

Article

Toxicity Assessment of Refill Liquids for Electronic Cigarettes

Vincent Varlet ¹, Konstantinos Farsalinos ^{2,3}, Marc Augsburger ¹, Aurélien Thomas ^{1,4}
and Jean-François Etter ^{5,*}

¹ Unit of Toxicology, University Center of Legal Medicine, 1000 Lausanne 25, Switzerland;
E-Mails: vincent.varlet@chuv.ch (V.V.); marc.augsburger@chuv.ch (M.A.);
aurelien.thomas@chuv.ch (A.T.)

² Department of Cardiology, Onassis Cardiac Surgery Center, Kallithea 17674, Greece;
E-Mail: kfarsalinos@gmail.com

³ Department of Pharmacology, University of Patras, Patras 26500, Greece

⁴ Swiss Center of Applied Human Toxicology, University of Lausanne, 1000 Lausanne 25, Switzerland

⁵ Institute of Global Health, Faculty of Medicine, University of Geneva, 1211 Geneva, Switzerland

* Author to whom correspondence should be addressed; E-Mail: Jean-Francois.Etter@unige.ch;
Tel.: +41-22-379-04-59.

Academic Editor: Paul B. Tchounwou

Received: 18 February 2015 / Accepted: 28 April 2015 / Published: 30 April 2015

Abstract: We analyzed 42 models from 14 brands of refill liquids for e-cigarettes for the presence of micro-organisms, diethylene glycol, ethylene glycol, hydrocarbons, ethanol, aldehydes, tobacco-specific nitrosamines, and solvents. All the liquids under scrutiny complied with norms for the absence of yeast, mold, aerobic microbes, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Diethylene glycol, ethylene glycol and ethanol were detected, but remained within limits authorized for food and pharmaceutical products. Terpenic compounds and aldehydes were found in the products, in particular formaldehyde and acrolein. No sample contained nitrosamines at levels above the limit of detection (1 µg/g). Residual solvents such as 1,3-butadiene, cyclohexane and acetone, to name a few, were found in some products. None of the products under scrutiny were totally exempt of potentially toxic compounds. However, for products other than nicotine, the oral acute toxicity of the e-liquids tested seems to be of minor concern. However, a minority of liquids, especially those with flavorings, showed particularly high ranges of chemicals, causing concerns about their potential toxicity in case of chronic oral exposure.

Keywords: electronic cigarette; electronic nicotine delivery systems; e-cigarette; e-liquid; quality control.

1. Introduction

Electronic cigarettes (e-cigarettes) are increasingly popular [1,2]. They comprise a battery-powered atomizer that produces vapor for inhalation from cartridges or tanks that usually contain propylene glycol or glycerol (or a mix of both), flavors, nicotine, water and ethanol [3]. Surveys show that 11% to 21% of adult smokers in the United States report having ever used e-cigarettes, which translates into several millions users [4–7]. Laboratory testing has shown that some refill liquids (e-liquids) for e-cigarettes contain impurities and toxic substances, or are not filled true to label [2], although a recent report showed that the quality of most e-liquids is compliant with norms [8]. Another concern is the lack of mandatory manufacturing standards for e-cigarettes and e-liquids. There are many manufacturers, but few, if any products are manufactured along standards imposed on medications. There is no guarantee that e-liquids do not contain impurities or toxic components. In addition, recent reports have revealed an increasing number of cases of accidental exposure to e-liquids, mainly through ingestion, and a few fatal cases were reported in the press, although not as case reports in peer-reviewed literature [9]. Nicotine may also oxidize in open containers to produce degradation products within the liquid itself, leading to the unintentional presence of products due to degradation processes in liquid refills [10].

Although vapors of e-cigarettes seem to be less toxic than tobacco smoke, relatively little is known about the content and toxicity of these vapors and of the liquids used to produce these vapors [2]. Thus, the objectives of this study were to assess levels of chemical and biological constituents, including micro-organisms, diethylene glycol, ethylene glycol, hydrocarbons, ethanol, aldehydes, tobacco-specific nitrosamines, and solvents, in a large set of commercial e-liquids purchased on the Internet (Table 1).

