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Aluminum in Tobacco Products Available in the United States

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Abstract

A quantitative method for the analysis of aluminum in tobacco products was developed, validated, and applied to select samples. Samples were prepared using standard microwave digestion of tobacco from various products. Detection and quantification utilized sector field inductively coupled plasma-mass spectrometry (SF-ICP-MS). Method applicability to analyze aluminum in a range of tobacco products was demonstrated with quantitative analyses of smokeless tobacco products, cigarette tobacco, little cigar tobacco, and roll your own/pipe tobacco. Though these products represent a convenience sampling, we observed that smokeless tobacco products, as a category, had the lowest average aluminum concentrations. Roll-your-own or pipe tobacco and little cigar tobacco had higher median and ranges of aluminum concentrations than cigarette and smokeless tobacco samples.

1. Introduction

Soluble aluminum compounds are neurotoxic to humans, and accumulate particularly in long-lived post mitotic cells such as neurons (1, 2, 3). Aluminum is toxic to plants as well. (2, 3, 4, 5). The aluminum solubility and plant bioavailability from soil aluminum depends on the soil pH, whether the soil has been amended with aluminum sulfate-containing fertilizers, and the relative propensities of plants to take up aluminum from minerals in the soil (4, 5, 6, 7).

The relative level of uptake depends on the route of exposure to aluminum and its form. Human exposure to aluminum may occur through different routes such as ingestion, dermal absorption, and inhalation. People can potentially be exposed to aluminum in municipal water treated with aluminum salts (8), consumer products such as antacids that contain aluminum hydroxide, some food additives with in which aluminum compounds have been added (9), and from food crops that have absorbed aluminum from soil. Many aluminum compounds are poorly soluble, so total aluminum uptake from the digestive tract can be low. Even though less than 0.01% of ingested aluminum is absorbed in the digestive tract, the absorbed aluminum accumulates in the brain, bone, and other tissues over time (2).

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Exposure to toxic substances from smokeless tobacco products is not the same as exposure by ingestion or inhalation since it is neither swallowed, nor inhaled, but retained predominantly in contact with oral epithelial tissues. The oral epithelium is not a significant barrier to the absorption of many substances (10, 11, 12, 13), but whether aluminum compounds may be absorbed via the oral cavity has not been determined.

Tobacco smoke inhalation is another route of human exposure to aluminum (14, 15, 16). Most aluminum compounds are not volatile, but when aluminum-containing substances such as tobacco undergo combustion, inhalation of the resulting mainstream smoke results in oral and pulmonary exposure to insoluble aluminum silicates (17). When combustible tobacco products are smoked, aluminum in the mainstream smoke aerosol is derived from soil-based insoluble aluminum silicates adhered to the leaf surfaces (17, 18) and possibly soluble and insoluble forms derived from internal uptake by the plant (4, 7). It is likely that non-volatile aluminum-containing compounds are transported in mainstream smoke in a manner similar to other non-volatile compounds in particulate liberated by combustion of the matrix (19).

The respiratory tract is not an effective barrier to many harmful substances (11), some soluble aluminum compounds may be absorbed in lung tissue. Insoluble fine particles including aluminum silicates from tobacco smoke may accumulate in the lung until ingested by phagocytic cells such as bronchoalveolar and interstitial macrophages (14, 15, 20) and contribute to pulmonary inflammation (21).

There is very little data available on aluminum-containing constituents of tobacco products (22) due to analytical interferences and difficulties in sample preparation, including dissolution of insoluble aluminum silicate species. Modern instrumentation including sector field inductively coupled plasma-mass spectrometry (SF-ICP-MS) together with clean sample preparation precautions have enabled the acquisition of quantitative information on aluminum. To provide accurate and measurements to fill a vitally underserved information gap, we measured aluminum concentrations in typical tobacco products and report results on select aluminum containing constituents from tobacco.

Experimental

Samples

Tobacco products were purchased from online retail outlets in the U.S. or from commercial establishments in the greater Atlanta, GA area between 2014 and 2016. Samples were assigned unique identifiers and were logged in to our LIMS data base when received. Samples were stored in their original packaging until analyzed. Only authorized lab personnel had access to the samples.

