Report on E-Cigarette Analytical Testing
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Report on E-Cigarette Analytical Testing

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1. Background

Since the introduction of e-cigarette in 2003, global use has grown exponentially. For instance, in the UK, number of users has increased from 700,000 in 2012 to 2.6 million in 2015 (Action on Smoking and Health, 2015). E-cigarette has become a rising trend, particularly among the youth. Some market estimations suggested that its global sales has reached US$3.5 billion in 2015, which was a 170-fold increase compared to 2008. E-cigarette was preliminarily used as an alternative for smoking. However, e-cigarette nowadays is gaining popularity and becoming widely-used in students and non-smokers.

There is the claim that e-cigarette can help people to quit smoking. Yet, many vapers also smoke traditional cigarettes, leading to the concern that such dual use is delaying or deterring smoking cessation. According to World Health Organization, there is no sufficient evidence to prove that e-cigarettes help smokers quit the habit and its safety remains unknown. Thus, e-cigarette has not yet been recognized as a legitimate tool for smoking cessation.

Several overseas research studies have identified toxic chemicals and carcinogenic substances in e-cigarettes, for instance, nicotine, various additives, heavy metals, propylene glycol, glycerin, formaldehyde and acetaldehyde. Most of these substances have been shown to cause various health problems, such as nausea, bradycardia, respiratory depression and lung diseases, etc. Other unknown ingredients and substances released during the vaping process may also cause negative effects on the health of users and other people. Unintended exposure of e-liquid to children could cause vomiting, cough, choke and death.

In order to obtain more information and raise public awareness on the safety and potential health hazards of e-cigarette products, Hong Kong Council on Smoking and Health (COSH) initiated a laboratory test on e-cigarette products. Hong Kong Baptist University (BU) was commissioned to conduct this analytical test to explore the chemical compositions of e-cigarette.

2. Objectives

With the aim to understand the chemicals in e-cigarette aerosol to safeguard public health, the analytical test was designed to identify the chemical substances in the aerosol of e-cigarettes and determine their concentrations. The results throw light on the chemical compositions and the associated health risks from e-cigarette use in Hong Kong.

3. Samples

BU received 13 e-cigarette samples from COSH in August 2015. Chemical tests and analyses on the samples have since started.

Out of the 13 samples provided by COSH, 4 are e-liquids (EC1 to EC4) and the rest are e-cigarettes with capsules. Details are stated in Table 1. To make comparison with the chemical compounds in the smoke of traditional cigarettes, two packs of conventional cigarettes were purchased by BU for this study.

Table 1. Sample details

	Forms	Flavours
EC1	Liquid	Tobacco
EC2		Mint
EC3		Mint
EC4		Cola
EC5	Capsule	Light Mint Marbo (imitate tobacco)
EC6		Black-ice Marbo (imitate tobacco)
EC7		Strawberry
EC8		Tobacco
EC9		Chocolate
EC10		Tobacco
EC11		Mint
EC12		Tobacco
EC13		Mint

4. Test method and Procedure

4.1 Setup and procedure

The experimental set-up (Figure 1) was designed to imitate human cigarette smoking. The set-up consisted of two 50 ml polypropylene conical test tubes connected together with glass tubes, and further connected to a pump (Model DQA-P104-AA, Volts:115, Amps:4.2, HZ 60, USA; connecting with CT-1000AC-AC, Converter, 1000 Watt, 50/60 HZ, USA) to suck air from the e-cigarette samples being tested. The pump was further connected to a CT-1000AC-AC Converter, 1000 Watt, 50/60 HZ, China. Each polypropylene conical test tube contained 25 ml of solvent mixture (hexane:dichloromethane,1:1). Sucked-in air will pass through the e-cigarette sample and the e-cigarette aerosol will pass the two absorption tubes.

Figure 1. A photo showing the experimental set-up. The pump is not shown

The solvent mixture in the absorption tubes will absorb the target analytes in the aerosol. By allowing the aerosol to go through two absorption tubes, the absorption of target analytes in vaping would be made more complete. Each sample was tested for 12 minutes to ensure maximal exposure of relevant chemicals in human lungs although a normal smoking activity does not last that long. A valid suck-in is indicated by the red or blue light at one end of the e-cigarette. About 111 mouthfuls of vaping would occur in the 12-minute duration (one bout). To set the correct pumping power, we began with the lowest power and slowly raised it to a level where it was strong enough to light the e-cigarette.

