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Flavour chemicals in electronic cigarette fluids

Peyton A Tierney,¹ Clarissa D Karpinski,² Jessica E Brown,¹ Wentai Luo,² James F Pankow^{1,2}

¹Department of Civil and Environmental Engineering, Portland State University, Portland, Oregon, USA
²Department of Chemistry, Portland State University, Portland, Oregon, USA

Correspondence to

Dr James F Pankow, Department of Civil and Environmental Engineering, Portland State University, PO 751, Portland, OR 97207-0751, USA; pankowj@pdx.edu

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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 confectionary 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.¹ 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'.² In April, US Food and Drug Administration issued a report in which it deemed that it has regulatory authority over e-cigarettes.³ 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-cigarettes⁴ and for those demanding regulatory control, including 29 Attorneys General.⁵ 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 2012.⁶ Supporters of regulation note that cigarettes with 'characterising flavours' (other than with menthol) were banned in 2009⁷ due to evidence that they were attracting youth to smoking. A recent report⁸ 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'.⁴

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 states⁹

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.

and

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 al*¹⁰ 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 al*¹¹ examined 41 e-cigarette refill fluids for cytotoxicity to human pulmonary fibroblasts, human embryonic stem cells and mouse neural stem cells, and concluded that



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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 by total flavour level	Flavour name (number for flavour) Brand	Refill bottle or disposable cartridge	Labelled nicotine (mg/mL)	Total for flavour chemicals determined (mg/mL)	(mg/mL)	Individual flavour chemicals	CAS Registry number	Class
1	'Double Dark Chocolate' (1 of 2 Chocolate/Cocoa) Zeus E-Juice	Refill bottle	12	43.0	33.0	Vanillin	121-33-5	Aldehyde
					4.7	Maltol	118-71-8	Alcohol
					3.5	Ethyl maltol	4940-11-8	Alcohol
					1.3	Ethyl vanillin	121-32-4	Aldehyde
2	'Cotton Candy' (1 of 2 Cotton Candy) Mt Baker Vapor	Refill bottle	12	31.4	27.1	Ethyl maltol	4940-11-8	Alcohol
					3.8	Ethyl vanillin	121-32-4	Aldehyde
					0.5	Vanillin	121-33-5	Aldehyde
3	'Wild Cherry' (1 of 3 Cherry) Mt Baker Vapor	Refill bottle	12	29.7	21.2	Benzaldehyde	100-52-7	Aldehyde
					2.8	<i>p</i> -Tolualdehyde	14-87-0	Aldehyde
					1.1	Benzyl alcohol	100-51-6	Alcohol
					0.7	Limonene	138-86-3*	Monoterpene
					0.5	Ethyl butyrate	105-54-4	Ester
					0.5	Benzyl acetate	140-11-4	Ester
4	'555 Menthol' (1 of 4 Menthol) Mt Baker Vapor	Refill bottle	12	28.5	21.6	Menthol	2216-51-5*	Alcohol
					5.2	Vanillin	121-33-5	Aldehyde
					1.2	Maltol	118-71-8	Alcohol
					0.5	γ -Undecalactone	104-67-6	Ketone
5	'Caught'n Pick'n Kid' (2 of 2 Cotton Candy) Rose City Vapors	Refill bottle	12	27.8	23.4	Ethyl maltol	4940-11-8	Alcohol
					4.0	Ethyl vanillin	121-32-4	Aldehyde
					0.4	Vanillin	121-33-5	Aldehyde
6	'Bubble Gum' (1 of 2 Bubble Gum) Mt Baker Vapor	Refill bottle	12	23.8	11.1	Ethyl butyrate	105-54-4	Ester
					7.1	Ethyl acetate	141-78-6	Ester
					1.9	Eugenol	97-53-0	Alcohol
					1.1	Limonene	138-86-3	Monoterpene
					0.9	2-Methylbutyl acetate	624-41-9	Ester
					0.8	Cinnamaldehyde	104-55-2	Aldehyde
					0.5	Ethyl hexanoate	6378-65-0	Ester
7	'Menthol' (2 of 4 Menthol) NJOY	Disposable cartridge	18	21.5	19.7	Menthol	2216-51-5*	Alcohol
					0.9	Menthone	14073-97-3*	Ketone
					0.6	Neomenthol	4919-01-0	Alcohol
8	'French Vanilla' (1 of 3 Vanilla) Mt Baker Vapor	Refill bottle	12	18.8	8.4	Ethyl vanillin	121-32-4	Aldehyde
					6.1	Vanillin	121-33-5	Aldehyde
					4.1	Maltol	118-71-8	Alcohol
9	'Cafe Mocha' (1 of 3 Coffee) Halo	Refill bottle	6	18.4	10.9	Vanillin	121-33-5	Aldehyde
					5.4	Ethyl vanillin	121-32-4	Aldehyde
					1.0	Ethyl maltol	4940-11-8	Alcohol
					0.7	Piperonal	120-57-0	Aldehyde
10	'Menthol Ice' (3 of 4 Menthol) Halo	Refill bottle	6	16.8	16.5	Menthol	2216-51-5*	Alcohol
					0.2	Carvone	6485-40-1*	Ketone
					0.1	Vanillin	121-33-5	Aldehyde
11	'Grape' (1 of 2 Grape) Taste E-Liquid	Refill bottle	24	13.4	6.2	Maltol	118-71-8	Alcohol
					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

