Arch Toxicol (2014) 88:1295–1308
DOI 10.1007/s00204-014-1294-7

SHORT COMMUNICATION

Chemical hazards present in liquids and vapors of electronic cigarettes

Christoph Hutzler · Meike Paschke · Svetlana Kruschinski · Frank Henkler · Jürgen Hahn · Andreas Luch

Received: 31 October 2013 / Accepted: 3 June 2014 / Published online: 11 June 2014

Abstract Electronic (e-)cigarettes have emerged in recent years as putative alternative to conventional tobacco cigarettes. These products do not contain typical carcinogens that are present in tobacco smoke, due to the lack of combustion. However, besides nicotine, hazards can also arise from other constituents of liquids, such as solvents, flavors, additives and contaminants. In this study, we have analyzed 28 liquids of seven manufacturers purchased in Germany. We confirm the presence of a wide range of flavors to enhance palatability. Although glycerol and propylene glycol were detected in all samples, these solvents had been replaced by ethylene glycol as dominant compound in five products. Ethylene glycol is associated with markedly enhanced toxicological hazards when compared to conventionally used glycerol and propylene glycol. Additional additives, such as coumarin and acetamide, that raise concerns for human health were detected in certain samples. Ten out of 28 products had been declared "free-of-nicotine" by the manufacturer. Among these ten, seven liquids were identified containing nicotine in the range of 0.1–15 µg/ml. This suggests that "carry over" of ingredients may occur during the production of cartridges. We have further analyzed the formation of carbonylic compounds in one widely distributed nicotine-free brand. Significant amounts of formaldehyde, acetaldehyde and propionaldehyde were only found at 150 °C by headspace GC–MS analysis. In addition, an enhanced formation of aldehydes was found in defined puff fractions, using an adopted machine smoking protocol. However, this effect was delayed and only observed during the last third of the smoking procedure. In the emissions of these fractions, which represent up to 40 % of total vapor volume, similar levels of formaldehyde were detected when compared to conventional tobacco cigarettes. By contrast, carbonylic compounds were hardly detectable in earlier collected fractions. Our data demonstrate the necessity of standardized machine smoking protocols to reliably address putative risks of e-cigarettes for consumers.

Keywords Electronic cigarette · Vapor · Liquids · Ethylene glycol · Flavors · Formaldehyde

C. Hutzler · M. Paschke · S. Kruschinski · F. Henkler · A. Luch (✉)
Department of Product Safety, German Federal Institute for Risk Assessment, Max-Dohrn-Strasse 8-10, 10589 Berlin, Germany
e-mail: Andreas.Luch@bfr.bund.de

J. Hahn
Official Chemical and Veterinary Surveillance Institute Sigmaringen, 72488 Sigmaringen, Germany

Introduction

Electronic (e-)cigarettes as battery-driven nicotine delivery devices emerged during the last 5 years (Caponnetto et al. 2012). Here, nicotine is dissolved in chemical "carriers", most frequently glycerol (i.e., glycerin) or propylene glycol. Usually, these liquids do also contain flavors, additives and a range of contaminants. E-cigarettes are designed to mimic conventional cigarettes. Upon suction, an electric element is operated to heat up the liquid to about 70–100 °C. Vapors are then formed on a fine meshed metal net and inhaled by consumers. In recent years, further innovations have been introduced, for example, glass fibers that replace metal meshes to direct liquids into to gas stream. Currently, the product spectrum differentiates into disposables and advanced refillables that are no longer shaped like conventional cigarettes and that can contain 10 ml of liquid or even more. The initial products investigated here

are still on the market and often used as "starter kits". A new variation of e-cigarettes was recently emerging, which are termed "electronic shisha cigarettes". These disposable e-cigarettes are designed in bright colors and advertised to target young people. Due to its high prevalence among children and youths the Dutch government recently expressed concerns about these products, which are nicknamed "kinder (children) cigarettes" in the Netherlands (see: http://nos.nl/artikel/502384-zorgen-bij-ggd-over-kindersigaret.html).

In general, e-cigarettes are considered less hazardous when compared to conventional tobacco products, since carcinogenic combustion products are unlikely to be produced. Some smokers might benefit from switching to such novel nicotine delivery devices, yet e-cigarettes are considered and marketed as stimulants in their own right. So, it remains unclear whether these products can appeal to non-smokers, or not. In a recent study, only 3.2 % of juvenile non-smokers in Poland reported to have ever used e-cigarettes (Goniewicz and Zielinska-Danch 2012a). Nevertheless, 20 % of Polish youths had already used e-cigarettes, indicating a considerable attractiveness to young people. Further, there was a higher prevalence in the 17–19 years age group, as compared with students of age 20–24. Still, only limited information is available on consumer behavior. Although e-cigarettes possibly could play a role in smoking cessation and the reduction of overall health risks for established smokers, these devices should also be regarded as novel options for first-time users and thus the induction of nicotine abuse.

Nicotine is a psychoactive and highly addictive compound (Jasinska et al. 2013). Initial studies indicated that comparatively low blood nicotine levels are reached by e-cigarette users (Bullen et al. 2010; Etter et al. 2011). It was also reported that lower nicotine doses are delivered through e-cigarette vapors (0.025–0.77 mg per 15 puffs), when compared with smoked tobacco cigarettes (1.54–2.60 mg per cigarette) (Goniewicz et al. 2012b). Still, such an exposure level could be sufficient for an overall inhalation of more than 5 mg nicotine per day, thus exceeding the estimated threshold for addictiveness that was proposed for tobacco cigarettes (Benowitz and Hennigfield 1994). However, it is uncertain whether this limit also applies to e-cigarettes. In more recent studies, nicotine blood plasma levels of 10–15 ng/ml had been demonstrated in e-cigarette users (Dawkins and Concoran 2014; Vansickel and Eissenberg 2013). These values are comparable with levels reached by tobacco cigarettes (15–30 ng/ml) at the lower end of the range (Hukkanen et al. 2005). Notably, analysis of cotinine, both in salvia (Etter and Bullen 2011a) and serum (Flouris et al. 2013), did suggest similar levels in e-smokers and tobacco smokers. These data indicate that the overall nicotine exposure is comparable and possibly higher than the level reached by nicotine replacement therapies (Etter and Bullen 2011a). The value of e-cigarettes to assist cessation is still discussed. A recent study demonstrated a reduced nicotine craving and improved working memory performance for one brand (Dawkins et al. 2012), whereas others observed reduced withdrawal symptoms without measurable nicotine effects, such as increased heart rates (Vansickel et al. 2010). A high proportion of users reported to apply e-cigarettes to support cessation (Etter and Bullen 2011b).

In animal experiments, nicotine can trigger a wide range of physiological effects via nicotinic acetylcholine receptors (nAChR). These receptors belong to a family of ligand gated ion channels, consisting of five subunits. Depending on tissue specificity, α (α1–10) and β (β1–4) subunits assemble homo- or heteropentamers (Changeux and Taly 2008) which then form a central pore to allow activation-induced influx of calcium into the cell. nAChR are expressed in the neuromuscular junctions, in the peripheral and central nervous system, as well as in endothelial cell, keratinocytes and other tissues. Physiological effects of nicotine include the release of catecholamines, increase of blood pressure and enhanced platelet aggregation. In combination with its capacity to induce endothelial cell proliferation, nicotine can promote atherosclerosis and is discussed as risk factor for tobacco-induced cardiovascular diseases (Cardinale et al. 2012). Nicotine-related angiogenesis and vascularization are also considered to promote cancer, macular degeneration and rheumatoid arthritis (Lee and Cooke 2012). In mice, nicotine was shown to enhance growth of atherosclerotic lesions and to accelerate progression of cancer cells into lung tumors (Heeschen et al. 2001). Notably, the latter effect was associated with a fourfold increase of capillary density in tumors of nicotine-treated mice.