2. Material and Methods

Previous research enabled us to identify the most popular brands of e-liquids used in several countries (USA, UK, France, Switzerland) [8,11,12]. We selected the brands that dominate the market in the USA and much of Western Europe, and we selected several other brands for convenience (e.g., from websites that sent products to Switzerland). We analyzed 42 bottles of 14 different brands purchased on the Internet in 2013 and received by mail. Upon receipt in Geneva, the bottles were kept at room temperature and protected from the light until they were sent for analysis to Helvic Laboratories (Stoke-on-Trent, UK) for the microbiological tests and to Hall Analytical Laboratories (Manchester, UK) for the chemical analyses. Analyses of ethylene glycol, conducted at a later time point by Hall Analytical Laboratories, were conducted on 32 bottles of the same 14 brands (Table 2). The liquids were kept at room temperature by these laboratories from the reception of the products to the analyses, which were performed in 2013–2014.

Table 1. Characteristics of 42 bottles of liquids for e-cigarettes, 2013.

| Brand | Model | Code | PG or VG | Bottle Capacity (mL) | Nicotine Label (mg/mL) | Batch Number “Exp” or “Use by” Date | Ordered on Website | Country Sent from |
|-------------|-----------------------------------|--------|----------|----------------------|------------------------|--|--------------------|-------------------|
| Janty | Classic Sahara, High “GSH H” | H60339 | PG | 10 | 16 | Exp 10 December 2014 | Eu.jantyworld.com | France |
| Janty | Vitaya eLiquid Texas, high | H60340 | PG | 10 | 16 | Pro: 22 November 2012 Exp: 21 November 2013 | Eu.jantyworld.com | France |
| Janty | Elixir PG eLiquid Havana, high | H60341 | PG | 15 | 16 | Pro: January 2013 Exp: January 2015 | Eu.jantyworld.com | France |
| Janty | Elixir VG eLiquid Tobacco | H60342 | VG | 15 | 16 | Pro: November 2012 Exp: November 2014. | Eu.jantyworld.com | France |
| Janty | Elixir VG Spearmint | H60343 | VG | 15 | 16 | Pro: November 2012 Exp: November 2014. | Eu.jantyworld.com | France |
| Janty | Elixir VG Texas | H60344 | VG | 15 | 16 | Pro: November 2012 Exp: November 2014 | Eu.jantyworld.com | France |
| Janty | Elixir VG Golden Sahara | H60345 | VG | 15 | 16 | Pro: May 2012. Exp: May 2014 | Eu.jantyworld.com | France |
| Ecigexpress | Minty Menthol | H60346 | PG | 10 | 18 | N/A | Ecigexpress.com | USA |
| Ecigexpress | Full Flavor Tobacco VG edit | H60347 | VG | 10 | 18 | N/A | Ecigexpress.com | USA |
| Ecigexpress | e-Juice Red USA Mix | H60348 | PG | 10 | 24 | N/A | Ecigexpress.com | USA |
| Vapor4Life | Gunslinger Nobacco Juice USA | H60349 | PG + VG | 30 | 18 | V0021001840. Best used by 31 December 2012 | Vapor4life.com | USA |
| Vapor4Life | VG 555 WOW Vapor Juice | H60350 | VG | 30 | 36 | PRD: 30 December 2012. Exp: 30. December 2014. | Vapor4life.com | USA |
| Vapor4Life | Wowboy Peppermint Wow Vapor Juice | H60351 | N/A | 30 | 18 | PRD: 30. December 2012. Exp: 30. December 2014. | Vapor4life.com | USA |

Table 1. Cont.

