Effects of pH/Nicotine, Refill Fluid, Cigarette Type, and Storage on Element/Metal Concentrations in Electronic Cigarette Fluids and Aerosols

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Key Words: Electronic cigarettes, ENDS, pH, acids, metals, elements, chemical elements, e-cigarettes, refill fluid, aerosol
Abstract

The concentrations of metals, acids, nicotine, and flavor chemicals were analyzed in four refill fluids before and after vaping in third-and-fourth-generation electronic cigarettes (ECs), and the effect of storing fluids in ECs on element/metal concentrations was determined. Metals and organic chemicals were analyzed using inductively coupled plasma optical emission spectroscopy, HPLC, and GC/MS. Maleic, benzoic, propionic, and citric acid were the refill fluid (0.032 to 5.498 mg/mL). Nickel, zinc, tin, selenium, silicon, copper, and lead were often in refill fluids before and after heating and in aerosols. Third generation ECs and low pH fluids generally had the highest concentrations of metals in their fluids and aerosols. Thirty flavor chemicals were in at least one refill fluid. During storage, metal concentrations in EC fluids increased significantly by 28 days and increased exponentially over 387 days. After storage, EC fluids with the lowest pH often had the highest concentrations of nickel, zinc, lead, and tin. The acids and increase in metals after heating and storage are a health concern that could contribute to respiratory diseases. These data support the need for providing date-of-manufacture on packaging, regulating acids/metals in ECs, and better understanding of the effects of EC metals/acids on human health.

Keywords: electronic cigarettes, metals, acids, storage, nickel, copper, zinc, aerosols, leachates, toxicity
Introduction

Electronic cigarettes (ECs) are popular battery-operated devices that deliver nicotine and other chemicals to consumers [1-5]. ECs have evolved from first-generation cig-a-likes, to second- and third- generation tank- or mod-style ECs that include powerful batteries and large fluid reservoirs, to fourth-generation pod-style ECs that are low power and sleek in design [2,6-9].

All ECs have atomizers that contain metal components, such as the filaments (nickel, chromium), wires (copper, silver, nickel, tin, aluminum, iron, chromium), and wicks (silicon, calcium, aluminum, magnesium) [7,10-12], and these elements/metal can leach into the fluid and transfer to the aerosol [10,13-19]. Prior to use, e-liquids (liquid in an EC) and EC refill fluids also contain selenium, tin, aluminum, calcium, silicon, manganese, chromium, and nickel [15] and flavor chemicals (e.g., ethyl maltol, maltol, vanillin, cinnamaldehyde, diacetyl, benzaldehyde) [20-23] that are often present in high concentrations [24].

Nicotine concentrations in e-liquids are quite variable [25-27] and can be as high as 60 mg/mL in JUUL products [24]. It has recently been appreciated that EC fluids also contain organic acids, such as benzoic acid, lactic acid, and citric acid [12,28], which form salts with nicotine making it more palatable to novice users [29]. The aerosol inhaled during vaping is thus a complex mixture of these chemical groups plus reaction products that form during heating and are often known toxicants [30-31].

The effects of vaping complex chemical mixtures on lung health are beginning to emerge [32-33]. Individual case reports have been reviewed and include adverse effects on the respiratory, neurological, gastrointestinal, and immune systems [34-35]. “E-cigarette or Vaping Use-Associated Lung Injury” (EVALI) is a serious lung disease that has been linked to EC use [36-38]. EVALI peaked in September 2019, and while fewer cases are being documented now,
EVALI is still occurring, mainly in the United States [39-40]. While vitamin E acetate likely contributes to EVALI [41-42], some EVALI patients did not use products with vitamin E acetate [40], and the cause of their sickness has not been established [43].

Metals and acids, which cause inflammation and oxidative stress [44-47], are abundant in some EC aerosols and are candidates for promoting lung diseases including EVALI, either alone or in conjunction with other EC aerosol chemicals. Storage also increases the concentration of elements/metals in the fluid and can expose consumers to higher concentrations of elements/metals than normal, with chronic exposure could lead to more adverse health effects. In our prior study of ECs that had been stored for 5-10 years, e-liquids with the lowest pHs had the highest concentrations of elements/metals in their fluids and heating usually increased these concentrations [12]. In the current study, we tested the hypothesis that acid content, pH, nicotine, EC fluid, and EC type affect the amount of element/metal leaching into e-liquids and subsequently passing into aerosol.

There were two specific purposes to this study. The first was to analyze the effect of variables including fluid pH, nicotine concentration, refill fluid brand (Apple Original and Atomic Apple), and EC type (third- versus fourth- generation) on element/metal concentrations in EC fluids before and after heating and in aerosols. The second purpose was to determine the effect of storage on the leaching of elements/metals from atomizer components into e-liquids in ECs.

Materials and Methods

Refill fluid and EC selection: “Reds Apple - Apple Original” (7 Daze MFG, Ontario, CA) and “Sour Vape Atomic Apple” (Sour Vape, Carlsbad, CA) refill fluids with 0 or 6 mg/mL of nicotine were selected to allow fluids with different pHs to be compared. Two ECs were used: a third-generation VooPoo with Drag S tanks and fourth-generation JUUL™ batteries with third-party
pods. The ECs were selected as they were the most popular in our location at the time of the study for each generation. All ECs and fluids were stored at room temperature, and all fluids were used within 6 months of purchase.

**Elemental analysis of EC atomizer components using scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS):** The atomizers from Drag S and third-party pods were dissected and the components of interest (filaments, connector pins, air tube, and atomizer shells) were mounted on aluminum pin stubs covered with carbon tape for SEM and EDS elemental analysis [48-51]. The morphology and elemental composition of each sample were analyzed using a NovaNano SEM 450 (ThermoFisher Scientific Co., Waltham, MA) equipped with Oxford Instruments NanoAnalysis, Aztec Synergy energy dispersive X-ray spectrometer fitted with a X-Max 50 50 mm² SDD detector with energy resolution of 129 eV at MnKα in the Central Facility for Advanced Microscopy and Microanalysis at the University of California at Riverside as previously described [11]. SEM images were acquired from uncoated samples using the secondary electron mode with a dedicated detector at 15 kV. The distribution of elements in the atomizing units was determined by generating elemental maps using Aztec software. EDS mapping provided a qualitative depiction of the spatial distribution of chemical elements in the atomizer components. The detection limit for the EDS method was about 0.1 wt%.

