Survey of metals present in the E-liquid of aged close pod-based and cigalike electronic-cigarettes from the North American market.

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Introduction

This study examined aged E-liquids for the presence of metals in Electronic Nicotine Delivery System (ENDS) cigalike and pod-based products purchased commercially within North America. The potential presence of metals and metalloids (e.g., Chromium, Lead, Nickel, Cadmium, Copper, Zinc, Antimony) in the aerosol or E-liquid are of interest because metals exposure may be linked to health effects such as cancer, cardiovascular disease, renal damage, and neurotoxicity. However, since metals have a low transfer efficiency from the e-liquid into the aerosol, the levels of metals in the aerosol will be lower than the levels measured in the E-liquid (Ref. Stephen Pappas et. al. for Toxic Metals in Liquid and Aerosol)

While there are variations in product design, metals may originate from the use of a metal coil (commonly nichrome) to heat an E-liquid as part of the aerosol generation process, or from soldered joints or other metallic parts of the device. The proximity of the metal components to the E-liquid can vary even when similar designs are used. In pods, the E-liquid is in direct contact with the heating element, and metals could leach from the heating coil into the E-liquid during storage.

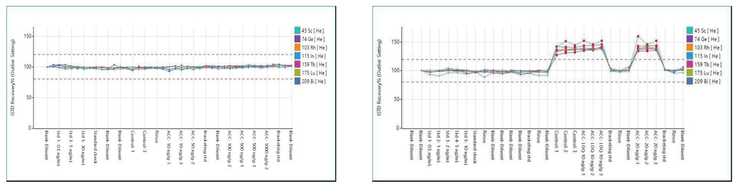
This study used single quadrupole inductively coupled plasma with mass spectrometer (ICP-MS) to examine six closed-pod and two cigalike devices, for a total of 27 unique combination of devices, E-liquid formulations, and batches. The products used in the study were purchased from retail channels and stored at ambient laboratory temperature and relative humidity (RH) condition for two years before testing to understand the impacts of worst-case long-term storage on the metals transfer to the E-liquid.

Experimental/Methods

Samples were analyzed using CORESTA Recommended Method No. 98 which was validated in-house and was used to perform the analysis to estimate the metals from the E-liquid. Analysis of all ENDS samples was performed by using Agilent 7800 ICP-MS with SPS4 autosampler. All the standards and samples were prepared by diluting 100-fold with diluent which contains 5% Nitric acid and 10% Methanol solution. Option gas as 20% oxygen in argon was used to eliminate carbon content which is generated by organic matrix. Helium gas was used as collision gas to remove argon related polyatomic interferences. For some of the element quantification, no gas mode was used due to low counts observed in gas mode. Sample dilution of 1:20 (w/v) was studied along with 1:100 (w/v) to optimize the method. Matrix effect was observed in internal standard response at 1:20 (w/v) due to high total dissolved solids (TDS). Conversely, using 1:100 dilution, the matrix effect was drastically reduced. The matrix effect is shown in Figure 1. A calibration curve was plotted at different levels of standards in the concentration range of 0.1 ng/mL to 100 ng/mL for all the elements. The calibration curve concentration range for each element is stated in Table1. The system suitability was assessed each day by checking the linear regression and % relative error at each concentration level for all metals. Continuous calibration verification solution (CCV) and secondary source standards as a part of system suitability were prepared at 30 ng/mL for each element. After every ten samples, a CCV and reagent blank sample were analyzed during the run. Acceptance criteria of 80-120% for CCV and concentration no more than lowest calibration standard for reagent blank samples were established.

Samples were stored at ambient laboratory temperature and RH after purchase and prior analysis. Two to seven replicate samples were prepared depending on the availability of the E-liquid. Due to limited quantity of some samples, preparations were scaled down. The final concentration of metals in E-liquid was calculated by applying factor of 100 to report the values in ng/g.

Figure 1. Internal standard response (1% TDS VS 5% TDS).



Validation

Validation parameters were evaluated as per Food and Drug Administration (FDA) draft guidance for validation of analytical testing methods for tobacco products. Accuracy was determined by using menthol and tobacco matrixes because most of the tested samples related to either menthol or tobacco flavor. Seven replicate preparations were spiked at low, mid, and high concentration ranges for accuracy. Accuracy results ranged from 83.3% to 109.4% of target concentration. Lowest level concentration met the acceptance criteria for recovery. Hence lowest level standard was determined as limit of quantitation (LOQ).

Linearity was confirmed at the calibration ranges stated in Table 1 to be between 0.1 ng/mL and 100 ng/mL. Linear regression (R^2) of all elements was considered acceptable by R^2 >= 0.995.

Method precision was evaluated by using seven replicate preparations at mid-level for all elements and intermediate precision was evaluated for 3 days at mid-level for all elements by two analysts. The method precision and intermediate precision results were observed below 8% for all elements. Repeatability was evaluated as within-run variation of five replicate aspirations. Repeatability was observed below 2.47% for majority of elements.