Continued

Table 1 Continued

Rank by total flavour level	Flavour name (number for flavour) Brand	Refill bottle or disposable cartridge	Labelled nicotine (mg/mL)	Total for flavour chemicals determined (mg/mL)	(mg/mL)	Individual flavour chemicals	CAS Registry number	Class
12	'Green Apple' (1 of 2 Apple) Mt Baker Vapor	Refill bottle	12	12.2	4.5	(E)-2-Hexen-1-ol	928-95-0	Alcohol
					4.3	(3Z)-3-Hexen-1-ol	928-96-1	Alcohol
					1.7	Hexyl acetate	142-92-7	Ester
					0.8	Ethyl acetate	141-78-6	Ester
					0.7	Ethyl butyrate	105-54-4	Ester
13	'Longhorn' (1 of 7 'Tobacco') Halo	Refill bottle	6	10.4	8.4	Benzyl alcohol	100-51-6	Alcohol
					1.6	Vanillin	121-33-5	Aldehyde
					0.2	Ethyl acetate	141-78-6	Ester
14	'555' (2 of 7 'Tobacco') Mt Baker Vapor	Refill bottle	12	9.3	7.1	Vanillin	121-33-5	Aldehyde
					1.7	Maltol	118-71-8	Alcohol
					0.3	Ethyl vanillin	121-32-4	Aldehyde
15	'Grape' (2 of 2 Grape) Mt Baker Vapor	Refill bottle	12	9.0	7.2	Ethyl acetate	141-78-6	Ester
					1.3	Methyl anthranilate	134-20-3	Ester
					0.6	Ethyl butyrate	105-54-4	Ester
					4.9	Ethyl vanillin	121-32-4	Aldehyde
16	'Vanilla' (2 of 3 Vanilla) Rose City Vapors	Refill bottle	12	7.5	1.7	Ethyl maltol	4940-11-8	Alcohol
					0.3	Ethyl butyrate	105-54-4	Ester
					2.9	Limonene	138-86-3	Monoterpene ester
17	'Bubblegum' (2 of 2 Bubble Gum) Viquid	Refill bottle	12	7.5	1.7	Ethyl butyrate	105-54-4	Aldehyde
					1.0	Benzaldehyde	100-52-7	Aldehyde
					0.6	2-Methylbutyl acetate	624-41-9	Ester
					5.7	Menthol	2216-51-5*	Alcohol
18	'Magnificent Menthol' (4 of 4 Menthol) BLU	Disposable cartridge	22†	7.1	0.6	Menthone	14 073 -97-3*	Ketone
					0.3	Ethyl maltol	4940-11-8	Alcohol
					4.7	Maltol	118-71-8	Alcohol
19	'Java Jolt' (2 of 3 Coffee) BLU	Disposable cartridge	22†	6.7	1.5	Ethyl maltol	4940-11-8	Alcohol
					0.3	Ethyl vanillin	121-32-4	Aldehyde
					5.0	Vanillin	121-33-5	Aldehyde
20	'First Step Tobacco' (3 of 7 'Tobacco') Rose City Vapors	Refill bottle	12	6.6	1.1	Maltol	118-71-8	Alcohol
					0.2	Ethyl vanillin	121-32-4	Aldehyde
					2.5	Hexyl acetate	142-92-7	Ester
21	'Granny Smith Apple' (2 of 2 Apple) Taste E-Liquid	Refill bottle	24	5.7	0.8	Ethyl acetate	141-78-6	Ester
					0.7	2-Methylbutyl acetate	624-41-9	Ester
					2.6	Ethyl vanillin	121-32-4	Aldehyde
22	'Vivid Vanilla' (3 of 3 Vanilla) BLU	Disposable cartridge	22†	4.7	1.5	Vanillin	121-33-5	Aldehyde
					0.3	Benzyl alcohol	100-51-6	Alcohol
					2.7	Ethyl maltol	4940-11-8	Alcohol
23	'Cherry' (2 of 3 Cherry) Taste E-Liquid	Refill bottle	6	4.3	0.9	Benzaldehyde PG acetal	2568-25-4	Acetal
					0.6	Benzaldehyde	100-52-7	Aldehyde
					2.3	Vanillin	121-33-5	Aldehyde
24	'Belgium Cocoa' (2 of 2 Chocolate/Cocoa) Halo	Refill bottle	6	3.7	0.8	Maltol	118-71-8	Alcohol
					0.6	2356-Tetramethylpyrazine	1124-11-4	Pyrazine
					2.3	Benzyl alcohol	100-51-6	Alcohol
25	'Coffee' (3 of 3 Coffee) Mt Baker Vapor	Refill bottle	12	2.6	0.3	Vanillin	121-33-5	Aldehyde
					NA	Unknown minor constituents	NA	Unknown

