Flavour chemicals in electronic cigarette fluids

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ABSTRACT

Background Most e-cigarette liquids contain flavour chemicals. Flavour chemicals certified as safe for ingestion by the Flavor Extracts Manufacturers Association may not be safe for use in e-cigarettes. This study identified and measured flavour chemicals in 30 e-cigarette fluids.

Methods Two brands of single-use e-cigarettes were selected and their fluids in multiple flavour types analysed by gas chromatography/mass spectrometry. For the same flavour types, and for selected confectionery flavours (eg, bubble gum and cotton candy), also analysed were convenience samples of e-cigarette fluids in refill bottles from local ‘vape’ shops and online retailers.

Results In many liquids, total flavour chemicals were found to be in the ∼1–4% range (10–40 mg/mL); labelled levels of nicotine were in the range of 0.6–2.4% (6 to 24 mg/mL). A significant number of the flavour chemicals were aldehydes, a compound class recognised as ‘primary irritants’ of mucosal tissue of the respiratory tract. Many of the products contained the same flavour chemicals: vanillin and/or ethyl vanillin was found in 17 of the liquids as one of the top three flavour chemicals, and/or at ≥0.5 mg/mL.

Conclusions The concentrations of some flavour chemicals in e-cigarette fluids are sufficiently high for inhalation exposure by vaping to be of toxicological concern. Regulatory limits should be contemplated for levels of some of the more worrisome chemicals as well as for total flavour chemical levels. Ingredient labeling should also be required.

INTRODUCTION

Use of electronic cigarettes (aka e-cigarettes, electronic nicotine delivery systems and ENDS) is expanding rapidly, with global sales estimated at US $1.5 billion in 2012 and US$3.5 billion in 2013; sales for 2014 were projected to be US$7 billion. Adoption of e-cigarettes has far out-paced our understanding of their implications for health, including the initial composition of the e-cigarette fluids as well as presence of harmful by-products formed during ‘vaping’. In April, US Food and Drug Administration issued a report in which it deemed that it has regulatory authority over e-cigarettes. No specific regulations were yet proposed, except that sales to those under 18 should be prohibited; final action is slated for June 2015. The use of flavourings in e-cigarette fluids has become a central focus for those marketing e-cigarettes and for those demanding regulatory control, including 29 Attorneys General. Centers for Disease Control and Prevention (CDC) reports that the percentage of high school students who acknowledged ever using an e-cigarette doubled from 4.7% in 2011 to 10% in 2012. Supporters of regulation note that cigarettes with ‘characterising flavours’ (other than with menthol) were banned in 2009 due to evidence that they were attracting youth to smoking. A recent report states that an astonishing 7764 unique flavour names were available online in January 2014, with 242 new flavours being added per month, and sales occurring under 466 brands. For the 7764 flavour names, only a small number relate to ‘tobacco’; the vast majority are confectionary in nature, for example, chocolate raspberry, cherry cheesecake, cotton candy, vanilla, grape, apple, coffee, bubble gum, etc. The NJOY brand had avoided explicitly labelled confectionary flavour names, but due to rapidly losing market share, it was recently reported to have plans to offer products in ‘butter crumble’ and ‘black and blue berry’.

Some manufacturers of e-cigarette fluids have cited that the ingredients, including the flavour chemicals used, are all ‘food grade’, and/or ‘generally recognised as safe’ (GRAS). However, GRAS certification by the Flavor Extracts Manufacturers Association (FEMA) pertains only to ingestion, not inhalation. FEMA currently states that the [FEMA] Expert Panel does not evaluate flavor ingredients for use in tobacco products including e-cigarettes or other products that are not human food, or products that result in exposures other than ingestion.

E-cigarette manufacturers should not represent or suggest that the flavor ingredients used in their products are safe because they have FEMA GRAS™ status for use in food because such statements are false and misleading.