**Organic acid identification and pH measurement of EC refill fluids:** To prepare organic acid samples and standards, authentic reference material for each target organic acid was dissolved in HPLC grade water to produce a stock solution. These stock solutions were combined in mobile phase A (see below) to produce a multipoint calibration standard ranging in
concentration from ~20 ng/µL to ~500 ng/µL for each target acid. Refill fluid samples were prepared for analysis by diluting 20 µL of sample in mobile phase A to 1 mL. The diluted refill fluid samples were shaken by hand until mixed, then run immediately.

Analyses were completed using an Agilent Technologies (Santa Clara, CA) Infinity 1260 HPLC with a UV-Vis absorbance detector cell. The wavelengths 210 nm and 230 nm (bandwidth 4 nm) were used for detection with 360 nm (bandwidth 80 nm) as the reference wavelength. An Agilent Infinity Lab Poroshell 120 SB-AQ column with the dimensions of 3.0 x 150 mm and 2.7-micron particle size was used for separation. The analytical column was protected by a 3.0 x 5 mm guard column of the same phase type and particle size. The column oven was kept at 35°C for the duration of the run. An injection volume of 2 µL was used for samples and standards. The total flow rate was 0.500 mL/min. Mobile phase A was composed of pH 2 phosphate buffer prepared in HPLC grade water with 1% HPLC grade acetonitrile and mobile phase B was 100% HPLC grade acetonitrile. The mobile phase gradient used was as follows: 100% A from start until 4.5 mins, then grade to 40% A at 11.5 mins until 16 mins, then 100% A at 16.1 mins until 20 mins.

For pH determination, CO$_2$-free water was first prepared for dilution of the refill fluids. Deionized water was sonicated for 20 mins then sparged with high purity nitrogen for another 20 mins and stored under nitrogen until needed. To prepare a sample for pH measurement, 0.5 mL of refill fluid was diluted with 4.5 mL of CO$_2$-free water and mixed for 30 seconds via stir bar before electrode insertion. Analyses were carried out using a Mettler Toledo T50 titrator equipped with an InLab® Micro pH electrode (Mettler, Toledo, Columbus, OH), which was calibrated prior to each sampling session. For each sample solution, the potential (mV) was monitored until the reading was stable. A solution pH was then calculated using the electrode calibration equation. During analyses the sample container was covered, and the headspace
was kept under a gentle nitrogen stream to minimize CO$_2$ infiltration. All pHs were measured in freshly purchased samples.

Preparation of samples for elemental analysis in the fluid and aerosol: The element concentrations in the EC fluids before use, after use, and in the aerosol were analyzed using inductively coupled optical emission spectroscopy (ICP-OES). “Fluid before” refers to fluid sampled directly from the refill fluid bottle. “Fluid after” refers to fluid sampled from the tank and pod following aerosol production. All fluid samples were prepared as previously described [15]. For all fluid types, 500 µL of e-liquid was dissolved in 9.5 mL of 2% nitric acid/98% deionized water. Fluid samples were stored in 15-mL nitric acid-washed conical tubes.

For aerosol analysis, each refill fluid was filled into an individual tank or pod. Aerosols were then generated on a smoking machine and collected in two glass impingers in tandem using an interval puffing protocol described previously [15,48]. The interval puffing protocol takes a 4.3 s puff every minute for 10 minutes, then followed by a 5–10-minute rest period. Aerosols were dissolved in a solution of 2% nitric acid/98% deionized water (Table S1). Room air samples were prepared similarly by bubbling room air through the impingers. All aerosol samples were stored in 15-mL nitric acid washed conical tubes.

Preparation of EC refill fluid samples for analysis of elemental leaching: To evaluate the effects of nicotine and pH on leaching of elements/metals with storage, each refill fluid at both nicotine concentrations was filled into individual Drag S tanks or third-party JUUL™ pods and allowed to age at room temperature. Samples were collected on Day 0 from the bottle, and from the tanks/pods on Day 28 and Day 56. For Drag S tanks, fluid was also analyzed on Day 387. Fluid was also sampled from the refill bottles on Day 387 to determine how the fluid aged in the bottle.
during storage. All leaching fluid samples were prepared for ICP-OES analysis as described above.

Elemental Analysis of EC refill fluid and aerosol samples: Twenty-two elements/metals were screened in the fluids and aerosols using ICP-OES on an Optima 7300 DV ICP-OES (Perkin-Elmer, Waltham, MA) as described previously [15,48-49]. In addition, standard curves (0.0005 to 10,000 mg/L) of the 22 elements were prepared and analyzed. A 2% nitric acid blank was analyzed, and concentrations in the blank were subtracted from all test samples. For every refill fluid and aerosol, the samples were analyzed in triplicates.

Analysis of flavor chemicals and nicotine in EC refill fluid: Both refill fluids were prepared for nicotine and flavor chemical analysis as described in detail previously [22,24]. All samples were prepared at 1:20 dilution by dissolving 50 µL of EC fluid into 950 µL of isopropyl alcohol (Table S1). For samples that may oversaturate the column, a 1:200 dilution was prepared by dissolving 5 µL of EC fluid into 995 µL of isopropyl alcohol. Samples were prepared and stored in amber GC vials. 180 flavor chemicals and nicotine were screened in the two refill fluids using gas chromatography and mass spectrometry (GC-MS) on an Agilent 5975 C GC-MS system (Santa Clara, CA). An isopropyl alcohol blank was also analyzed. All of the samples were analyzed for flavors and nicotine in March and November of 2021.