Method LOD was determined as per CORESTA Guide N° 28 by performing 23 aspirations of reagent blanks. The calculated LOD results were listed in **Table 1**.

$$LD = (T0.99 \times \sigma Blanks) + x$$

Where:

LD = Method Limit of Detection

T0.99 = Student's t-value appropriate for the single-tailed 99th percentile t statistic and a standard deviation estimate with sample size (n) minus 1 degrees of freedom.

σ = Standard deviation of Blank Values or lowest Calibration curve standard.

x = Average blank value

Table 1. Validation data for elements for interest

Element	Isotope	Linearity range	LOD	LOQ	%Accuracy		%Precision		%Intermediate Precision	
		ng/mL	ppb	ppb	Menthol	Tobacc	Menthol	Tobacco	Menthol	Tobacco
Lithium	^{7}Li	0.1- 100	5.7	50	100.0	89.4	1.2	1.4	2.3	3.0
Aluminum	^{27}Al	5-100	56.2	500	99.6	96.5	0.8	2.2	4.2	4.9
Vanadium	^{51}V	0.1-100	1.0	50	98.7	92.2	0.3	1.8	3.0	3.6
Chromium	^{52}Cr	0.5-100	6.8	50	103.7	97.3	0.5	1.6	2.2	2.7
Manganese	^{55}Mn	0.1-100	4.4	50	99.5	95.9	0.6	1.5	3.1	3.3
Iron	^{56}Fe	5-100	15.3	500	108.8	105	0.6	1.8	3.2	3.8
Cobalt	^{59}Co	0.1-100	2.1	50	96.2	93.4	0.5	1.7	3.1	3.4
Nickel	^{60}Ni	0.1-100	6.8	50	97.0	94.6	0.5	1.3	3.1	3.4
Copper	^{63}Cu	0.1-100	8.4	50	96.4	93.4	0.5	1.3	3.2	3.7
Zinc	^{66}Zn	5-100	88.3	500	109.4	91.9	1.5	2.5	7.9	5.5
Gallium	^{71}Ga	0.1-100	0.6	50	97.6	96.5	0.8	2.1	2.7	2.9
Arsenic	^{75}As	0.5-100	1.9	50	90.8	95.1	0.9	1.2	3.1	4.4
Zirconium	^{90}Zr	0.1-100	4.8	50	98.4	94.7	0.3	1.4	1.8	2.0
Molybdenum	^{95}Mo	0.1-100	3.8	50	99.2	92.6	0.3	1.0	1.8	1.5
Cadmium	^{111}Cd	0.1-100	7.2	50	100.3	97.8	0.3	1.3	2.6	2.7
Tin	^{118}Sn	0.5-100	5.0	50	89.0	94.1	0.8	1.2	1.9	1.4
Antimony	^{121}Sb	0.1-100	5.3	50	99.6	96.1	0.2	1.5	1.6	1.7
Barium	^{137}Ba	0.5-100	1.2	50	98.9	98.6	1.1	2.1	1.9	2.1
Tungsten	^{182}W	0.5-100	6.3	50	83.3	93.3	1.8	0.9	4.7	2.6
Platinum	^{195}Pt	0.1-100	10.1	50	88.4	91.6	1.5	1.1	6.5	4.1
Lead	^{208}Pb	0.1-100	7.4	50	91.0	95.4	1.3	1.2	6.3	4.0

References:

1 Validation and Verification of Analytical Testing Methods Used for Tobacco Products, U.S Department of Health and Human Services Food and Drug Administration Center for Tobacco Products, December 2021.

2 Steven Pappas et. al. Toxic metals in Liquid and Aerosol from Pod-Type Electronic Cigarettes. J Anal Toxicol. 2022 Feb 14; 46(1): 69-75.

3 CORESTA Recommended Method No. 98. Determination of select metals in E-liquid by ICP-MS.

4 CORESTA Guide No 28, Technical Guide for setting Method LOD and LOQ values for the determination of Metals in E-Liquid and E-Vapour Aerosol by ICP-MS.

Results and Discussion

The study included a total of 27 samples comprising several brands, flavors, and batches of both closed pod and cigalike ENDS devices. Table 2 contains the list of samples from different manufacturers that are represented by A, B, C, D, E and F. In that, cigalike devices are obtained from manufacturer A, cigalike and pod devices are obtained from manufacturer B and rest of the pod devices are obtained from manufacturers C, D, E & F. The number represents different flavors and batches. Method's limits of detection (LOD) and limit of quantitation (LOQ) are stated in Table 1 for each element. Samples and calibration curve were prepared as per the method stated in experiment section and analyzed using ICP-MS with single quadrupole mass analyzer. Rhodium, Lutetium and Terbium elements are used as internal standards to quantify all elements based on their ionization potential. The results show a lot of variation for metals in different lots and different flavors of the same manufacturers. Nickel, Copper, and Zinc were observed in most of the E-liquids in the range of 0.066 to 299.048 mcg/g, 0.099 to 352.225 mcg/g, and 0.589 to 184.417 mcg/g respectively. Lead, Chromium, Iron, Antimony, and Tin were observed in some of the E-liquids in the range of 0.226 to 21.213 mcg/g, 0.067 to 13.252 mcg/g, 0.725 to 46.393 mcg/g, 0.051 to 1.119 mcg/g and 0.1 to 1.85 mcg/g, respectively. Lithium, Manganese, Gallium, Tin, and Barium are found in a few samples at below 3 mcg/g levels. Vanadium, Arsenic, Zirconium, Molybdenum, Platinum, and Tungsten are detected below the quantitation limit. Cadmium was not detected for all liquids. Refer to Table 2 for the results.