While it is likely that virtually all flavour ingredients that are popular in confectionary and food products have been included in multiple e-cigarette products, very little has been published on the levels of flavour chemicals in e-cigarette fluids. Farsalinos et al10 analysed e-cigarette refill fluids from seven countries for diacetyl (aka butanedione, often described as giving a buttery flavour), and acetyl propionyl (aka pentane-2,3-dione, often described as giving a caramel or buttery flavour). Both compounds were reported to be found in 74% of the samples tested, and the authors concluded that 47% of the diacetyl-containing samples and 42% of the acetyl propionyl-containing samples could lead to exposures higher than NIOSH safety limits. Bahl et al11 examined 41 e-cigarette refill fluids for cytotoxicity to human pulmonary fibroblasts, human embryonic stem cells and mouse neural stem cells, and concluded that...
Table 1  Concentrations of flavour chemicals measured at ≥0.5 mg/mL and/or for top three flavour chemicals in 30 e-cigarette products including e-cigarettes with disposable cartridges (NJOY and BLU) and refill bottles (six brands)

| Rank | Flavour name (number for flavour) | Refill bottle or disposable cartridge | Labelled nicotine (mg/mL) | Total for flavour chemicals determined (mg/mL) | Individual flavour chemicals | CAS Registry number | Class |
|------|----------------------------------|---------------------------------------|---------------------------|-----------------------------------------------|-----------------------------|-------------------|-------|
| 1    | 'Double Dark Chocolate' (1 of 2 Chocolate/Cocoa) | Refill bottle | 12 | 43.0 | Vanillin 121-33-5 | Aldehyde |
|      |                                  |                                       |                           | 33.0 | Maltol 118-71-8 | Alcohol |
|      |                                  |                                       |                           | 7.0 | Ethyl maltol 4940-11-8 | Alcohol |
|      |                                  |                                       |                           | 4.7 | Ethyl vanillin 121-32-4 | Aldehyde |
| 2    | 'Cotton Candy' (1 of 2 Cotton Candy) | Refill bottle | 12 | 31.4 | Ethyl maltol 4940-11-8 | Alcohol |
|      | Mt Baker Vapor                    |                                       |                           | 27.1 | Ethyl vanillin 121-32-4 | Aldehyde |
|      |                                  |                                       |                           | 3.8 | Vanillin 121-33-5 | Aldehyde |
| 3    | 'Wild Cherry' (1 of 3 Cherry)     | Refill bottle | 12 | 29.7 | Benzaldehyde 100-52-7 | Aldehyde |
|      | Mt Baker Vapor                    |                                       |                           | 21.2 | p-Tolualdehyde 14-87-0 | Alcohol |
|      |                                  |                                       |                           | 2.8 | Benzyl alcohol 100-51-6 | Alcohol |
|      |                                  |                                       |                           | 1.1 | Ethyl butyrate 105-54-4 | Ester |
|      |                                  |                                       |                           | 0.5 | Benzyl acetate 140-11-4 | Ester |
|      |                                  |                                       |                           | 0.5 | γ-Undecalactone 104-67-6 | Ketone |
| 4    | '555 Menthol' (1 of 4 Menthol)    | Refill bottle | 12 | 28.5 | Menthol 2216-51-5* | Alcohol |
|      | Mt Baker Vapor                    |                                       |                           | 21.6 | Ethyl maltol 121-33-5 | Alcohol |
|      |                                  |                                       |                           | 5.2 | Ethyl vanillin 121-32-4 | Aldehyde |
|      |                                  |                                       |                           | 1.2 | Vanillin 121-33-5 | Aldehyde |
| 5    | 'Caught’n Pick’n Kid' (2 of 2 Cotton Candy) | Refill bottle | 12 | 27.8 | Ethyl maltol 4940-11-8 | Alcohol |
|      | Rose City Vapors                  |                                       |                           | 23.4 | Ethyl vanillin 121-32-4 | Aldehyde |
