Simultaneous Collection of Volatile Organic Compounds and Carbonyls in Mainstream Cigarette Smoke from Japanese Cigarettes Using a Sorbent Cartridge Followed by GC/MS and HPLC

Rina IZU[1,2], Shigehisa UCHIYAMA®[1], Kanae BEKKI[1], Yohei INABA[1],
Hideki NAKAGOME[2] and Naoki KUNUGITA[1]

® E-mail : uchiyama@niph.go.jp

[1] Department of Environmental Health, National Institute of Public Health, 2-3-6, Minami, Wako-shi, Saitama 351-0197

[2] Graduate School of Engineering, Chiba University, 1-33, Yayoi-cho, Inage-ku, Chiba-shi, Chiba 263-8522

(Received June 10, 2014; Accepted July 25, 2014)

Volatile organic compounds (VOCs) and carbonyl compounds in mainstream cigarette smoke from Japanese cigarettes were measured using solid-sorbent collection, followed by GC/MS and HPLC. As a result, it was shown that the major carbonyl compounds in mainstream cigarette smoke were acetaldehyde (48–56 %) and acetone (20–24 %); the major VOCs in mainstream cigarette smoke were isoprene (40–57 %) and toluene (9–12 %), respectively. A tobacco-like product, such as Neo Cedar, is the second kind pharmaceutical product; however, it generated many kinds of carcinogen, such as benzene and furans. Moreover, the total carbonyl compounds generated from Neo Cedar was 1.1–2.0-fold higher than those from commercially available cigarettes. During the collection of mainstream cigarette smoke, generation of VOCs and carbonyl compounds was varied by the condition of the smoking machine. On every puff collection, the collected amount by the 1st puff was different from that of the 2nd puff; therefore, it was suggested that the filter structure of the cigarette influenced the chemical compounds in the mainstream cigarette smoke.

***Keywords*:** mainstream cigarette smoke; carboxen 572; volatile organic compounds; carbonyl compounds; Neo Cedar.

Int. J. Environ. Res. Public Health **2014**, *11*, 11192-11200; doi:10.3390/ijerph111111192

OPEN ACCESS

International Journal of
**Environmental Research and
Public Health**
ISSN 1660-4601
www.mdpi.com/journal/ijerph

Review

Carbonyl Compounds Generated from Electronic Cigarettes

Kanae Bekki [1], Shigehisa Uchiyama [1,*], Kazushi Ohta [2], Yohei Inaba [1], Hideki Nakagome [2] and Naoki Kunugita [1]

[1] Department of Environmental Health, National Institute of Public Health, 2-3-6 Minami, Wako-shi, Saitama 351-0197, Japan; E-Mails: bekki.kanae@niph.go.jp (K.B.); yohei_inaba@niph.go.jp (Y.I.); kunugita@niph.go.jp (N.K.)
[2] Graduated School of Engineering, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba-shi, Chiba 263-8522, Japan; E-Mails: uchiyama.s@chiba-u.jp (K.O.); nakagome@tu.chiba-u.ac.jp (H.N.)

* Author to whom correspondence should be addressed; E-Mail: uchiyama@niph.go.jp; Tel.: +81-48-458-6262; Fax: +81-48-458-6270.

External Editor: Konstantinos Farsalinos

Received: 24 June 2014; in revised form: 2 October 2014 / Accepted: 16 October 2014 / Published: 28 October 2014

Abstract: Electronic cigarettes (e-cigarettes) are advertised as being safer than tobacco cigarettes products as the chemical compounds inhaled from e-cigarettes are believed to be fewer and less toxic than those from tobacco cigarettes. Therefore, continuous careful monitoring and risk management of e-cigarettes should be implemented, with the aim of protecting and promoting public health worldwide. Moreover, basic scientific data are required for the regulation of e-cigarette. To date, there have been reports of many hazardous chemical compounds generated from e-cigarettes, particularly carbonyl compounds such as formaldehyde, acetaldehyde, acrolein, and glyoxal, which are often found in e-cigarette aerosols. These carbonyl compounds are incidentally generated by the oxidation of e-liquid (liquid in e-cigarette; glycerol and glycols) when the liquid comes in contact with the heated nichrome wire. The compositions and concentrations of these compounds vary depending on the type of e-liquid and the battery voltage. In some cases, extremely high concentrations of these carbonyl compounds are generated, and may contribute to various health effects. Suppliers, risk management organizations, and users of e-cigarettes should be aware of this phenomenon.

Keywords: electronic cigarette; e-cigarette aerosol; carbonyl compounds

---

1. Introduction

An electronic cigarette (e-cigarette) is a battery-powered device designed to deliver nicotine to a smoker. It was first developed by Herbert A. Gilbert, who patented a device described as "a smokeless non-tobacco cigarette" that involved "replacing burning tobacco and paper with heated, moist, flavored air" in 1963 [1]. However, the invention of the e-cigarette in 2003 is attributed to Hon Lik, a Chinese pharmacist, and e-cigarettes were introduced to the Chinese market as a smoking cessation device in 2004 [2]. There are several types of e-cigarettes, which include nicotine or are nicotine-free liquid-holding cartridges. E-cigarettes are presented as low-risk products, with a realistic look, feel, and taste when compared with traditional cigarettes [3]. Among major carcinogens and toxic compounds such as nitrosamines and polycyclic aromatic hydrocarbons (PAHs) in traditional cigarette smoke, several combustion products are included in the e-cigarette aerosol, too. Nitrosamines are present at levels almost similar to nicotine replacement therapies (NRTs) [4], and PAHs are completely absent from e-cigarettes. E-cigarette vendors have marketed their products as a cheaper and safer smokeless alternative to traditional cigarettes and a possible smoking cessation tool. Consequently, many cigarette smokers have turned to e-cigarettes, and the number of e-cigarette smokers is increasing [5–7]. According to a report by UBS Securities LLC (Union Bank of Switzerland, Zurich, Switzerland), e-cigarette market sales doubled from $250–$500 million between 2011 and 2012 and are expected to quadruple by 2014 [8]. In recent years, on the international market, e-cigarettes have been widely advertised via television, radio, magazines, newspapers, and the Internet. This mass marketing and commercialization of e-cigarettes is estimated to increase consumer awareness and the future use of e-cigarettes [9]. Additionally, the legal situation may be contributing to the widespread use of e-cigarettes. The World Health Organization (WHO) raised the alarm with regard to e-cigarettes that include nicotine and issued a Technical Report Series 955 in 2009 which states the following: the safety of e-cigarettes is not confirmed, and e-cigarettes are not an appropriate tool for smoking cessation therapy [10]. The Food and Drug Administration (FDA) reported that e-cigarettes contain carcinogens and toxic chemicals, such as nitrosamines and diethylene glycol, which have potentially harmful effects on humans [11]. Furthermore, the FDA found that nicotine was detected in the e-cigarette cartridges labeled as nicotine-free [10,12], and carcinogens and toxic chemicals, such as carbonyl compounds, were detected in the aerosols from e-cigarettes [7,13,14]. Evaluating the source and amount of carbonyl compounds released is crucial for regulators as well as consumers and manufacturers, and ways to reduce such emissions need to be investigated. This paper presents an overview of our research in this field, as well as a comparison with other relevant studies.

In this article, we review the results of our research over the past four years, and incorporate the current literature found in Science Direct, PubMed, and Google Scholar databases from journal articles published between 2010 and 2014. Various combinations of keywords, such as "e-cigarette", "electronic cigarette", "chemical components" and "carbonyl compounds" were used to find the relevant literature.

2. Carbonyl Compounds Emitted from Japanese E-Cigarettes

Uchiyama *et al.* measured carbonyl compounds in e-cigarette aerosols using cartridges impregnated with hydroquinone (HQ) and 2,4-dinitrophenylhydrazine (DNPH), followed by high-performance liquid chromatography (HPLC) [13–15]. Before collecting the aerosol from the e-cigarettes, an HQ-cartridge and a DNPH-cartridge were connected. The coupled cartridges were then connected between the mouthpiece of the e-cigarette and the smoking machine, and the aerosol from the e-cigarette was drawn into the coupled cartridges (from the HQ-cartridge to the DNPH-cartridge) according to the Canadian intense regimen (55 mL puff volume, 2-s puff duration, 30-s puff interval, and 10 puffs) [16]. After collection, the coupled cartridges were extracted using acetonitrile containing 1% phosphoric acid in the direction opposite to that of the air sampling until a 4.5 mL total volume was attained. After 10 min, ethanol (0.5 mL) was added to the eluate, and the solution was analyzed by HPLC.

Thirteen brands of Japanese e-cigarettes were measured, and several derivative peaks of carbonyl compounds, such as formaldehyde, acetaldehyde, acetone, acrolein, propanal, crotonaldehyde, butanal, glyoxal, and methylglyoxal, were detected [13,14]. Table 1 shows the mean amounts of the major carbonyl compounds generated from the Japanese e-cigarettes. For a typical cigarette smoking experience of 10 puffs, these values were translated into µg/10 puffs. The top entry in each cell indicates the mean value for the high-amount group, and the bottom entry indicates the mean value for the low-amount group. The indices N_{high} and N_{low} indicate the number of e-cigarettes that generated high and low concentrations of carbonyl compounds, respectively. As described below, carbonyl compounds were incidentally generated by touching the nichrome wire with e-liquid and increasing the battery output voltage. Therefore, concentrations showed bimodal distributions and were divided in extremely high and low groups. For clarity, the failure rate (FR) is indicated in Table 1. FR was calculated by the following equation: $FR = N_{\text{high}}/(N_{\text{high}} + N_{\text{low}}) \times 100$.

Table 1. Amounts (µg/10 puff) of major carbonyl compounds generated from 13 brands of Japanese e-cigarettes. Smoking machine was performed at 10 puffs (reproduced from [13] with permission from The Japan Society for Analytical Chemistry).