Statistical analysis: The raw data were used to statistically analyze the total and individual concentrations of the elements/metals measured in four refill fluids (two with nicotine and two without nicotine in both Apple Original and Atomic Apple), two pH/nicotine concentrations, and
in the two EC types. All analysis of variance (ANOVA) with the Fisher post-hoc test and Bonferroni correction was performed for each refill fluid, pH/nicotine, and EC using Minitab (Minitab, Inc., State College, PA), and results were graphed using Prism (GraphPad, La Jolla, CA) (in storage experiment lines were fitted to data). When p values were less than 0.013, the comparison was considered significantly different. When a concentration was below the limit of detection, it was treated as a zero for the statistical analysis.

For the relationship between refill fluid brand, pH/nicotine, and EC type on total and individual element/metal concentrations in fluids and aerosols: Total and individual element/metal concentrations, a three-way ANOVA was performed as described above. If the three factors (refill fluid brand, pH/nicotine, EC type) did not yield significance, the model was refit and a two-way ANOVA was performed. In the case of concentration in the fluid before use, when the three- and two- factor ANOVA did not yield significance, the model was refitted to include only refill fluid brand. For the concentrations in the fluid before use, when p values were less than 0.05, the comparison was considered significantly different.

The individual element/metal concentrations in fluids during storage: For individual element/metal concentrations, a two-way ANOVA was performed as described above. Factors included day, pH/nicotine.

Additional Information on Methods

Additional information on EDS, running conditions and instrument information for the ICP-OES, and details on the interactions of the ANOVA for the experiments comparing the relationship between refill fluid brand, pH/nicotine, and EC type, and leaching of elements/metals over time are listed in the Supplemental Material.
Results

*Chemical and morphological analysis of EC atomizing components.*

To determine the elemental composition of the Drag S and third-party pod atomizers, each component was analyzed using SEM and EDS (Figure 1). The filaments in both brands were comprised of chromium, iron, and aluminum (kanthal), with the third-party pod also containing nickel (Figure 1A-D, M-P, T, W). The filament support in the Drag S and the air tube in the third-party pod was comprised of only nickel (Figure 1E-F, U-W). The Drag S atomizing shell was nickel and tin (Figure 1G-H, W). The Drag S battery interfacing and third-party pod connector pins were nickel with gold plating (Figure 1I-K, Q-S, W).
Figure 1. SEM analysis and EDS elemental maps of Drag S and third-party pod atomizing units. (A) Mesh filament from Drag S was comprised of chromium (B), iron (C), and aluminum (D). (E) Filament support in the Drag S was comprised of nickel (F). G. The atomizing unit shell was comprised of nickel (H) and tin (L). (I) The battery interface plate of Drag S was nickel (J) plated with gold (K). (M) The filament from the third-party pod contained nickel (N), chromium
(O), iron (P), and aluminum (T). Q. Battery connector pins were nickel (R) plated with gold (S).

(U) The air-tube in the third-party pod contained nickel (V). (W) Heat map showing all elements identified in Drag S and third-party pod atomizing units. Red squares indicate the element was a major peak in the EDS analysis. Pink squares indicate the element was a minor peak in the EDS analysis. White squares indicate the element was not detected in the component.

**Organic acids and pH of the EC refill fluids.**

The concentrations of the organic acids and the measured pHs in the two EC refill fluids are summarized in Table 1. Of 12 organic acids measured in Apple Original and Atomic Apple refill fluids, five were above the limit of quantification (> 0.012 mg/mL) (Table 1), and seven were either not detected or < LOQ (Table S10). Maleic, benzoic, and citric acids were present in all refill fluid samples, while propionic acid was only in Atomic Apple. The concentration of organic acids ranged from 0.032 to 5.498 mg/mL. The highest concentration was maleic acid was greater than 2 mg/mL in all but one fluid. In contrast, benzoic, propionic, and citric acid concentrations ranged from 0.032 to 0.766 mg/mL. When nicotine was present in the refill fluid, the concentrations of maleic acid increased, while the concentrations of benzoic, propionic, and citric acid decreased. All refill fluids had relatively low pHs (< 3.59), except for Apple Original with 6 mg of nicotine, which was pH ~7.85.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Flavor</th>
<th>Solvent</th>
<th>Nicotine Conc</th>
<th>Maleic Acid</th>
<th>Benzoic Acid</th>
<th>Propionic Acid</th>
<th>Citric Acid</th>
<th>pH</th>
</tr>
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<tbody>
<tr>
<td>Daze Red Apple</td>
<td>Apple Original</td>
<td>VG/PG</td>
<td>0</td>
<td>0.473</td>
<td>0.766</td>
<td>0.040</td>
<td>3.26</td>
<td></td>
</tr>
<tr>
<td>Daze Red Apple</td>
<td>Apple Original</td>
<td>VG/PG</td>
<td>6</td>
<td>2.067</td>
<td>0.283</td>
<td>0.032</td>
<td>7.85</td>
<td></td>
</tr>
<tr>
<td>Sour Vape</td>
<td>Atomic Apple</td>
<td>70 VG/30 PG</td>
<td>0</td>
<td>3.182</td>
<td>0.145</td>
<td>0.082</td>
<td>0.063</td>
<td>2.58</td>
</tr>
<tr>
<td>Sour Vape</td>
<td>Atomic Apple</td>
<td>70 VG/30 PG</td>
<td>0</td>
<td>4.240</td>
<td>0.129</td>
<td>0.098</td>
<td>0.063</td>
<td>2.59</td>
</tr>
<tr>
<td>Sour Vape</td>
<td>Atomic Apple</td>
<td>70 VG/30 PG</td>
<td>6</td>
<td>5.498</td>
<td>0.094</td>
<td>0.047</td>
<td>0.056</td>
<td>3.59</td>
</tr>
</tbody>
</table>

Abbreviations:VG; Vegetable Glycerin, P; Propylene Glycol. Color coding: red indicate highest concentrations, green indicate the lowest concentrations.
The relationship between pH/nicotine, refill fluid brand, and EC type on total element/metal concentrations in fluids and aerosols.