Unfortunately, there is only limited experimental data on the effects of nicotine inhalation in rodents. In one long-term study of 68 rats that were exposed to high levels of nicotine over a 2-year period, no increase in mortality, atherosclerosis or tumor frequency had been found (Waldum et al. 1996). In humans, health risks of nicotine are usually considered in the context of tobacco smoke. However, transdermal nicotine patches used in replacement therapy are not associated with increased cardiovascular risks (Greenland et al. 1998). In addition, Swedish studies on snus failed to show an association between smokeless tobacco and acute myocardial infarction (Hansson et al. 2012), or strong connections to cardiovascular diseases (Hansson et al. 2009). However, it remains unclear whether similar conclusions do apply for nicotine inhalation by humans. According to the assessment of Benowitz and Gourlay (1997), the risks of cardiovascular toxicity are small in nicotine replacement therapies and less relevant than potential benefits of cessation. Nevertheless, e-cigarettes are usually neither designed nor authorized as

therapeutic applications, but should be regarded as novel consumer products, which can be used for pleasure, relaxation and hedonistic reasons.

Besides nicotine, little is known about the ingredients that belong to the groups of flavors and additives. These compounds remain often undeclared on the packages, but might comprise also substances of sensitizing, toxic or irritating potency. Additional health risks had been discussed for glycerol or propylene glycol, which are frequently used as vaporizing solvents. Previous studies on e-cigarette smokers indicated only moderate toxicological effects, such as an increased respiratory flow resistance (Vardavas et al. 2012). However, such an effect was not confirmed by Flouris et al. (2013). Further, consequences of long-term exposure to glycerol and propylene glycol have not been investigated at all. Both compounds might pyrolyze, leading to the formation of aldehydes. A Japanese study (Uchiyama et al. 2010) reported a significant formation of formaldehyde, acetaldehyde and acrolein, although the machine smoking regime was not adopted to human smoking behavior. The question whether e-cigarettes can release carcinogenic compounds remains highly important. Our data confirm a significant release of carbonylic compounds including formaldehyde and thus a potential health concern for e-cigarette smokers. Our findings further illustrate a strong necessity to regulate the major compounds in e-cigarette liquids, including vaporizing agents, flavors, additives and contaminants that are likely to pose risks to the health of consumers.

Results

Characterization of vaporizing agents, flavors and additives in 28 e-cigarette liquids

We have purchased 28 different liquids from German retailers or via the internet. These products were provided by seven manufacturers. Ten among these liquids were explicitly declared "free-of-nicotine". However, seven out of these ten liquids were identified containing nicotine in the range of 0.1–15 µg/ml. For the other 18 liquids, no declaration regarding nicotine was provided by the manufacturers at all, indicating that these products were likely perceived as nicotine free. However, in 16 of these samples, nicotine levels found were in the range 0.1–324 µg/ml.

Our first analyses were focused on vaporizing agents that constitute the major ingredients of such liquids. Although glycerol and propylene glycol are reported to be frequently used, precise information on its contents was not available for the products analyzed. Applying a gas chromatography method, in conjunction with a flame ionization detector (GC–FID), we could confirm both glycerol and propylene glycol in all 28 samples (Table 1; Fig. 1). In addition, ethylene glycol was detected in 13 samples as well. Unexpectedly, in an e-cigarette liquid from one particular manufacturer, the ethylene glycol content was as high as 76 %, indicating that this compound occasionally is used to replace glycerol and propylene glycol. Four out of five products from this particular manufacturer revealed with >70 % ethylene glycol, whereas only 2 % were detectable in the fifth. Seven products that came from three

Table 1 GC–FID analysis of solvents (vaporizing agents) in a total of 28 e-cigarette liquids

	1,2-Propylene glycol	Glycerol	Ethylene glycol
Positive samples	28	28	13
Range (%)	2–79	7–42	1–76
Average value (%)	53	26	26
Median (%)	61	25	5

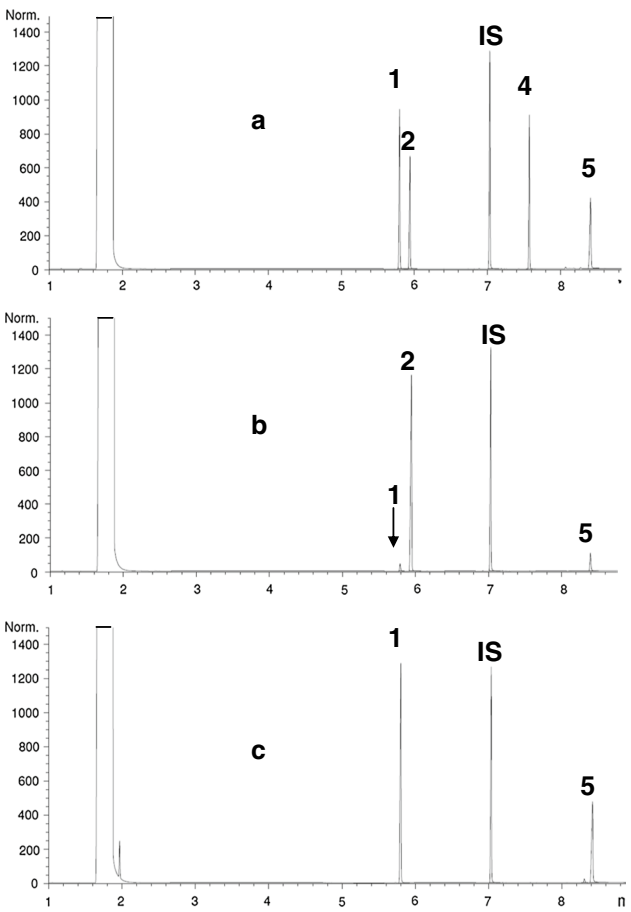

Fig. 1 GC–FID chromatograms of analytical standards. **a** Mixture of standard compounds: *1* 1,2-propylene glycol; *2* ethylene glycol, *IS* internal standard: 1,4-butanediol; *4* triacetine (1,2,3-propanetriol triacetate); *5* glycerol. **b** E-cigarette liquid containing ethylene glycol as main component. **c** E-cigarette liquid without ethylene glycol