4.2 Analytical methods

After 12 minutes of absorption, the solvents in the two test tubes would be mixed together and be concentrated in a Rotary Reduced Pressure Evaporator or through natural evaporation to reduce the solvent mixture volume to around 1 ml and then be transferred to a 1.5ml vial which needed to be washed by pure hexane. This was followed by Gas Chromatography – Mass Spectrum (GC-MS) determination.

GC-MS determination was carried out by Agilent Technologies 7890A GC System connecting with 5975 C Inert Mass Spectrum Detector (triple-Axis Detector), Agilent Company, USA. Using the Scan model in GC-MS, the embedded computer would compare the structure and M/Z ratio of the chemical compounds in the e-liquid and e-cigarette aerosol with the stored database which contained information of more than 20,000 species of volatile compounds. Thus, through the Scan model (in which the MS detector can identify M/Z ratio from 50-550) the molecular structure of the chemical compounds would be compared and be identified after chromatographic separation. The quantities of each chemical compound were indicated by the size of their relevant peaks in the chromatograph.

The selected ion monitoring (SIM) model was deployed to measure the concentration of all target analytes in the aerosol but the detailed determination of four target analytes (PAHs, PBDEs, formaldehydes and nicotine) through SIM model was carried out by spiking each sample with the standards of the target analytes. SIM, as opposed to Scan, was used to identify newly emerging chemical compounds, such as polybrominated diphenyl ethers (PBDEs), as the Scan library usually would not contain these newly emerged chemical compounds. Also, SIM was the standard method to accurately determine chemical compounds as it is 20 times more sensitive than Scan. The standards used for the 4 target analytes were:

- ^{13}C-PBDE standards (Wellington Laboratories Inc. ON, Canada);
- (S)-(-)-Nicotine 98% 18637-6 & 1521 SDO EO 5g 98%;
- ULTRA Scientific SMA-300 aromatic hydrocarbons standard 4X1 ml. ULTRA pak 401-294-9400; and
- Fluka Analytical 00071 – 100ml >99.0 % pure at 2-8^{o}C formaldehyde (GC).

4.3 Collection of aerosol

To prepare the e-liquids for the aerosol generating experiment, we took 0.5ml from each of the four samples and have it dissolved in an acetone and hexane (1:1) mixture. Then the mixture with e-liquid was added to an e-cigarette cartridge and the aerosol was generated in the manner described in section 4.1.

Separate aerosol samples were collected using the same methods described above for heavy metal determination with the exception that the solution in the two test tubes was an acid mixture consisting hydrochloric acid, nitric acid and water (HCl:HNO3:Milli Q water in a ratio of 1:1:5). This mixture would completely and effectively capture any metal molecules or their ions passing through it. After each bout, the acid solutions in each test tube would be combined together (50ml in total) in another tube and be determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The M/Z ratio of each metal in standard mixture metal solution was used to identify the heavy metal species and at the same time have the quantities determined.

5. Results

Table 2 lists the results of the 4 analytes, namely, PAHs, PBDEs, nicotine and formaldehydes.

Table 2. Concentration of targeted analytes in samples

	PAHs	PBDEs	Nicotine	Formaldehydes
unit	ppb or ng/ml			ppm or µg/ml
replicates	2	2	3	1
EC1	8.4 (0.28)	2.5 (0.28)	28.5 (0.28)	3,711.8
EC2	7.9 (0.07)	2.1 (0.07)	12.5 (0.07)	5,782.4
EC3	11.3 (0.42)	9.0 (0.28)	10.5 (0.14)	6,676.5
EC4	23.5 (0.78)	191.9 (0.07)	8.5 (0.21)	6,217.6
EC5	10.8 (0.28)	7.2 (0.57)	5.0 (0.07)	6,329.4
EC6	504.5 (7.78)	1,490.0 (7.07)	5.5 (0.07)	5,276.5
EC7	15.6 (0.14)	149.3 (3.82)	6.5 (0.28)	5,129.4
EC8	5.1 (0.42)	46.8 (0.28)	8.4 (0.57)	4,911.8
EC9	6.7 (0.07)	145.7 (4.24)	9.9 (0.07)	4,594.1
EC10	2.9 (0.57)	3.5 (0.57)	4.5 (0.00)	3,841.2
EC11	4.3 (0.14)	158.1 (2.97)	7.4 (0.00)	3,482.4
EC12	27.8 (0.14)	2.3 (0.07)	14.0 (0.07)	4,182.4
EC13	5.8 (0.14)	1.7 (0.07)	3.5 (0.07)	n.a.
Traditional cigarette A	134.5 (0.49)	6.3 (0)	266.0 (5.29)	110
Traditional cigarette B	182.0 (1.41)	5.6 (0.42)	270.3 (5.03)	130
Control	0.0 (0)	0.0 (0)	0.03 (0.06)	8.7