To evaluate the effects of pH/nicotine, refill fluid brand, and EC type on total and individual element/metal concentrations, Apple Original refill fluid with a low nicotine/low pH (0 mg, pH ~3.26) and high nicotine/high pH (6 mg, pH ~7.85) and Atomic Apple with a low nicotine/low pH (0 mg, pH ~2.58) and high nicotine/low pH (6 mg, pH ~3.59) were filled into Drag S tanks and third-party pods. Aerosols were generated and the concentrations of 22 elements were measured in the fluid before heating, the fluid after heating, and in the aerosol (Figures 2-7, Figure S1-S2, Table S2-S5). The effects of pH/nicotine, refill fluid product, and EC type on element/metal concentrations were analyzed to determine statistical significance as described in the Materials and Methods.
Figure 2. Average total element/metal concentrations in ECs. (A, B) The total concentration of 22 elements/metalts in refill fluids before and after heating. (C) The effect of refill fluid brand and EC type on total element/metal concentrations in fluids after heating. (D) The concentration
of total elementals/metals transferred to the aerosol for each refill fluid brand and EC type. (E)
The effect of refill fluid brand and EC type on transfer of total elements/metals to the aerosol. In
A, B, and D, each bar is the mean ± standard deviation of three independent measurements. In
C and E, data were analyzed using a two-factor ANOVA (refill fluid brand, EC type). When
means were significant, a Fisher post hoc test with Bonferroni correction was applied; * = p ≤ 
0.013, ** = p ≤ 0.003, *** = p ≤ 0.000. The statistical data for fluid before heating were not
significantly different and were not plotted.

In both the Apple Original and Atomic Apple, the total element/metal concentrations in
the fluid before use (directly out of the bottle) were very low (< 0.5 mg/L) in both brands (Figure
2A). The concentrations of total elements/metals in the fluid after heating were much higher
than in the fluid before heating, and measured up to 8 mg/L, as shown in Figure 2B for each
refill fluid in both EC types. In a two factor ANOVA, both the refill fluid product and the EC type
affected total element/metal concentrations (Figure 2C). The Atomic Apple refill fluids had
significantly higher total element concentrations than the Apple Original refill fluids (Figure 2C).
The fluids filled into the Drag S had significantly higher total concentrations than fluids filled into
the third-party pods (Figure 2C). There was transfer of elements/metals into the aerosol in both
Drag S and third-party pods containing both the Apple Original and Atomic Apple refill fluids
(Figure 2D). Both the refill fluid and the EC type affected total element/metal concentrations in
the aerosol (Figure 2E). The total concentration of elements/metals that transferred to the
aerosol made with Apple Original in the third-party pod was significantly higher than the Atomic
Apple counterpart (Figure 2E). The total concentration of elements/metals in Drag S filled with
Atomic Apple was significantly higher than the total concentrations in the third-party pods
(Figure 2E).
The relationship between pH/nicotine, refill fluid brand, and EC type on individual element/metal concentrations in fluids and aerosols.

Nickel concentrations in the fluid before use (directly out of the bottle) were very low (< 0.02 mg/L) in all refill fluids, with Apple Original having a significantly higher concentration than Atomic Apple (Figure 3A-B). The concentrations of nickel in the fluid after heating are much higher than in the fluid before heating, measuring up to 3 mg/L in the Atomic Apple fluids (Figure 3C). The refill fluid and the EC type significantly affected nickel concentrations in the fluid after heating (Figure 3D). In both ECs, Atomic Apple fluids had significantly higher concentrations of nickel than Apple Original fluids (Figure 3D). The fluids filled in the Drags S had significantly higher nickel concentrations than third-party pods (Figure 3D). Nickel transfer to the aerosol was variable with the highest concentration being about 0.8 mg/L in both refill fluid brands and EC types (Figure 3E). The refill fluid and the EC type affected nickel concentrations in the aerosol (Figure 3F). The Atomic Apple had significantly higher concentrations of nickel in the aerosol than the Apple Original (Figure 3F). The concentration of nickel in Atomic Apple aerosols was significantly higher in the Drag S than in the third-party pods (Figure 3F).
Figure 3. Average concentrations of nickel in ECs. (A) The concentrations of nickel in the fluid before heating. (B) The effect of refill fluid brand on nickel concentrations in fluids before heating. (C) The concentrations of nickel in the fluid after heating. (D) The effect of refill fluid brand and EC type on nickel concentrations in fluids after heating. (E) The concentrations of nickel that transferred to the aerosol for each refill fluid brand and EC type. (F) The effect of refill
fluid brand and EC type on transfer of nickel to the aerosol. In A, C, and E, each bar is the mean ± standard deviation of three independent measurements. In B, data were analyzed using a one-way ANOVA (refill fluid brand). * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001. In D and F, data were analyzed using a two-factor ANOVA (refill fluid brand, EC type). When means were significant, a Fisher post hoc test with Bonferroni correction was applied; * = p ≤ 0.013, ** = p ≤ 0.003, *** = p ≤ 0.000.

Zinc concentrations in the fluid before use (directly out of the bottle) were very low (< 0.04 mg/L) in both Atomic Apple and Apple Original fluids, and there was no significant difference between the means (Figure 4A). The concentrations of zinc in the fluid after heating were much higher than in the fluid before heating, measuring up to 6 mg/L in Atomic Apple in the Drag S (Figure 4B). The refill fluid brand and the EC type significantly affected zinc concentrations (Figure 4C). The concentration of zinc in the Atomic Apple fluids after heating were significantly higher than in the Apple Original (Figure 4C). The fluids in the Drags S had significantly higher concentrations of zinc than the fluids in the third-party pods (Figure 4C). Zinc did transfer into the aerosol in both refill fluid brands and both EC types, although transfer efficiency was variable with a maximum concentration of 0.4 mg/L in Drag S with Atomic Apple (Figure 4D). There was no significant difference between the means (Figure 4D).
Figure 4. **Average concentrations of zinc in ECs.** (A) The concentrations of zinc in the fluid before heating. (B) The concentrations of zinc in the fluid after heating. (C) The effect of refill fluid brand and EC type on nickel concentrations in fluids after heating. (D) The concentration of zinc that transferred to the aerosol for each refill fluid brand and EC type. In A, B, and D, each bar is the mean ± standard deviation of three independent measurements. In C, data were
analyzed using a two-factor ANOVA (refill fluid brand, EC type). When means were significant, a Fisher post hoc test with Bonferroni correction was applied; \(* = p \leq 0.013, \** = p \leq 0.003, \*** = p \leq 0.000\). The statistical data for fluid before heating and transfer to the aerosol were not significantly different and were not plotted.