| Brand | Model | Code | PG or VG | Bottle Capacity (mL) | Nicotine Label (mg/mL) | Batch Number “Exp” or “Use by” Date | Ordered on Website | Country Sent from |
|------------------|------------------------------------|--------|----------|----------------------|------------------------|-------------------------------------|------------------------------|-------------------|
| Totally Wicked | American red tobacco | H60352 | PG + VG | 10 | 6 | Lot # TW1215 | totallywicked-liquid.co.uk | UK |
| Totally Wicked | Titanium Ice | H60353 | VG | 50 | 72 | Batch No. NGLY034 | totallywicked-liquid.co.uk | UK |
| Totally Wicked | Platinum Ice | H60354 | VG | 50 | 54 | Batch No. NGLY0034 | totallywicked-liquid.co.uk | UK |
| Totally Wicked | Glycerine (to dilute «Ice» models) | | VG | | | | totallywicked-liquid.co.uk | UK |
| Sedansa | American Blend Premium | H60355 | PEG 400 | 10 | 0 | N/A | Sedansa.be | Belgium |
| Sedansa | Mint Premium | H60356 | VG | 10 | 0 | N/A | Sedansa.be | Belgium |
| Sedansa | Turkish Tobacco Premium | H60357 | PG + VG | 10 | 0 | N/A | Sedansa.be | Belgium |
| Sedansa | 7 Star | H60358 | N/A | 10 | 0 | N/A | Sedansa.be | Belgium |
| Sedansa | RY4 | H60359 | N/A | 10 | 0 | N/A | Sedansa.be | Belgium |
| Johnson Creek | Domestic | H60360 | PG + VG | 15 | 18 | Lot B1221811 | johnsoncreeksmokejuice.com | USA |
| Johnson Creek | JC Original | H60361 | PG + VG | 15 | 18 | Lot B1131801 | johnsoncreeksmokejuice.com | USA |
| Johnson Creek | Espresso | H60362 | PG + VG | 15 | 18 | Lot B0221807 | johnsoncreeksmokejuice.com | USA |
| Johnson Creek | Read Oak Tennessee Cured | H60363 | VG | 15 | 18 | Lot B1231817 | johnsoncreeksmokejuice.com | USA |
| TECC Titan fluid | American red high | H60364 | PG | 10 | 18 | April 2013–October 2014 | Theelectroniccigarette.co.uk | UK |
| TECC Titan | Apple, High Blended | H60365 | N/A | 10 | 18 | March 2013–September 2014 | Theelectroniccigarette.co.uk | UK |
| TECC Titan | Virginia high | H60366 | N/A | 10 | 18 | April 2013–October 2014 | Theelectroniccigarette.co.uk | UK |
| TECC Titan | Cappucino high | H60367 | N/A | 10 | 18 | March 2012–September 2013 | Theelectroniccigarette.co.uk | UK |
| TECC Titan | American red Super High | H60368 | N/A | 10 | 36 | April 2013–October 2014 | Theelectroniccigarette.co.uk | UK |

Table 1. Cont.

| Brand | Model | Code | PG or VG | Bottle Capacity (mL) | Nicotine Label (mg/mL) | Batch Number “Exp” or “Use by” Date | Ordered on Website | Country Sent from |
|-----------------|--------------------------------------|--------|----------|----------------------|------------------------|---------------------------------------|--------------------|-------------------|
| Intellicig | ECOpure E-Liquid | H60369 | VG | 10 | Rich 45 | Lot: ZBAX Use by April 2015 | Intellicig.com | UK |
| e-cigarettes.fr | Kyozen. E-liquide MLB high | H60370 | PG + VG | 10 | 18 | N/A | e-cigarettes-fr | France |
| e-cigarettes.fr | Kyozen. E-liquide Sahara high | H60371 | PG + VG | 10 | 18 | N/A | e-cigarettes-fr | France |
| e-cigarettes.fr | Kyozen. E-liquide Ruyan n° 4 high | H60372 | PG + VG | 10 | 18 | N/A | e-cigarettes-fr | France |
| CigLib | Tobacco, fort 16 mg/mL | H60373 | N/A | 10 | 16 | February 2012– August 2013 | Ciglib.fr | France |
| V2 Cigs | V2 Platinum | H60374 | PG + VG | 25 | 18 | Batch YSBA Best before 30 May 2015 | V2cigs.com | USA |
| e-liquide.com | Liqua Mints | H60375 | PG + VG | 10 | 18 | Batch B133 Exp: December 2014. | e-liquide.com | France |
| Tasty Vapor | Amaretto Stone Sour | H60376 | 30% VG | 29.6 mL | 18 | Born on 6 June 2013 | Tastyvapor.us | USA |
| Tasty Vapor | Black Licorice | H60377 | 30% VG | 29.6 mL | 18 | Born on 6 June 2013 | Tastyvapor.us | USA |
| Tasty Vapor | Apple Pie Candy | H60378 | 30% VG | 29.6 mL | 18 | Born on 6 June 2013 | Tastyvapor.us | USA |
| e-cig.com | “LIQ” e-liquid base VG | H60379 | VG | 50 | 48 | N/a | e-cig.com | China |
| e-cig.com | Pure nicotine “LIQ” | H60380 | VG | 10 | 18 | N/a | e-cig.com | China |

PG: propylene glycol; VG: vegetable glycerine; PEG: Polyethylene glycol; PRO/PRD: date produced; EXP: date expires; N/A: not available.