Continued

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Table 1 Continued

Rank by total flavour level	Flavour name (number for flavour) Brand	Refill bottle or disposable cartridge	Labelled nicotine (mg/mL)	Total for flavour chemicals determined (mg/mL)	Individual flavour chemicals	CAS Registry number	Class
26	'True Tobacco' (4 of 7 'Tobacco') Taste E-Liquid	Refill bottle	6	2.2	Ethyl maltol Vanillin Unknown minor constituents	4940-11-8 121-33-5 NA	Alcohol Aldehyde Unknown
27	'Cherry Crush' (3 of 3 'Cherry') BLU	Disposable cartridge	24†	1.2	Benzyl alcohol Piperonal Vanillin	100-51-6 120-57-0 121-33-5	Alcohol Aldehyde Aldehyde
28	'Torque 56' (5 of 7 'Tobacco') Halo	Refill bottle	6	1.2	Benzyl alcohol β-Damascene Ethyl butyrate	100-51-6 23726-91-2 105-54-4	Alcohol Ketone Ester
29	'Classic Tobacco' (6 of 7 'Tobacco') BLU	Disposable cartridge	22†	~0.1	Benzyl alcohol Unknown minor constituents	100-51-6 NA	Alcohol Unknown
30	'Traditional' (7 of 7 'Tobacco') NJOY	Disposable cartridge	18	low	Unknown minor constituents	NA	Unknown

Labelled Levels for Nicotine Given.

* Chirality of analytes not determined here. Most were probably L form.

†Based on labelled value for 'mg nicotine per cartridge' and fluid volume estimated here.

‡Measured here.

CAS, Chemical Abstracts Service; NA, not applicable; PG, propylene glycol.

when present, the cytotoxicity was related to the flavour chemicals, especially for cinnamon-flavoured refill fluids. A recent opinion piece in *JAMA*¹² states

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*¹³ 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 al*^{14 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 UI, 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,¹⁷ and the results were compared with data previously obtained for flavoured tobacco products.¹⁶ 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 al*¹⁶ plus 20 others, namely aromadendrene, 1,4-cineol, trans-cinnamaldehyde, citronellal,