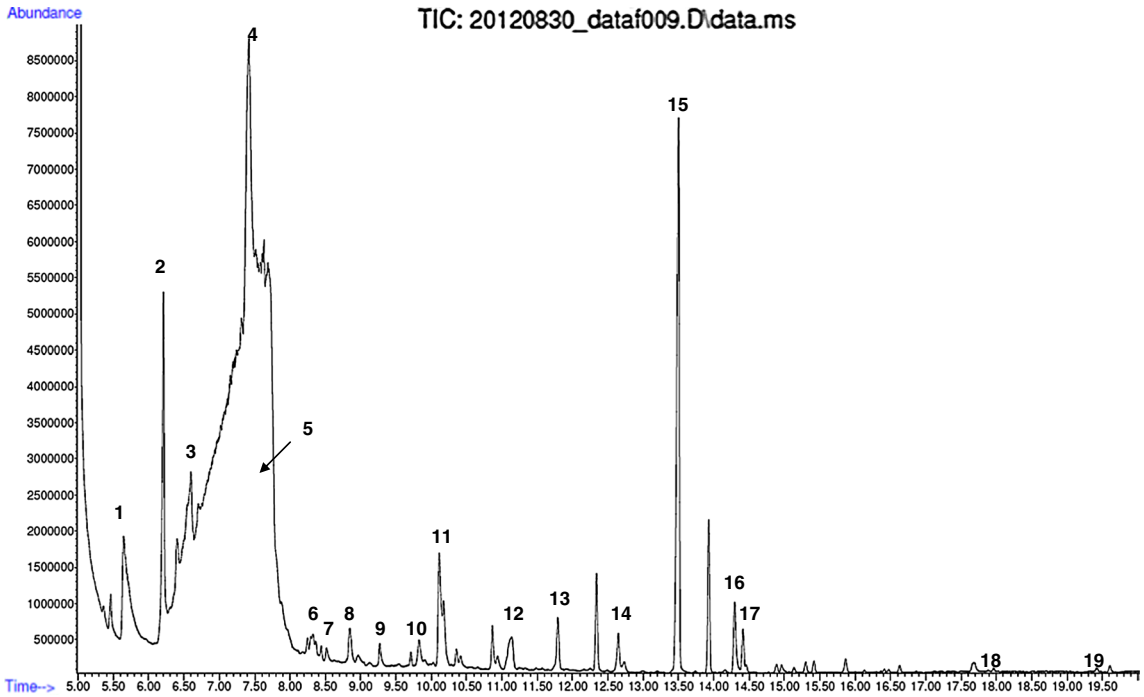

Fig. 2 Analysis of additives in nicotine-free e-cigarette liquids. High variations of flavors, additives and contaminants are identified in e-cigarette liquids by library search upon GC–MS analysis, including some putatively allergenic and genotoxic compounds. Representative chromatogram containing the following compounds: *1* linalool; *2* dipropylene glycol; *3* 3-methyl-1,2-cyclopentanedione; *4* acetine (1,2,3-propanetriol monoacetate); *5* glycerol; *6* citral (geranial and neral); *7* ethyl-α-toluate; *8* (2,2-diethoxyethyl)benzene; *9* geraniol acetate; *10* damascenone; *11* α-inone; *12* β-inone; *13* γ-decalactone; *14* δ-decalactone; *15* persicol (5-heptyloxolan-2-one); *16* δ-undecalactone; *17* ethyl myristate; *18*, *19* unidentified phthalates

different manufacturers contained 1–6 % ethylene glycol, and in one additional sample again >30 % was detected. Conversely, altogether fifteen samples produced by three other manufacturers were tested negative. Ethylene glycol is an irritant that is not permitted to be used as humectant in conventional tobacco products.

To initiate a more reliable and profound assessment of products that are currently marketed in Germany, we have analyzed the samples further to identify flavors, additives and putative contaminations. To this end, liquids were extracted using both alkaline and acid conditions and subjected to GC–MS analysis (Fig. 1). Compounds were identified via alignments of retention time and mass number according to the NIST GC database. A representative chromatogram of an e-cigarette liquid containing several flavor compounds is shown in Fig. 2. All compounds identified via library search upon GC–MS analysis of 28 e-cigarette liquids are compiled in Table 2. Our data confirm a wide spectrum of ingredients that include characteristic flavors such as vanillin, menthol or rheosmin (raspberry ketone). We also identified allergens, as for example cinnamic aldehyde and coumarin, the latter being prohibited in Germany as an additive in tobacco products. We further indentified eugenol, linalool, benzyl alcohol and anis alcohol, all of which are regulated by the European Cosmetics Directive based on their known allergenic properties in skin (Schnuch et al. 2007). In four samples, acetamide was detectable, a compound which is regarded as possible human carcinogen (IARC 1999). At the moment, it remains unclear whether this compound constitutes a contaminant or was added intentionally by the manufacturers. Because of the wide range of identified ingredients, it is difficult to identify typical patterns of e-cigarette liquid formulations and to generalize the toxicological risks that may be associated.

Formation of formaldehyde, acetaldehyde and propionaldehyde in e-cigarette liquids

Although the heating and vaporization of liquids do not involve combustion, formation of carbonylic compounds, especially formaldehyde, has been reported previously (Uchiyama et al. 2010). However, it was not clarified whether these compounds were initially present as contaminants or formed through thermolysis upon the heating of e-cigarette liquids. Thermodegradation of glycerol can lead to various carbonyls, including acrolein (Carmines and Gaworski 2005) and formaldehyde (Nimlos et al. 2006). To exclude the possibility that liquids were contaminated

Table 2 Volatile flavor substances detected in a total of 28 e-cigarette liquids upon extraction and GC/MS analysis

No.	Compound name	Frequency in 28 e-cigarette liquids	MW (g/mol)	BP (°C)	Case no.
1	Vanillin	22	152	285–286	121-33-5
2	Ethyl maltol	16	140	161	4940-11-8
3	Ethyl Vanillin	14	166	295	121-32-4
4	Menthol	12	156	212	89-78-1
5	Piperonal	7	150	263	120-57-0
6	Damascenone (α or β)	7	190	276	23696-85-7
7	3-Methyl-1,2-cyclopentanedione	6	112	178	765-70-8
8	Acetamide	6	59	221	60-35-5
9	Linalool	6	154	198–199	78-70-6
10	Trimethylpyrazine	6	122	171	14667-55-1
11	Terpineol	5	154	219	7785-53-7
12	Eugenol	5	164	252	97-53-0
13	Piperonal propyleneglycol acetal	5	208	299	61683-99-6
14	Citral	5	152	229	5392-40-5
15	Corylon	5	112	245	80-71-7
16	Anisaldehyde propylene glycol acetal	4	194	287–289	6414-32-0
17	Benzaldehyde	4	106	178	100-52-7
18	Benzyl benzoate	4	212	323	120-51-4
19	Coumarin	4	146	302	91-64-5
20	γ-Octalactone	4	142	239	104-50-7
21	1,2-Hexanediol	3	118	153–160	629-11-8
22	Acetylpyrazine	3	122	188–190	22047-25-2
23	Anisaldehyde	3	136	247–249	123-11-5
24	Benzophenone	3	182	305	119-61-9
25	Benzyl alcohol	3	108	206	100-51-6
26	Diisobutyl phthalate	3	278	295	84-69-5
27	Phenylethyl alcohol	3	122	218	60-12-8
28	Benzyl acetate	3	150	212	140-11-4
29	Pulegone	3	152	224	89-82-7
30	(2,2-Diethoxyethyl) benzene	2	166	237	101-48-4
31	1,8-Cineol	2	154	174–177	470-82-6
32	4-Chloro-2,5-dimethoxy-aniline	2	187	163	6358-64-1
33	4-Methyl-2-pentyl-1,3-dioxolane	2	158	186	1599-49-1
34	Anatabin	2	160	145–146	581-49-7
35	Benzaldehyde propylene glycol acetal	2	164	242	2568-25-4
36	Carvone	2	150	230–231	6485-40-1
37	Cinnamaldehyde	2	132	251	104-55-2
38	Diacetin	2	176	259	25395-31-7
39	Ethyl mandelate	2	180	254	774-40-3
40	Ethyl phenylacetate	2	164	227–229	202-993-8
41	Hydrocoumarine	2	148	272	119-84-6
42	Isoamyl butylate	2	158	176	106-27-4
43	Isobornyl acetate	2	196	229–233	125-12-2
44	Limonene	2	136	177	138-86-3
45	Methyl cinnamate	2	162	262	103-26-4
46	Methyl dihydrojasmonate	2	226	109–112	14851-98-7
47	Miosmine	2	146	142–143	532-12-7
48	*n*-Decanoic acid	2	172	270	334-48-5
49	Piperitone	2	152	233	89-81-6