Table 2. Content of 42 bottles of liquids for e-cigarettes, 2013.

| Brand | Model | Code | Diethylene Glycol (µg/g) | Ethylene Glycol (µg/g) | Hydrocarbons, (µg/g) | Ethanol (µg/g) |
|------------------|--------------------------------|----------|--------------------------|------------------------|----------------------|----------------|
| Janty | Classic Sahara, High “GSH H” | H60339 | <0.5 | 9.27 | <25 | -- |
| Janty | Vitaya eLiquid Texas, high | H60340 | <0.5 | 6.19 | <25 | 399 |
| Janty | Elixir PG eLiquid Havana, high | H60341 | <0.5 | 4.29 | <25 | 60 |
| Janty | Elixir VG eLiquid Tobacco | H60342 | <0.5 | 6.59 | <25 | 241 |
| Janty | Elixir VG Spearmint | H60343 | <0.5 | 7.02 | 790 (Limonene) | 118 |
| Janty | Elixir VG Texas | H60344 | <0.5 | 7.57 | <25 | 445 |
| Janty | Elixir VG Golden Sahara | H60345 | 1.6 | 6.70 | <25 | 1157 |
| Ecigexpress | Minty Menthol | H60346 | <0.5 | -- | 1829 (Limonene) | 2455 |
| Ecigexpress | Full Flavor Tobacco VG edition | H60347 | 0.6 | 2.91 | <25 | -- |
| Ecigexpress | e-Juice Red USA Mix | H60348 | <0.5 | -- | <25 | 1931 |
| Vapor4Life | Gunslinger Nobacco Juice USA | H60349 | <0.5 | -- | <25 | 1196 |
| Vapor4Life | VG 555 WOW Vapor Juice | H60350 | <0.5 | -- | <25 | - |
| Vapor4Life | Wowboy Peppermint Wow Juice | H60351 | <0.5 | 4.12 | <25 | - |
| Totally Wicked | American red tobacco | H60352 | <0.5 | 6.49 | <25 | 548 |
| Totally Wicked | Titanium Ice, nicotine base | H60353 | <0.5 | 3.60 | <25 | - |
| Totally Wicked | Platinum Ice, nicotine base | H60354 | <0.5 | 2.90 | <25 | - |
| Sedansa | American Blend Premium | H60355 | <0.5 | 4.80 | <25 | 390 |
| Sedansa | Mint Premium | H60356 | <0.5 | 5.72 | <25 | 31 |
| Sedansa | Turkish Tobacco Premium | H60357 | <0.5 | 3.68 | <25 | 375 |
| Sedansa | 7 Star | H60358 | <0.5 | -- | <25 | - |
| Sedansa | RY4 | H60359 | <0.5 | -- | <25 | - |
| Johnson Creek | Domestic | H60360 | 4.0 | 9.43 | <25 | 1211 |
| Johnson Creek | JC Original | H60361 | 0.6 | 20.28 | <25 | 359 |
| Johnson Creek | Espresso | H60362 | <0.5 | -- | <25 | 212 |
| Johnson Creek | Read Oak Tennessee Cured | H60363 | 3.5 | 17.71 | <25 | 1840 |
| TECC Titan fluid | American red high | H60364 | <0.5 | 4.17 | <25 | - |
| TECC Titan fluid | Apple, High Blended | H60365 | <0.5 | 4.02 | <25 | 336 |
| TECC Titan fluid | Virginia high | H60366 | 0.8 | -- | <25 | 2915 |
| TECC Titan fluid | Cappucino high | H60367 | <0.5 | 4.75 | <25 | 6 |
| TECC Titan fluid | American red Super High | H60368 | <0.5 | -- | <25 | - |
| Intellicig | ECOpure E-Liquid | H60369 | 1.0 | 7.48 | <25 | 3453 |
| e-cigarettes.fr | E-liquide MLB high | H60370 | <0.5 | 5.79 | <25 | 1695 |
| e-cigarettes.fr | E-liquide Sahara high | H60371 | <0.5 | 3.78 | <25 | 2074 |
| e-cigarettes.fr | E-liquide Ruyan n° 4 high | H60372 | <0.5v | -- | <25 | 2694 |
| CigLib | Tobacco, fort 16 mg/mL | H60373 | <0.5 | 6.03 | <25 | - |
| V2 Cigs | V2 Platinum | H60374 | <0.5 | 5.13 | <25 | 1335 |
| e-liquide.com | Liqua Mints | H60375 | <0.5 | 4.48 | 779 (Limonene) | - |
| Tasty Vapor | Amaretto Stone Sour | H60376 * | <0.5 | 4.40 | 106,479 | 404 |