Tin concentration in the fluid before use (directly out of the bottle) was very low (<0.08 mg/L) in both fluids, but the concentrations in Atomic Apple were significantly higher than in Apple Original (Figure 5A-B). The concentrations of tin in the fluid after heating was higher than the fluid before heating, measuring up to 0.2 mg/L in Atomic Apple in Drag S (Figure 5C). The refill fluid product and the EC type affected tin concentrations in fluid after heating (Figure 5D). The Atomic Apple fluids had significantly higher concentrations of tin than Apple Original fluids (Figure 5D). The refill fluids in the Drags S had significantly higher concentrations of tin than fluids in the third-party pods (Figure 5D). Tin did not transfer well into the aerosol, except in Atomic Apple 6 mg in Drag S, which had 0.15 mg/L in the aerosol (Figure 5E). The refill fluid brand and EC type affected tin concentrations in the aerosol (Figure 5F). The concentration of tin in the Atomic Apple fluid was significantly higher than the concentration in the Apple Original (Figure 5F). The Atomic Apple filled into the Drag S had a significantly higher concentration of tin than the third-party pods (Figure 5F).
Figure 5. Average concentrations of tin in ECs. (A) The concentrations of tin in the fluid before heating. (B) The effect of refill fluid brand on tin concentrations in fluids before heating. (C) The concentrations of tin in the fluid after heating. (D) The effect of refill fluid brand and EC type on tin concentrations in fluids after heating. (E) The concentration of tin that transferred to the aerosol for each refill fluid brand and EC type. (F) The effect of refill fluid brand and EC type
on transfer of tin to the aerosol. In A, C, and E, each bar is the mean ± standard deviation of three independent measurements. In B, data were analyzed using a one-way ANOVA (refill fluid brand). * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001. In D and F, data were analyzed using a two-factor ANOVA (refill fluid brand, EC type). When means were significant, a Fisher post hoc test with Bonferroni correction was applied; * = p ≤ 0.013, ** = p ≤ 0.003, *** = p ≤ 0.000.

Selenium concentrations in the fluid before use (directly out of the bottle) measured 0.1 to 0.2 mg/L (Figure 6A), which was within the concentration range reported in previously [15], and the concentration in Apple Original was significantly higher than Atomic Apple (Figure 6B). Selenium in the fluid after heating was similar to the concentrations in the fluid before heating, measuring on average 0.144 mg/L (Figure 6C). There was transfer of selenium to the aerosol in both ECs and in all refill fluid groups, although transfer efficiencies were variable (Figure 6D). The refill fluid brand and EC type affected selenium concentrations in the aerosol (Figure 6E). The mean concentration of selenium was significantly higher only in the Atomic Apple filled into the Drag S in comparison to the Atomic Apple into the third-party pod (Figure 6E).
Figure 6. Average concentrations of selenium in ECs. (A) The concentrations of selenium in the fluid before heating. (B) The effect of refill fluid brand on selenium concentrations in fluids before heating. (C) The concentrations of selenium in the fluid after heating. (D) The concentration of selenium that transferred to the aerosol for each refill fluid brand and EC type. (E) The effect of refill fluid brand and EC type on transfer of selenium to the aerosol. In A, C,
and D, each bar is the mean ± standard deviation of three independent measurements. In B, data were analyzed using a one-way ANOVA (refill fluid brand). * = p ≤ 0.05, ** = p ≤ 0.01, *** = p ≤ 0.001. In E, data were analyzed using a two-factor ANOVA (refill fluid brand, EC type). When means were significant, a Fisher post hoc test with Bonferroni correction was applied; * = p ≤ 0.013, ** = p ≤ 0.003, *** = p ≤ 0.000. The statistical data for fluid after heating were not significantly different and were not plotted.

Silicon was only found in Atomic Apple with no nicotine (directly out of the bottle) (<0.08 mg/L) (Figure 7A). Silicon was measured in the fluid after heating at concentrations up to 0.2 mg/L (Figure 7B). The EC type affected silicon concentrations (Figure 7C) with Drag S having significantly higher concentrations of silicon in its fluids than the third-party pods (Figure 7C). Silicon did transfer into the aerosol in most groups and was measured at concentrations up to 0.25 mg/L (Figure 7D), but there was no significant difference between the concentration means.
Figure 7. Average concentrations of silicon in ECs. (A) The concentration of silicon in the fluid before heating. (B) The concentrations of silicon in the fluid after heating. (C) The effect of refill fluid brand and EC type on silicon concentrations in fluids after heating. (D) The concentration of silicon that transferred to the aerosol for each refill fluid brand and EC type. In
A, B, and D, each bar is the mean ± standard deviation of three independent measurements. In C, data were analyzed using a two-factor ANOVA (refill fluid brand, EC type). When means were significant, a Fisher post hoc test with Bonferroni correction was applied; * = p ≤ 0.013, ** = p ≤ 0.003, *** = p ≤ 0.000. The statistical data for fluid before heating and transfer to the aerosol were not significantly different and were not plotted.