Tobacco sample and procedural blank preparation for analysis

Tobacco samples from commercial cigarettes, Quality Control moist snuff tobacco samples 1S3 and CRP2 (North Carolina State University, Raleigh, NC, USA) little cigar filler tobacco, and most smokeless tobacco products were dried for a minimum of 1 hour at 90°C. Syrupy moist smokeless tobacco products were dried for up to 16 hours to render moisture contribution to sample mass negligible. Dried tobacco was rendered more homogeneous by grinding for 20 seconds with a Smart Grind coffee grinder (Black and Decker, Middleton, WI, USA).

Dried tobacco samples (0.100 to 0.125 g) were digested in TFM vessels with 9 mL environmental grade nitric acid (GFS, Powell, OH, USA) that was further purified by distillation in a perfluoroalkoxy (PFA) sub-boiling still (CEM, Matthews NC, USA), 2 mL 30–35% double distilled hydrochloric acid, and 0.5 mL 46–51% double distilled hydrofluoric acid (GFS, Powell, OH, USA) to assure complete dissolution of silica and aluminum silicates. Procedural blanks were prepared by adding the same digestion reagents to a TFM digestion vessel. The procedural blanks were then processed through the microwave digestion and dilution procedures as if they were samples. Sample digestion was accomplished by a 10 minute ramp from ambient temperature to 190°C, and maintaining 190°C for 15 minutes with a Milestone Ethos microwave system (Shelton, CT, USA). Digested samples were rinsed from digestion vessels into acid-cleaned 50 mL polymethylpentene (PMP) class A volumetric flasks and diluted to volume with ultrapure water (>18 MΩ·cm). 5.00 mL aliquots of the diluted digestates were further diluted with 1% v/v nitric acid and 1% v/v hydrochloric acid to 100 mL in PMP volumetric flasks.

Sector Field-ICP-MS Instrument Parameters for Tobacco Analysis

Analyses of digested tobacco were performed using an Element XR Sector Field Inductively Coupled Plasma-Mass Spectrometer (ThermoFisher, Bremen, Germany). Aliquots (10 mL) of calibration blanks, standards, procedural blanks, QCs and diluted samples were transferred to acid-cleaned 15 mL "Metal Free" polypropylene sample tubes (VWR, Atlanta, GA, USA) and placed in a FAST autosampler (ESI, Omaha, NE, USA) for analysis. The sample introduction system included a PFA-ST nebulizer, Peltier-cooled PC3 PFA spray chamber, o-ring free sapphire injector and high performance torch (ESI). Samples were teed with internal standard 10 μg/L gallium in 1% v/v nitric acid and 1% v/v hydrochloric acid ahead of the nebulizer and pumped simultaneously at 17 rpm through 0.76 mm i.d. peristaltic pump tubing to the nebulizer for approximately 600 μL/minute liquid flow rate. Plasma gas flow was 16 L/min with 1210 Watts forward power. Sample gas and auxiliary gas were optimized for highest possible stable aluminum signal while maintaining oxide formation at 1% or less, generally near 1.10 L/minute with auxiliary gas near 0.80 L/minute. Other parameters were optimized for optimum signal. Platinum-tipped nickel sampler and H skimmer cones were obtained from Spectron (Ventura, CA, USA). Data was acquired in medium resolution (r ≥ 4,000, 10% valley definition) with 3 runs and 10 passes, 0.010 second sample time, 20 samples per peak in Mass Accuracy mode using auto-lock mass with mass offsets determined for ^{27}Al and ^{69}Ga (internal standard) after mass calibration. Mass windows were 100%, with 50% search windows and 60% integration windows.