|      |                                  |                                       |                           | 4.0 | Vanillin 121-33-5 | Aldehyde |
| 6    | 'Bubble Gum' (1 of 2 Bubble Gum) | Refill bottle | 12 | 23.8 | Ethyl butyrate 105-54-4 | Ester |
|      | Mt Baker Vapor                    |                                       |                           | 11.1 | Ethyl acetate 141-78-6 | Ester |
|      |                                  |                                       |                           | 7.1 | Ethyl hexanoate 6378-65-0 | Ester |
|      |                                  |                                       |                           | 1.9 | Limonene 138-86-3* | Alcohol |
|      |                                  |                                       |                           | 1.1 | 2-Methylbutyl acetate 624-49-1 | Alcohol |
|      |                                  |                                       |                           | 0.9 | Cinnamaldehyde 104-55-2 | Alcohol |
|      |                                  |                                       |                           | 0.8 | Ethyl hexanoate 6378-65-0 | Ester |
|      |                                  |                                       |                           | 0.5 | Ethyl hexanoate 6378-65-0 | Ester |
| 7    | 'Menthol' (2 of 4 Menthol)        | Disposable cartridge | 18 | 21.5 | Menthol 2216-51-5* | Alcohol |
|      | NJOY                             |                                       |                           | 19.7 | Neomenthol 4919-01-0 | Alcohol |
|      |                                  |                                       |                           | 0.9 | Neomenthol 4919-01-0 | Alcohol |
| 8    | 'French Vanilla' (1 of 3 Vanilla) | Refill bottle | 12 | 18.8 | Ethyl vanillin 121-32-4 | Aldehyde |
|      | Halo                             |                                       |                           | 8.4 | Vanillin 121-33-5 | Aldehyde |
|      |                                  |                                       |                           | 6.1 | Maltol 118-71-8 | Alcohol |
| 9    | Cafe Mocha (1 of 3 Coffee)        | Refill bottle | 6  | 18.4 | Vanillin 121-33-5 | Aldehyde |
|      | Halo                             |                                       |                           | 10.9 | Ethyl vanillin 121-32-4 | Aldehyde |
|      |                                  |                                       |                           | 5.4 | Ethyl maltol 4940-11-8 | Alcohol |
|      |                                  |                                       |                           | 1.0 | Ethyl maltol 4940-11-8 | Alcohol |
| 10   | 'Menthol Ice' (3 of 4 Menthol)    | Refill bottle | 6  | 16.8 | Menthol 2216-51-5* | Alcohol |
|      | Halo                             |                                       |                           | 16.5 | Ethyl vanillin 121-32-4 | Aldehyde |
|      |                                  |                                       |                           | 0.2 | Ethyl vanillin 121-33-5 | Aldehyde |
|      |                                  |                                       |                           | 0.1 | Ethyl vanillin 121-33-5 | Aldehyde |
| 11   | 'Grape' (1 of 2 Grape)            | Refill bottle | 24 | 13.4 | Maltol 118-71-8 | Alcohol |
|      | Taste E-Liquid                   |                                       |                           | 6.2 | Ethyl acetate 141-78-6 | Ester |
|      |                                  |                                       |                           | 3.5 | Ethyl acetate 141-78-6 | Ester |
|      |                                  |                                       |                           | 1.3 | Ethyl maltol 4940-11-8 | Alcohol |
|      |                                  |                                       |                           | 1.0 | Methyl anthranilate 134-20-3 | Ester |
|      |                                  |                                       |                           | 0.7 | Ethyl isovalerate 108-64-5 | Ester |
|      |                                  |                                       |                           | 0.3 | Ethyl isovalerate 108-64-5 | Ester |