Product	N_{high} N_{low}	FR	Formaldehyde	Acetaldehyde	Acrolein	Propanal	Glyoxal	Methylglyoxal
A	16	31	34 ± 35	26 ± 28	4.1 ± 3.8	8.8 ± 11	2.5 ± 3.6	2.9 ± 3.1
	35		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B	06	20	13 ± 5.8	0.2 ± 0.1	6.6 ± 2.4	1.1 ± 0.7	16± 6.6	11 ± 4.3
	24		1.4 ± 0.9	n.d.	1.2 ± 0.9	n.d.	n.d.	2.0 ± 1.2
C	08	27	22 ± 15.4	0.9 ± 1.4	5.3 ± 5.5	3.4 ± 3.5	9.9 ± 5.2	12.1 ± 5.5
	22		1.7 ± 1.4	n.d.	0.6 ± 0.6	n.d.	0.7 ± 0.7	1.2 ± 1.0
D	12	24	15 ± 6.6	13.8 ± 6.6	20 ± 9.9	13.2 ± 10.5	4.2 ± 2.3	6.1 ± 4.1
	37		0.8 ± 1.0	n.d.	n.d.	n.d.	n.d.	n.d.
E	14	40	17 ± 7.7	15 ± 6.1	18 ± 6.6	15 ± 8.3	4.5 ± 2.4	4.7 ± 4.3
	21		0.7 ± 0.8	n.d.	0.7 ± 0.9	n.d.	n.d.	n.d.
F	02	40	6.6 ± 0.9	1.5 ± 0.1	1.1 ± 0.1	0.4 ± 0.1	1.5 ± 0.4	3.2 ± 0.5
	03		2.0 ± 1.7	0.9 ± 0.2	0.7 ± 0.3	n.d.	n.d.	0.9 ± 0.8
G	01	4	29	10	10	3.5	9.4	20
	25		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H	05	17	10 ± 4.9	4.6 ± 2.4	4.5 ± 2.2	n.d.	2.5 ± 0.5	4.6 ± 3.1
	25		0.9 ± 1.4	n.d.	n.d.	n.d.	n.d.	n.d.
I	06	20	3.2 ± 1.0	6.1 ± 3.2	6.1 ± 2.3	7.7 ± 2.3	n.d.	n.d.
	24		1.5 ± 1.4	2.6 ± 2.9	2.8 ± 2.7	3.3 ± 3.4	n.d.	n.d.
J	00	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	04		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
K	00	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	30		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L	00	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	30		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M	00	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	13		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 1. *Cont.*

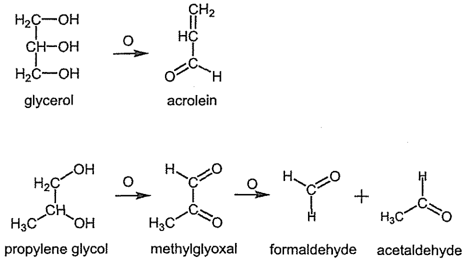
Notes: Resulting data were divided into two groups based on the formaldehyde concentration (10 mg/m^3). The upper line indicates the mean value for the high-concentration group, and the lower line indicates the mean value for the low-amount group. Indices N_{high} and N_{low} indicate the number of e-cigarettes that generated high and low amounts of carbonyl compounds, respectively. FR indicates the failure rate, which was calculated using the equation as follows: $FR = N_{\text{high}}/(N_{\text{high}} + N_{\text{low}}) \times 100$. Values are mean ± SD; n.a., not available; n.d., not detected.

Four (J, K, L, and M) out of the 13 e-cigarette brands did not generate any carbonyl compounds. The other nine e-cigarette brands (A, B, C, D, E, F, G, H, and I) generated various carbonyl compounds. The amount of carbonyl compounds obtained for the high-amount group was significantly higher than that obtained for the low-amount group. The maximum concentrations of formaldehyde, acetaldehyde, acrolein, propanal, glyoxal, and methylglyoxal were 140, 120, 40, 46, 23, and 21 µg/10 puffs, respectively. Most notably, very high amounts of formaldehyde were measured in e-cigarette aerosols. Glyoxal and methylglyoxal, which show mutagenicity, are specific to e-cigarette aerosols and have been minimally detected in the mainstream smoke from traditional cigarettes. The amount of carbonyl compounds in these brands of e-cigarettes varied significantly not only among different brands but also among different samples of the same products.

3. Mechanism for Generation of Carbonyl Compounds from E-Cigarettes

The design of most e-cigarettes includes a plastic tube holding a battery, an air flow sensor, a vaporizer, and a nicotine/flavor cartridge with a chemical component, such as glycerols or glycols, which turn the liquid to aerosol [17]. The function of e-cigarettes has changed from disposable and rechargeable to "tank systems" that can hold a large volume of e-liquid. This e-liquid incidentally touches the heated nichrome wire and is oxidized to formaldehyde, acetaldehyde, acrolein, glyoxal, and methylglyoxal in the presence of oxygen in the surrounding air [13,14]. There is a great variety of commercial e-liquids manufactured and distributed by various companies. Figure 1 shows the reaction of e-liquid with heated nichrome wire in the study of Uchiyama *et al.* [13] and Ohta *et al.* [14]. Glycerol in e-liquid is oxidized with heated nichrome wire to form acrolein, while propylene glycol in e-liquid is oxidized to form methyl glyoxal, formaldehyde, and acetaldehyde [13,14]. This e-liquid inadvertently touches the heated nichrome wire to form these oxidation products.

Figure 1. Oxidation of e-liquid (glycerol and propylene glycol) with nichrome wire (reproduced from [14] with permission from The Japan Society for Analytical Chemistry).



Furthermore, battery output voltage affects the concentration of the carbonyl compounds in the emission [7]. Some new e-cigarettes allow users to increase vapor production and nicotine delivery by changing the battery output voltage. Kosmider *et al.* showed that increasing the voltage from 3.2–4.8 V resulted in a 4 to >200 times increase in the formaldehyde, acetaldehyde, and acetone levels [7]. In fact, the levels of formaldehyde in vapors from high-voltage devices were almost identical to those in traditional cigarette smoke (1.6–52 μg per cigarette) [18]. Ohta *et al.* further reported that increasing levels of carbonyl compounds, such as formaldehyde and acetaldehyde, were observed for a voltage over 3 V [13,14]. Consequently, commercial e-cigarettes with 4–5 V batteries are sufficient to generate carbonyl compounds, with the battery output voltage significantly affecting the concentration of carbonyl compounds in the e-cigarette aerosol. As such, high-voltage e-cigarettes may expose users to high levels of carbonyl compounds.

4. Discussion

E-cigarettes are advertised as less harmful products because they are believed to contain fewer and less toxic inhaled compounds than traditional cigarettes. Consequently, e-cigarettes are considered to be an appropriate tool for tobacco harm reduction, which describes actions taken to lower the health risks associated with using nicotine delivered through combustible tobacco [19]. However, e-cigarettes have not been theoretically or experimentally proven to be safer products. In fact, there are some case reports of health damage induced by e-cigarettes in many countries, including the USA and in Europe. The most common symptom is a dry mouth and throat [12,20] , which is considered to originate from the water-absorbing property of propylene glycol and glycerol, the main constituents of e-liquids. Furthermore, several health impacts such as hypertension, asthma, chronic obstructive pulmonary disease, lipoid pneumonia, cardiac arrhythmias, eosinophilic pneumonitis, congestive heart failure, disorientation, and hypotension are considered to be caused by e-cigarette use [21–24]. On the other hand, benefits of e-cigarette use such as smoking abstinence and a reduction in asthmatic smokers is reported in the survey of e-cigarette use [25].

However, regulation of the e-cigarette should be considered on the basis of reported adverse health effects. In some countries and regions, such as Europe and the USA, regulations regarding nicotine content, labeling, advertising, and sale of e-cigarettes are already in effect [26,27]. Some countries do not accept e-cigarettes as a cessation tool for smokers, yet regulate it as a medical product [28,29]. Recently, direction about manufacture, presentation, ingredients, sale, and certain aspects of labeling and packaging were adopted to regulate e-cigarette users and companies in Europe [30]. However, the chemical compounds generated from the e-cigarettes themselves are yet to be regulated. To promote e-cigarette regulation, we need to show more substantial scientific data about the impacts of e-cigarettes. In recent years, several studies, including those of our group, reported that carbonyl compounds such as formaldehyde, acetaldehyde, acrolein, and glyoxal are often found in e-cigarette aerosols, which are considered to have toxic effects on human health. Formaldehyde is classified as a human carcinogen (Group 1) by the International Agency for Research on Cancer (IARC), and acetaldehyde is classified as possible carcinogenic to humans (Group 2B) [31]. Acrolein causes irritation of the nasal cavity and damages the lining of the lungs [16]. These compounds in e-cigarettes are potentially hazardous and induce various health effects on its users.

Some carbonyls, such as formaldehyde, acetaldehyde, and acrolein in e-cigarette emissions have also been reported in other countries [32–34]. Table 2 shows the amount of formaldehyde, acetaldehyde, and acrolein in the aerosols of Polish e-cigarettes [35]. According to these data, the emissions from e-cigarettes without propylene glycol were almost 100-fold lower than those from traditional cigarettes [36].

Table 2. Amounts (μg) of major carbonyl compounds generated from 12 brands of Polish e-cigarettes. Smoking machine was performed at 150 puffs (reproduced from [35] with permission from BMJ Publishing Group Ltd.).

Product	Formaldehyde	Acetaldehyde	Acrolein
EC01	44.2 ± 4.1	4.6 ± 0.2	41.9 ± 3.4
EC02	23.6 ± 8.7	6.8 ± 3.2	4.4 ± 2.5
EC03	30.2 ± 2.3	8.2 ± 2.5	16.6 ± 2.5
EC04	47.9 ± 0.2	11.5 ± 2.0	30.1 ± 6.4
EC05	56.1 ± 1.4	3.0 ± 0.2	22.0 ± 1.6
EC06	35.3 ± 2.7	13.6 ± 2.1	2.1 ± 0.4
EC07	19.0 ± 2.7	11.1 ± 3.3	8.5 ± 3.6
EC08	6.0 ± 2.0	8.8 ± 1.6	0.7 ± 0.4
EC09	3.2 ± 0.8	3.5 ± 0.3	ND
EC10	3.9 ± 1.5	2.0 ± 0.1	2.7 ± 1.6
EC11	23.9 ± 11.1	3.7 ± 1.5	1.1 ± 0.6
EC12	46.3 ± 2.1	12.0 ± 2.4	7.4 ± 3.2

Kosmider *et al.* reported that formaldehyde and acetaldehyde were detected in eight of 13 samples [7]. The amounts of formaldehyde and acetaldehyde in e-cigarette aerosols at a lower voltage were on average 13 and 807-fold lower than those in traditional cigarette smoke, respectively. The highest levels of carbonyls were observed in e-cigarette aerosols generated from propylene-glycol-based solutions.