Table 2. Cont.

| Brand | Model | Code | Diethylene Glycol (µg/g) | Ethylene Glycol (µg/g) | Hydrocarbons, (µg/g) | Ethanol (µg/g) |
|-------------|------------------------|-----------|--------------------------|------------------------|----------------------|----------------|
| Tasty Vapor | Black Licorice | H60377 ** | <0.5 | 4.20 | 2082 | 3675 |
| Tasty Vapor | Apple Pie, Candy | H60378 | <0.5 | -- | <25 | 77 |
| e-cig.com | “LIQ” e-liquid base VG | H60379 | 2.2 | 66.97 | <25 | 2250 |
| e-cig.com | Pure nicotine “LIQ” | H60380 | 0.8 | 6.12 | <25 | 3623 |

* Sample H60376: alpha-Pinene (isomer) (4790 µg/g); beta-pinene (isomer) (27,137 µg/g); limonene (50,936 µg/g); 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl) [gamma terpinene] (11,438 µg/g); benzene, 1-methyl-2-(1-methylethyl)-[para-cymene] (5498 µg/g); cyclohexane, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-(6950 µg/g). ** Sample H60377: alpha-pinene (641 µg/g) and limonene (1441 µg/g).

2.1. Microbiological Tests

We tested the e-liquids for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* according to methodology described in the European Pharmacopoeia Section 2.6.13, and proceeded to microbial enumeration for total aerobic microbial count (TAMC) and total yeast and mold count (TYMC) according to the methodology described in the European Pharmacopoeia Section 2.6.12 [13]. These tests are required by the European Pharmacopoeia for oromucosal products. For inhalation use and for aqueous preparations intended for oral use, TAMC should be ≤ 100 colony forming unit (CFU) per mL and TYMC should be ≤ 10 CFU/mL [13]. The liquids were diluted at 1:100 for the TAMC and TYMC tests, but we report results for the undiluted concentrations. For microbiological tests only, two batches of each liquid (purchased at different dates and with different batch numbers) were analyzed.

2.2. Chemical Tests

For each e-liquid tested, diethylene glycol and hydrocarbons analyses were performed after methanolic dilution via gas chromatography-mass spectrometry (GC-MS), and ethylene glycol analyses were performed via chemical ionisation GC-MS (selected ion monitoring). Solvents and ethanol analyses were done through headspace GC-MS, and tobacco-specific nitrosamines analyses (TSNA) through liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). For aldehydes monitoring, a known sample weight of each sample was placed directly onto a LpDNPH tube and eluted with 5 mL of acetonitrile, then analysed by LC coupled with ultra-violet detection and MS (LC-UV/MS). The reference solutions, used for identification and quantification of the substances, contained known levels of each substance under scrutiny.

2.3. Toxicity Assessment

We determined whether the concentrations of each of the molecules detected in the liquids were within a normal range for food or pharmaceutical products, based on the ICH guidelines for new drug products, the European Pharmacopoeia for active ingredients, and other relevant literature [13,14].

We also assessed the conformity of the e-liquids by comparing the observed concentrations to the acceptable limits defined in the strictest food residue regulations available [15], and to the standards for good manufacturing practices (GMP) used in the flavor and fragrance industry [16].

To assess the potential toxicity of the e-liquids, we compared the concentrations measured to parameters available for human exposure in the environment (air, water) or in food: Estimated Human Exposure (EHE), Acceptable Daily Intake (ADI), Maximized Survey-Derived Intake (MSDI), and Tolerable Daily Intake (TDI). For the conformity assessment of toxicity, we selected the lowest values of acceptance available (in the EU, US or in various national regulations such as Germany, Japan and France) to investigate the toxicity of e-liquids. Two separate assessments were performed: (a) potential acute oral toxicity was assessed following a hypothetical scenario of ingestion of 10 grams of liquid; (b) potential chronic toxicity associated with an assumed average daily consumption of 3 grams of e-liquid. The daily consumption of 3 grams was based on evidence from surveys of dedicated e-cigarette users [17].