Copper was found only in Atomic Apple fluids before use (directly out of the bottle) (<0.02 mg/L) (Figure S1A) and was significantly higher than in Apple Original (Figure S1B). Copper was measured in the fluid after heating at concentrations reaching up to 0.06 mg/L (Figure S1C). The refill fluid brand and EC type affected copper concentrations in the fluid after heating (Figure S1D). After heating, the Atomic Apple fluids had significantly higher concentrations of copper than the Apple Original fluids in the third-party pods (Figure S1D). The Apple Original fluids in the Drag S had significantly higher concentrations of copper than the Apple Original in the third-party pods (Figure S1D). Copper did not transfer well to the aerosol (generally <0.015 mg/L), except for Apple Original (6 mg) in the third-party pod, which had 0.15 mg/L of copper in its aerosol (Figure S1E).

Lead was not detected in either refill fluid before use (directly out of the bottle) (Figure S2A). The concentrations of lead in the fluids after heating were relatively low (maximum = 0.08 mg/L) (Figure S2B) and were higher in the ECs with Atomic Apple fluid. The refill fluid brand did affect lead concentrations after heating (Figure S2C). The Atomic Apple refill fluids had significantly higher concentrations of lead than the Apple Original fluids (Figure S2C). Lead did transfer into the aerosols but was variable and yielded the highest (0.04 mg/L) in Apple Original fluid in the third-party pods (Figure S2D).
Effect of pH/nicotine, refill fluid brand and ED type on leaching of elements/metals into refill fluids during storage.

To determine how metal concentrations change over time during storage in ECs, Drag S tanks and third-party pods were filled with Apple Original refill fluid containing 0 mg of nicotine/low pH (pH ~3.26) or 6 mg of nicotine/high pH (pH ~7.85) or with Atomic Apple with 0 mg of nicotine/low pH (pH ~2.58), or 6 mg of nicotine/low pH (pH ~3.59) and allowed to age at room temperature. After 28 and 56 days, fluids were sampled from each tank and pod, and the metal concentrations were analyzed. Samples were aged for 387 days in the Drag S. Day 0 samples were taken directly from the bottle. The metal concentrations for nickel, zinc, lead, tin, and copper are presented for both ECs and all refill fluids in Figure 8 and Supplemental Tables S6-S9.
Figure 8. Effects of pH/nicotine, refill fluid brand and EC type on leaching of metals from Drag S tanks and third-party pods during storage. The concentration of nickel (A-D), zinc (E-H), lead (I-L), tin (M-P), and copper (Q-T) were measured during storage in Drag S tanks and third-party pods filled with Apple Original and Atomic Apple refill fluids. Each point is the mean ± standard deviation of three independent samples. Concentrations were measured in both EC brands on Day 0, Day 28, and Day 56, and on Day 387 in Drag S only. All data were analyzed using a two-way ANOVA (day, pH/nicotine) was performed. When means were significant, a Fisher post hoc test with Bonferroni correction was applied. For Drag S: * = p ≤ 0.007, ** = p ≤ 0.001, *** = p ≤ 0.000; third-party pods: * = p ≤ 0.010, ** = p ≤ 0.002, *** = p ≤ = 0.000.
By 28 days, there were significant increases in nickel, zinc, lead, tin, and copper concentrations in some of the fluids that had aged in both brands of ECs (Figure 8A-H, J-N, Q-T). In the Drag S containing Apple Original, there was an exponential increase in nickel, zinc, lead, tin, and copper concentrations in the low pH fluids during storage (Figure 8A, E, I, M, Q), with the high pH counterpart always having lower concentrations of these elements, except for copper. In Drag S containing Atomic Apple fluids (which both had low pHs while one also had nicotine), there was also an exponential increase in the concentrations of nickel, zinc, lead and tin in the fluids without nicotine during storage (Figure 8A, E, I, M, Q). However, zinc, lead, tin and copper also had an exponential increase in concentration in fluids with low pH and contained nicotine (Figure 8G, K, O, S).

In the third-party pods containing Apple Original fluids with low and high pH, nickel, zinc, lead, and copper concentrations increased exponentially in fluids over 56 days of storage, with concentrations usually being higher in the low pH fluid, except for copper (Figure 8B, F, J, N, R). In the third-party pods containing low pH Atomic Apple fluids with or without nicotine, nickel, zinc, lead, and copper concentrations in fluids increased exponentially over 56 days of storage, with concentrations of zinc and copper being higher in the low pH fluid that contained nicotine (Figure 8D, H, P, T). The absence of significant changes in tin concentration in the third-party pods may be due to an absence of tin in the atomizers of these pods (Figure 8L) or could be due to tin depositing on surfaces [52].
Concentration of nicotine and flavor chemicals in EC refill fluids

The concentrations of nicotine and the flavor chemicals in the four refill fluids are summarized in Table 2. The concentration of nicotine in Apple Original was 6.77 mg/mL, which is 13% higher than the labeled concentration (6 mg). In Atomic Apple, the concentration was 6.23 mg/mL, which was 4% higher than the label (6 mg/L). Eight months later, the nicotine concentration in both refill fluids had decreased by 12% to 5.28 mg/mL (Apple Original) and 5.28 mg/mL (Atomic Apple). The refill fluids labeled 0 mg nicotine did not contain nicotine.