Table 2 continued

No.	Compound name	Frequency in 28 e-cigarette liquids	MW (g/mol)	BP (°C)	Case no.
50	*p*-Menthane-1,2-diol	2	172	268	33669-76-0
51	Syringol	2	154	261	91-10-1
52	*trans*-Carane	2	138	169	18968-23-5
53	α-Damascenone	2	192	267	57549-92-5
54	γ-Heptalactone	2	128	226	105-21-5
55	γ-Nonalactone	2	156	267	104-61-0
56	Butyl carbitol	2	152	231	112-34-5
57	Persicol	1	184	286	104-67-6
58	(*R*)-(+)-Citronellal	1	154	230	2385-77-5
59	1-(2-Butoxyethoxy)-ethanol	1	162	231	112-34-5
60	2,6-Di-*tert*-butyl-*p*-cresol	1	220	263	28-37-0
61	2-Ethoxy-4(2-propenyl)-phenol	1	178	312	94-86-0
62	2-Ethylhexyl fumarate	1	340	185	141-02-6
63	2-Hydroxyethyl silicylate	1	182	150–155	87-28-5
64	2-Isopropyl-5-methyl-3-cyclohexene-1-one	1	152	244	535-86-4
65	2-Nitro-thiophene	1	129	224	609-40-5
66	2-Phenyl-1,3-dioxan-5-ol	1	180	334	1708-40-3
67	2-*sec*-Butylcyclohexanone	1	154	236–239	14765-30-1
68	2-Thujene	1	136	151	2867-05-2
69	3,4-Xylenol	1	122	226	95-65-8
70	3-Hexen-1-ol	1	100	157	928-96-1
71	4-Acetylanisol	1	150	263	100-06-1
72	4-Acetyltoluene	1	166	289	1634-34-0
73	4-Anisaldehyde	1	136	247–249	123-11-5
74	4-*tert*-Octylphenol	1	206	282	140-66-9
75	6-Methylcoumarine	1	160	303	92-48-8
76	Acetophenon	1	120	202	98-86-2
77	Acetovanillin	1	194	289	881-68-5
78	Acetylvanillin	1	194	288	881-68-5
79	Allylcaproate	1	156	190–191	123-68-2
80	Anabasin	1	162	270–272	13078-04-1
81	Anis alcohol	1	138	259	105-13-5
82	Benzyl cinnamate	1	238	195–200	103-41-3
83	Benzyl propionate	1	164	222	122-63-4
84	Benzyl salicylate	1	228	300	118-58-1
85	Bis-(2-furfuryl)-disulfide	1	226	229–230	4437-20-1
86	Butyl buturyl lactate	1	216	272	7492-70-8
87	Caryophyllene	1	204	260–261	87-44-5
88	Caryophyllene oxide	1	220	280	1139-30-6
89	Cinnamic acid methylester	1	162	260–262	103-26-4
90	Creosol	1	138	220	93-51-6
91	Dicyclopentenyl alcohol	1	150	246	27137-33-3
92	Diethyl carbitol	1	162	189	112-36-7
93	Diethyl malonate	1	160	199	105-53-3
94	Diethyl succinate	1	174	230	68989-39-9
95	Dimethyltriglykol	1	178	216	112-49-2
96	Elemol	1	222	289–290	8024-27-9
97	Ethoxytriglycol	1	178	256	112-50-5
98	Ethyl acetoacetate	1	130	181	141-97-9

Table 2 continued

No.	Compound name	Frequency in 28 e-cigarette liquids	MW (g/mol)	BP (°C)	Case no.
99	Ethyl benzoate	1	150	211–213	93-89-0
100	Ethyl caproate	1	144	168	123-66-0
101	Ethyl cinnamate	1	176	271	103-36-6
102	Ethyl heptanoate	1	158	188	106-30-9
103	Ethyl hexadecanoate	1	284	303	628-97-7
104	Ethyl myristate	1	256	295	124-06-1
105	Ethyl nonanoate	1	186	220	123-29-5
106	Ethyl-3-methyl-3-phenylglycidate	1	206	274	77-83-8
107	Ethylcaprylare	1	172	208	106-32-1
108	Ethyl-α-methylbutyrate	1	130	133	7452-79-1
109	Geraniol	1	154	229–230	106-24-1
110	Geraniol acetate	1	196	137–139	105-87-3
111	Geraniol butyrate	1	224	151–153	106-29-6
112	Glutaric acid dimethyl ester	1	160	210–215	1119-40-0
113	Glyceryl monocaprate	1	246	269	26402-22-2
114	Hexyl acetate	1	144	172	142-92-7
115	Hexyl butyrate	1	172	207	2639-63-6
116	Hexyl hexanoate	1	200	246	6378-65-0
117	Isoamyl isovalerate	1	172	194	659-70-1
118	Isobutyl benzoate	1	178	241	120-50-3
119	Isomenthol	1	156	218	3623-52-7
120	Isopentyl isobutyrate	1	158	165	2050-01-3
121	Maltol	1	126	285	118-71-8
122	Methyl acetate	1	74	44	79-20-9
123	Methyl-α-isoionone	1	206	285	127-51-5
124	Monoacetin	1	134	258	26446-35-5
125	*n*-Hexanoic acid	1	116	206	142-62-1
126	Nonyl-cyclopropane	1	168	293	74663-85-7
127	Nootkatone	1	218	319	4674-50-4
128	Propyl thiocyanate	1	85	83–84	110-78-1
129	Rheosmin	1	164	140–146	5471-51-2
130	Styrolyl propionate	1	178	245	120-45-6
131	Tetraethylene glycol dimethyl ether	1	222	275–276	143-24-8
132	α-Decalactone	1	170	267	18436-37-8
133	α-Hexyl-cinnamaldehyde	1	216	308	101-86-0
134	α-Terpineol acetate	1	196	240	80-26-2
135	α-Terpinolen	1	136	182	586-62-9
136	β-Bourbonene	1	204	256	5208-59-3
137	β-Cadinene	1	204	272	523-47-7
138	γ-Decalactone	1	170	267	706-14-9
139	γ-Octalactone	1	142	240	104-50-7
140	γ-Terpinen	1	136	183	99-85-4
141	σ-Decalactone	1	170	268	5579-78-2

MW molecular weight, *CAS* chemical abstracts service registry number, *BP* boiling point (taken from: http://www.lookchem.com, http://topuunion.guidechem.com, and http://www.dguv.de/ifa/Gefahrstoffdatenbanken/GESTIS-Stoffdatenbank/index.jsp)

with carcinogenic aldehydes, we looked into 5 among the above-mentioned 28 samples lacking nicotine declaration and 2 additional liquids which were explicitly labeled with "contains nicotine" These 7 liquids were analyzed regarding their aldehyde contents. Further, one of the presumably "free-of-nicotine" liquids, which is widely distributed and easily accessible in Germany, was also used for additional experiments applying a standardized machine smoking protocol. The samples were first analyzed at room temperature, showing only traces of formaldehyde, acetaldehyde and

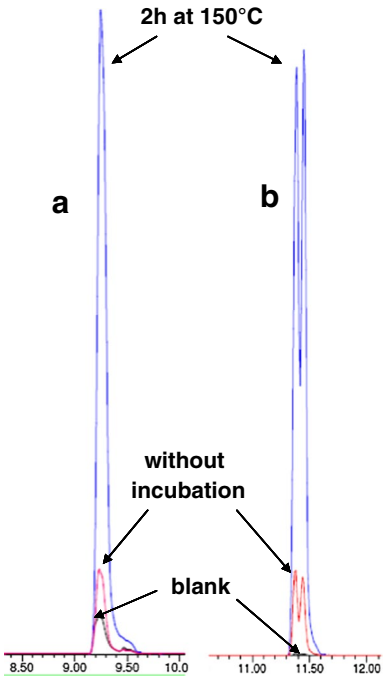

Fig. 3 Influence of the temperature on the levels of formaldehyde and acetaldehyde. GC–MS analysis of the corresponding PFBHA derivatives demonstrating an up to tenfold increase of the levels of formaldehyde (**a**) and acetaldehyde (**b**, two isomers) in an e-cigarette liquid upon incubation at 150 °C for 2 h

proprionaldehyde, all in the range of µg/ml or below. We applied headspace GC–MS to enable incubation at various temperatures. At the temperature of 150 °C, but not 100 °C, the levels of acetaldehyde and formaldehyde were found up to tenfold–20-fold higher when compared to ambient temperatures for samples containing 1,2-propylene glycol as main component (Fig. 3). For one sample containing ethylene glycol as main component factor, the acetaldehyde content increased from 0.50 to 348 µg/ml (factor 700) under these conditions. These incubations were performed in a simple way during the period of 2 h. Since there was just the liquid, and no e-cigarette item used for this experiment, we conclude that the aldehydes were generated in the absence of any air flow or independent from the formation of vapors at the surface of the fine structured metal mesh. Yet, the levels of acetaldehyde detected were no higher than 86 µg per ml and thus comparatively low when compared to the corresponding numbers obtained with cigarette or water pipe tobacco (Schubert et al. 2012).