Calibration standards and reportable concentration range

Instrument calibration was established with High Purity Standards aluminum standard 10001–1 (Charleston, SC, USA) diluted into 1% v/v nitric acid and 1% v/v hydrochloric acid to prepare a 5 calibration standard range from 12.5 to 300 μg/L. Assuming a minimum of 100 mg dried tobacco per digestion, the calibrated reportable range is from 0.125 μg/mg tobacco to 3.00 μg/mg tobacco. The calibration blank consisted of the acid solution in which calibration standards had been prepared. Gallium internal standard (10 µg/L, NIST 3119a, Gaithersburg, MD, USA,) was prepared in the same acid solution. The calibration blank and standards were analyzed at the beginning of each analytical run. Calibration was considered acceptable if the correlation coefficients were ≥ 0.999. The internal standard corrected calibration blank background was subtracted from calibration standards. The procedural digestion blank was subtracted from quality controls (QCs) and samples.

Calculation of Aluminum in Tobacco Method Precision, Accuracy, and LOD

Results obtained from twenty duplicate analyses of Smokeless Tobacco Reference Products 1S3 and CRP2 used as tobacco quality control samples and quintuplicate run results from Standard Reference Materials BCR-482 (lichen), NIST 1570a spinach Leaves, and NIST 1573a tomato leaves) were used to calculate a plant matrix specific method Limit of Detection (LOD) using a Taylor plot of standard deviations versus mean results to determine S_0 (23). Three times the standard deviation obtained by extrapolation of the regression line to 0 µg/mg concentration was used to calculate the method LOD. The results of the analyses of the same reference products were also used to determine precision and accuracy of the method.

Quality Control

Quality control was maintained by analysis of Smokeless Reference Tobacco Product (STRP) 1S3 and CRP2 before and after each group of samples. The analytical QC samples were evaluated using a modified Westgard evaluation approach (24). When a QC was determined to be out of control according to the modified Westgard criteria, results in the respective batch were not used and analyses were repeated.

Results and Discussion

Aluminum in Tobacco Method Performance

The calculated LOD for tobacco aluminum was 0.012 µg/mg dried tobacco. Only concentrations greater than the lowest calibration standard concentration (12.5 µg/L) divided by 100 mg, the lower sample mass limit for sample preparation, were reported as greater than the Lowest Reportable Level (LRL), providing a more conservative lower limit for reportable concentrations. The LRL based on the lowest calibration standard was 0.125 µg/mg tobacco, ten times higher than the calculated LOD. Similar calculation for the highest calibration standard resulted in a 3.00 µg/mg tobacco Highest Reportable Level (HRL). All sample concentrations were greater than the LRL and lower than the HRL.

Precision based on five analyses of QCs, CRMs, STRPs, and SRMs was ± 11.7% at 0.568 µg Al / mg leaf material for NIST SRM 1573a and ± 3.2% at 0.819 µg/mg for STRP 1S3. Acceptable accuracy was validated based on comparison of analytical results at two concentration levels with certified aluminum concentrations in SRMs (Table 1).

Sample Analysis Results and Discussion

The results from quintuplicate analyses of smokeless tobacco, little cigar, roll your own/pipe tobacco, and cigarette tobacco for aluminum concentrations are shown in Table 2. Based on the inclusion of cigarette, little cigar, pipe, roll-your-own tobacco (which constitute the majority of "any combustible tobacco product" category), as well as smokeless tobacco products in this study, these samples are representative of approximately 98% of tobacco products used by over 60,000 respondents in the United States National Adult Tobacco Survey, 2013–2014 (25). All sample concentration results were within the reportable range. The tobacco aluminum concentrations were similar to those reported for a more limited range of products (22). It should be noted that the concentrations are "total aluminum," not speciated as soluble or insoluble forms.

Though the sample number is small, it is apparent that as a product group, commercial smokeless tobacco samples had the lowest aluminum concentrations. Though there was some overlap between the other product groups, the mean aluminum concentrations and concentration ranges in little cigar and roll your own/pipe tobacco group were higher than in cigarette tobacco.

