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Toxic Metals Transfer from Heating Coils to e-liquids: Safety Assessment of Popular e-cigarettes in Jordan

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ABSTRACT

The rate of smokers in Jordan has been among the highest globally. Electronic Nicotine Delivery systems (ENDs) are considered helpful in smoking cessation but also have the potential for metals exposure resulting from their transfer from the metallic coils to the e-liquid upon use. Metal exposure is associated with severe health outcomes. We sought to assess the levels of toxic metals (Cr, Cd, Pb, Ni and Al) transfer from two of the most popular coils used in ENDs among users in Jordan. The validated inductively coupled plasma-optical emission spectroscopy (ICP-OES) with limit of detections (LODs) of 0.10, 0.90, 0.15, 0.13 and 1.00 $mg.kg^{-1}$ was employed to measure the levels of toxic metals in the e-liquid samples. Following a repetitive usage of coils in both tank and pod systems for five continuous days, the cumulative amount of toxic metals; Ni, Cr, Al and Pb levels were significantly increased in all e-liquids used ($p < 0.0001$) compared to the fresh unheated samples. The obtained results showed a time-dependent increase of metals transfer from coils to e-liquids, thus highlighting the need for additional studies to re-assess the safety claims of using ENDs for smoking cessation.

Keywords: Metals, ENDs, e-liquid, ICP-OES, safety assessment.

1. INTRODUCTION

Smoking rates in Jordan are documented to be one of the highest rates globally. Reports stated that 66% of males above 18 years consume tobacco products while 16.5% are using Electronic Nicotine Delivery systems (ENDs) in 2019[1] and raised to 18% in 2021[2]. This rate is expected to reveal further increase since ENDs are considered a beneficial tool in smoking cessation by 69.1% of the adult population aged ≥ 18 years who participated in a national survey [3]. Therefore, tobacco smokers in Jordan are changing their smoking habit to consumption of ENDs at the expense of regular tobacco smoking. It is estimated that one out of ten regular tobacco smokers has either attempted or switched to ENDs [4]. The situation in Jordan is not different from other countries worldwide since ENDs use is increasing worldwide to approximately 68 million users in 2020 [5]. Claims about the safety of ENDs compared to tobacco smoking did not eliminate the potentially harmful effects of ENDs exposure, especially after long-term use [6].

ENDs are electronic devices that produce aerosols by resistance heating of an electronic-liquid (e-liquid) solution via a metallic coil[7]. Frequent metallic coils used include Kanthal (an iron/chromium/aluminium alloy) and Nichrome (a chromium and nickel alloy) [8]. Through the vaporization of e-liquids, toxic metals may be released by the metallic coils and inhaled by the user [9]. ENDs have the potential for metals exposure resulting from their transfer from the metallic coils to the e-liquid[10]. Unprotected exposure to elevated concentrations of heavy

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metals is associated with severe health consequences. For instance, exposure to the neurotoxic lead (Pb) may increase the risk of neurological disorders, such as Parkinson's disease and Alzheimer's disease [11, 12]. Other diseases might also arise upon exposure to metals including nephrotoxicity for Pb and cadmium (Cd) [13]; neurotoxicity for aluminium (Al) [14] and lung cancer for chromium (Cr) and nickel (Ni) [15].

In Jordan, tanks and pods are two of the most used delivery devices for e-liquids that are supplied to users from different sources (brands). They are connected to refillable ENDs provided with different internal designs, components, and materials [16]. The e-liquid mostly contains nicotine, water, propylene glycol, glycerol, flavours, and pH modifiers [17], and mostly are free from toxic metals. However, metals can transfer to e-liquid from the housing and vapor path that is usually made out of stainless steel or the heating element that could be manufactured from kanthal or nichrome, in which a cotton wick is located inside [18].