Furthermore, the data revealed large variations in carbonyl levels for different e-cigarettes. However, in general, there is an insufficient amount of data on the hazardous carbonyl compounds emitted from e-cigarettes, thus warranting continued broad monitoring of these compounds.

5. Conclusions

Studies have shown that e-cigarettes emit toxic carbonyl compounds, generated from thermal decomposition. These substances can have adverse health effects; however, in most cases, the levels are lower than those in tobacco cigarette smoke. It is important to expand the research in this field, to better understand the source of carbonyls emitted from e-cigarettes and find ways to reduce them.

Acknowledgments

This research was partially supported by JSPS KAKENHI Grant Numbers 25560361 and 23650425.

Author Contributions

Kanae Bekki reviewed articles on e-cigarette study and wrote the paper. Shigehisa Uchiyama improved the analytical method (HQ-DNPH method) for e-cigarette. Kazushi Ohta and Shigehisa Uchiyama measured 363 e-cigarettes (13 brands) using HQ-DNPH method. Kazushi Ohta performed additional experiments to understand the generation mechanism of carbonyl compounds from e-cigarette. Yohei Inaba prepared experimental equipment and all e-cigarettes tested in this study. Shigehisa Uchiyama, Naoki Kunugita and Hideki Nakagome provided writing assistance and technical advice on the regulatory aspects of the paper. All contributors approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Gilbert, H.A. Smokeless Non-Tobacco Cigarette. Patent No. 3200819, 17 August 1965.
2. Cummings, K.M.; Dresler, C.M.; Field, J.K.; Fox, J.; Gritz, E.R.; Hanna, N.H.; Ikeda, N.; Jassem, J.; Mulshine, J.L.; Peters, M.J.; *et al.* E-cigarettes and cancer patients. *J. Thorac. Oncol.* **2014**, *9*, 438–441.
3. Farsalinos, K.E.; Romagna, G.; Tsiapras, D.; Kyrzopoulos, S.; Voudris, V. Characteristics, perceived side effects and benefits of electronic cigarette use: A worldwide survey of more than 19,000 consumers. *Int. J. Environ. Res. Public Health* **2014**, *11*, 4356–4373.
4. Farsalinos, K.E.; Polosa, R. Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: A systematic review. *Ther. Adv. Drug Saf.* **2014**, *5*, 67–86.
5. Palazzolo, D.L. Electronic cigarettes and vaping: A new challenge in clinical medicine and public health. A literature review. *Front. Public Health* **2013**, *1*, doi:10.3389/fpubh.2013.00056.
6. Ayers, J.W.; Ribisl, K.M.; Brownstein, J.S. Tracking the rise in popularity of electronic nicotine delivery systems (electronic cigarettes) using search query surveillance. *Amer. J. Prev. Med.* **2011**, *40*, 448–453.

7. Kosmider, L.; Sobczak, A.; Fik, M.; Knysak, J.; Zaciera, M.; Kurek, J.; Goniewicz, M.L. Carbonyl compounds in electronic cigarette vapors-effects of nicotine solvent and battery output voltage. *Nicotine Tobacco Res.* **2014**, doi:10.1093/ntr/ntu078.
8. Modi, N.; Schmid, B.; Miller, R. Clearing the Smoke on E-Cigarettes. Available online: http://www.ubs.com/investmentresearch (accessed on 10 May 2014).
9. Kim, A.E.; Arnold, K.Y.; Makarenko, O. E-cigarette advertising expenditures in the U.S., 2011–2012. *Amer. J. Prev. Med.* **2014**, *46*, 409–412.
10. World Health Organization. WHO study group on tobacco product regulation report on the scientific basis of tobacco product regulation. *WHO Techn. Report Ser.* **2009**, *955*, 1–41.
11. U.S. Food and Drug Administration (FDA). FDA Warns of Health Risks Posed by E-Cigarettes. Available online: http://www.fda.gov/downloads/ForConsumers/ConsumerUpdates/Updates/UCM173430.pdf (accessed on 22 May 2014).
12. Etter, J.F.; Bullen, C. Electronic cigarette: Users profile, utilization, satisfaction and perceived efficacy. *Addiction* **2011**, *106*, 2017–2028.
13. Uchiyama, S.; Ohta, K.; Inaba, Y.; Kunugita, N. Determination of carbonyl compounds generate from the e-cigarette using couples silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography. *Anal. Sci.* **2013**, *29*, 1219–1222.
14. Ohta, K.; Uchiyama, S.; Inaba, Y.; Nakagome, H.; Kunugita, N. Determination of carbonyl compounds generated from the electronic cigarette using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine. *Bunseki Kagaku* **2011**, *60*, 791–797.
15. Uchiyama, S.; Inaba, Y.; Kunugita, N. Determination of acrolein and other carbonyls in cigarette smoke using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine. *J. Chromatogr. A* **2010**, *1217*, 4383–4388.
16. Health Canada Official Method T-115, Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke; Health Canada: Ottawa, ON, Canada, 1999.
17. Cobb, N.K.; Byron, M.J.; Abrams, D.B.; Shields, P.G. Novel nicotine delivery systems and public health: the rise of the "e-cigarette". *Amer. J. Public Health* **2010**, *100*, 2340–2342.
18. Counts, M.E.; Morton, M.J.; Laffoon, S.W.; Cox, R.H.; Lipowicz, P.J. Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. *Regul. Toxicol. Pharmacol.* **2005**, *41*, 185–227.
19. Zeller, M.; Hatsukami, D. The strategic dialogue on tobacco harm reduction: A vision and blueprint for action in the U.S. *Tob. Control* **2009**, *18*, 324–332.
20. Dawkins, L.; Turner, J.; Roberts, A.; Soar, K. "Vaping" profiles and preferences: An online survey of electronic cigarette users. *Addiction* **2013**, *108*, 1115–1125.
21. Monroy, A.E.; Hommel, E.; Smith, S.T.; Raji, M. Paroxysmal atrial fibrillation following electronic cigarette use in an elderly woman. *Clin. Geriatr.* **2012**, *20*, 28–32.
22. Thota, D.; Latham, E. Case Report of electronic cigarettes possibly associated with eosinophilic pneumonitis in a previously healthy active-duty sailor. *J. Emerg. Med.* **2014**, *47*, 15–17.
23. Chen, I.L. FDA summary of adverse events on electronic cigarettes. *Nicotine Tob. Res.* **2013**, *15*, 615–616.

24. Hua, M.; Alfi, M.; Talbot, P. Health-related effects reported by electronic cigarette users in online forums. *J. Med. Internet Res.* **2013**, *15*, doi:10.2196/jmir.2324.
25. Polosa, R.; Morjaria, J.; Caponnetto, P.; Caruso, M.; Strano, S.; Battaglia, E.; Russo, C. Effect of smoking abstinence and reduction in asthmatic smokers switching to electronic cigarettes: Evidence for harm reversal. *Int. J. Environ. Res. Public Health* **2014**, *11*, 4965–4977.
26. Paradise, J. Electronic cigarettes: Smoke-free laws, sale restrictions, and the public health. *Amer. J. Public Health* **2014**, *104*, 17–18.
27. Benowitz, N.L.; Goniewicz, M.L. The regulatory challenge of electronic cigarettes. *J. Amer. Med. Assoc.* **2013**, *310*, 685–686.
28. German Cancer Research Center. Electronic Cigarettes—An Overview. Available online: http://www.dkfz.de/en/presse/download/RS-Vol19-E-Cigarettes-EN.pdf (accssed on 18 May 2014).
29. Jimenez Ruiz, C.A.; Solano Reina, S.; de Granda Orive, J.I.; Signes-Costa Minaya, J.; de Higes Martinez, E.; Riesco Miranda, J.A.; Altet Gomez, N.; Lorza Blasco, J.J.; Barrueco Ferrero, M.; de Lucas Ramos, P. The electronic cigarette. Official statement of the Spanish society of pneumology and thoracic surgery (separ) on the efficacy, safety and regulation of electronic cigarettes. *Arch. Bronconeumol.* **2014**, *50*, 362–367.
30. EUR-Lex Access to European Union Law. Directive 2014/40/EU of The European Parliament and of The Council. Available online: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:JOL_2014_127_R_0001 (accessed on 5 June 2014).
31. *Monographs on the Evaluation of Carcinogenic Risks to Humans*; International Agency for Research on Cancer (IARC): Lyon, France, 1999; Volume 71, pp. 319–335.
32. Leaderer, B.P.; Naeher, L.; Jankun, T.; Balenger, K.; Holford, T.R.; Toth, C.; Sullivan, J.; Wolfson, J.M.; Koutrakis, P. Indoor, outdoor, and regional summer and winter concentrations of PM_{10}, $PM_{2.5}$, SO_4^{2-}, H^+, NH_4^+, NO_3^-, NH_3, and nitrous acid in homes with and without kerosene space heaters. *Environ. Health Perspect.* **1999**, *107*, 223–231.
33. Laugesen, M. *Safety Report on the Ruyan® E-Cigarette Cartridge and Inhaled Aerosol*; Health New Zealand Ltd.: Christchurch, New Zealand, 2008.
34. Schripp, T.; Markewitz, D.; Uhde, E.; Salthammer, T. Does e-cigarette consumption cause passive vaping? *Indoor Air* **2013**, *23*, 25–31.
35. Goniewicz, M.L.; Knysak, J.; Gawron, M.; Kosmider, L.; Sobczak, A.; Kurek, J.; Prokopowicz, A.; Jablonska-Czapla, M.; Rosik-Dulewska, C.; Havel, C.; *et al.* Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob. Control* **2014**, *23*, 133–139.
36. McAuley, T.R.; Hopke, P.K.; Zhao, J.; Babaian, S. Comparison of the effects of e-cigarette vapor and cigarette smoke on indoor air quality. *Inhal. Toxicol.* **2012**, *24*, 850–857.