Formation of carbonylic compounds in a standardized machine smoking procedure correlates with decreased liquid levels in cartridges

To investigate whether the formation of carbonylic compounds can occur under vaporizing conditions, we have adapted a standardized machine smoking protocol to mimic human smoking behavior. In these experiments, a first-generation device operating with prefilled cartridges was used. The puff volume of 55 ml and frequency of 2 puffs/min were adapted from the Health Canada machine smoking regime (WHO 2004). A slightly higher puff duration of 3 s was used in these experiments. An initial fraction of 50 puffs was collected, followed by consecutive fractions that contained 10 puffs each. The experiment was continued until no visible smoke was released from cartridges any more. The data obtained demonstrate an increased formation of selected aldehydes of up to threefold–fourfold higher when compared to similar samples that were heated for 2 h at 150 °C (Fig. 4). Further, the release of aldehydes does not occur continuously, but is strongly enhanced in the second half of the smoking period. An interruption of the smoking regime for 15 min did not show a strong effect on the formation of carbonylic compounds (Fig. 4). Therefore, the occurrence of aldehydes seems to be associated with lower liquid levels within the cartridges. This leads to an increased air flow and could promote overheating of the wire in case no safety features are incorporated to maintain a constant temperature claimed to be approximately 60–70 °C. Further, formation of combustion products was well apparent before the liquid levels reached exhaustion. In the affected fractions of 10 puffs, high amounts of aldehydes can be reached that are comparable or even higher as in conventional cigarettes. For example, a maximal release of 30–50 µg formaldehyde per fraction was observed. As each fraction represents 10 puffs, the level of exposure against formaldehyde becomes similar to conventional cigarettes during the last third of the smoking procedure (formaldehyde in tobacco smoke is estimated up to 52 µg per cigarette or 8–10 puffs; Counts et al. 2005). Although the formation of carbonyls remains low at the beginning, a steep increase occurs after 50–100 puffs (Fig. 5). Eventually, the levels of formaldehyde (blue bars) start decreasing again, but remain high until exhaustion of the liquid reservoir.

Discussion

Although e-cigarettes are less harmful than conventional cigarettes, the assessment of putative benefits and risks remains still controversial. These products might be of some value for smokers who may otherwise be unable to quit (Caponnetto et al. 2012). However, recent studies also indicate that high proportions of American (CDC 2013) and Korean (Lee et al. 2014) high school students who use e-cigarettes also smoke tobacco. Although the prevalence among youths is still low, it had doubled during the period of 2011–2012 (CDC 2013). Recent data further suggest that e-cigarettes do not play a significant role in harm

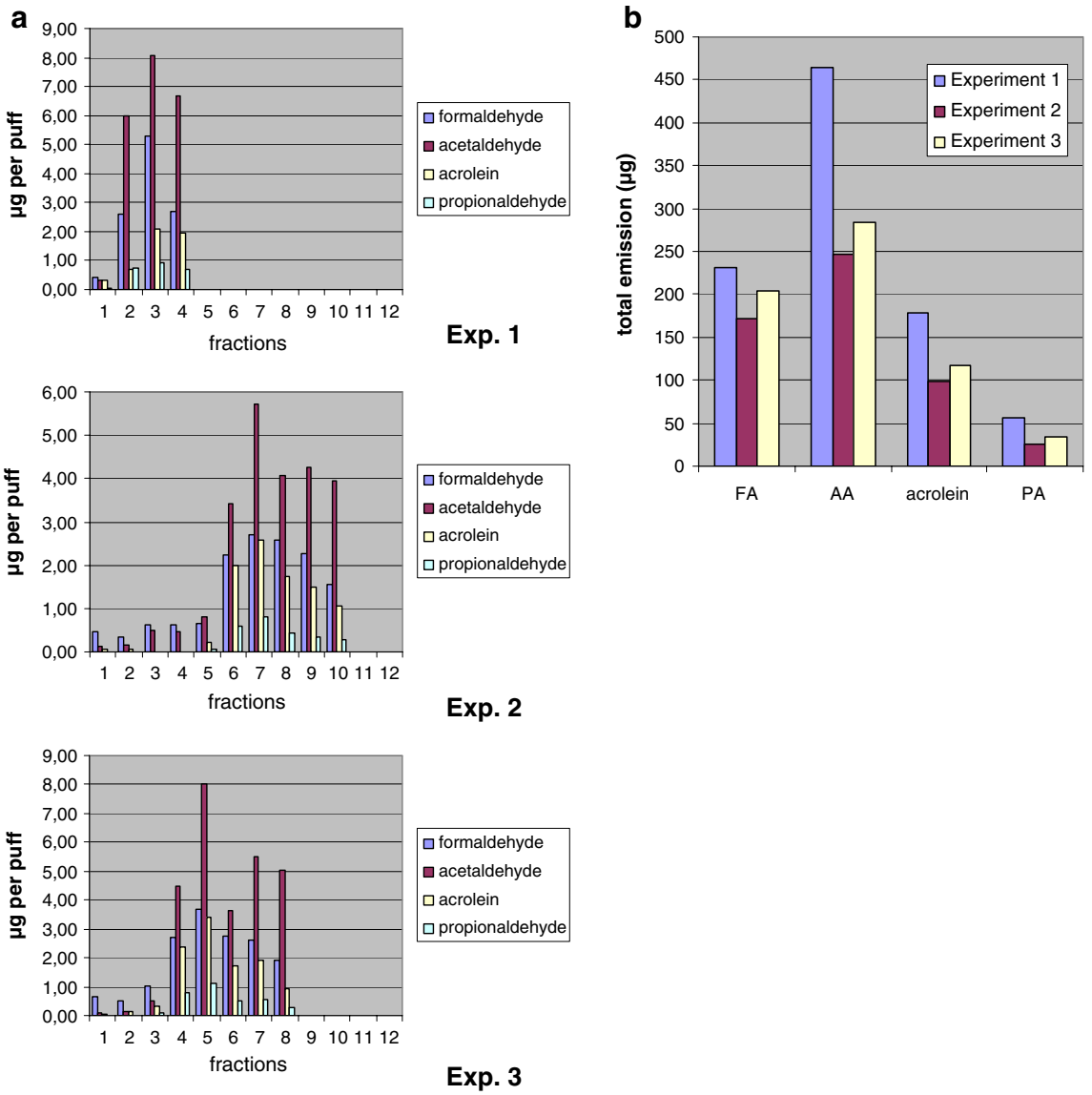

Fig. 4 Carbonyls released from e-cigarettes using a standardized machine smoking protocol. **a** Formation of carbonylic compounds was analyzed by using an e-cigarette brand that is widely distributed by German retail stores. The first fraction contains the initial 50 puffs, whereas 10 puffs were collected in each consecutive fraction (cf. "Materials and methods" section). Three independent experiments are shown. **b** Total numbers of formaldehyde (FA), acetaldehyde (AA), acrolein and propionaldehyde (PA) for each experiment

reduction for adolescents who were used to smoke cigarettes (Lee et al. 2014).

An appropriate regulation of e-cigarettes remains an urgent issue. In the initial proposal for a revised Tobacco Product Directive (TPD), the European Commission proposed an upper limit of 2 mg nicotine per product for exemption from regulation as medical product (see: http://ec.europa.eu/health/tobacco/docs/com_2012_788_en.pdf). However, the European Parliament took a different approach to dispense any restriction for nicotine content per product and suggested only a concentration limit of 30 mg nicotine per milliliter (European Parliament 2013; Amendment 170 in respect to article 18). This would have allowed to market products that could contain 300 mg or more nicotine, without restrictions of sale to address the high toxicity of nicotine. Remarkably, toxicity was not even stated as hazard in the amended health warning. The human toxicity of nicotine is still a matter of discussion. Recently, the human lethal dose was estimated to range between 6.5 and 13 mg per kg body weight (b.w.) and is possibly higher than the widely accepted 1 mg per kg b.w. (Mayer 2014). Adopting these higher values still illustrates serious toxicological concerns in respect to non-restricted distribution of high level nicotine-containing products. In addition, these