3. Results

3.1. Microbiological Analyses

All the liquids under scrutiny complied with European Pharmacopoeia norms for the absence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Four samples had total aerobic microbial count = 1 CFU/mL (H60348, H60355, H60357 and H60379). All the other investigated samples had total aerobic microbial count <1 CFU/mL. All the samples except one had total yeast and mold counts <1 CFU/mL. A glycerin bottle for mixing the liquids, purchased from *Totally Wicked*, had total yeast and mold count = 1 CFU/mL.

3.2. Diethylene Glycol

All the samples analyzed had concentrations of diethylene glycol below 4 µg/g (the limit of detection (LOD) was 0.5 µg/g).

3.3. Ethylene Glycol

With the exception of 3 samples (H60361: 20.3 µg/g, H60363: 17.7 µg/g, and H60379: 67 µg/g), all samples contained less than 10 µg/g ethylene glycol.

3.4. Hydrocarbons

The concentrations of hydrocarbons were below the LOD of 25 µg/g for all except five samples (H60344: 790 µg/g, H60346: 1830 µg/g, H60375: 780 µg/g, H60376: > 100,000 µg/g and H60377: 2080 µg/g). Most of these hydrocarbons were terpenic compounds, which were probably used as flavoring agents. Limonene was identified in all these cases as the main component, followed by pinene isomers and gamma-terpinene (Table 2).

3.5. Ethanol

All the samples had concentrations of ethanol below 3.7 mg/g.

3.6. Aldehydes

Formaldehyde concentrations ranged from 0.1 to 9.0 $\mu\text{g/g}$ and acetaldehyde concentrations from 0.05 to 10.2 $\mu\text{g/g}$ (Table 3). Acrolein content was below the limit of detection (LOD: 0.111 $\mu\text{g/g}$) in all except three products (H60360: 0.18 $\mu\text{g/g}$, and H60363: 0.21 $\mu\text{g/g}$ and H60380: 1.03 $\mu\text{g/g}$). Propionaldehyde was below the LOD (0.043 $\mu\text{g/g}$) in all except seventeen products (Table 3). Butyraldehyde was below the LOD (0.077 $\mu\text{g/g}$) in all except eight products. Valeraldehyde (LOD: 0.281 $\mu\text{g/g}$) and 2,5-dimethylbenzaldehyde (LOD: 0.027 $\mu\text{g/g}$) were below their respective LOD in all products. Crotonaldehyde was below the LOD (0.053 $\mu\text{g/g}$) in all except two products (H60363: 0.067 $\mu\text{g/g}$, and H60377: 0.084 $\mu\text{g/g}$). For benzaldehyde, twelve products were below the LOD (0.035 $\mu\text{g/g}$) (Table 3). Isovaleraldehyde levels were below the LOD (0.194 $\mu\text{g/g}$) in all except four samples: H60349 (1.54 $\mu\text{g/g}$), H60351 (1.09 $\mu\text{g/g}$), H60360 (3.14 $\mu\text{g/g}$), and H60379 (1.43 $\mu\text{g/g}$). *O*-tolualdehyde levels were under the LOD (0.017 $\mu\text{g/g}$) except in one product (H60378: 0.043 $\mu\text{g/g}$), and *m*- and *p*-tolualdehyde levels, measured as the sum of these two isomers, were below the LOD (0.018 $\mu\text{g/g}$) except for H60377 (0.069 $\mu\text{g/g}$). Twelve liquids had hexaldehyde concentrations above the LOD (0.036 $\mu\text{g/g}$) (Table 3).

3.7. Tobacco-Specific Nitrosamines

All the samples had nitrosamines concentrations below the LOD (1 $\mu\text{g/g}$).