Forty-one flavor chemicals were identified in the refill fluids (Table 2). Thirty were above the limit of quantifications (> 0.01 mg/mL). The 11 flavor chemicals that were below the limit of quantification are in Supplemental Table S11. Apple Original contained 22-23 flavor chemicals at both pHs, while Atomic Apple 0 mg had only 3-6 flavor chemicals, and Atomic Apple 6 mg had 16-17 flavor chemicals. The concentrations of the individual flavor chemicals ranged from 0.011 to 6.980 mg/mL. The five flavor chemicals with the highest concentrations were: 1-hexanol (6.980 mg/mL), ethyl 2-methylbutanoate (6.334 mg/mL), triacetin (4.373 mg/mL), hexyl acetate (3.914 mg/mL), and amyl acetate (2.744 mg/mL) (located at the top of the table). Over 8 months of storage, all but six of the flavor chemicals decreased in concentration with the largest percentage decrease (64%) being isoamyl isovalerate.
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<td>3.667</td>
<td>0.808</td>
<td>0.766</td>
<td>6.980</td>
<td>5.107</td>
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<td>0.042</td>
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<td>0.549</td>
<td>0.188</td>
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<td>Ethyl Isobutyrate</td>
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<td>γ-Decalactone</td>
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<td>0.025</td>
<td>0.023</td>
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<tr>
<td>cis-Linalool oxide</td>
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<td>0.022</td>
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</table>

Color coding: red indicate highest concentrations, green indicate the lowest concentrations.
Discussion

This study examined the elements/metals in atomizers from Drag S and 3rd party JUUL compatible pods, the acids in two popular brands of refill fluids (Apple Original and Atomic Apple), the effect of heating on element/metal concentrations in the EC fluids and aerosols, and the effect of storage of fluids in the ECs on metals in the fluids. In both the heating and storage experiments, the variables included pH/nicotine, EC type, and refill fluid brand/flavor. The atomizers from the Drag S tank and third-party pods contained elements/metals that were also present in the refill fluids and aerosols. Of six organic acids in the refill fluids, maleic acid was the highest in concentration.

The major findings of the experiment that involved heating different fluids in two different ECs were: (1) Total and individual element/metal concentrations generally increased in fluids after heating, and these increases were significantly affected by both the EC type and refill fluid used. Concentrations were generally higher in the fluids of high-powered Drag S ECs than in the 3rd party pods operated on a low powered JUUL battery, and concentrations were significantly higher in the “Atomic Apple” than in the “Apple Original” fluids. A similar relationship between power and metal concentration has been reported in other studies [15-17,48,53-54]; (2) In general, transfer to the aerosol was greater for “Atomic Apple” in Drag S ECs than for “Atomic Apple” in 3rd party pods, in agreement with studies showing low transfer efficiency with lower power devices (e.g., first- and fourth- generation ECs) [7,49-51,55-59]. In the 3rd party pods, element/metal transfer to aerosols was better for the “Apple Original” refill fluid than for “Atomic Apple”, indicating that refill fluid per se can affect transfer; (3) pH/nicotine did not significantly affect total or individual element/metal concentrations in refill fluids before heating, after heating, or in aerosols, perhaps because the pH and nicotine were not identical in the two commercial products. Future studies with laboratory made fluids with precisely controlled pH may be better able to identify effects of acids/nicotine on metal release into fluid.
The major conclusions from the storage experiment were: (1) Most individual element/metal concentrations increased exponentially with storage time, often reaching significance by 28 days of storage (e.g., nickel and zinc) and increasing many fold by day 387 for some metals, such as nickel; (2) Sometimes element/metal release was much higher in the refill fluid with low pH (e.g., nickel release from Drag S with “Apple Original” at pH 3.26 versus “Apple Original” at pH 7.85); (3) For some elements/metals, release from atomizer components was similar in both low and high pH fluid (e.g., zinc in Drag S with Atomic Apple); (4) In both EC brands with “Atomic Apple”, copper release was significantly higher in the fluids that contained nicotine; since the fluid pHs were similar, these data suggest that the nicotine rather than pH increased copper release, and (5) in one case (Atomic Apple fluid in the third party pod), tin did not increase during storage, probably indicating tin was not present in this atomizer.

Source of Elements/Metals. Elements/Metals in both the heating and storage experiments likely came from the refill fluid solvents (PG and VG) and the atomizer components. PG and VG contain elements, including selenium, zinc, chromium, lead, iron, aluminum, and tin [10,15-16,57,60], and third- and fourth- generation atomizers contain metal components [7,10-13,61]. Nickel, which was in the filaments, atomizing shells, and air-tubes of Drag S and third-party pods, was the most frequently identified metal in the heated fluids and in the aerosols. While nickel could originate from any of these components, a prior dual element particle analysis suggested it comes from the stainless steel in other fourth generation pods [61]. Lead and zinc were measured in the fluids and aerosols but were not detected in any of the atomizer components, perhaps because their atomizer concentrations were below the limit of detection by SEM/EDS or because they were in atomizer components that we did not analyze. Tin could be a component of the solvents, but also could originate from the atomizing components in the Drag S. Chromium, iron, and aluminum were in the atomizing units, but their concentrations were low in heated fluids, suggesting they are not readily released into fluids, in
agreement with other studies [12,16]. EC users have reported a metallic taste in some products [62]. There is clear variability in the elements/metals in aerosols, which may explain why metallic taste is not found with all EC products.

**pH and organic acid in EC fluids.** In 2014, the pH of EC fluids was reported to vary among products [63]. The pHs of the refill fluids in our study ranged from 2.58 to 7.85 and were similar to the range (3.60 to 9.60) reported in other products [13,29,64-65], including JUUL™ pod e-liquids (4.02 to 6.79) [13,29,66]. The use of benzoic acid in JUUL™ e-liquids increased interest in acids and pHs of e-liquids [28-29,67-68]. In EC cartomizers that were purchased 5-10 years ago, the e-liquids had varying concentrations of seven acids (citric, lactic, succinic, levulinic, tartaric, butyric, malic) [12] and were similar to products purchased more recently (benzoic, lactic, levulinic, tartaric, butyric, malic and salicylic acids) [29]. These studies indicate that manufacturers have been adding acids to e-liquids for the past 10 years. Long-term (5-10 years) aged e-liquids with the highest organic acid concentrations had the lowest pHs and the highest total concentration of elements/metals [12]. The two refill fluids in the current study, which were purchased in 2020, contained maleic, benzoic, propionic, and citric acid. Some of these acids were low in concentration and may have formed from PG/VG or from degraded flavor chemicals [69]. Maleic acid, which had the highest concentration in the unused e-liquids, was likely added intentionally or may have formed from malic acid, which is used as an apple flavoring [70-75].