Continued
| Rank by total flavour level | Flavour name (number for flavour) | Brand | Refill bottle or disposable cartridge | Total for flavour chemicals determined (mg/mL) | Individual flavour chemicals (mg/mL) | CAS Registry number | Class |
|----------------------------|----------------------------------|-------|--------------------------------------|---------------------------------------------|----------------------------------------|-------------------|-------|
|                            | ‘Green Apple’ (1 of 2 Apple)     | Mt Baker Vapor | Refill bottle 12 | 12.2 | (E)-2-Hexen-1-ol 4.5 | 928-95-0 | Alcohol |
|                            |                                  |       |                                      | 4.3 | (3Z)-3-Hexen-1-ol 4.3 | 928-96-1 | Alcohol |
|                            |                                  |       |                                      | 1.7 | Hexyl acetate 1.7 | 142-92-7 | Ester |
|                            |                                  |       |                                      | 0.8 | Ethyl acetate 0.8 | 141-78-6 | Ester |
|                            |                                  |       |                                      | 0.7 | Ethyl butyrate 0.7 | 105-54-4 | Ester |
|                            | ‘Longhorn’ (1 of 7 ‘Tobacco’)   | Halo | Refill bottle 6 | 10.4 | Benzyl alcohol 8.4 | 100-51-6 | Alcohol |
|                            |                                  |       |                                      | 1.6 | Vanillin 1.6 | 121-33-5 | Aldehyde |
|                            | ‘555’ (2 of 7 ‘Tobacco’)        | Mt Baker Vapor | Refill bottle 12 | 9.3 | Ethyl acetate 1.5 | 121-32-4 | Aldehyde |
|                            | ‘Grape’ (2 of 2 Grape)          | Mt Baker Vapor | Refill bottle 12 | 9.0 | Ethyl acetate 1.3 | 141-76-6 | Ester |
|                            | ‘Vanilla’ (2 of 3 Vanilla)      | Rose City Vapors | Refill bottle 12 | 7.5 | Ethyl acetate 0.6 | 105-54-4 | Ester |
|                            | ‘Bubblegum’ (2 of 2 Bubble Gum) | Liquid | Refill bottle 12 | 7.5 | Ethyl acetate 0.6 | 105-54-4 | Ester |
|                            | ‘Magnificent Menthol’ (4 of 4 Menthol) | BLU | Disposable cartridge 22† | 7.1 | 2-Methylbutyl acetate 0.6 | 624-41-9 | Ester |
|                            | ‘Java Jolt’ (2 of 3 Coffee)     | BLU | Disposable cartridge 22† | 6.7 | Menthol 5.7 | 2216-51-5* | Alcohol |
|                            | ‘First Step Tobacco’ (3 of 7 Tobacco) | Rose City Vapors | Refill bottle 12 | 6.6 | Menthone 0.6 | 14073-97-3* | Ketone |
|                            | ‘Granny Smith Apple’ (2 of 2 Apple) | Taste E-Liquid | Refill bottle 24 | 5.7 | Ethyl maltol 0.3 | 4940-11-8 | Alcohol |
|                            | ‘Vivid Vanilla’ (3 of 3 Vanilla) | BLU | Disposable cartridge 22† | 4.7 | Ethyl maltol 0.3 | 4940-11-8 | Alcohol |
|                            | ‘Cherry’ (2 of 3 Cherry)        | Taste E-Liquid | Refill bottle 6 | 4.3 | Ethyl maltol 1.5 | 4940-11-8 | Alcohol |
|                            | ‘Belgium Cocoa’ (2 of 2 Chocolate/Cocoa) | Halo | Refill bottle 6 | 3.7 | Benzyl alcohol 0.3 | 100-51-6 | Alcohol |
|                            | ‘Coffee’ (3 of 3 Coffee)        | Mt Baker Vapor | Refill bottle 12 | 2.6 | Benzyl alcohol 0.3 | 100-51-6 | Alcohol |

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Research is needed to characterize both the presence of toxic chemicals in ENDS flavorings and the potential adverse respiratory effects of exposure to e-liquids, especially flavorings.