3.8. Solvents

1,3-butadiene was detected only in H60348 (10 $\mu\text{g/g}$). The chromatographic resolution was not sufficiently efficient to separate acetaldehyde and ethylene dioxide, but a peak corresponding to these compounds was noticeable in two samples (H60360: 9 $\mu\text{g/g}$, and H60363: 13 $\mu\text{g/g}$). Acetone was found in H60348 (20 $\mu\text{g/g}$) and in H60365 (9 $\mu\text{g/g}$). The following compounds were found in the following samples only: 1-propanol in H60379 (16 $\mu\text{g/g}$), 3-hydroxy-2-butanone in H60363 (16 $\mu\text{g/g}$), 2-methylpropyl acetate (26 $\mu\text{g/g}$) and methyl, 2-methyl butyrate (12 $\mu\text{g/g}$) in H60378. 2,3-butanedione was found in three liquids (H60360: 9 $\mu\text{g/g}$, H60363 : 43 $\mu\text{g/g}$, and H60378: 12 $\mu\text{g/g}$). Cyclohexane was detected in two liquids: H60351 (11 $\mu\text{g/g}$) and in H60367 (6 $\mu\text{g/g}$). 3-methylbutanal was detected in two products (H60360: 14 $\mu\text{g/g}$, and H60363: 6 $\mu\text{g/g}$). 2-methyl-1,3-dioxane and isomers were found in two products (H60378: 41 $\mu\text{g/g}$, and H60377: 57 $\mu\text{g/g}$). 1-butanol was detected in two products (H60360: 10 $\mu\text{g/g}$, and H60363: 6 $\mu\text{g/g}$), whereas ethyl propanoate was detected in three products: H60361 (6 $\mu\text{g/g}$), in H60378 (123 $\mu\text{g/g}$), and in H60379 (88 $\mu\text{g/g}$). 1,1-diethoxyethane was found in H60378 (40 $\mu\text{g/g}$), H60351 (11 $\mu\text{g/g}$) and H60380 (23 $\mu\text{g/g}$). Finally, ethyl acetate was the most important residual solvent present in several samples, in concentrations lower than 100 $\mu\text{g/g}$ except for H60378 (253 $\mu\text{g/g}$) (Table 4).

Table 3. Aldehydes (in µg/g) in 42 bottles of e-liquids, 2013.

| Brand | Code | Formaldehyde | Acetaldehyde | Propionaldehyde | Crotonaldehyde | Butyraldehyde | Benzaldehyde | Hexaldehyde |
|----------------|--------|--------------|--------------|-----------------|----------------|---------------|--------------|-------------|
| LOD | | 0.060 | 0.030 | 0.043 | 0.053 | 0.077 | 0.035 | 0.036 |
| Janty | H60339 | 0.497 | 0.728 | 0.043 | <0.053 | <0.077 | <0.035 | <0.036 |
| Janty | H60340 | 0.450 | 0.545 | <0.043 | <0.053 | 0.101 | 0.036 | <0.036 |
| Janty | H60341 | 0.389 | 0.591 | <0.043 | <0.053 | <0.077 | <0.035 | <0.036 |
| Janty | H60342 | 0.244 | 0.425 | <0.043 | <0.053 | <0.077 | <0.035 | <0.036 |
| Janty | H60343 | 0.884 | 0.310 | <0.043 | <0.053 | <0.077 | 0.330 | <0.036 |
| Janty | H60344 | 0.617 | 0.132 | <0.043 | <0.053 | <0.077 | <0.035 | <0.036 |
| Janty | H60345 | 0.138 | 2.03 | <0.043 | <0.053 | <0.077 | <0.035 | <0.036 |
| Ecigexpress | H60346 | 0.161 | 1.74 | <0.043 | <0.053 | 0.186 | 0.160 | <0.036 |
| Ecigexpress | H60347 | 2.51 | 0.498 | 0.066 | <0.053 | 0.178 | <0.035 | <0.036 |
| Ecigexpress | H60348 | 0.303 | 0.539 | <0.043 | <0.053 | <0.077 | <0.035 | <0.036 |
| Vapor4Life | H60349 | 0.776 | 0.507 | 0.089 | <0.053 | 0.217 | 40.0 | <0.036 |
| Vapor4Life | H60350 | 0.522 | 0.737 | <0.043 | <0.053 | <0.077 | <0.035 | <0.036 |
| Vapor4Life | H60351 | 0.269 | 1.49 | <0.043 | <0.053 | <0.077 | 0.072 | <0.036 |
| Totally Wicked | H60352 | 0.532 | 0.129 | <0.043 | <0.053 | <0.077 | 0.821 | <0.036 |
| Totally Wicked | H60353 | 1.13 | 0.040 | <0.043 | <0.053 | <0.077 | <0.035 | <0.036 |
| Totally Wicked | H60354 | 1.25 | 0.055 | <0.043 | <0.053 | <0.077 | <0.035 | <0.036 |
| Sedansa | H60355 | 0.813 | 1.25 | 0.167 | <0.053 | 0.164 | <0.035 | <0.036 |
| Sedansa | H60356 | 0.409 | 0.086 | <0.043 | <0.053 | <0.077 | 0.422 | <0.036 |
| Sedansa | H60357 | 0.590 | 0.896 | 0.152 | <0.053 | 0.172 | 0.247 | <0.036 |
| Sedansa | H60358 | 0.865 | 0.923 | 0.074 | <0.053 | <0.077 | 0.133 | <0.036 |
| Sedansa | H60359 | 0.681 | 0.944 | 0.067 | <0.053 | <0.077 | 0.063 | <0.036 |
| Johnson Creek | H60360 | 0.356 | 2.58 | 0.122 | <0.053 | <0.077 | 0.291 | <0.036 |
| Johnson Creek | H60361 | 2.92 | 3.08 | 0.231 | <0.053 | <0.077 | 0.245 | <0.036 |
| Johnson Creek | H60362 | 1.97 | 3.21 | 0.166 | <0.053 | <0.077 | 0.116 | <0.036 |
| Johnson Creek | H60363 | 0.651 | 2.35 | 0.261 | 0.067 | <0.077 | 0.175 | <0.036 |
| TECC | H60364 | 0.467 | 0.235 | <0.043 | <0.053 | <0.077 | 0.078 | <0.036 |