BUNSEKI KAGAKU Vol. 60, No. 10, pp. 791–797 (2011)

報　文

ハイドロキノンと2,4-ジニトロフェニルヒドラジンを含浸させた二連シリカカートリッジを用いる電子タバコから発生するカルボニル化合物の分析

太田　和司[1]，内山　茂久®[2]，稲葉　洋平[2]，中込　秀樹[1]，欅田　尚樹[2]

電子タバコから発生する煙の成分をハイドロキノン（HQ）と2,4-ジニトロフェニルヒドラジン（DNPH）を用いた二連カートリッジ法（HQ-DNPH法）で分析し，発生する化学物質，生成メカニズム等の検討を行った．市販されている電子タバコの煙を分析した結果，ホルムアルデヒド，アセトアルデヒド，アクロレイン，グリオキサール，メチルグリオキサール等，多くの有害なカルボニル化合物が高濃度で検出された．電子タバコ専用カートリッジに含まれる液体を分析した結果，主成分はグリセロールやグリコール類であった．そこで，様々なグリコール類をコイル状のニクロム線に塗布した模擬電子タバコを作製し，一定の電圧を印加し，そこから発生する気体を分析した．その結果，3 V以上の電圧を印加すると，エチレングリコールからグリオキサールが，プロピレングリコールからメチルグリオキサールが，グリセロールからアクロレインが発生することが明らかになった．

1　緒　　言

電子タバコは，香港に所在する北京SBT如煙科技発展有限公司が2003年に世界で初めて開発したとされ，現在，世界各国に普及している．それはバッテリー，蒸発ユニット，液体カートリッジで構成され「煙草」に模した気体吸引機であり，実際に吸引することで液体カートリッジに入れられた液体が蒸発ユニットを通過するとき霧状となりそれを吸引する仕組みである．しかし，世界保健機関WHOは，2008年9月に海外で販売されているニコチン入り電子タバコの利用について，「安全性は確認されておらず，禁煙療法とは考えられない」と警鐘を鳴らし，2009年にTechnical Report Series 955 1)を発行している．またアメリカ食品医薬品局FDAは，2009年7月，米国で販売されている電子タバコから不凍液成分であるジエチルグリコールや発がん物質であるニトロサミン類が検出されたと報告している2)．日本国内では，国民生活センターの調べで，個人輸入されている海外販売のニコチン含有電子タバコだけでなく，国内販売している商品からもニコチンがわずかながら検出され問題となった（平成22年8月18日）．しかし，電子タバコから発生する気体の化学分析が困難であったため，現在までに十分に報告されていない．

ホルムアルデヒドやアセトアルデヒドなどのカルボニル化合物は人体に有害な物質である．比較的高濃度のホルムアルデヒドやアセトアルデヒドの長期間暴露は，喘息3)やガン4)～6)のリスクを高めることも知られている．また，アクロレインやクロトンアルデヒドなどのα,β-不飽和アルデヒドは反応性が高く，人への影響が懸念されている物質である．2006年にFengらは，煙草煙中のアクロレインが肺がんのリスクを増加させることを報告している7)．したがって，カルボニル化合物の人に対する健康影響や生成機構を解明することは重要である．

2010年にUchiyamaらは，α,β-不飽和アルデヒドを含む広範囲のカルボニル化合物の分析方法として，ハイドロキノン（HQ）含浸シリカと2,4-ジニトロフェニルヒドラジン（DNPH）8)含浸シリカを用いたHQ-DNPH cartridge法を報告した9)．これは，ラジカル捕捉剤であるHQによりDNPHシリカ中のアクロレインのヒドラゾン誘導体の分解や重合を防ぎ，α,β-不飽和アルデヒドを含む広範囲のカルボニル化合物の分析を可能とする方法である．

本研究ではHQ-DNPH cartridge法を用いて，電子タバコから発生する気体に含まれる有害なカルボニル化合物の分析を行い，その発生量や発生メカニズムを検討した．

2　実　　験

2・1　装置と試薬

高速液体クロマトグラフィー（HPLC）は，LC-20AD送液ポンプを二台，SPD M20Aフォトダイオードアレー検出

---

[1] 千葉大学大学院工学研究科：263-8522　千葉県千葉市稲毛区弥生町1-33
[2] 国立保健医療科学院生活環境研究部：351-0197　埼玉県和光市南2-3-6

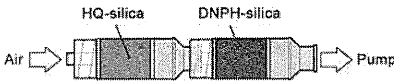


Fig. 1　Schematic drawing of the HQ-DNPH-cartridge

器を備えた島津製作所製 Prominence LC-20 を使用した．分離カラムは Ascentis Express RP-Amide（2.7 µm particle size, 150 mm × 4.6 mm i.d., Supelco 製）を用い，カラムオーブン温度を 40 ℃，注入量を 10 µL とした．グラジェントモードの HPLC 分析には，移動相にアセトニトリル（40 %）と水（60 %）の混合溶液（A 溶液）と，5 mmol/L の酢酸アンモニウムを含むアセトニトリル（75 %）と水（25 %）混合溶液（B 溶液）を用いた．カラム流量 0.7 mL/min で A 溶液 100 % を 8 分間保ち，37 分間で B 溶液 100 % にした後，15 分間 B 溶液 100 % を保った．

ガスクロマトグラフィー質量分析（GC/MS）は，ヒューレットパッカード製 5890/5972 型を使用し，カラムはスペルコ製 SLB-5ms（0.25 mm i.d. × 30 m，0.25 µm）を用いた．分析条件は，カラム温度 40 ℃（5 分保持）40 ℃～300 ℃（昇温速度 10 ℃/min），300 ℃（26 分保持）とした．質量分析条件は m/z 40～350 のスキャン測定，GC と MS のインターフェイス温度は 250 ℃，イオン源温度 280 ℃，電子イオン化（EI）電圧は 70 eV，注入条件は高圧スプリットレス法（2 min）とした．

自動喫煙装置は Borgwaldt Technik GmbH 製（model LM1/PLUS）を使用し，HCI（Health Canada Intensive）法[10]に準拠して，一回の吸煙量を 55 mL，吸煙時間を 2 秒，吸煙間隔を 28 秒，吸煙回数を 10 回に設定した．

HPLC 及び試料調整用の純水は Millipore 製 Milli-Q システムを使用した．2,4-ジニトロフェニルヒドラジン塩酸塩は東京化成工業製，ハイドロキノン，リン酸（85 %），エタノール（99.5 %），グリセロール（99 %），エチレングリコール（99.5 %），プロピレングリコール（99 %）は和光純薬工業製を使用した．シリカゲルは AGC SI-Tech 製の 60/80 mesh，120 Å を使用した．電子タバコは，日本国内で一般に市販されている 10 銘柄（A, B, C, D, E, F, G, H, I, J）について検討を行った．

2･2　DNPH-cartridge および HQ-cartridge の作製

DNPH-cartridge：高濃度測定用と低濃度測定用の二種類の DNPH-cartridge を作製した．シリカゲル 50 g を純水 500 mL で 3 回洗浄する．次に 500 mL のメタノールで 2 回洗浄した後，500m L のアセトニトリルで 2 回洗浄する．2,4-ジニトロフェニルヒドラジン塩酸塩を，低濃度測定用の場合は 0.25 g，高濃度測定用の場合は 1 g，及びリン酸 1 mL をアセトニトリル 300 mL に溶かす．この溶液を洗浄したシリカに添加した後，40 ℃ に設定したロータリーエバポレーターでアセトニトリルを留去した．この DNPH-silica を 280 mg 秤（ひょう）量し，1 mL のレゾリアンチューブに充填して DNPH-cartridge とした．

HQ-cartridge：ハイドロキノンを 0.05 g 秤量し，50 mL のアセトニトリルに溶かす．この溶液を洗浄シリカ 50 g に添加した後，40 ℃ に設定したロータリーエバポレーターでアセトニトリルを留去した．この HQ-silica を 280 mg 秤量し，1 mL のレゾリアンチューブに充填して HQ-cartridge とした．

2･3　電子タバコから発生するカルボニル化合物の捕集と分析

DNPH-cartridge の前段に HQ-cartridge を接続し HQ-DNPH-cartridge（Fig. 1）を作製する．主流煙を測定するときは，電子タバコのカートリッジ（吸引口）に HQ-DNPH-cartridge の HQ-cartridge 側を接続し，DNPH-cartridge 側を自動喫煙装置の試料口に接続する．HCI 法で捕集した後，直ちに抽出，分析を行わない場合は再び HQ-DNPH-cartridge を DNPH-cartridge と HQ-cartridge に分割し，冷暗所に保管した．捕集終了後，HQ-DNPH-cartridge の DNPH-cartridge 側から，リン酸を 1 % 含むアセトニトリル溶液 4 mL で溶出し，誘導体化のため 10 分間放置した後，過剰な付加反応を抑制するために，エタノールを 1 mL 添加した．この溶液 10 µL を HPLC に導入し，各種のカルボニル化合物 DNPH 誘導体を分析した．