The transfer of toxic metals to ENDs e-liquid has been documented in the literature [19, 20]. Furthermore, previous studies demonstrated the presence of several metals including Cr, Ni, Pb, Zn, and Mn in ENDs aerosol that originated from ENDs pod system. The designated metals were found in varying levels [10, 21]. Several agencies have set exposure limits for inhalation of such toxic metals including the Agency for Toxic Substance and Disease Registry (ATSDR), the National Institute for Occupational Safety and Health (NIOSH), the Occupational Safety and Health Administration, and the American Conference of Governmental Industrial Hygienists (ACGIH) [22]. However, no permissible limits for toxic metals have been set by various global and local authorities. Additionally, a continuous exposure to e-liquids with more precise and accurate quantification of metal transfer from ENDs coils to e-liquid is still needed.

Studying the toxicity of substances is very important [23-27]. In our previous studies, we investigated the levels of toxic metals in various pharmaceutical preparations from a local market in Jordan, which is claimed to be a relatively safe option for users [28, 29]. Here we report the presence of high levels of toxic metals in e-liquids upon heating ENDs, resulting from the continuous transfer of metals from internal coils used in Jordan market into the e-liquids. Providing an accurate and precise method for time-dependent analysis, the levels of potentially toxic metals were detected using an inductively coupled plasma optical emission spectrometer ICP-OES due to its high accuracy.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

A solution consisted of 10% Nitric acid (HNO_3) (69%, w/v% trace metal concentration, Fluka Analytical, France) was used for sample digestion. Standard solutions of Pb, Al, Ni, Cd and Cr, each of 1000 ppm, (Merck, Germany) were used for the preparation of working standard solutions within the linear range for each element and were prepared at 6 concentration levels using the appropriate dilutions of stock solutions for the construction of calibration curves. All plastic and glassware used in experiments were firstly cleaned with proper detergent, washed thoroughly with distilled water, and deionized water, soaked overnight in 10% (v/v) HNO_3, and finally rinsed several times with deionized water immediately before use to remove any traces of contamination by metals. Nicotine was obtained from (Alfa Aesar, UK).

2.2. Sample Collection

ENDs products were obtained from a local market in Amman, Jordan. The products investigated were tank systems (voopoo drag s pro) and pod systems (wenax h1). E-liquid products were purchased from local stores (**Table 1**)

Table 1. The description of brands of e-liquid used for analysis.

Brand of E-liquid	Date of Purchase	Date of Expiration	Labelled Level of Nicotine (mg/mL)	Flavour
e-Liquid 1	10- June-21	Oct-23	25	Apple
e-Liquid 2	10- June -21	May-23	25	Watermelon
e-Liquid 3	10- June -21	Jul-23	25	Mango

E-Liquids were sampled directly from tanks and pods in 24 h sampling periods for consecutive 5 days. In which the ENDs products were filled with e-liquids and were turned on for puffing every 15 minutes for 5 h each day. After 24 h, the tank/pod systems were evacuated and the same procedure was repeated the next day for successive five days.

2.3. Sample preparation

Matrix digestion is vital in all analytical processes for determining trace elements. In this work, 0.5 mL e-liquids were accurately withdrawn from the tank/pod systems into 2 mL PTFE sample cups (PerkinElmer, Waltham, MA, USA) followed by the addition of a freshly prepared 1 mL 10% HNO_3 (w/v) and left for 15 min then shaking for 5 min. to allow matrix digestion. Then 0.5 mL of deionised water was added to the resultant mixture. Then, samples were filtered using a 0.45 µm syringe filter and the filtrate was completed to 5 mL with deionised water. The procedure was repeated three times for the same samples and the control was prepared from the acid mixture as a referee.

2.4. Instrumentation

The determination of metal levels was determined using Optima 2000 DV inductively coupled plasma optical emission spectrometer (ICP-OES, PerkinElmer Corporation, USA). The instrument settings were prepared to work to its optimum operating conditions: the incident power was 1300 W, the Argon gas flow rate of plasma was 15 L.min^{-1}, the auxiliary 0.5 L.min^{-1} and the nebulizer was 0.8 L.min^{-1}. The flow rate of the sample was set at 1.5 L.min^{-1}. The temperature heater temperature was adjusted to 30.5 °C. To detect LOD for each metal, serial dilutions of standards were prepared for each designated metal. The repeatability of the method was calculated by the analysis of one standard solution (0.1 mg.L^{-1}) of each metal five times. Furthermore, five standard solutions of each metal were analyzed in-between runs with the RSD values in a range from 1.6 to 2.2%.