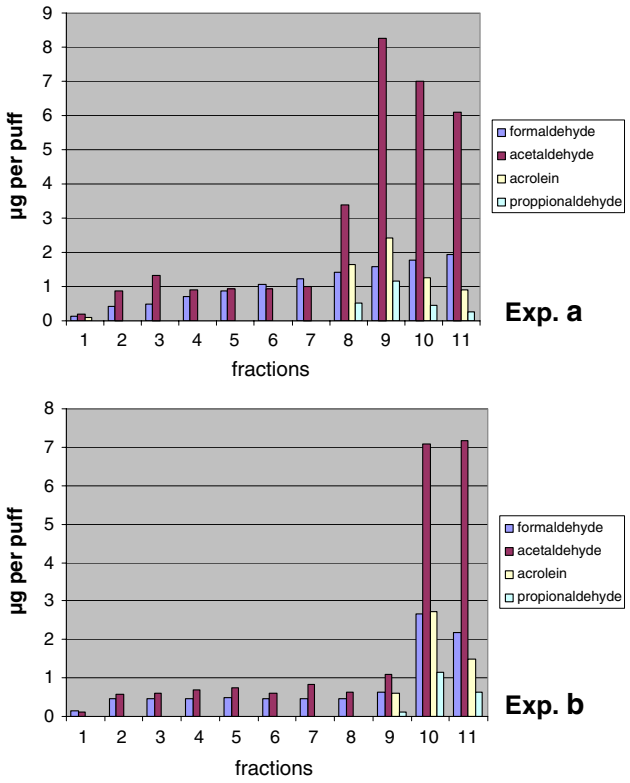

Fig. 5 Formation of carbonyls is not affected by interruption of the smoking procedure. The first fraction contains the initial 60 puffs, whereas 10 puffs were collected in each consecutive fraction (cf. "Materials and methods" section). **a** The smoking procedure was interrupted for 15 min after the initial 60 puffs, in order to adjust the liquids to room temperature after approximately 50 % of total puffs were completed. **b** Machine smoking was continued without interruption. The formation of formaldehyde (FA), acetaldehyde (AA), acrolein and propionaldehyde (PA) per puff and fraction is indicated for both experiments

highly toxic solutions will be supplemented with flavors that are typical for food and might mask the unpleasant taste of nicotine. The TPD has recently been adopted after final negotiations between the European institutions. Upper limits were set to 20 mg/ml and 200 mg nicotine per refill bottle, and thus far beyond the levels proposed by the Commission in 2012.

Although nicotine must be regarded as dominant risk for human health, further hazards can arise from additives and contaminants. Several studies that assessed glycerol and propylene glycol as vaporizing agents did conclude that these substances are relatively safe in vapors (Robertson et al. 1947; Werley et al. 2011; Wieslander et al. 2001), although mild effects have been described and little is known about long-term effects. However, the relevance of this conclusion is further limited, since no regulation exists to specify compounds that can be used in legally traded products. We observed that both glycerol and propylene glycol are being replaced by ethylene glycol in some products. It is not clear whether this replacement was intended for some technological or for palatable reasons. Ethylene glycol is widely used as an anti-freezing agent and associated with pronounced toxicological risks (Hess et al. 2004; Wills et al. 1974). This illustrates that a regulatory framework is required that also covers the major ingredients and additives in low-nicotine or nicotine-free e-cigarette liquids as well. This would also help to enable sufficient product surveillance by state authorities. Our data also confirm the presence of a wide range of flavors and additives in e-cigarette liquids. These include some potentially allergenic compounds, as for example linalool, cinnamic aldehyde, coumarin and eugenol that should be declared by manufacturers to enable for avoidance by sensitized people. Additional methods need to be developed and applied for the surveillance of products. This might also include cytotoxicity tests (Farsalinos et al. 2013a) for an initial screening.

Until now, no well-established methods are available to generate vapors in a machine smoking regime that model the vaping topography of typical users. This could differ from tobacco cigarettes (Farsalinos et al. 2013b). The experiments presented here can only be regarded as an approximation in order to identify putative risks. Analysis of e-cigarette vapors revealed a significant formation of carbonyls, especially carcinogenic formaldehyde, acetaldehyde, as well as acrolein and propionaldehyde in a brand that is widely distributed by German retailers. This effect was clearly established using an adopted Canada Intense machine smoking regime. The data presented are for three independent experiments. We only observed the formation a significant level of carbonyls in the second half of the smoking protocol, starting not before puff 60 (Fig. 3a). As already mentioned, it is possible that this is due to overheating, as decreasing liquid levels are insufficient to cool the heating element. This cause for overheating might be preventable in novel refillable products, where liquid levels can be monitored by the consumer himself. Perhaps, experienced users can avoid inhaling contaminated vapors via recognizing an altered taste. However, this effect was observed well before the liquid became exhausted. Exposure to vapors that are enriched with combustion products is therefore feasible for all types of consumers. In this case, exposure to formaldehyde can even be comparable with conventional cigarettes, as 20–50 µg formaldehyde was measured per 10 puffs in the final fractions. This corresponds roughly to an exposure that can be expected by smoking one cigarette. Our data are in line with a recent study conducted by Goniewicz et al. (2013), though the levels of carbonyls were somewhat higher in our experiments. For instance, we found an approximately fourfold–fivefold higher release of formaldehyde per analyzed liquid. Compared to formaldehyde, the levels of acetaldehyde were

even higher, reaching a level of up to 80 µg in individually collected fractions. Again, no acetaldehyde was observed in the early time fractions, and only traces of this compound in control liquids (3 µg/ml). Intriguingly, the heating of liquids up to 150 °C for 2 h, only led to comparatively low levels of acetaldehyde (75 µg per ml liquid, as compared to 250–450 µg/ml in machine smoking experiments). At temperatures below 100 °C, only traces of carbonyls were observed.

Besides temperature and possible overheating effects due to decreasing liquid levels, the occurrence of decomposition products might be affected by technical parameters, as for example fluid content in liquids, air flow and possibly catalytic properties of the metal mesh. Mandatory safety standards, such as control systems that prevent overheating or maintain a minimum fluid level in cartridges, might therefore be required for all products, independent from the necessity of restrictions of the nicotine contents.

Materials and methods

Chemicals and reagents

All chemicals, analytical standards and solvents used were of analytical or LC–MS grade. Acetaldehyde, propionaldehyde, *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine-hydrochloride (PFBHA), (−)-nicotine solution (1.0 mg/ml), D,L-nicotine-(methyl-d_3) were obtained from Fluka/Sigma-Aldrich (Steinheim, Germany). All standards used for the determination of vaporizing solvents were purchased from Sigma-Aldrich, that is, propylene glycol, 1,3-butanediol, 1,4-butanediol (used as internal standard), diethylene glycol, glycerol, ethylene glycol, triethylene glycol and glyceryl triacetate. The 1-bromo-4-fluorobenzene solution (1,000 µg/ml in methanol) was from Supelco/Sigma-Aldrich (Steinheim, Germany). Methanol, ethyl acetate, sodium chloride, hydrochloric acid and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Dichloromethane, ammonium hydroxide and hexane were from Sigma-Aldrich (Steinheim, Germany).

Determination of aldehydes

An aldehyde standard stock solution (100 mg/l) was prepared by dilution of acetaldehyde and propionaldehyde with methanol. Standard solutions for calibration were prepared by diluting of a standard stock solution in the concentration range from 0.1 to 5.0 µg/ml with methanol.

In 20 ml headspace vials, 2 g sodium chloride was weight and 5 ml water (with 0.05 µg/ml 1-bromo-4-fluorobenzene as internal standard) added. These aqueous internal standard solutions were freshly made every day. A total of 30 ml of an aqueous solution of PFBHA (8.4 mg/ml) was added to 50 µl of each standard solution or e-cigarette liquid. The vial was then immediately sealed with an aluminum screw cap and analyzed by headspace GC–MS.