3　結果と考察

3･1　電子タバコから発生する気体に含まれるカルボニル化合物

国内で市販されている 10 銘柄の電子タバコ（A～J）から発生する煙に含まれるカルボニル化合物を HQ-DNPH 法で測定した．代表的なクロマトグラムを Fig. 2 に示す．未反応の DNPH のほかに，ホルムアルデヒド，アセトアルデヒド，アセトン，アクロレイン，プロパナール，クロトンアルデヒド，ブタナール，グリオキサール，メチルグリオキサール，ヘキサナールの誘導体のピークが検出されたが，特にホルムアルデヒド，アセトアルデヒド，アクロレイン，プロパナールグリオキサール，メチルグリオキサールなどの，炭素数が 1～3 のアルデヒド類が高濃度で発生することが明らかになった．なお，これらのピークは，合成した各 DNPH 誘導体の保持時間から同定した．WHO が策定したホルムアルデヒドの室内指針値[11]は 0.1 mg/m^3 であるが，A～J のホルムアルデヒド発生量の平均値は，それぞれ 11, 13, 4.6, 0.3, 1.6, 20, 8.1, 2.8, 6.9, 0.5 mg/m^3 であり，いずれの銘柄も指針値以上の濃度を示した．ホルムアルデヒド以外のアセトアルデヒド，アクロレイン，グリオキサール，メチルグリオキサールなどの物質についても，非常に高濃度で発生した．しかし，それらの発生量は同一銘柄でも大きく変動していた．例えば，銘柄 F では，ホルムアルデヒド発生量の平均値は 20 mg/m^3 であるが，最大値は 260 mg/m^3，最小値は 0.1 mg/m^3 であり，変動幅が非常に大きい．それぞれの銘柄間でも大きな変動が認められ，中には，D, E, J 銘柄のようにカルボニル化合物発生量が全体的に低い電子タバコも存在した．また，カルボニル化合物を多く発生した変霧器は，捕集（喫煙）後，ニクロム線の周囲が黒褐色に変色する傾向が認められた．

次に，A, B, C, D, E の銘柄について，それぞれ 10 サンプルロットずつ購入し，1 サンプルロットにつき 3 回ずつの測定を行った．Fig. 3 に各銘柄における，ホルムアルデヒド，アクロレイン，グリオキサール，メチルグリオキサールの発生量を示す．測定したすべての電子タバコから様々なカルボニル化合物が発生したが，銘柄ごとに発生量，物

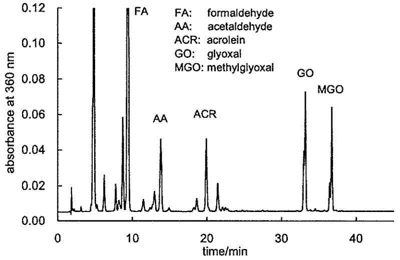


Fig. 2　Representative chromatogram of carbonyl-DNPH derivatives generated from electronic cigarette

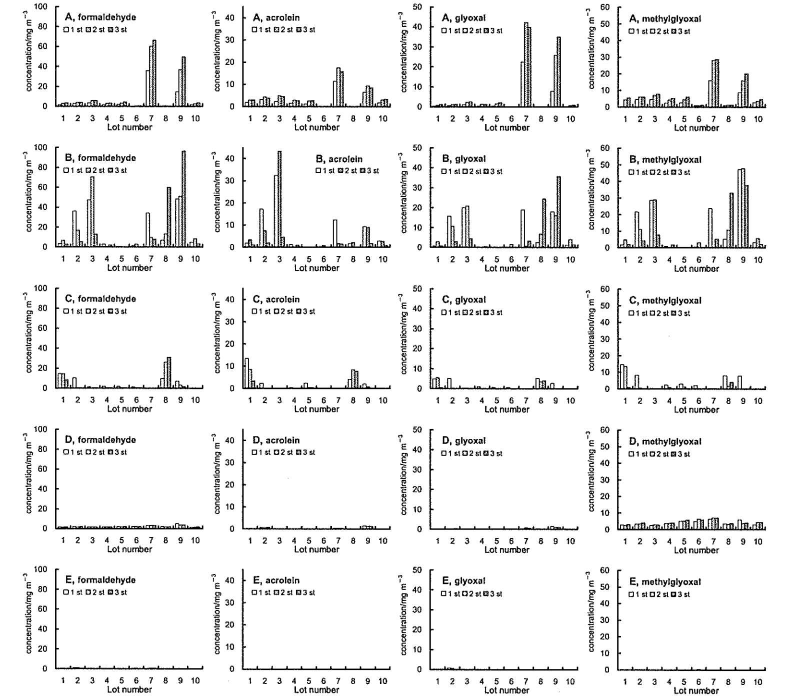


Fig. 3　Concentrations of formaldehyde, acrolein, glyoxal and methylglyoxal generated from various brands of electronic cigarettes

質の種類が異なった．また，同一銘柄でも，個々のサンプルロット間で大きなばらつきが認められた．例えば，銘柄Aでは，10個のサンプルロット中，No.7とNo.9の2個のサンプルロットがホルムアルデヒド，アクロレイン，グリオキサール，メチルグリオキサールを多く発生した．ホルムアルデヒド，グリオキサールは，発生量の低いサンプルロットと比較すると，おおむね10倍程度高濃度である．また，同一サンプルロット内でも測定回数による濃度差が大きく，サンプルロットNo.9のグリオキサール濃度は1回目と3回目では5倍程度発生量に差が生じている．銘柄Bでは，全10個のサンプルロット中，No.2，No.3，No.7，No.8，No.9の5個のサンプルロットがホルムアルデヒド，アクロレイン，グリオキサール，メチルグリオキサールを多く発生した．これらの発生量は，A銘柄と同程度であるが，ホルムアルデヒドは97 mg/m^{3}の高い値を示した．また，銘柄Aと同様に，同一サンプルロット内の測定回数による濃度差は大きかった．銘柄Cでは，全体的にカルボニル化合物の発生量は少ないが，全10個のサンプルロット中，No.1とNo.8のサンプルロットが比較的多く発生した．銘柄Dでは，すべてのサンプルロットで2.6～7.1 mg/m^{3}のメチルグリオキサールを発生したが，ホルムアルデヒド，アクロレイン，グリオキサールの発生量は少なかった．銘柄Eでは，いずれのサンプルロットにおいてもカルボニル化合物の発生量は非常に少なかった．

以上の結果から，カルボニル化合物の発生量には三つの大きなばらつきがあることが明らかになった．すなわち，銘柄間のばらつき，サンプルロットごとの発生量のばらつき，サンプルロット内のばらつきである．HQ-DNPH法によるホルムアルデヒド，アセトアルデヒド，アクロレイン測定時の相対標準偏差は，それぞれ1.9, 1.8, 1.2, 2.1 %である[9]ので測定法のばらつきは非常に小さい．したがって，ばらつきの原因は，電子タバコの製品自体にあると思われる．

3·2　電子タバコのカートリッジに含まれる液体中の化学物質の分析

カルボニル化合物の分析：銘柄F, G, H, Iの電子タバコカートリッジの液体をカートリッジからピペットでバイアルに移し，DNPH溶出液と混合させ誘導体化を行った後，HPLCで分析した．その結果，変霧器によって気化される前のカートリッジには，カルボニル化合物がほとんど含まれていないことが明らかになった．

揮発性有機化合物の分析：銘柄F, G, H, Iの電子タバコカートリッジ2，3本分の液体をカートリッジからピペットでバイアルに移し，メタノールで希釈した後，GC/MSで分析した．その結果，電子タバコカートリッジに含まれる液体の主成分は，エチレングリコール，プロピレングリコール，ジプロピレングリコール，グリセロール，トリエチレングリコール，ペンタエチレングリコールなどのグリセロール，グリコール類であった．また，これらのグリセロール，グリコール類は，成分比率は異なっているものの，どの銘柄にも含有していた．これらの結果から，電子タバコカートリッジに含まれる液体の成分はグリセロール，グリコール類であり，電子タバコを吸煙するとき，すなわち，煙を発生させるとき，カルボニル化合物が生成することが示唆される．

3·3　電子タバコから発生するカルボニル化合物の生成メカニズム

前述したように，電子タバコは，バッテリー，変霧器，液体カートリッジで構成され，カートリッジ内の液体がニクロム線により霧状となる仕組みである．そこで，ニクロム線と乾電池で構成される市販の電子タバコに模した“霧発生器”を作製し，発生するカルボニル化合物を検討した．ニクロム線をコイル状に巻き，エチレングリコール，プロピレングリコール，グリセロールを，それぞれ10 μL塗布した後，一定の電圧を印加し，“煙”を発生させる．この煙をHQ-DNPH-cartridgeを用いて，Uchiyamaらの方法[9]に従い0.5 L/minの流量で3分間捕集し，前述の分析方法で各種のカルボニル化合物を分析した．4.5 Vの電圧を印加したときに発生したカルボニル化合物誘導体のクロマトグラムをFig. 4に示す．様々なカルボニル化合物が検出されたが，ホルムアルデヒド（FA），アセトアルデヒド（AA）はすべてのグリセロール，グリコール類から発生したが，グリセロールからはアクロレイン（ACR）が，エチレングリコールからはグリオキサール（GO），プロピレングリコールからはメチルグリオキサール（MGO）が多く発生する傾向が認められた．これらの物質はScheme 1の酸化反応により発生したことが推測される．前述したように，市販されている電子タバコには，グリセロールと数種類のグリコール類の混合物が使用されているが，これらの混合比により発生するカルボニル化合物が異なることが推測される．

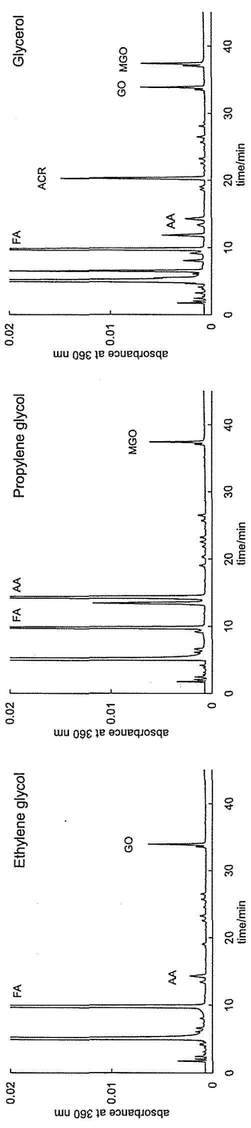
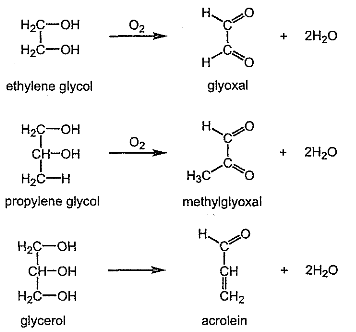


Fig. 4　Chromatographic profiles of carbonyl compounds generated by heating ethylene glycol (left panel), propylene glycol (middle panel) and glycerol (right panel)