**Effects of storage and pH on leaching metals.** In the storage experiment, the presence of acids and low pHs in the EC refill fluids correlated with increased element/metal concentrations in most fluids, an effect that was element/metal dependent. Apple Original (0 mg) and Atomic Apple (0 and 6 mg) had low pHs and elevated concentrations of nickel and zinc in their fluids. Acids in these products may have corroded metal components leading to increases in nickel and zinc [13]. Citric acid, which was measured in both refill fluids, is a key ingredient in metal
etching [76-78], causes corrosion [13], and could elevate element/metal concentrations in e-liquids.

*Flavor chemicals and nicotine in EC fluids.* Flavor chemical and nicotine concentrations decreased during 5-10 years of storage in ECs [12,26-27,79]. A similar decrease was observed in Apple Original and Atomic Apple refill fluids (in the bottles) during storage for a much shorter period of time (8 months). Changes in flavor chemical concentrations likely affect the taste of EC aerosols, which may deteriorate with time. In the storage experiment, the flavor chemicals in refill fluid bottles varied with product, as shown in other studies [12,21,23,80]. Three of the four refill fluids contained apple flavorants (butyl acetate, hexyl acetate, and ethyl 2-methylbutanoate) [81-83] at concentrations of 0.047 to 6.334 mg/mL. Atomic Apple (0 mg) had two apple flavorants (1-hexanol, 2-hexen-1-ol, (E)-), and their concentrations were very low (0.042 to 0.086 mg/mL).

*Health effects of acid and metal inhalation/health concerns.* There are potential health hazards associated with handling and inhalation of acids and metals [84-93]. Most elements/metals and acids in the EC refill fluids can cause skin irritation [84-86,90-93], and skin exposure does occur during handling of products or spills [1]. Prolonged inhalation of elements/metals can increase lung inflammation, respiratory irritation, coughing, chest pains, and shortness of breath [84-89]. Inhalation of acids can also produce coughing, wheezing, and headaches, as well as irritation of the nose, throat, and lung [90-93]. All of these symptoms have been reported online by EC users [34,62] or diagnosed by physicians treating patients who vaped [35], including those with EVALI [36,38]. The relationship between respiratory diseases, such as EVALI, and the elements/metals and acids in EC aerosols is not well understood. Inhalation of high concentrations of elements/metals and/or acids should be considered in evaluating EVALI patients as well as other patients who present with symptoms linked to EC use. Our data show that element/metal and acid concentrations vary in unused products,
elements/metals can increase exponentially in used or stored products, reaching levels that could be harmful. As an example, vaping Apple Original or Atomic Apple in Drag S produced nickel containing aerosols averaging about 0.4 mg/L. This concentration is quite high for nickel and exceeds the regulatory exposure limits for nickel inhalation [94]. Moreover, nickel would be inhaled with other heavy metals, which may in aggregate be more harmful than a single element/metal. Elements/Metals and acids either alone or in conjunction with vitamin E acetate could produce the symptoms of EVALI and/or other respiratory diseases.

Our data show that various factors should be considered in evaluating diseased patients who use ECs and that linkage between acids, elements/metals, and respiratory disease is difficult to demonstrate. Element/Metal concentrations in aerosols varied significantly with refill fluid and EC type such that certain combinations yielded high metal concentrations in aerosols, while other were negligible (e.g., aerosols produced from Atomic Apple in Drag S had high levels of nickel, but aerosols made in the third-party pods did not). The length of storage of liquids in disposable or prefilled pods can also affect element/metal exposure. Finding a causal relationship between vaping metals and respiratory disease will depend on determining the exact e-liquid that patients vaped and the EC model that was used. Not all EC aerosols are the same, and some may be far more dangerous than others. To advance understanding of disease caused by EC use, it is essential that the products (fluids and ECs) be documented, and lab tested to determine what components are in the aerosol. If this is not done, the heterogeneity in EC aerosols will likely produce very “noisy” data and preclude identifying disease causative agents.

Regulations and methods to reduce metal and acid inhalation. Consumers do not know if they will be exposed to high concentrations of acids or toxic metals when they use ECs. Our study and others support regulating the use of acids in EC fluids [29], operating ECs at low powers [54,94], using non-metallic components in the atomizer when possible, and providing an
expiration date on EC packaging to avoid buildup of leached elements/metals in e-liquids over time. The presence of nicotine was more important than low pH in promoting leaching of copper into e-fluid in the storage experiment, suggesting that factors affecting leaching are complex and vary for different elements/metals. Premarket evaluations of EC products by the FDA could also include information on metal and acids in aerosols of products being evaluated. Other factors that should be considered in regulation include nicotine concentration [18,25,27,79], concentrations of other chemicals in e-liquids, such as flavor chemicals and synthetic coolants [20-24], bottle safety, and manufacturing and EC design [7,51,96].

**Conclusions.** The release of elements/metals into e-liquids and subsequent transfer into aerosols is a complex process that depends on the refill fluid, EC-style and components, metal per se, and the length of storage of fluid in an EC before use. Acids can affect element/metal release during storage of disposable EC products, and acids themselves may adversely affect respiratory tissues. Metal release and acid content vary within and between EC brands. In a given test group, metals behaved differently, e.g., more nickel was released at low pH than at high pH in Apple Original in Drag S, while the amounts of copper were similar at both low and high pH in the same group. Measuring transfer of element/metal to the aerosol is important as efficiency of transfer is variable and can depend on the element/metal and the EC used. Storage of fluids in ECs increased metal concentrations, indicating that date-of-manufacture and shelf-life information should be provided on labels. pH, nicotine, refill fluid type, and EC brands were factors that increased element/metal concentrations in the fluid during storage. Not all EC aerosols had high concentrations of elements/metals. Those that do may pose a health risk to the users of such products. When evaluating EC-related disease, such as EVALI, it will be important to determine what product and fluid were vaped as some may have high concentrations of elements/metals that could cause or contribute to the disease, while other
products may be less harmful. These data can benefit consumers, healthcare providers, vape shop owners, and regulatory agencies.
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