scatter dot plot showing geometric mean and range for Al ($\mu\text{g g}^{-1}$) in each placental tissue component. Each data point represents the average Al concentration measured by duplicate analysis of a sample. Black horizontal lines indicate geometric mean concentrations for each sample component. Numbers in parentheses indicate the number of placenta samples analyzed for each component.

Table 1

ETAAS instrument and method parameters, and optimized furnace temperature program, for the determination of Al in placental tissues.

Method Parameters					
Background correction	Inverse Longitudinal Zeeman				
Atomizer design	THGA				
Wavelength (nm)	309.3				
Injection temp (°C)	100				
Pipette speed (%)	100				
Read time (s)	3.5				
Read delay (s)	0				
BOC time (s)	2				
Program time (s)	110				
Step	Temp (°C)	Ramp (s)	Hold (s)	Int Flow (mL min ⁻¹)	
Drying	130	1	20	250	
Pyrolysis 1	530	10	30	250	
Pyrolysis 2	1400	10	20	250	
Atomization	2300	0	5	0	
Clean	2450	1	3	250	
Cool	20	5	5	250	

Table 2

Determination of Al in QC materials and spike bovine liver digests.⁴⁵ Al levels in caprine liver RMs reflect endogenous concentrations in $\mu\text{g g}^{-1}$ dry weight.

CRM	Units	n	a _{Cert}	b _{U_{exp}}	c _{Found}	SD	d _{% Rec}
NIST SRM 1547 Peach Leaves	$\mu\text{g g}^{-1}$	70	249	8	236	10	95
NIST SRM 1515 Apple Leaves	$\mu\text{g g}^{-1}$	70	286	9	265	20	93
NIST SRM 1640 "Natural Water"	$\mu\text{g L}^{-1}$	57	52	1.5	58	2	111
HPS CRM Bovine Liver Solution	$\mu\text{g L}^{-1}$	53	200	1	212	6	106
Spiked Digest	Units	n	Target	Found	SD	% Rec	
Bovine Liver Powder	$\mu\text{g g}^{-1}$	42	5	5.2	0.5	103	
Caprine Liver RM	Units	n	Found	SD	e _{U_{exp}}		
G99-3	$\mu\text{g g}^{-1}$	44	0.40	0.06	0.1		
G99-13	$\mu\text{g g}^{-1}$	48	0.34	0.08	0.2		
G99-14	$\mu\text{g g}^{-1}$	43	0.47	0.07	0.1		
G2000-1	$\mu\text{g g}^{-1}$	35	1.9	0.1	0.2		

^aCert = value from the CRM certificate accompanied by an uncertainty statement.

^bU_{exp} = CRM expanded uncertainty ($\pm$ 95% confidence interval).

^cFound = mean value obtained using ETAAS. An additional (uncertain) significant figure has been added to found data for informational purposes.

^d% Rec = found per cent recovery.

^eU_{exp} = found expanded uncertainty.⁴⁵

Table 3

Descriptive statistics for each placental tissue component analyzed. Geometric mean, minimum and maximum values, and percentile values are reported in $\mu\text{g g}^{-1}$ dry weight. The % detectable values refer to the percentage of samples per component in which Al levels were detectable.

	Tissue Component		
	Placenta Body (n = 165)	Placenta Membrane (n = 155)	Umbilical Cord (n = 154)
Geometric Mean	0.56	0.53	0.27
Minimum Value	<0.25	<0.25	<0.25
25th Percentile	0.40	0.30	<0.25
Median Value	0.55	0.47	<0.25
75th Percentile	0.77	0.96	0.41
Maximum Value	4.3	9.2	1.8
% Detectable	95	81	46

Table 4

Comparison between geometric mean Al concentrations found in human placental tissue in the present study, and arithmetic mean Al concentrations previously reported for other human biological tissues and fluids.

Technique	Sample	Units	n	Al Concentration	Reference
ETAAS	Placenta Body	µg g ⁻¹	165	0.56	This work
ETAAS	Placenta Membrane	µg g ⁻¹	155	0.53	This work
ETAAS	Umbilical Cord	µg g ⁻¹	154	0.27	This work
^a NAA	^c Placenta	µg g ⁻¹	100	1.51	50
ETAAS	Liver	µg g ⁻¹	30	1.24	23
ETAAS	Heart	µg g ⁻¹	21	0.666	23
ETAAS	Muscle	µg g ⁻¹	30	0.635	23
ETAAS	Bone	µg g ⁻¹	5	1.67	24
ETAAS	Bone	µg g ⁻¹	30	1.81	23
ETAAS	^d Brain	µg g ⁻¹	30	0.369	23
ETAAS	^e Kidney	µg g ⁻¹	30	0.787	23
ETAAS	Hair	µg g ⁻¹	30	8.37	23
^b ICP-MS	Serum	µg L ⁻¹	110	11.00	51
ETAAS	Blood	µg L ⁻¹	10	12.1	25

^aNAA = neutron activation analysis.

^bICP-MS = inductively coupled plasma-mass spectrometry.

^c1-2 g sections of placenta.

^dMean concentration of Al in gray and white brain matter.

^eMean concentration of Al in cortex and medulla.