Scheme 1　Formations of aldehydes from glycerol and glycols

次に，印加電圧によるカルボニル化合物発生量の変化をFig. 5に示す．ホルムアルデヒドの発生量は，他の物質に比較して際立って多い．また，炭素数が2のエチレングリコールからはホルムアルデヒド，グリオキサールが多く発生したが，炭素数が3以上のアクロレイン，メチルグリオキサールの発生はほとんど無かった．一方，炭素数3のプロピレングリコールからはホルムアルデヒド，アセトアルデヒドのほかに，メチルグリオキサールが多く発生した．これに対し，同じ炭素数3のグリセロールからはホルムアルデヒド，グリオキサール，アクロレインが多く発生したが，アセトアルデヒド，メチルグリオキサールの発生は少なかった．このように同じ炭素数のグリコール類でも発生する物質は異なっているが，いずれの物質も3 Vを超える電圧を印加するとカルボニル化合物が発生し始めている．市販の電子タバコに使用されているバッテリーの電圧は4～5 Vであるため，十分にカルボニル化合物を生成することが可能である．また，これらの発生成分と電子タバコからの発生成分はおおむね一致していた．

カルボニル化合物を多く発生した変霧器のニクロム線の周囲が黒褐色に変色していたことから，電子タバコカートリッジに含まれる液体（グリセロール，グリコール類）が，何らかの要因によりニクロム線と接触したために，液体が異常に高温になり，カルボニル化合物を発生したことが推測される．

4　結　言

本研究で，電子タバコから有害なカルボニル化合物が，非意図的に高濃度で発生することが明らかになった．また，これら有害物質の発生量にはばらつきがあり，銘柄間のばらつき，サンプルロットごとの発生量のばらつき，サンプルロット内のばらつきが認められた．これらのばらつきについて，カルボニル化合物を多く発生した変霧器のニクロム線の周囲が黒褐色に変色していた．

ニクロム線にグリセロール，グリコール類を塗布し，電圧を印加したところ，実際に電子タバコで使用されている4～5 V付近で，カルボニル化合物の発生量が急増した．電子タバコから発生するカルボニル化合物は，グリコール類，グリセリンのニクロム線による加熱が原因である．元来，グリコール類やグリセリンは毒性の低い物質とされているが，加熱されることで有害な物質を発生することが明らかになった．現在，電子タバコは国内でも普及し始めているので，さらなる安全性の検討が必要である．

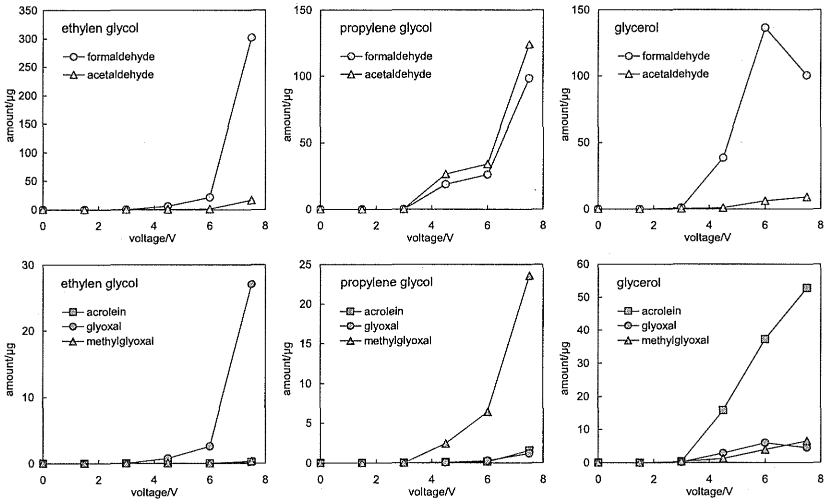


Fig. 5 Generation of carbonyl compounds from ethylene glycol, propylene glycol and glycerol heated by using a Nichrome wire

謝　辞

本研究は，厚生労働科学研究費（H21-3次がん-一般-005），科研費［挑戦的萌芽研究課題番号23650425，基盤研究（C）課題番号22300255］の助成を受けたものである．

文　献

1) World Health Organization : WHO Technical Report Series, No. 955, "*The Scientific Basis of Tobacco Product Regulation*", Third Report of a WHO Study Group, 955, (2010).
2) U.S. Food and Drug Administration, FDA and Public Health Experts Warn About Electronic Cigarettes : FDA News Relese, July 22, (2009).
3) G. McGwin, J. Lienert, J. I. Kennedy : *Environ. Health Perspect.*, **118**, 313 (2010).
4) R. Golden, D. Pyatt, P. G. Shields : *Crit. Rev. Toxicol.*, **36**, 135 (2006).
5) National Toxicology Program : Final Report on Carcinogens Background Document for Formaldehyde. Rep. Carcinog. Backgr. Doc. 10-5981, i-512 (2010).
6) T. Salthammer, S. Mentese, R. Marutzky : *Chem. Rev.*, **110**, 2536 (2010).
7) Z. Feng, W. Hu, Y. Hu, M. S. Tang : *Proc. Natl. Acad. Sci. USA*, **103**, 15404 (2006).
8) S. Uchiyama, Y. Inaba, N. Kunugita : *J. Chromatogr. B*, **879**, 1282 (2011).
9) S. Uchiyama, Y. Inaba, N. Kunugita : *J. Chromatogr. A*, **1217**, 4383 (2010).
10) Health Canada, Method T-115, "*Determination of Tar, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke*", (1999), (Ottawa, Canada).
11) World Health Organization (WHO) Regional Office for Europe, "*Air quality guidelines for Europe*", WHO Regional Publications, European Series, No. 23, (1987).

Determination of Carbonyl Compounds Generated from the Electronic Cigarette Using Coupled Silica Cartridges Impregnated with Hydroquinone and 2,4-Dinitrophenylhydrazine

Kazushi OHTA[1], Shigehisa UCHIYAMA[2], Yohei INABA[2],
Hideki NAKAGOME[1] and Naoki KUNUGITA[2]

[1] Graduate School of Engineering, Chiba University, 1-33, Yayoicho, Inage-ku, Chiba-shi, Chiba 263-8522
[2] Department of Environmental Health, National Institute of Public Health, 2-3-6, Minami, Wako-shi, Saitama 351-0197

(Received 25 May 2011, Accepted 4 July 2011)

The electronic cigarette, introduced recently to the marketplace, is a battery-powered device that provides tobacco-like smoke by heating a chemical solution into a vapor. There is, however, little information available regarding the safety of the electronic cigarette, because analysis of the smoke produced is very difficult due to the nature of the chemical components, *e.g.* acrolein and other carbonyls. Recently, an effective method for the determination of acrolein and other carbonyls using a dual-cartridge system has been developed. Each cartridge consists of reagent-impregnated silica particles ; the first contains hydroquinone (HQ) for the inhibition of acrolein polymerization, while the second contains 2,4-dinitrophenylhydrazine (DNPH) for the derivatization of carbonyls. Samples were drawn through the cartridge, first through the HQ-impregnated silica and then the DNPH-impregnated silica. During extraction, excess DNPH was washed into the HQ bed, where it reacted with acrolein and other trapped carbonyls to form the corresponding hydrazone derivatives. All of the hydrazones derived from airborne carbonyls were completely separated and measured using HPLC. In this study, we analyzed carbonyl compounds generated by the electronic cigarette using the HQ-DNPH technique. Results showed that formaldehyde, acetaldehyde, acrolein, glyoxal and methyl glyoxal were contained in the electronic cigarette smoke. The maximum concentration of formaldehyde was 260 mg/m^3. Depending on the brand, cartridges usually contain humectants to produce the vapor (*e.g.* ethylene glycol, propylene glycol or glycerol) and flavors (*e.g.* tobacco, mint, fruit and chocolate). Therefore, a simple electronic cigarette was made, comprising a coiled Nichrom wire and glycols ; a voltage of 1.5 ~ 7.5 V was applied to the Nichrom wire. It was found that when the voltage exceeded 3 V, a mist containing carbonyl compounds was generated. From the results, it was elucidated that ethylene glycol was oxidized to formaldehyde and glyoxal ; propylene glycol was oxidized to formaldehyde, acetaldehyde and methylglyoxal ; and glycerol was oxidized to formaldehyde, acrolein, glyoxal and methylglyoxal.

Keywords **:** electronic cigarette ; hydroquinone ; 2,4-dinitrophenylhydrazine ; acrolein ; carbonyl compounds.

ANALYTICAL SCIENCES DECEMBER 2013, VOL. 29

Notes

Determination of Carbonyl Compounds Generated from the E-cigarette Using Coupled Silica Cartridges Impregnated with Hydroquinone and 2,4-Dinitrophenylhydrazine, Followed by High-Performance Liquid Chromatography

Shigehisa UCHIYAMA[†], Kazushi OHTA, Yohei INABA, and Naoki KUNUGITA

Department of Environmental Health, National Institute of Public Health, 2-3-6 Minami, Wako, Saitama 351-0197, Japan

Carbonyl compounds in E-cigarette smoke mist were measured using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine, followed by high-performance liquid chromatography. A total of 363 E-cigarettes (13 brands) were examined. Four of the 13 E-cigarette brands did not generate any carbonyl compounds, while the other nine E-cigarette brands generated various carbonyl compounds. However, the carbonyl concentrations of the E-cigarette products did not show typical distributions, and the mean values were largely different from the median values. It was elucidated that E-cigarettes incidentally generate high concentrations of carbonyl compounds.