Table 2. Metals analysis results for marketed formulations:

Formulation	Concentration (ng/g)										
	^{7}Li	^{52}Cr	^{56}Fe	^{59}Co	^{60}Ni	^{63}Cu	^{66}Zn	^{111}Cd	^{118}Sn	^{121}Sb	^{208}Pb
LOD	5.7	6.8	15.3	2.1	6.8	8.4	88.3	7.2	5.0	5.3	7.4
LOQ	50	50	500	50	50	50	500	50	50	50	50
A-1	BQL	BQL	BQL	BQL	65.8	219.1	BDL	BDL	BQL	715.4	BDL
A-2	BDL	67.4	1523.6	BQL	216.1	4231.0	4415.7	BDL	BQL	1118.5	BQL
B-1	BQL	145.0	5338.6	BQL	508.3	156,025	98,517	BDL	73.4	283.8	454.9
B-2	BQL	133.6	3787.4	BQL	637.7	208,614	115,732	BDL	99.9	447.0	322.6
B-3	BQL	125.1	4947.2	BQL	2001.6	207,337	112,612	BDL	136.1	63.8	385.4
B-4	BDL	369.6	7723.4	BQL	1191.1	352,225	184,417	BDL	1027.7	327.2	4960.1
B-5	2708.4	13,252	46,393	218.5	157,946	99.0	BQL	BDL	BDL	BDL	BDL
B-6	BQL	158.0	5507.1	BQL	341.3	164,394	91,514	BDL	166.6	375.9	707.5
B-7	2658.8	110.9	1313.3	135.3	299,048	BQL	BQL	BDL	BDL	BDL	BDL
B-8	BDL	BQL	725.3	BQL	BQL	BQL	589.2	BDL	BDL	BDL	BDL
C-1	113.2	165.4	1864.0	BQL	5651.4	5091.6	17,358	BDL	BQL	BDL	427.6
C-2	93.5	158.9	2145.5	BQL	5860.0	3719.9	25,362	BDL	BQL	BDL	225.9
C-3	106.0	264.7	3179.4	BQL	31,644	73,059	108,470	BDL	947.4	BQL	6753.6
C-4	96.2	155.5	1617.2	BQL	489.7	734.5	21,293	BDL	BQL	BDL	BQL
C-5	135.7	281.0	3854.0	BQL	113,763	152,038	174,212	BDL	1850.3	50.9	14,141
C-6	125.5	271.3	3364.2	BQL	1975.6	21,222	41,890	BDL	172.2	BQL	1088.8
C-7	105.8	298.5	3249.6	BQL	25,098	60,465	83,728	BDL	570.3	BQL	3916.0
D-1	BQL	BDL	BQL	BQL	887.5	7790.9	6317.5	BDL	BQL	BQL	BDL
D-2	BQL	BQL	805.3	BQL	1774.9	8773.5	10,500	BDL	91.2	BDL	BQL
D-3	BQL	BQL	BQL	BQL	1785.5	14,633	15,860	BDL	BQL	BDL	BDL
D-4	BQL	BQL	1115.6	BQL	3636.4	124,611	102,655	BDL	511.8	BDL	BQL
E-1	BQL	BQL	BQL	BQL	693.4	3436.4	4384.5	BDL	BQL	BDL	BDL
E-2	BQL	BQL	BQL	BQL	2898.7	10,035	9645.2	BDL	57.6	BDL	BQL
E-3	BDL	BQL	BQL	BQL	1116.9	47,807	35,941	BDL	206.2	BDL	BQL
E-4	BDL	BQL	BQL	BQL	1013.1	3479.9	3491.0	BDL	BQL	BDL	BDL
E-5	BDL	BQL	BQL	BQL	2217.7	99,563	74,995	BDL	344.5	BDL	BQL
F-1	BDL	245.3	5238.6	BQL	34,928	157,616	169,876	BDL	1720.7	BQL	21,213

BDL= Below Detection Limit. BQL= Below Quantitation Limit

Conclusion

The primary purpose of this study was to survey metals presents under worst-case long-term storage conditions for aged, commercially marketed products using a validated method. The data demonstrates that some metals can leach at high levels into E-liquids following prolonged storage at ambient temperature and relative humidity in some of the formulations due to extended contact between the liquid and metal components. Further, the results indicate that the amount of leaching varies among the manufacturers and formulations due to heater components of the devices are made with different compositions of elements. The metal concentrations in the liquid can be helpful in predicting storage time, improvements to device designs, and optimize the formulations to reduce exposure to these metals.

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