In addition to possible differences in soil preparation for crops that were used for the different products, another possible reason for lower aluminum concentrations in the smokeless tobacco group could be product preparation. Many smokeless, cigarette, roll your own/pipe tobacco, and little cigar tobacco products are cut into strips or finer pieces as evident from examination of the products. The fines resulting from cutting are recovered as reconstituted tobacco sheet that is mixed with filler for many cigarettes, and used to manufacture wrapping paper for many little cigars. Reconstituted tobacco is not typically a component of traditional smokeless tobacco products (26). We have previously shown that superficial aluminum silicates from soil are visibly higher in reconstituted tobacco than on smokeless or cigarette filler tobacco surfaces (18). It is possible that many superficial aluminum silicate particles are dislodged as fines during cutting and recovered along with tobacco leaf fines in reconstituted sheet. This is one possible reason that the small number of loose leaf (Southern Pride, Taylor's Pride) and moist snuff (Copenhagen, Red Seal) smokeless tobacco products tested in this study collectively had lower total aluminum concentrations than the other types of tobacco products.

We have previously shown using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS), that aluminum silicate particles are present in mainstream smoke (17). Insoluble aluminum silicate particles have been described as "smokers' inclusions," and as the principal particles visible in pulmonary macrophages of smokers (14, 15, 16). While insoluble particles such as kaolin and halloysite aluminum silicates can be imaged using SEM-EDS, soluble forms of aluminum dispersed in the leaf tissue would not be visible. Therefore, it is likely that a substantial portion of the total aluminum transferred from tobacco into mainstream smoke that is inhaled by smokers is in insoluble form, although a small amount may be transported in soluble form.

Whether aluminum in mainstream tobacco smoke is in soluble or insoluble form determines the mechanism of toxicity. In soluble form, aluminum could be taken up by pinocytosis at the pulmonary epithelium, or by other mechanisms. Ultimately, aluminum in soluble form could enter circulation, accumulate in tissues, and exert toxic effects including neurotoxicity effects (2). Insoluble forms greater than 100 nm diameter would generally be ingested by macrophages and induce inflammatory response, whereas insoluble nanoparticles smaller than 100 nm could be taken up by bronchoalveolar macrophages, or enter interstitial macrophages and other cells and cause inflammation (20, 21, 27, 28). Therefore, further work is necessary to characterize the proportions of aluminum in tobacco and mainstream smoke that might be in soluble form, and the predominant size ranges of the insoluble forms.

Conclusions

This work resulted in a validated method for the analysis of an analytically challenging metallic element in tobacco. The new method was used to determine aluminum concentrations in various tobacco products, and complements our earlier work using SEM-EDS (17, 18). However, it must be emphasized that the route of exposure to aluminum depends on whether the tobacco product is smokeless or combustible. The data reported here formed a basis for future work on characterization of the forms and concentrations of aluminum in mainstream tobacco smoke. Aluminum exposure via mainstream tobacco smoke is an important but incompletely characterized potential health risk, whether the inhalation exposure to aluminum from tobacco smoke represents exposure to a soluble form, an insoluble form, or both.

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Abbreviations:

ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Centers for Disease Control and Prevention
CRM	Certified Reference Material
SRM	Standard Reference Material
STRP	Smokeless Tobacco Reference Product

References

1. Ganrot PO (1986) Metabolism and possible health effects of aluminum. Environmental Health Perspectives, 65, 363–441. [PubMed: 2940082]
2. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Aluminum http://www.atsdr.cdc.gov/toxprofiles/tp22.pdf, 2008, 14–126, viewed 2 February, 2018.
3. Exley C (2016) The toxicity of aluminium in humans. Morphologie, 100, 51–55. [PubMed: 26922890]
4. Delhaize E, Ryan PR Aluminum toxicity and tolerance in plants. (1995) Plant Physiology, 107, 315–321. [PubMed: 12228360]
5. Grevenstuk T, Romano A (2013) Aluminium speciation and internal detoxification mechanisms in plants: where do we stand? Metallomics, 5, 1584–1594. [PubMed: 24185904]
6. Epstein E (1994) The anomaly of silicon in plant biology. Proceedings of the National Academy of Sciences USA, 91, 11–17.

7. Hobara S, Fukunaga-Yoshida S, Suzuki T, Matsumoto S, Matoh T, Ae N. (2016) Plant silicon uptake increases active aluminum minerals in root-zone soil: Implications for plant influence on soil carbon. Geoderma, 279, 45–52.