Keywords E-cigarette, carbonyl compounds, acrolein, glyoxal, methylglyoxal, glycerol, propylene glycol

(Received September 11, 2013; Accepted October 25, 2013; Published December 10, 2013)

Introduction

E-cigarettes (electronic cigarettes or e-cigs) are battery-powered devices designed to deliver nicotine to a smoker in the form of a vapor, and were first introduced into the Chinese market in 2004. Currently, they are widely used around the world. In the United States, as of 2011, approximately 21% of adults who smoked traditional cigarettes had used electronic cigarettes, which was an increase from 10% in 2010, according to a study released by the Centers for Disease Control and Prevention.[1] Overall, approximately 6% of all adults have tried E-cigarettes, and these estimates are nearly double those from 2010.[1] It was reported in the news media in 2013 that electronic cigarettes were beginning to gain cultural acceptance, and sales were growing rapidly.[2]

An electronic cigarette contains three essential components: a plastic cartridge that serves as a mouthpiece and a reservoir for a liquid, an "atomizer" that vaporizes the liquid, and a battery. The liquid used to produce the vapor in electronic cigarettes is a solution of propylene glycol and/or glycerin and/or polyethylene glycol mixed with concentrated flavors and, optionally, a variable percentage of liquid nicotine concentrate. These base liquids have been widely used as food additives, as base solutions in personal care products, such as toothpaste, and in medical devices, such as asthma inhalers. However, there are few reports on chemical compounds in E-cigarette smoke mist; moreover, the health effects of inhaling nicotine vapor into the lungs are uncertain.

We have developed a new method (the HQ-DNPH method) for the determination of acrolein and other carbonyl compounds in cigarette smoke using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine[3] (DNPH), and we reported that E-cigarettes sometimes accidentally generate various carbonyl compounds, such as formaldehyde, acetaldehyde, acrolein, glyoxal, and methyl glyoxal.[3,4] In these previous studies, we concluded that ethylene glycol was oxidized to formaldehyde and glyoxal; propylene glycol was oxidized to formaldehyde, acetaldehyde, and methylglyoxal; and glycerol was oxidized to formaldehyde, acrolein, glyoxal, and methylglyoxal.[4] In this study, we determined the concentration of various carbonyl compounds generated from a total of 363 E-cigarettes (13 brands). The results are presented herein.

Experimental

Apparatus and reagents

An HPLC system (Shimadzu, Kyoto, Japan) with two LC-20AD pumps, an SIL-20AC autosampler and an SPD M20A photodiode array detector, was used. The analytical column was an Ascentis Express RP-Amide (2.7 μm particle size, 150 mm × 4.6 mm i.d., Supelco Inc., Bellefonte, PA). The column temperature was 40°C, and the injection volume was 10 μL. Solution A of the mobile phase mixture was composed of acetonitrile/water (40/60 v/v) containing 5 mmol/L ammonium acetate; solution B was composed of acetonitrile/water (75/25 v/v). HPLC elution was carried out with 100% A for 8 min, followed by a linear gradient from 100% A to 100% B in 37 min, and then maintained constant for 15 min using 100% B. The flow rate of the mobile phase was 0.7 mL/min.

An LM1/PLUS (Borgwaldt Technik GmbH, Hamburg,

[†] To whom correspondence should be addressed.
E-mail: uchiyama@niph.go.jp

Germany) smoking machine was used for the collection of cigarette smoke.

The water used for the HPLC analysis and sample preparation was deionized and purified using a Milli-Q Water System equipped with a UV lamp (Millipore, Bedford, MA). 2,4-Dinitrophenylhydrazine hydrochloride (>98%) was obtained from Tokyo Kasei Co., Ltd. (Tokyo, Japan). Acetonitrile (HPLC grade, >99.9%), ethanol (>99.5%), hydroquinone (>99%), phosphoric acid (85% solution in water), and ammonium acetate (99.999%) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). The silica gel (spherical, 60/80 mesh, 120 Å mean pore size) was acquired from AGC Si-Tech. Co., Ltd. (Fukuoka, Japan).

The DNPH-impregnated silica cartridge (DNPH-cartridge) and the hydroquinone-impregnated silica cartridge (HQ-cartridge) were prepared according to previous reports.[3,4]

Collection and analysis of E-cigarette smoke

Before collecting smoke from the E-cigarettes, an HQ-cartridge and a DNPH-cartridge were connected. The coupled cartridges were then connected between the mouthpiece of the E-cigarette and the smoking machine, and the smoke from the E-cigarette was drawn into the coupled cartridges from the HQ-cartridge to the DNPH-cartridge according to the Canadian intense regimen;[5] (55 mL puff volume, 2-s puff duration, 30-s puff interval, and 10 puffs). After collection, the coupled cartridges were extracted using acetonitrile containing 1% phosphoric acid in a direction opposite to the air sampling direction until the total volume of the solution was 4.5 mL. After 10 min, ethanol (0.5 mL) was added to the eluate, and the solution was analyzed by HPLC. If the extraction was not performed immediately, the HQ-DNPH cartridge set was decoupled, and the individual cartridges were capped with stoppers.

Results and Discussion

Analysis of E-cigarette smoke by the HQ-DNPH method

Various types of carbonyl compounds were detected in the E-cigarette smoke. Figure 1 shows a representative chromatogram of a sample eluate by HPLC analysis with UV (360 nm) detection. In the HQ-DNPH method, it is possible to analyze C1 - C10 carbonyl compounds, and C1 - C3 carbonyl compounds, such as formaldehyde, acetaldehyde, acetone, acrolein, propanal, glyoxal, and methylglyoxal, were detected.

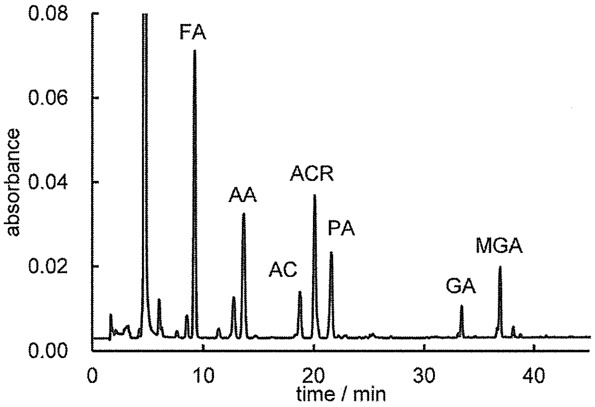


Fig. 1 Representative chromatogram of carbonyl DNPhydrazones derivatized from DNPH with carbonyls found in E-cigarette smoke. FA, formaldehyde; AA, acetaldehyde; AC, acetone; ACR, acrolein; PA, propanal; GA, glyoxal; MGA, methylglyoxal.

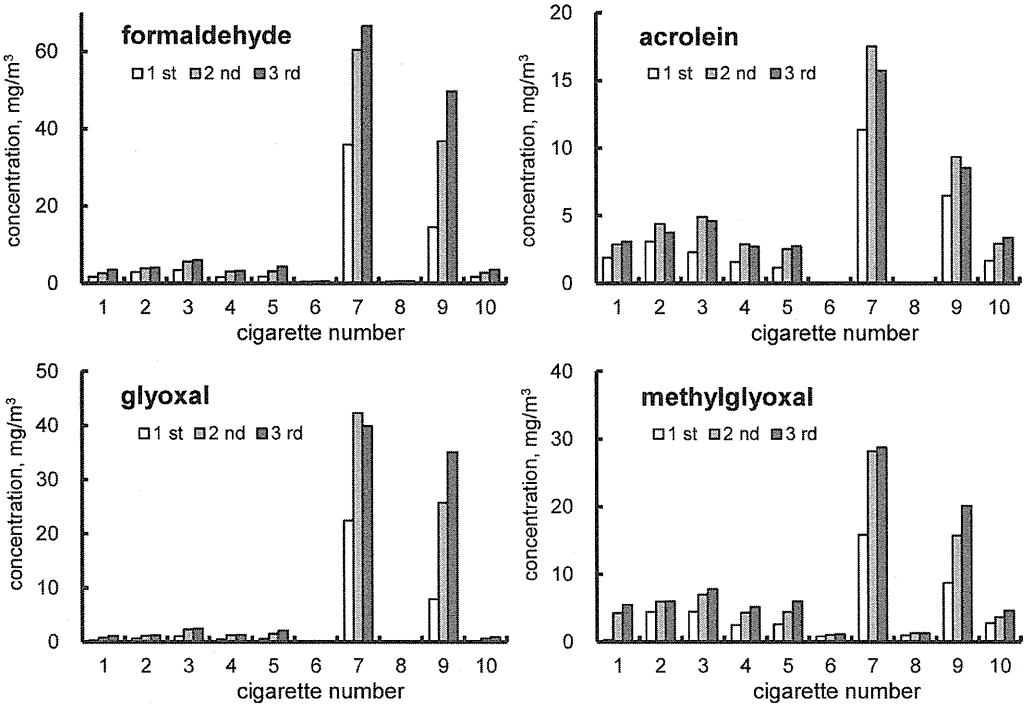


Fig. 2 Concentrations of formaldehyde, acrolein, glyoxal, and methylglyoxal generated from 10 different E-cigarettes of the same brand. Reproduced with permission from Fig. 3 in Ref. 4.

Table 1 Concentrations (mg/m^3) of major carbonyl compounds generated from 13 brands of E-cigarettes

Product	N_{high} N_{low}	FR	Formaldehyde	Acetaldehyde	Acrolein	Propanal	Glyoxal	Methylglyoxal
A	16	31	61 ± 64	48 ± 51	7.5 ± 6.9	16 ± 19	4.6 ± 6.5	5.3 ± 5.7
	35		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
B	6	20	44 ± 19	0.3 ± 0.1	12 ± 4.3	2.0 ± 1.2	29 ± 12	20 ± 7.8
	24		2.6 ± 1.6	n.d.	2.2 ± 1.6	n.d.	n.d.	3.7 ± 2.2
C	8	27	40 ± 28	1.7 ± 2.5	9.7 ± 10	6.1 ± 6.3	18 ± 9.5	22 ± 10
	22		3.1 ± 2.6	n.d.	1.1 ± 1.1	n.d.	1.3 ± 1.4	2.1 ± 1.9
D	12	24	28 ± 12	25 ± 12	36 ± 18	24 ± 19	7.7 ± 4.1	11 ± 7.5
	37		1.5 ± 1.8	n.d.	n.d.	n.d.	n.d.	n.d.
E	14	40	31 ± 14	27 ± 11	34 ± 12	27 ± 15	8.2 ± 4.4	8.6 ± 7.9
	21		1.3 ± 1.5	n.d.	1.2 ± 1.7	n.d.	n.d.	n.d.
F	2	40	12 ± 1.7	2.8 ± 0.2	2.0 ± 0.1	0.7 ± 0.1	2.8 ± 0.7	5.8 ± 0.9
	3		3.6 ± 3.1	1.6 ± 0.4	1.2 ± 0.5	n.d.	n.d.	1.6 ± 1.5
G	1	4	53	19	19	6.3	17	37
	25		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
H	5	17	19 ± 8.9	8.3 ± 4.3	8.1 ± 4.0	n.d.	4.6 ± 0.9	8.4 ± 5.7
	25		1.7 ± 2.6	n.d.	n.d.	n.d.	n.d.	n.d.
I	6	20	5.8 ± 1.9	11 ± 5.9	11 ± 4.2	14 ± 4.1	n.d.	n.d.
	24		2.8 ± 2.6	4.8 ± 5.2	5.0 ± 4.9	6.0 ± 6.2	n.d.	n.d.
J	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	4		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
K	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	30		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
L	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	30		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
M	0	0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	13		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

The upper line indicates the mean value for the high-concentration group, and the lower line indicates the mean value for the low-concentration group. Indices N_{high} and N_{low} indicate the number of E-cigarettes that generated high and low concentrations of carbonyl compounds, respectively. FR indicates the failure rate, which was calculated using the following equation: FR = $N_{\mathrm{high}}/(N_{\mathrm{high}} + N_{\mathrm{low}}) \times 100$. Values are mean ± SD. n.a., not available; n.d., not detected.