Notes: average value of all replicates (s.d.)

5.1 Poly-nuclear Aromatic Hydrocarbons

Poly-nuclear Aromatic Hydrocarbons (PAHs) is a group of chemicals consisting of hundreds of individual species. Most PAHs have high molecular weights and low volatility. In this study, 15 marker species of PAHs were selected for analysis. They are considered most significant in terms of their health effects on humans by major environmental authorities, such as the Environmental Protection Agency in the US (USEPA) and other scientific groups in the world. The PAHs concentration reported in Table 3 shows these 15 marker species of PAHs.

Table 3. PAH species analyzed in this study

1. Naphthalene	9. Chrysene
2. Acenaphthylene	10. Benzo(b)fluoranthene ++
3. Acenaphthene	11. Benzo(k)fluoranthene
4. Fluorene	12. Benzo(a)pyrene ++++
5. Phenanthrene	13. Dibenzo(a,h)anthracene +++
6. Anthracene	14. Benzo(g,h,i)perylene
7. Fluoranthene	15. Indeno(1.2.3-cd)pyrene+
8. Pyrene	

PAHs mainly come from petroleum hydrocarbon contamination or combustion of organic materials. They are toxic and have deleterious impacts on human health. Benzo(a)pyrene is a very strong and well-known carcinogenic compound. Exposure to PAHs would substantially increase human risk of mutagenic and malformation and even result in cancer. It is shown in Table 2 that traditional cigarettes have high concentrations of PAHs. Although most e-cigarette products had much lower concentrations of PAHs, one was very high at 504.5 ppb and a few were within the 10-30 ppb range. Our test results also showed that although no real combustion occurs, heating of e-liquid in e-cigarette cartridge still produced PAHs (Zheng and Richardson, 1999).

Since only the reference dose (RfD)[1] for some species of PAHs could be found, pyrene was used as an example. USEPA established in 2012 a RfD of 0.003mg/kg/day for non-carcinogenic pyrene. If it was assumed that all measured PAHs in the aerosols were represented by pyrene, and using EC6 (highest in PAHs) as an illustration, it is “safe” to vape 357 bouts per day for an adult of 60 kg in weight. However, this “safe” limit had not taken into account the health risk from the other carcinogenic compounds, such as Benzo(a)pyrene. In addition, e-cigarette was not the only source of PAH for the majority of human population. Thus, the appropriate question to ask is if it is sensible to add to the health risk from this group of carcinogenic substances in exchange for the “enjoyment” from vaping.

5.2 Polybrominated Diphenyl Ethers (PBDEs)

BDE-209 is an efficient flame retardant with about 80,000 tonnes being produced each year during the 20th century. They have been used in polymer products, furniture, car cabins, textiles and plastic products, electronic and electric products. PBDE contamination exists everywhere. All our environmental media such as air, water, soil, sediment etc., contain some levels of PBDEs. The concentration of PBDEs in indoor environment tends to be higher than that at outdoor owing to the existence of multiple sources of PBDEs in an indoor setting (Zheng, et al., 2012).