Hutzler et al.11 analysed 28 e-cigarette liquids from seven manufacturers by gas chromatography/mass spectrometry (GC/MS) and used comparisons with known compound-specific MS patterns to tentatively (and qualitatively) identify the presence of 141 flavour chemicals in one or more of the products. Vanillin, ethyl maltol, ethyl vanillin and menthol were the four most frequently found flavour chemicals, reported to be present in 79%, 57%, 50% and 43% of the 28 samples, respectively. However, since authentic standards were not used, actual concentrations could not be deduced. As follow-up to Bahl et al,11 Behar et al14,15 examined cytotoxicity and measured levels of cinnamaldehyde, 4-methoxycinnamaldehyde and vanillin for 10 ‘cinnamon’ flavoured refill fluids. For the three compounds, the highest concentrations were ~40, 3 and 8 mg/mL, respectively (~4%, 0.3% and 0.8% by weight or volume).

Product labels rarely provide ingredient information beyond the level of nicotine, and the inclusion of propylene glycol and/or glycerol. To provide additionally needed information, we describe determinations of the levels of flavour chemicals in the fluids of a convenience sample of disposable e-cigarettes and refill bottles over a range of flavour types.

### METHODS

We assumed that meaningful conclusions could be obtained by analysing 30 products. The e-cigarette fluids examined were selected from a vast and rapidly changing array of products. BLU and NJOY, two brands of disposable-cartridge e-cigarettes, were purchased in five flavours: tobacco, menthol, vanilla, cherry and coffee. Also purchased in the same flavours (from online retailers and local ‘vape’ shops in Portland, Oregon) were refill bottles for tank systems. Refill bottles in five other confectionary flavours (chocolate/cocoa, grape, apple, cotton candy and bubble gum) were also purchased. After dilution with methanol, the fluids were analysed by GC/MS. Using internal standard-based calibration procedures similar to those described elsewhere,16 analyses were performed using an Agilent (Santa Clara, California, USA) 7693 autosampler, Agilent 7890A GC and Agilent 5975C MS. The GC column type was Agilent DB-5MS U, of 30 m length, 0.25 mm id and 0.25 mm film thickness. For each replicate sample, ~50 mg of each fluid was dissolved in 1 mL of methanol. One microlitre of the methanol solution was then injected on the GC with a 25:1 split. The GC temperature programme for all analyses was: 35°C hold for 5 min; 10°C/min to 300°C; then hold for 3.5 min at 300°C. No analyses of aerosols generated from the fluids were carried out.

Qualitative analyses of the 30 e-cigarette fluids were first carried out here using the NIST 14 MS library,16 and the results were compared with data previously obtained for flavoured tobacco products.17 Quantitative analyses of the 30 fluids were then undertaken, using authentic standards, for a specific list of compounds, which formed the ‘target analyte list’. If reported here, the presence of each target analyte was confirmed by matching GC retention times and MS patterns with results obtained with the authentic standards; the level was determined by comparison with calibration standard runs. The target analyte list included the 70 compounds listed in Brown et al16 plus 20 others, namely aramadendrene, 1,4-cineol, trans-cinnamaldehyde, citronellal,
Assuming respiration at 0.83 m³/h (20 m³/day), these values give recommended work-place exposure limits of 60 and 67 mg/day, respectively. For e-cigarette liquid consumption rates, ~5 mL/day is commonly self-reported in online ‘vaping’ forums. In our data, the brand with rank 3 in total flavour chemicals contained benzaldehyde at 21 mg/mL; the rank 1 brand contained vanillin (4-hydroxy-3-methoxybenzaldehyde) at 33 mg/mL; 5 mL/day then suggests possible inhalation rates of ~105 and ~165 mg/day, respectively, twice the recommended limits. Although the group of fluids analysed here represents only a small sample of the available products, the data suggest that a small number of flavour chemicals are particularly popular among manufacturers: for example, vanillin and ethyl vanillin, maltol and ethyl maltol, benzaldehyde and benzyl alcohol, and ethyl butyrate and ethyl acetate. Regulatory actions that should be considered include requiring ingredient identification, limiting levels of some individual flavour chemicals, and limiting total levels of flavour chemicals.

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