Concentration of carbonyl compounds in E-cigarette smoke

The concentration of carbonyl compounds in the smoke mist from 13 brands of E-cigarettes sold in Japan was determined by the HQ-DNPH method. The analysis of these actual brands of E-cigarettes revealed very large variations in the carbonyl concentrations among not only different brands, but also different examples of the same products. Typical distributions of the carbonyl concentrations were not observed for any of the E-cigarettes tested, and the mean values were largely different from the median values. These concentration variations were not caused by the analytical method, because the HQ-DNPH method has good reproducibility (RSD less than 2.1%).[3] We previously reported that the smoke mist generated from E-cigarettes unexpectedly contains carbonyl compounds.[4] This conclusion is based on the fact that for the same E-cigarette products, it was found that some E-cigarettes generated high concentrations of carbonyl compounds, while others did not. Figure 2 shows the concentrations of formaldehyde, acrolein, glyoxal, and methylglyoxal generated from 10 electronic cigarettes of the same brand. These results represent triplicate measurements for 10 samples. As can be seen in the figure, the number 7 and 9 E-cigarettes generated peculiarly high concentrations of carbonyl compounds. Therefore, the resulting data were divided into two groups based on the formaldehyde concentration (10 mg/m^3): a high concentration group and a low concentration group. Table 1 shows the concentrations of the major carbonyl compounds generated from 13 brands of E-cigarettes. In the table, the top entry in each cell indicates the mean value for the high-concentration group, and the lower entry indicates the mean value for the low-concentration group. The indices N_{high} and N_{low} indicate the number of E-cigarettes that generated high and low concentrations of carbonyl compounds, respectively. FR indicates the failure rate, which was calculated by the following equation: FR = $N_{\mathrm{high}}/(N_{\mathrm{high}} + N_{\mathrm{low}}) \times 100$.

Four (J, K, L, M) out of the 13 E-cigarette brands did not generate any carbonyl compounds. The other nine E-cigarette brands (A, B, C, D, E, F, G, H, I) generated various carbonyl compounds. The concentrations of carbonyl compounds obtained for the high concentration group were significantly higher than that determined for the low concentration group. The maximum concentrations of formaldehyde, acetaldehyde, acrolein, propanal, glyoxal, and methylglyoxal were 260, 210, 73, 83, 42, and 38 mg/m^3, respectively. For a typical cigarette smoking experience of 10 puffs, these values translate to maximum concentrations of 140 μg formaldehyde/cigarette, 120 μg acetaldehyde/cigarette, 33 μg acrolein/cigarette, 46 μg propanal/cigarette, 23 μg glyoxal/cigarette, and 21 μg methylglyoxal/cigarette. Most notably, very high concentrations of formaldehyde were measured in the smoke from the E-cigarettes. Glyoxal and methylglyoxal are peculiar to E-cigarette smoke, and have not been detected in the mainstream smoke from normal cigarettes. Glyoxal is known to be mutagenic to *Salmonella typhimurium* strains TA100, TA102, and TA104.[6,7] It has been shown that glyoxal reacts with guanine residues in DNA.[8] Its tumor promoting activity has also been reported.[9,10] Methylglyoxal, the most mutagenic of all aldehydes, is known to inhibit formaldehyde metabolism, thus

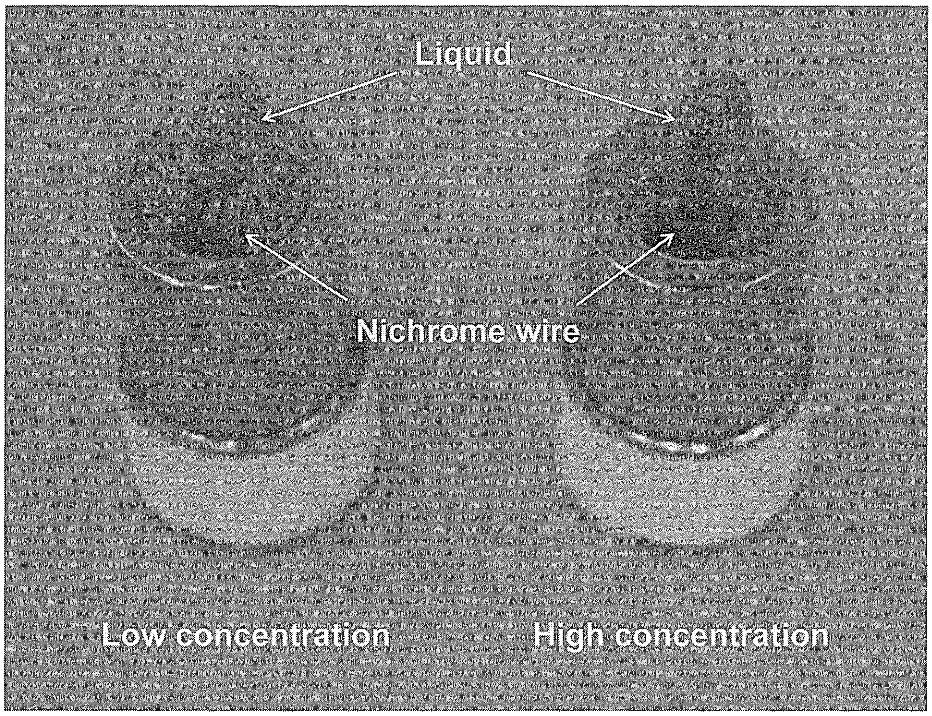


Fig. 3 E-cigarette atomizers that generated low and high concentrations of carbonyl compounds.

enhancing formaldehyde-induced cytotoxicity.[11]

After smoking an E-cigarette, the atomizer that generated the high concentrations of carbonyl compounds was burned black. Figure 3 shows atomizers after smoking 10 puffs. The left atomizer generated a low concentration. The right atomizer generated a high concentration of carbonyl compounds, and the color around Nichrome wire changed from white to black. These results suggest that the compounds in the E-cigarette liquid, such as glycerol and glycols, incidentally touch the heated Nichrome wire and are oxidized to formaldehyde, acetaldehyde, acrolein, glyoxal, and methylglyoxal.

Conclusions

E-cigarettes incidentally generate carbonyl compounds in the E-cigarette smoke mist. A possible cause for carbonyl generation is the oxidation of liquids in the E-cigarette, such as glycerol and glycols, when they incidentally touch the heated Nichrome wire in the atomizer, and are oxidized to formaldehyde, acetaldehyde, acrolein, glyoxal, and methylglyoxal. In some cases, these hazardous compounds are generated with extremely high concentrations. Suppliers and users of E-cigarettes should pay attention to this phenomenon.

Acknowledgements

The authors thank Ms. Sayuri Yanai for her technical assistance. This research was partially supported by Ministry of Health, Labour, and Welfare of the Japanese Government, the Health and Labour Sciences Research Grants (Research on Health Security Control and Third Term Comprehensive Control Research for Cancer).

References

1. Centers for Disease Control and Prevention (CDC), "*CDC Newsroom, About one in five U.S. adult cigarette smokers have tried an electronic cigarette*", February 28, **2013**, http://www.cdc.gov/media/releases/2013/p0228_electronic_cigarettes.html.
2. L. Alderman, "*E-Cigarettes Are in Vogue and at a Crossroads*", The New York Times, **2013**, http://www.nytimes.com/2013/06/13/business/e-cigarettes-are-in-vogue-and-at-a-crossroads.html?_r=1&.
3. S. Uchiyama, Y. Inaba, and N. Kunugita, *J. Chromatogr., A*, **2010**, *1217*, 4383.
4. K. Ohta, S. Uchiyama, Y. Inaba, H. Nakagome, and N. Kunugita, *Bunseki Kagaku*, **2011**, *60*, 791.
5. Health Canada Official Method T-115, "*Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke*", **1999**, Ottawa, Canada.
6. Y. Sayato, K. Nakamuro, and H. Ueno, *Mutat. Res., Genet. Toxicol. Environ. Mutagen.*, **1987**, *189*, 217.
7. L. Dorado, M. Ruiz Montoya, and J. M. Rodríguez Mellado, *Mutat. Res., Fundam. Mol. Mech. Mutagen.*, **1992**, *269*, 301.
8. R. Shapiro and J. Hachmann, *Biochemistry*, **1966**, *5*, 2799.
9. C. Furihata, S. Yoshida, and T. Matsushima, *Jpn. J. Cancer Res.*, **1985**, *76*, 809.
10. M. Takahashi, H. Okamiya, F. Furukawa, K. Toyoda, H. Sato, K. Imaida, and Y. Hayashi, *Carcinogenesis*, **1989**, *10*, 1925.
11. S. Teng, K. Beard, J. Pourahmad, M. Moridani, E. Easson, R. Poon, and P. J. O'Brien, *Chem. Biol. Interact.*, **2001**, *130 - 132*, 285.