The accuracy of the analytical wet digestion procedure should be determined especially after optimizing the conditions. This was carried out through analysis of a reference e-liquid. The reference in concern was prepared in the laboratory comprising propylene glycol, glycerol and pure nicotine in addition to known concentrations of each analyzed metal using the developed method. The results obtained are presented in **Table 2**.

Table 2. Method accuracy assessment using the prepared reference e-liquid

Toxic metal	standardized value mg.kg^{-1}	Determined value mg.kg^{-1}	Recovery (%)
Cr	4	3.82 ± 0.43	95%
Cd	4	3.75 ± 0.89	94%
Pb	4	3.89 ± 0.39	97%
Ni	4	3.91 ± 0.66	98%
Al	4	3.88 ± 0.80	97%

2.5. Statistical analysis

Results were evaluated using Graph Pad Prism v9.5.1. Representative Figures are shown as mean ± standard deviation (SD). Two-way ANOVA statistical analyses and Dunnett's multiple comparisons test was used to assess significant differences and defined as * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), **** ($P < 0.0001$). All experiments were repeated 3 times.

Table 3 and **Table 4** along with their representative

3. RESULTS AND DISCUSSION

In order to accurately report the levels of potentially toxic metals in e-liquids, ICP-OES was used following the optimizing and validating method of analysis. The detected levels of tested toxic metals including Ni, Cr, Cd, Al, and Pb are shown in

Figure 1a and **Figure 1b** for both tank and pod systems, respectively.

Table 3. Levels of metals in e-liquids in tank systems (n = 3).

#	Products no. and use	Ni mg/kg	Cr mg/kg	Cd mg/kg	Al mg/kg	Pb mg/kg
1.	e-Liquid 1 after 5 days use	11.92 ± 2.6	15.8 ± 4.8	<DL	1.554 ± 0.85	0.951 ± 0.62
2.	e-Liquid 1 after 4 days use	17.72 ± 3.9	12.76 ± 2.4	<DL	1.42 ± 0.38	0.57 ± 0.36
3.	e-Liquid 1 after 3 days use	0.62 ± 0.22	3.38 ± 0.90	<DL	1.56 ± 0.67	0.638 ± 0.53
4.	e-Liquid 1 after 2 days use	0.568 ± 0.17	10.9 ± 2.10	<DL	2.712 ± 0.82	1.082 ± 0.56
5.	e-Liquid 1 after 1 days use	0.052 ± 0.02	0.762 ± 0.36	<DL	1.566 ± 0.79	0.42 ± 0.17
6.	e-Liquid 1 after 0 days use	<DL	<DL	<DL	<DL	<DL
7.	e-Liquid 2 after 5 days use	3.572 ± 1.16	4.8 ± 0.54	<DL	1.56 ± 0.53	0.852 ± 0.24
8.	e-Liquid 2 after 4 days use	0.552 ± 0.11	2.392 ± 0.77	<DL	2.07 ± 0.48	0.032 ± 0.02
9.	e-Liquid 2 after 3 days use	0.54 ± 0.26	2.234 ± 0.93	0.09 ± 0.03	1.984 ± 0.84	0.398 ± 0.18
10	e-Liquid 2 after 2 days use	0.346 ± 0.12	3.952 ± 1.02	<DL	2.76 ± 0.92	1.67 ± 0.67
11	e-Liquid 2 after 1 days use	0.044 0.02	1.954 ± 0.85	<DL	1.204 ± 0.46	0.166 ±0.06
12	e-Liquid 2 after 0 days use	<DL	<DL	<DL	<DL	<DL
13	e-Liquid 3 after 5 days use	1.577 ± 0.77	3.395 ± 1.56	0.02 ± 0.1	1.421 ± 0.60	0.611 ± 0.26
14	e-Liquid 3 after 4 days use	0.733 ± 0.32	1.873 ± 0.95	<DL	1.294 ± 0.49	0.547 ± 0.32
15	e-Liquid 3 after 3 days use	0.441 ± 0.25	2.012 ± 1.04	<DL	1.318 ± 0.72	0.423 ± 0.18
16	e-Liquid 3 after 2 days use	0.036 ± 0.02	1.816 ± 1.01	<DL	1.597 ± 0.61	0.595 ± 0.39
17	e-Liquid 3 after 1 days use	0.029 ± 0.01	0.595 ± 0.74	<DL	0.536 ± 0.48	0.317 ± 0.17
18	e-Liquid 3 after 0 days use	<DL	<DL	<DL	<DL	<DL