8. Hopkins ES (1940) Colloidal chemistry in water treatment. Industrial and Engineering Chemistry, 32(2), 263–267.

9. Kapoor R, Metzger LE (2008) Process cheese: Scientific and technological aspects—a review. Comprehensive reviews in Food Science and Food Safety, 7, 194–214.

10. Adams D (1975) The mucus barrier and absorption through the oral mucosa. Journal of Dental Research, 54, B19–B26. [PubMed: 1055742]

11. ATSDR. Routes of Exposure, Toxicology Curriculum for Communities Trainer's Manual, 2002, Module II, pp. 95–100, http://www.atsdr.cdc.gov/training/toxmanual/pdf/module-2.pdf, Viewed 10 December 2017.

12. Squier CA (1991) The permeability of oral mucosa. Critical Reviews in Oral Biology and Medicine, 2, 13–32. [PubMed: 1912142]

13. Mauro M, Crosera M, Bianco C, Bellomo F, Bovenzia M, Adami G, Filon FL (2015) In vitro permeability of silver nanoparticles through porcine oromucosal membrane. Colloids and Surfaces B: Biointerfaces, 132, 10–16. [PubMed: 26001797]

14. Brody AR, Craighead JE (1975) Cytoplasmic inclusions in pulmonary macrophages of cigarette smokers. Laboratory Investigation, 32, 125–132. [PubMed: 163418]

15. Choux R, Pautrat G, Viallat J, Farisse P, Boutin C (1978) Inorganic cytoplasmic inclusions in alveolar macrophages. Archives of Pathology and Laboratory Medicine, 102, 79–83. [PubMed: 203246]

16. Girod CE, King TE Jr. (2005) COPD A Dust-Induced Disease? Chest, 128, 3055–3064. [PubMed: 16236986]

17. Pappas RS, Halstead MM, Watson CH (2016) Electron microscopic analysis of silicate and calcium particles in cigarette smoke tar. International Journal of Respiratory and Pulmonary Medicine, 3, 039 (DOI: 10.23937/2378-3516/1410039).

18. Halstead MM, Watson CH, Pappas RS (2015) Electron microscopic analysis of surface inorganic substances on oral and combustible tobacco products. Journal of Analytical Toxicology, 39, 698–701. [PubMed: 26286581]

19. Pappas RS, Fresquez MR, Martone N, Watson CH (2014) Toxic metal concentrations in mainstream smoke from cigarettes available in the USA. Journal of Analytical Toxicology, 38, 204–211. [PubMed: 24535337]

20. Sébastien P, Chamak B, Gaudichet A, Bernaudin J-F, Pinchon MC, Bignon J (1994) Comparative study by transmission electron microscopy of particles in alveolar and interstitial human lung macrophages. Annals of Occupational Hygiene, 38(Supplement 1), 243–250.

21. Shaykhiev R, Krause A, Salit J, Strulovici-Barel Y, Harvey B-G, O'Connor TP, Crystal RG (2009) Smoking-dependent reprogramming of alveolar macrophage polarization: Implication for pathogenesis of COPD. Journal of Immunology, 183, 2867–2883.

22. Exley C,Begum A, Woolley MB, Bloor RN (2006) Aluminum in tobacco and cannabis and smoking-related disease. The American Journal of Medicine, 119, 276.e9–276.e11.

23. Taylor JK (1987) Quality Assurance of Chemical Measurements 1st ed. Boca Raton, FL: CRC Press, pp. 79–81, 194.

24. Caudill SP, Schleicher RL, Pirkle JL (2008) Multi-rule quality control for the age-related eye disease study. Statistics in Medicine, 27, 4094–4106. [PubMed: 18344178]

25. Hu SS, Neff L, Agaku IT, Cox S, Day AR, Holder-Hayes E, King BA (2016) Tobacco product use among adults – United States, 2013–2014. Morbidity and Mortality Weekly Report, 65(27), 685–691. [PubMed: 27416365]

26. North Carolina State University. Smokeless Tobacco Reference Products 2018; https://strp.wordpress.ncsu.edu/product-information/ (Viewed 29 January 2018).