The headspace GC–MS analysis was performed on an HP 6890 gas chromatograph equipped with an Agilent MSD 5975C mass spectrometer (Agilent Technologies), a Gerstel Multi Purpose Sampler (MPS-2), and a Gerstel Cold Injection System (CIS) (Gerstel, Mühlheim an der Ruhr, Germany). Separation was achieved with a DB-17MS (30 m × 0.25 mm i.d. × 0.25 µm film) capillary column (Agilent Technologies). Headspace and GC–MS conditions were as follows. Incubation time: 60 min; incubation temperature: 60 °C, syringe temperature: 105 °C, flush time: 1,200 s; injection mode: splitless; CIS temperature program: initial temperature of 45 °C increased up to 300 °C by a rate of 12 °C/s in the Ramp 1 an then increased up to 350 °C by a rate of 10 °C/s in the Ramp 2 and hold for 10 min; helium as carrier gas at a constant flow of 1,0 ml/min. The initial oven temperature of 45 °C increased by 7 °C/min up to 150 °C and then by 15 °C/min up to 310 °C, which was kept constant for further 10 min. The total run time was 37.67 min. MSD parameters: solvent delay: 4 min; MSD transfer line: 295 °C, ion source temperature: 230 °C; quadrupole temperature: 150 °C; acquisition mode: SIM with m/z = 95, 174, 176 (for 1-bromo-4-fluorobenzene); 181, 209, 239 (for acetaldehyde-*O*-[(2,3,4,5,6-pentafluorophenyl)methyl]oxime); 181, 236, 253 (for propionaldehyde-*O*-[(2,3,4,5,6-pentafluorophenyl)methyl]oxime).

Determination of nicotine

(−)-Nicotine standard solutions for calibration were prepared by diluting a (−)-nicotine solution (1.0 mg/ml) with methanol to reach a concentration range of 0.1–5.0 µg/ml. Then, a 50 µl aliquot of each standard solution and e-cigarette liquid was added into a 1.5 ml vial. Each sample was alkalinized with 100 µl of 1.0 M sodium hydroxide. Then, 50 µl of an internal standard solution (1.0 µg/ml D,L-nicotine-(methyl-d_3) in methanol) and 200 µl dichloromethane/hexane (1:1 v/v) used for one-step single extraction was added. All samples were mixed at 250 rpm for 60 min. The organic layer was transferred to a new vial with an insert after extraction. Finally, a 2 µl aliquot was injected into GC–MS and analyzed.

The GC–MS analysis was performed on an HP 6890 gas chromatograph equipped with an Agilent MSD 5975C mass spectrometer (Agilent Technologies), a Gerstel Multi Purpose Sampler (MPS-2), and a Gerstel Cold Injection System (CIS) (Gerstel, Mühlheim an der Ruhr, Germany). Separation was achieved with a DB-17MS (30 m × 0.25 mm i.d. × 0.25 µm film) capillary column (Agilent Technologies). The GC–MS conditions were as follows. Injection

mode: splitless; CIS temperature program: initial temperature of 45 °C increased up to 320 °C by a rate of 12 °C/s and hold for 5 min; helium as carrier gas at a constant flow of 1,0 ml/min. The initial oven temperature of 40 °C increased by 25 °C/min up to 310 °C which was kept constant for further 10 min. The total run time was 20.80 min; solvent delay: 5 min; MSD transfer line: 295 °C, ion source temperature: 230 °C; quadrupole temperature: 150 °C; acquisition mode: SIM with $m/z = 84$, 133, 162 [(−)-nicotine) and $m/z = 87$, 136, 165 (D,L-nicotine-methyl-d3)].

Determination of vaporizing solvents

E-cigarette liquids were analyzed by diluting a sample solution of approx. 5 mg/ml (precisely weighed) with methanol containing 1,4-butanediol in a concentration of 2 mg/ml as internal standard. One microliter aliquot of this sample solution was injected into the GC–FID and analyzed. GC–FID analysis was performed on an HP 6890 gas chromatograph equipped with an FID detector and an autosampler (Agilent Technologies). Separation was achieved on a forte SolGel-WAX (30 m × 0.25 mm i.d. × 0.25 µm film) capillary column (SGE Analytical Science Pty Ltd., VIC, Australia). GC–FID conditions were as follows. Split mode, split ratio: 20:1; injector temperature: 220 °C; hydrogen as carrier gas at a constant flow of 1.0 ml/min; detector temperature: 300 °C. Initial oven temperature of 50 °C was held for 1 min, then raised by 30 °C/min up to 280 °C and hold for 15 min. Total run time was 23.67 min.

Qualitative screening analysis

In the optimized qualitative screening method, each e-cigarette liquid was analyzed under two different conditions. A 100 µl aliquot of e-cigarette liquid was added into a 1.5 ml vial. Then, 200 µl of ethyl acetate and 100 µl of 0.1 M hydrochloric acid were added for the acidic extraction, and 100 µl of 0.2 M ammonia for the basic extraction, respectively. Then, the vials were immediately sealed and mixed at 250 rpm for 10 min. The organic layer was transferred to a new vial with an insert after extraction. A 2 µl aliquot was injected into the GC–MS and analyzed. The GC–MS instrumentation was the same as for the determination of nicotine. The GC–MS conditions were as follows. Injection mode: splitless; CIS temperature program: initial temperature of 45 °C increased up to 350 °C by a rate of 12 °C/s and hold for 5 min; helium as carrier gas at a constant flow of 1.0 ml/min. The initial oven temperature of 40 °C increased by 20 °C/min up to 100 °C, then by 10 °C/min up to 170 °C, and hold for 2 min, then 170 °C raised by 8 °C/min up to 250 °C and then by 25 °C/min up to 320 °C, which was kept constant for further 5 min. Total run time was 29.80 min; MSD transfer line: 295 °C, ion source temperature: 230 °C; quadrupole temperature: 150 °C; acquisition mode: scan with $m/z = (30–450)$. For peak identification, the NIST08 spectral library was used.

Machine smoking protocol

E-cigarette vapors were generated using a Borgwaldt RM20H smoking machine that was operated under following conditions: 55 ml puff volume, 3 s puff duration, 2 puffs/min. An initial fraction of 50 or 60 puffs and consecutive fractions of 10 puffs each were collected. In addition, five empty puffs were also sampled between each fraction to avoid cross contamination and overheating of the e-cigarettes investigated. The machine smoking was continued for up to 160 puffs, until no further vapors were observed. The lighting source was switched off during the procedure. To allow for quantification of carbonylic compounds, the vapors of each fraction were led into two bottles that both contained freshly prepared 35 ml of 8.58 mM 2,4-dinitrophenylhydrazine (DNPH, 2 g into 200 ml H_2O and 800 ml acetonitrile, v/v). Then, the contents of the bottles were mixed and kept at room temperature for 30 min to allow for derivatization. This solution was then diluted 1:5 into 16.51 mM Tris base and analyzed by HPLC, using a Sulpelco Ascentis RP-Amide 100 mm × 2.1 mm × 3 µm column. DNPH derivatives of formaldehyde, acetaldehyde, acrolein and propionaldehyde were detected via a diode array detector (DAD) and quantified according to commercial standards (Sigma-Aldrich).

Acknowledgments This study was financially supported by intramural funding of the German Federal Institute for Risk Assessment (BfR), SFP Grant No. 1322-535.

References

Benowitz NK, Gourlay SG (1997) Cardiovascular toxicity of nicotine: implications for nicotine replacement therapy. J Am Coll Cardiol 29:1422–1431

Benowitz NL, Hennigfield JE (1994) Establishing a nicotine threshold for addiction. The implications for tobacco regulation. N Engl J Med 331:123–125

Bullen C, McRobbie H, Thornley S, Glover M, Lin R, Laugesen M (2010) Effect of an electronic nicotine delivery device (e cigarette) on desire to smoke and withdrawal, user preferences and nicotine delivery: randomised cross-over trial. Tob Control 19:98–103

Caponnetto P, Campagna D, Papale G, Russo C, Polosa R (2012) The emerging phenomenon of electronic cigarettes. Expert Rev Respir Med 6:63–74

Cardinale A, Nastrucci C, Cesario A, Russo P (2012) Nicotine specific role in angiogenesis, proliferation and apoptosis. Crit Rev Toxicol 42:68–89

Carmines EL, Gaworski CL (2005) Toxicological evaluation of glycerin as a cigarette ingredient. Food Chem Toxicol 10:1521–1539

CDC (2013) Notes from the field: electronic cigarette use among middle and high school students—United States, 2011–2012. MMWR Morb Mortal Wkly Rep 62:729–730