DL (Detection limit)

Table 4. Levels of metals in e-liquids in pod systems (n = 3).

#	Products no. and use	Ni mg/kg	Cr mg/kg	Cd mg/kg	Al mg/kg	Pb mg/kg
1.	e-Liquid 1 after 5 days use	1.804 ± 0.99	1.714 ± 0.74	0.004	0.826 ± 0.62	0.83 ± 0.46
2.	e-Liquid 1 after 4 days use	1.609 ± 0.56	1.183 ± 0.30	<DL	0.621 0.36	0.318 0.25
3.	e-Liquid 1 after 3 days use	0.372 ± 0.43	0.338 ± 0.06	<DL	0.702 ± 0.43	0.231 ± 0.09
4.	e-Liquid 1 after 2 days use	0.231 ± 0.39	0.942 ±0.36	<DL	0.775 ± 0.87	0.609 ± 0.16
5.	e-Liquid 1 after 1 days use	0.012 ± 0.01	0.166 ±0.09	<DL	0.365 ± 0.33	0.026 ± 0.01
6.	e-Liquid 1 after 0 days use	<DL	<DL	<DL	<DL	<DL
7.	e-Liquid 2 after 5 days use	2.062 ± 0.88	2.404 ± 0.69	<DL	1.512 ± 0.62	1.71 ± 0.43
8.	e-Liquid 2 after 4 days use	0.552 ± 0.23	2.392 ± 0.85	<DL	2.07 ±0.71	0.032 ± 0.01
9.	e-Liquid 2 after 3 days use	0.54 ± 0.19	2.234 ± 0.52	0.09	1.984 ±0.37	0.398 ± 0.07
10	e-Liquid 2 after 2 days use	0.346 ± 0.15	3.952 ±1.07	<DL	2.76 ± 0.48	1.67 ± 0.49
11	e-Liquid 2 after 1 days use	0.044	1.954 ±0.45	<DL	1.204 ± 0.77	0.166 ± 0.02
12	e-Liquid 2 after 0 days use	<DL	<DL	<DL	<DL	<DL
13	e-Liquid 3 after 5 days use	1.66 ± 0.53	0.474 ± 0.03	<DL	0.5 ± 0.14	1.032 ± 0.61
14	e-Liquid 3 after 4 days use	0.599 ± 0.17	1.177 ± 0.67	<DL	1.046 ± 0.41	0.188 ± 0.04
15	e-Liquid 3 after 3 days use	0.287 ± 0.07	0.919 ± 0.31	<DL	0.814 ± 0.16	0.286 ± 0.01
16	e-Liquid 3 after 2 days use	0.03	1.021 ± 0.81	<DL	0.739 ±0.20	0.402 ± 0.07
17	e-Liquid 3 after 1 days use	0.018	0.405 ± 0.10	<DL	0.202 ±0.04	0.293 ± 0.11
18	e-Liquid 3 after 0 days use	<DL	<DL	<DL	<DL	<DL

During the experimental time of five days, higher but various levels of toxic metals were found in all tested e-liquids (**Figure 1a**, **Figure 1b**), indicating that transfer of toxic metals out of both tank and pod systems started from the first day of using coils with heating. For example, the levels of Cr and Al were significantly higher in e-liquid 2 after the first day of using the coil of both tank and pod systems ($p < 0.0001$ and $p < 0.05$ respectively), while with liquid 1 and 3, such metals started to significantly increase after the second day of usage. This agrees with the finding of Olmedo *et al.* who reported that metal concentrations in the e-liquid increased markedly after it was added to the ENDs device when brought into contact with the heating coil in the generated aerosol and in the liquid that remained in the tank [10].