[1] *It is an estimate of a daily exposure to the human population, including the vulnerable groups, that is likely to be without significant risk of harmful effects during a human’s lifetime while taking into account uncertainties.*

As an electronic product, adding flame retardants on the cartridge of an e-cigarette is not surprising. If PBDEs are added, they will easily be vaporized together with the e-liquid aerosols and be inhaled directly by vapers. PBDEs are associated with thyroid hormone disruption, reproductive developmental toxicity including neurotoxicity and carcinogenicity. Informed by literature, 27 typical species of PBDEs were selected for testing in this study. Based on Table 2, elevated levels of total PBDEs were found with 5 samples and in general, total PBDEs levels in e-cigarettes were higher than those in traditional cigarettes.

USEPA (2014) has established RfDs for several congeners of PBDEs only. Based on the RfDs of the most commonly found congener of PBDEs and using the sample with the highest concentration of total PBDEs (EC6), the safe limit for vaping for a person with 60kg of body weight is 282 bouts per day. If the RfDs for the most harmful congener of PBDEs is used to represent total PBDEs, then the safe limit is reduced to 4 bouts per day of EC6 per person of 60 kg in body weight.

5.3 Nicotine

Pyridine-3-(1-methyl-2-pyrolidinyl)-(s), commonly called nicotine, was extensively found in the samples. It is concluded that nicotine was found in all e-cigarette samples even though some products were labelled with "0 nicotine" on packaging. Yet, in comparison, the levels of nicotine in e-cigarette were much lower than those in traditional cigarettes. While nicotine is an addictive substance, with the low levels found in e-cigarette, it will take longer for vapers to be addicted to the nicotine when compared to tobacco cigarettes. Still, people (especially youths) may be attracted to vaping because of the availability of a large range of flavours in e-cigarettes.

5.4 Formaldehyde

Formaldehyde is an important precursor to many other materials and chemical compounds. In 1996, the installed capacity for the production of formaldehyde was estimated to be 8.7 million tonnes per year. It is mainly used in the production of industrial resins, e.g., for particle board and coatings. It is also often used in everyday items especially in keeping aquatic food fresh. International Agency for Research on Cancer (IARC) has confirmed that formaldehyde is a Group 1 human carcinogen. A Group 1 human carcinogen is one that "there is sufficient evidence of carcinogenicity in humans" for this substance. This means that "a causal relationship has been established between" this substance "and an increased incidence of malignant" or "an appropriate combination of benign and malignant neoplasms in animals" (IARC, 2006). It can also cause coughing and is an irritant to eye and skin. In view of its widespread use, toxicity, and volatility, absorption of formaldehyde is a significant threat for human health.

Based on Table 2, elevated levels of formaldehyde were found in all e-cigarette samples and all of them were much higher than those found in tobacco cigarettes. USEPA established an oral reference dose (RfD) of 0.2mg/kg (of body weight)/day for formaldehydes (1989). The oral RfD is used in this case because the concentration of formaldehydes is measured using 1 ml of solvent as basis. Assuming a human being of 60 kg, the "safe" dose for formaldehydes from EC11 (lowest in formaldehyde) is 3.45 bouts per day and for EC3 (highest in formaldehyde), the "safe" dose is 1.8 bouts.

5.5 Heavy metals in e-cigarettes products

Most heavy metals are harmful to human. Among them, nickel (Ni) could cause tumor and even cancer. Cadmium (Cd) would cause osteoporosis as Cd replaces calcium to form CdCO3 which is weaker than CaCO3. Other heavy metals such as mercury and lead are well-known toxicants (Williams et al., 2013). Lead poisoning could lead to bilateral wrist drop in adults and cognitive impairment in children. Chronic exposure to lead is associated with chronic renal failure and interstitial nephritis (Gordon et al., 2002). The most infamous mercury poisoning, Minamata disease, occurred in Japan last century. Patients with Minamata disease typically have ataxia, sensory disturbances, dysarthria, auditory disturbances, constriction of the visual field, and tremor (Harada, 1995). Table 4 shows the averaged duplicate concentrations of heavy metals in e-cigarette aerosol.