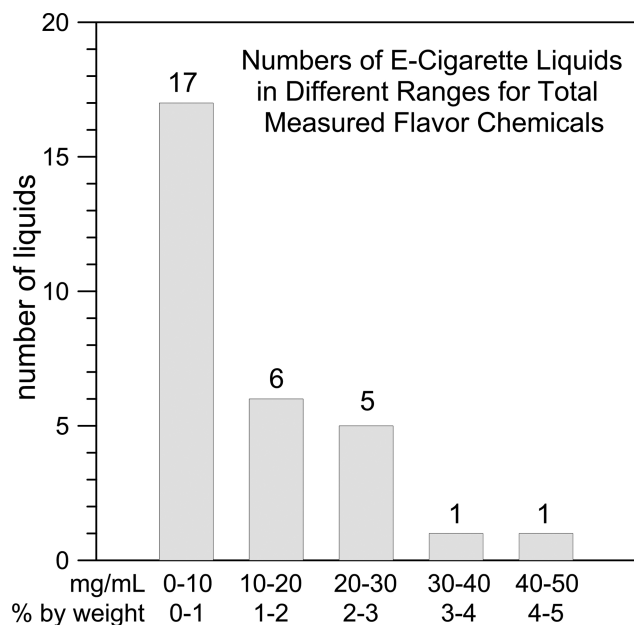


Figure 1 Bar plot of number of e-cigarette fluids out of 30 with total determined flavour chemical levels in five concentration ranges.

citronellol propionate, coumarin, decanal, ethyl acetate, ethyl hexanoate, fenchol, limonene oxide, *trans*-linalyl propionate, maltol, 3'-methylacetophenone, neomenthol, 2-nonanone, pentyl propionate, pulegone, γ -terpineol and 2,3,5,6-tetramethylpyrazine. The vicinal diketone compounds diacetyl and 2,3-pentanedione were not in the target analyte list.

RESULTS

Total flavour chemicals were greater than 1% by weight in 13 of the liquids analysed (table 1). Concentration values in mg/mL are nearly equivalent to values with units of mg/g; 10 mg/mL corresponds to ~1% by weight. Six of the 24 compounds in table 1 are aldehydes, a compound class recognised toxicologically to be 'primary irritants' of the mucosa of the respiratory tract.¹⁸ For the 'tobacco' flavoured fluids, none of the flavour chemicals reported are obtainable at the levels found by adding a tobacco extract to the e-cigarette fluid; while extracts of tobacco may be used in some 'tobacco' flavoured fluids, a majority of the 'tobacco' flavoured products were found to contain confectionary flavour chemicals. Figure 1 provides a bar plot for numbers of fluids versus per cent by weight for the 30 e-cigarette liquids. Thirteen of the liquids (43%) contained total determined flavour chemical levels greater than 1% by weight. Seven of the liquids (23%) contained levels greater than 2% by weight. Two of the liquids (7%) contained levels greater than 3% by weight.

LIMITATIONS

The array of e-cigarette products is vast and growing daily. As such, this study was unable to provide a comprehensive overview of the levels of flavour chemicals in such products currently on the market. Nevertheless, the results obtained are likely to be similar to what a broad survey would have revealed, and in any case strongly suggest that very high levels of some flavour chemicals are undoubtedly present in a great number of the thousands of products currently available.

DISCUSSION

Recommended 8 h occupational exposure limits by inhalation for benzaldehyde and vanillin are ~9 and 10 mg/m³, respectively.¹⁹

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 flavor chemicals, and limiting total levels of flavor chemicals.

What this paper adds

- ▶ Flavour chemicals are present in almost all e-cigarette fluids currently on the market in the USA and globally. Concerns are rising among public health professionals that flavoured e-cigarette products may make e-cigarette use attractive to youth. Second, high doses of some flavour chemicals may be safe when ingested, but quite unsafe when inhaled. Third, toxic degradation products may be produced by reaction of the flavour chemicals at the high temperatures present during e-cigarette use (aka 'vaping').
- ▶ Flavoured e-cigarette products do not typically list the levels of specific flavour chemicals present, and most do not identify the major flavour chemicals present.
- ▶ The analyses of 30 products on the US market revealed that 13 were more than 1% by weight flavour chemicals. Chemicals identified included aldehydes (eg, benzaldehyde and vanillin) which could cause respiratory irritation.

Contributors JFP and PAT planned the study. JFP supervised the study. PAT selected the e-cigarette fluids to be analysed. PAT, CDK, JEB and WL carried out the analyses. WL managed the data QA/QC processes, and was assisted by PAT and CDK. JFP and PAT drafted the manuscript. All authors reviewed the manuscript.

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Updated information and services can be found at:

<http://tobaccocontrol.bmj.com/content/early/2015/03/27/tobaccocontrol-2014-052175>

These include:

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