27. Ferin J, Oberdorster G (1992) Translocation of particles from pulmonary alveoli into the interstitium. Journal of Aerosol Medicine, 5, 179–187.

28. Lu S, Duffin R, Poland C, Daly P, Murphy F, Drost E, MacNee W, Stone V, Donaldson K (2009) Efficacy of simple short-term in vitro assays for predicting the potential of metal oxide

nanoparticles to cause pulmonary inflammation. Environmental Health Perspectives, 117, 241–247. [PubMed: 19270794]

Table 1.

Aluminum analytical method accuracy at two concentration levels relative to SRM certified values.

SRM	Mean ± S (μg/mg)	Certified ± U	Recovery
BCR-482	1.072 ± 0.061	1.103 ± 0.024	97.2%
NIST1573a	0.568 ± 0.066	0.598 ± 0.012	95.0%

Table 2.

Aluminum concentrations in various tobacco products. All brands are ® trademarks of the respective manufacturers.

Tobacco Source	Mean ± S, μg/mg
Copenhagen Pouches	0.501 ± 0.028
Red Seal Wintergreen Long Cut	0.474 ± 0.045
Southern Pride	0.271 ± 0.027
Taylor's Pride	0.387 ± 0.045
Smokeless Tobacco Average	**0.408 ± 0.104**
Cheyenne Full Flavor 100	1.26 ± 0.17
Cheyenne Green Xtreme Menthol 100	1.55 ± 0.36
Cheyenne Menthol Green	1.25 ± 0.41
Hav-a-Tampa Natural	0.937 ± 0.237
Phillies	0.901 ± 0.21
Remington Red 100	1.27 ± 0.37
Santa Fe Red	0.834 ± 0.19
Smokers Best Green Menthol 100	1.74 ± 0.22
Smokers Best Lights	1.29 ± 0.22
Swisher Red Sweets	0.944 ± 0.359
Vaquero Natural Black 100	1.57 ± 0.30
Vendetta 9mm Black	0.903 ± 0.220
Little Cigar Average	**1.20 ± 0.30**
Bold Largo Pipe Tobacco	1.24 ± 0.36
Bugler Pipe Tobacco	0.777 ± 0.157
C.W. Obel Bali Shag	0.813 ± 0.174
Gambler Tube Cut Cigarette Tobacco	1.30 ± 0.72
Red Cap Pipe Tobacco	0.716 ± 0.084
Top Regular	1.44 ± 0.25
Pipe/Roll Your Own Average	**1.05 ± 0.31**
American Spirit Blue	0.648 ± 0.082
American Spirit Black	0.522 ± 0.077
American Spirit Yellow	0.666 ± 0.036
American Spirit Orange	0.588 ± 0.056
Basic Blue 100	0.786 ± 0.128
Camel Crush	0.718 ± 0.146
Camel Blue Turkish	0.763 ± 0.073
Camel Wides Turkish	0.802 ± 0.060
Camel Yellow Filter 99	0.862 ± 0.189
Carlton 100s	0.614 ± 0.059

Tobacco Source	Mean ± S, μg/mg
Fortuna Red 100	0.846 ± 0.057
L and M Blue 100	0.778 ± 0.092
L and M Red	0.826 ± 0.314
Lucky Strike	1.06 ± 0.32
Marlboro 27	0.640 ± 0.086
Marlboro NXT	0.578 ± 0.085
Marlboro Black 100	0.776 ± 0.148
Marlboro Red 100	0.717 ± 0.050
Marlboros 72	0.754 ± 0.087
Marlboro Silver Virginia 100	0.369 ± 0.027
Marlboro Skyline Menthol	0.347 ± 0.033
Marlboro Smooth Menthol	0.641 ± 0.144
Marlboro Southern Cut Gold	0.994 ± 0.330
Merit Gold	0.735 ± 0.124
Pall Mall	0.823 ± 0.180
Parliament Blue 100	0.783 ± 0.112
Pyramid Red 100	0.628 ± 0.075
Rave Red 100	0.849 ± 0.100
Cigarette Tobacco Average	**0.719 ± 0.157**