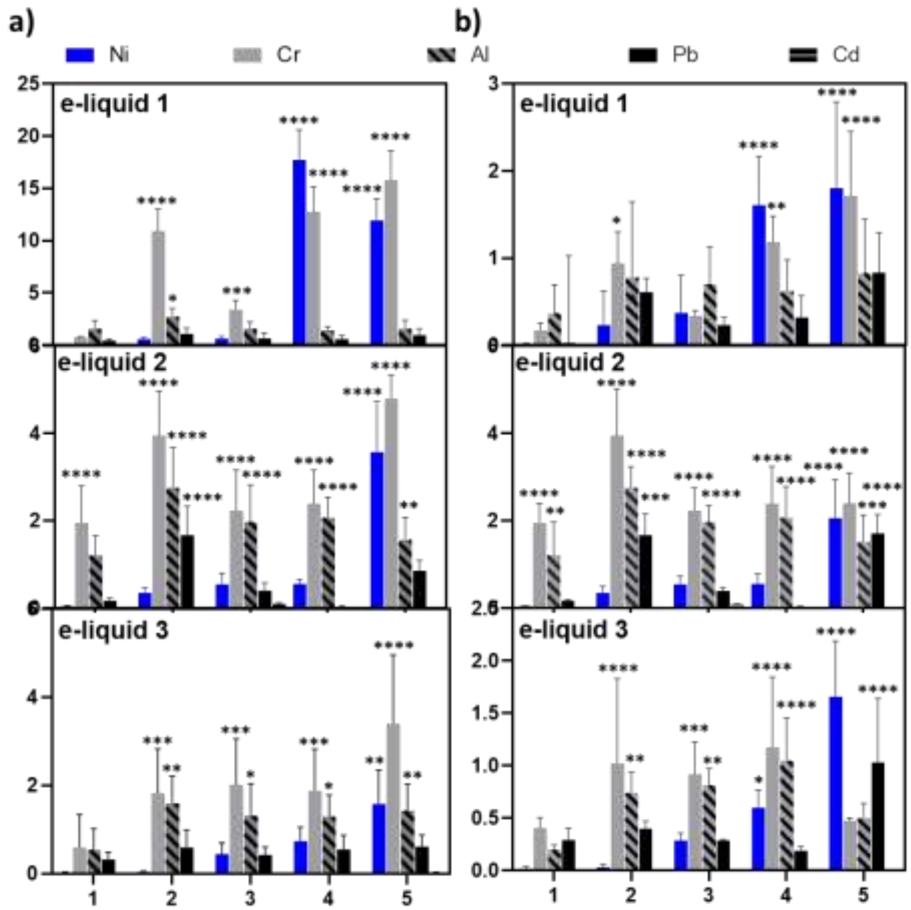

Figure 1: Toxic metals transfer study from tank
(a, and pod **(b** system ENDs' coil into three tested e-liquids for five continuous days. The levels of Ni, Cr, Cd, Al and Pb are reported in mg/kg. Data are reported as mean ± SD (n=3 internal repeats). Significant differences from pre-heated e-liquids (day 0) are expressed as *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

However, the results also indicate the unpredictable and uncontrollable health problems that users might develop upon using different brands of ENDs. This comes from the point that metals are transferred from the ENDs' coils to the e-liquid and from the e-liquid to the aerosol which is eventually inhaled by the user. Indeed, ENDs are considered a significant source of generating toxic metals that might lead to end-user exposure [30]. Chronic exposure to such metals as continuous usage of ENDs provide the user's body with a dangerous amount of toxic metals that ultimately adversely affect various body system, especially the cardiovascular system [31].