Changeux JP, Taly A (2008) Nicotine receptors, allosteric proteins and medicine. Trends Mol Med 14:93–102

Counts ME, Morton MJ, Laffoon SW, Cox RH, Lipowicz PJ (2005) Smoke composition and predicting relationships for international commercial cigarettes smoked with three machine-smoking conditions. Regul Toxicol Pharmacol 41:185–227

Dawkins L, Concoran O (2014) Acute electronic cigarette use: nicotine delivery and subjective effects in regular users. Psychopharmacology 231:401–407

Dawkins L, Turner J, Hasna S, Soar K (2012) The electronic-cigarette: effects on desire to smoke, withdrawal symptoms and cognition. Addict Behav 37:970–973

Etter JF, Bullen C (2011a) Salvia cotinine levels in users of electronic cigarettes. Eur Respir J 38:1219–1236

Etter J, Bullen C (2011b) Electronic cigarette: users profile, utilization, satisfaction and perceived efficacy. Addiction 106:2017–2028

Etter JF, Bullen C, Flouris AD, Laugesen M, Eissenberg T (2011) Electronic nicotine delivery systems: a research agenda. Tob Control 20:243–248

European Parliament (2013) Amendments adopted by the European Parliament on 8 October 2013 on the proposal for a directive of the European Parliament and of the Council on the approximation of the laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation and sale of tobacco and related products [COM(2012)0788–C7-0420/2012–2012/0366(COD)]

Farsalinos KE, Romagna G, Allifranchini E, Ripamonti E, Bocchietto E, Todeschi S, Tsiapras D, Kyrzopoulos S, Voudris V (2013a) Comparison of the cytotoxic potential of cigarette smoke and electronic cigarette vapour extract on cultured myocardial cells. Int J Environ Res Public Health 10:5146–5162

Farsalinos KE, Romagna G, Tsiapras D, Kyrzopoulos S, Voudris V (2013b) Evaluation of electronic cigarette use (vaping) topography and estimation of liquid consumption: implications for research protocol standards definition and for public health authorities' regulation. Int J Environ Res Public Health 10:2500–2514

Flouris AD, Chorti MS, Poulianiti KP, Jamurtas AZ, Kostikas K, Tzatzarakis MN, Wallace Hayes A, Tsatsakis AM, Koutedakis Y (2013) Acute impact of active and passive electronic cigarette smoking on serum cotinine and lung function. Inhal Toxicol 25:91–101

Goniewicz ML, Zielinska-Danch W (2012) Electronic cigarette use among teenagers and young adults in Poland. Pediatrics 130:e879–e885

Goniewicz ML, Kuma T, Gawron M, Knysak J, Kosmider L (2012) Nicotine levels in electronic cigarettes. Nicotine Tob Res 15:158–166

Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J, Prokopowicz A, Jablonska-Czapla M, Rosik-Dulewska C, Havel C, Jacob P III, Benowitz N (2013) Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. Tob Control 23:133–139

Greenland S, Satterfield MH, Lanes SF (1998) A meta-analysis to assess the incidence of adverse effects associated with the transdermal nicotine patch. Drug Saf 18:297–308

Hansson J, Pedersen NL, Galanti MR, Andersson T, Ahlbom A, Hallqvist J, Magnusson C (2009) Use of snus and risk for cardiovascular disease: results from the Swedish Twin Registry. J Intern Med 265:717–724

Hansson J, Galanti MR, Hergens MP, Fredlund P, Ahlbom A, Alfredsson L, Bellocco R, Eriksson M, Hallqvist J, Hedblad B, Janssom JH, Nilsson P, Pedersen N, Lagerros YT, Östergren PO, Magnusson C (2012) Use of snus and acute myocardial infarction: pooled analysis of eight prospective observational studies. Eur J Epidemiol 27:771–779

Heeschen C, Jang JJ, Weis M, Pathak A, Kaji S, Hu RS, Tsao PS, Johnson FL, Cooke JP (2001) Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. Nat Med 7:833–839

Hess R, Bartels MJ, Pottenger LH (2004) Ethylene glycol: an estimate of tolerable levels of exposure based on a review of animal and human data. Arch Toxicol 78:671–680

Hukkanen J, Jacob P, Benowitz NL (2005) Metabolism and disposition kinetics of nicotine. Pharmacol Rev 57:79–115

IARC monographs on the evaluation of carcinogenic risks to humans (1999) Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide, vol 71. International Agency for Research on Cancer, Lyon

Jasinska AJ, Zorick T, Brody AL, Stein EA (2013) Dual role of nicotine in addiction and cognition: a review of neuroimaging studies in humans. Neuropharmacology. doi:10.1016/j.neuropharm.2013.02.015

Lee J, Cooke JP (2012) Nicotine and pathological angiogenesis. Life Sci 91:1058–1064

Lee S, Grana RA, Glantz SA (2014) Electronic cigarette use among Korean adolescents: a cross-sectional study of market penetration, dual use, and relationship to quit attempts and former smoking. J Adolesc Health 54:684–690

Mayer B (2014) How much nicotine kills a human? Tracing back the generally accepted lethal dose to dubious self-experiments in the nineteenth century. Arch Toxicol 88:5–7

Nimlos MR, Blanksby SJ, Qian X, Himmel ME, Johnson DK (2006) Mechanisms of glycerol dehydration. J Phys Chem A 110:6145–6156

Robertson OH, Loosli CG, Puck TT, Wise H, Lemon HM, Lester W (1947) Tests for the chronic toxicity of propylene glycol and triethylene glycol on monkeys and rats by vapor inhalation and oral administration. J Pharmacol Exp Ther 91:52–76

Schnuch A, Uter W, Geier J, Lessmann H, Frosch PJ (2007) Sensitization to 26 fragrances to be labelled according to current European regulation. Results of the IVDK and review of the literature. Contact Dermat 57:1–10

Schubert J, Heinke V, Bewersdorff J, Luch A, Schulz TG (2012) Waterpipe smoking: the role of humectants in the release of toxic carbonyls. Arch Toxicol 86:1309–1316

Uchiyama S, Inaba Y, Kunugita N (2010) Determination of acrolein and other carbonyls in cigarette smoke using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine. J Chromatogr A 1217:4383–4388

Vansickel AR, Eissenberg T (2013) Electronic cigarettes: effective nicotine delivery after acute administration. Nicotine Tob Res 15:267–270

Vansickel AR, Cobb CO, Weaver MF, Eissenberg TE (2010) A clinical laboratory model for evaluating the acute effects of electronic "cigarettes": nicotine delivery profile and cardiovascular and subjective effects. Cancer Epidemiol Biomark Prev 19:1945–1953

Vardavas CI, Anagnostopoulos N, Kougias M, Evangelopoulou V, Connolly GN, Behrakis PK (2012) Short term pulmonary effects of using an e-cigarette: impact on respiratory flow resistance, impedance and exhaled nitric oxide. Chest 141:1400–1406

Waldum HJ, Nilsen OG, Nilsen T, Rorvik H, Syversen U, Sandvik AK, Haugen OA, Torp SH, Brenna E (1996) Long term effects of inhaled nicotine. Life Sci 58:1339–1346

Werley MS, McDonald P, Lilly P, Kirkpatrick D, Wallery J, Byron P, Venitz J (2011) Non-clinical safety and pharmacokinetic evaluations of propylene glycol aerosol in Sprague–Dawley rats and Beagle dogs. Toxicology 287:76–90

WHO (2004) WHO Study Group on tobacco product regulation. Guiding principles for the development of tobacco research and testing capacity and proposed protocols for the initiation of tobacco product testing, p 11. http://www.who.int/tobacco/global_interaction/tobreg/goa_2003_principles/en/index.html. (Accessed 8 Aug 2013)

Wieslander G, Norbäck D, Lindgren T (2001) Experimental exposure to propylene glycol mist in aviation emergency training: acute ocular and respiratory effects. Occup Environ Med 58:649–655

Wills JH, Coulston F, Harris ES, McChesney EW, Russell JC, Serrone DM (1974) Inhalation of aerosolized ethylene glycol by man. Clin Toxicol 7:463–476