Table 4. Heavy metals in e-cigarette aerosol

	Cu	Zn	Ag	Cd	Hg	Pb	Ni
	ng/ml (of acid mixture)						
EC1	14.5	126.0	2.4	14.0	11.5	1.0	12.4
EC2	13.1	133.8	7.0	11.2	21.7	2.0	12.9
EC3	18.9	126.4	4.5	15.0	11.2	1.0	15.4
EC4	11.3	135.2	8.4	11.6	1.1	3.0	17.5
EC5	4.5	96.1	4.0	11.0	30.2	1.3	5.3
EC6	29.0	81.4	3.7	14.4	20.2	7.0	6.1
EC7	11.4	78.7	3.1	10.2	10.1	6.0	8.4
EC8	13.6	115.9	5.3	11.8	0.5	4.0	2.7
EC9	10.8	107.8	5.6	10.9	0.0	1.0	3.8
EC10	11.6	88.9	2.8	8.1	0.6	3.0	2.3
EC11	10.6	71.8	3.2	10.0	0.8	4.8	8.1
EC12	10.7	75.9	4.9	9.0	0.3	2.8	11.1
EC13	21.4	65.3	1.8	11.3	0.0	2.5	10.8

Cu:copper, Zn:zinc, Ag:silver, Cd:cadmium, Hg:mercury, Pb:lead, Ni:nickel
Note: composition of the acid mixture is mentioned in section 4.3.

On the whole, cadmium, mercury, lead and nickel are more toxic than cooper, zinc and silver to humans. It should be reminded that while one bout will not bring acute poisoning to the vapers with the tested levels of heavy metals, the long term cumulative toxicity effects should never be overlooked, in particular, e-cigarettes are getting more popular in youth and children.

5.6 Other chemicals found in e-cigarette aerosol

Other volatile chemical compounds found in e-cigarette aerosols included but do not limit to 1,2,3-propanetriol diacetate; triacetin; proparedioc acid dierthyester; cyclohexanenone 5-methyl-2-(1-methyl ethyl)-trans, methane diethoxy-; propanol, dibutyl phthalate; and glycerin. Accurate concentrations of these compounds could not be determined by Scan model alone. Owing to the lack of resource and time, they were not determined by the SIM model.

6. Conclusion

Findings from this study showed that a whole array of harmful and even carcinogenic substances was found in the aerosols of e-cigarette. E-liquid was the main source of chemical compounds in e-cigarette aerosols. E-liquids usually contained a mix of propylene glycol, glycerin, nicotine, and flavorings. Some e-liquids may even contain ingredients such as cannabis, tobacco extract, or adulterants (Giroud et al., 2015). The materials used to make e-capsules may also contain harmful substances that may escape to the aerosols during vaping.

In sum, this study showed that e-cigarette aerosols contains
- varying to elevated levels of PBDEs and PAHs;
- generally low but varying levels of nicotine;
- elevated levels of formaldehyde;
- varying to moderate levels of various heavy metals; and
- a myriad of many other chemical compounds.

The concentrations of PAHs, PBDEs and nicotine varied widely among samples. When compared with the aerosol from traditional cigarettes, high levels of PBDE was present only in e-cigarette aerosols and this can be explained by use of this popular flame-retardant in the e-cigarette cartridges. Also, exclusive to e-cigarette aerosols, the concentration of formaldehydes in the aerosols of e-cigarette was consistently much higher than that from traditional cigarette. However, when compared to the smoke from traditional cigarettes, e-cigarettes aerosol generally contained lower levels of nicotine. Regarding the “safe” consumption limit of e-cigarette, it appeared that the most severe health damage comes from the high concentration of formaldehydes in the aerosol.

World Health Organization recommends organizations or governments to consider prohibiting or regulating e-cigarettes to prevent the initiation of e-cigarettes by non-smokers and youth, minimize its possible health risk and prevent unproven health claims and advertising (World Health Organization, 2014). At least 46 countries have restricted the sale of e-cigarettes, 26 have banned or restricted the use and 18 have implemented a complete ban on sale, advertising, promotion and sponsorship, import, distribution and manufacturing of e-cigarettes. As a result, Hong Kong Government proposed a total ban in May 2015 in order to safeguard public health. It remains controversial whether e-cigarette is a gateway of smoking or a less harmful alternative tool to quit smoking. Thus, a stringent approach on e-cigarettes should be adopted for preventing its rapid spread in the same manner as tobacco in the last century. To take a precautionary approach in public health, e-cigarettes should not be allowed until sufficient evidence to prove the safety of e-cigarette use is established.

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