The transfer of most detected metals was not following a specific pattern. This reveals that other factors play an important role in the transfer process of metals from the coil to e-liquids, some might be related to the composite of the used system.

One interesting finding was related to the transfer levels of Ni into the tested e-liquids, as it follows an increasing pattern with time. For instance, the levels of Ni of the three different e-liquids used on day 5 of the experiment were ~55 to 230-fold higher than on day 1 of the experiment as determined in the used tank system, and ~47 to 150-fold higher in the used pod system. This indicates that Ni is more susceptible to transfer with ageing and repetitive usage of the coil. This exhibits more risk for users who do not change the coil continuously. Indeed, exposure to Ni has been found to be carcinogenic and might predispose bronchitis [20].

The cumulative levels of each toxic metal following five days of usage were compared between tank and pod systems for the same e-liquid brand (**Figure 2**).

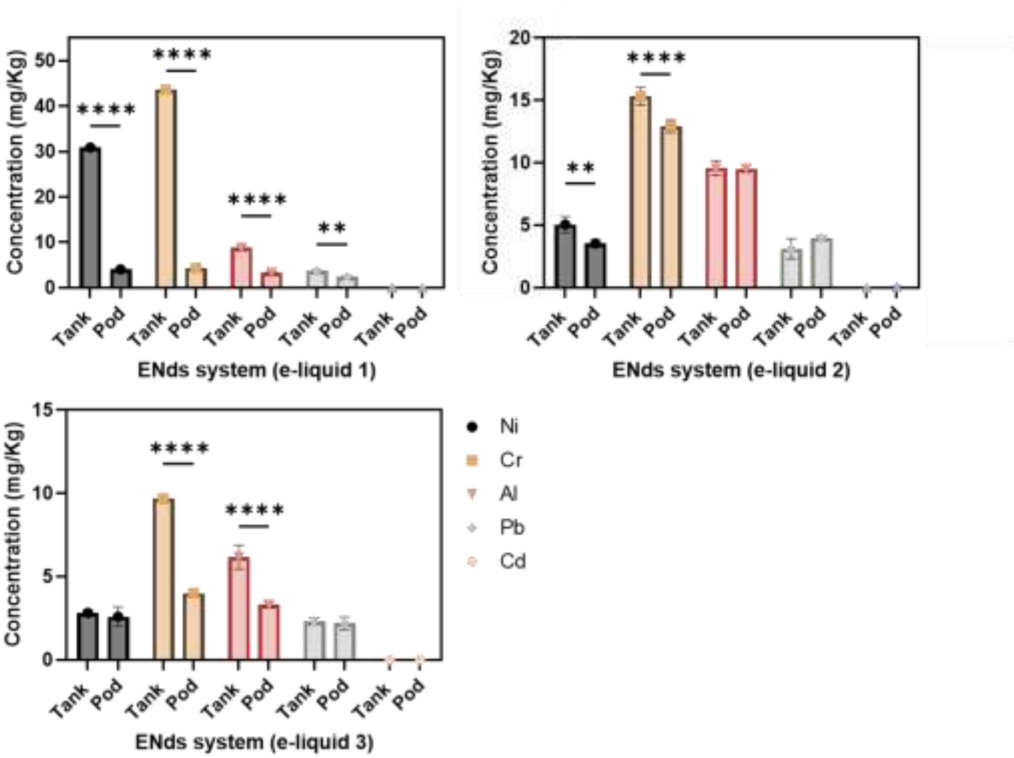

Figure 2: Comparative cumulative concentrations of Ni, Cr, Cd, Al, and Pb for continuous five days in all tested e-liquids between both tank and pod systems ENDs.

Statistical analyses revealed significant higher accumulation of Cr in all tested e-liquids and of Ni and Al of tank compared to pod. Values are reported as mean ± SD (n = 3). ** P < 0.01 and *** P < 0.001.

Interestingly significantly higher levels of Ni and Cr metals were found in e-liquid 1 with tank usage compared to pod ($p < 0.0001$) and in e-liquid 3 ($p < 0.05$) for Cr. This might be attributed to that coils used in tank systems contains more metals in comparison to pod systems [32]. Besides, this highlights that the type of e-liquid used can also contribute to the amount and type of metals leaching from the same coil.

Such results might offer a relatively safer option for users using pod systems ENDs. However inclusive studies comprising a higher number of ENDs and liquid brands are needed before concluding this.

Taking into consideration that ENDs are used in a continuous rather than occasional manner, and except for Cd, the cumulative levels of all detected toxic metals; Ni, Cr, Al and Pb levels after five continuous days of usage were significantly higher in all e-liquids used ($p< 0.0001$) for both systems compared to the fresh unheated samples. Our results support findings obtained by Gray *et al.* who confirm that detectable metals in e-liquids are resulting from contact between the e-liquid and ENDs device components [19], and Hess *et al.* who found that when ENDs are used, direct contact of the e-liquid comes to the heating coil, resulting in leaching of the coil metals into the e-liquid at higher temperatures [20]. Our findings also emphasise the urge for additional safety studies of using ENDs with chronic users as a safe method for smoking cessation.

4. CONCLUSION

An accurate and precise analytical method based on ICP-OES for the analysis of 5 toxic metals in ENDs e-liquids was developed and validated. Analyses of ENDS e-liquids before any contact with the metallic ENDs coils revealed no metal concentrations and were below LODs. While e-liquids in contact with the metallic coils showed significantly increased metals concentrations including Ni, Cr, Al and Pb ($p < 0.0001$). The increase in metal concentrations was correlated with the increased time of e-liquid contact with the metallic coils in both tank and pod systems. The obtained results confirm that detectable metals in e-liquids are resulting from contact between the e-liquid and heated device metallic coil. The presence of the investigated metals in e-liquid might cause a potential adverse effect to ENDs users and more regulations should be put to ensure the quality of ENDs products to ensure safety to users.

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Competing interests

The authors declare no competing interests.

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انتقال المعادن السامة من ملفات التسخين إلى السائل الإلكتروني: تقييم الأمان للسجائر الإلكترونية الشائعة الاستعمال في الأردن

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[1] قسم الصيدلة، كلية الصيدلة، جامعة الزيتونة الاردنية، الأردن.

ملخص

معدل المدخنين في الأردن من بين أعلى المعدلات على مستوى العالم. تعتبر أجهزة توصيل النيكوتين الإلكترونية مفيدة في الإقلاع عن التدخين وفقًا لمسح وطني حديث. هذه الأجهزة الإلكترونية لديها احتمالية التعرض للمعادن السامة الناتج عن نقلها من الملفات المعدنية إلى السائل الإلكتروني اثناء عملية التسخين. يرتبط التعرض للمعادن بنتائج صحية خطيرة للمستعملين لهذه الأنظمة. الهدف من هذه الدراسة هو تقييم انتقال المعادن السامة وتشمل الالمنيوم، النيكل، الرصاص، الكروم، والكادميوم من ملفات اثنين من أنظمة توصيل النيكوتين الإلكترونية الأكثر استخدامًا في الأردن بين المستخدمين كل 24 ساعة لمدة 5 أيام متواصلة من الأستعمال. تم قياس تراكيز المعادن السامة في عينات السائل الإلكتروني باستخدام جهاز مطياف الانبعاث البصري البلازمي المقترن حثيًا، مع حدود دنيا من التراكيزبعد تطوير طريقة ذات درجة تحقق عالية ومثبتة. وجدت معظم المعادن السامة في اغلب العينات المحللة، بينما تم الكشف عن الكادميوم في عينتين فقط تم تحليلهما. أظهرت النتائج التي تم الحصول عليها على وجود علاقة بين زيادات نقل المعادن من الملفات إلى السوائل الإلكترونية مع طول فترة استخدام أنظمة توصيل النيكوتين الإلكترونية.

الكلمات الدالة: المعادن، ENDs، السائل الإلكتروني، ICP-OES، تقييم السلامة.

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