Table 3. Cont.

| Brand | Code | Formaldehyde | Acetaldehyde | Propionaldehyde | Crotonaldehyde | Butyraldehyde | Benzaldehyde | Hexaldehyde |
|-----------------|--------|--------------|--------------|-----------------|----------------|---------------|--------------|-------------|
| TECC | H60365 | 1.04 | 0.209 | 0.189 | <0.053 | 0.478 | 0.146 | 0.096 |
| TECC | H60366 | 0.297 | 0.299 | <0.043 | <0.053 | <0.077 | 0.145 | 0.076 |
| TECC | H60367 | 0.547 | 0.559 | <0.043 | <0.053 | 1.03 | 0.582 | 0.046 |
| TECC | H60368 | 0.776 | 0.389 | <0.043 | <0.053 | <0.077 | <0.035 | <0.036 |
| Intellicig | H60369 | 0.114 | 4.05 | 0.083 | <0.053 | <0.077 | 0.581 | <0.036 |
| e-cigarettes.fr | H60370 | 0.257 | 0.413 | <0.043 | <0.053 | <0.077 | 0.104 | 0.068 |
| e-cigarettes.fr | H60371 | 0.565 | 0.803 | 0.049 | <0.053 | <0.077 | 0.039 | 0.105 |
| e-cigarettes.fr | H60372 | 0.205 | 0.381 | <0.043 | <0.053 | <0.077 | 0.060 | <0.036 |
| CigLib | H60373 | 0.274 | 0.421 | <0.043 | <0.053 | <0.077 | 0.035 | 0.089 |
| V2 Cigs | H60374 | 0.411 | 0.332 | 0.045 | <0.053 | <0.077 | 0.146 | 0.115 |
| e-liquide.com | H60375 | 9.00 | 3.14 | <0.043 | <0.053 | <0.077 | 0.145 | 0.100 |
| Tasty Vapor | H60376 | 3.52 | 2.37 | <0.043 | <0.053 | <0.077 | 305 | 0.532 |
| Tasty Vapor | H60377 | 0.441 | 10.2 | 0.063 | 0.084 | <0.077 | 3.70 | 0.192 |
| Tasty Vapor | H60378 | 1.88 | 1.44 | <0.043 | <0.053 | <0.077 | 9.61 | <0.036 |
| e-cig.com | H60379 | 0.226 | 0.393 | 0.047 | <0.053 | <0.077 | 0.062 | 0.132 |
| e-cig.com | H60380 | 1.95 | 2.03 | <0.043 | <0.053 | <0.077 | 0.068 | 0.081 |

Table 4. Solvents (in µg/g) in bottles of e-liquids, 2013 *.

| Code | 1,3- Butadiene | Acetaldehyde/ Ethylene Oxide | Acetone | 1- Propanol | 2,3- Butanedione | Ethyl Acetate | Cyclohexane | 3- Methyl Butanal | 2-Methyl- 1,3- Dioxane | 1- Butanol | Propanoic Acid, Ethyl Ester | 1,1- Diethoxy Ethane | 3-Hydroxy- 2-Butanone | Acetic Acid, 2-Methylpropyl Ester | Butanoic Acid, 2-Methyl-, Methyl Ester |
|--------|-------------------|------------------------------------|---------|----------------|---------------------|------------------|-------------|-------------------------|------------------------------|---------------|-----------------------------------|----------------------------|--------------------------|---|--|
| H60343 | | | | | | 72 | | | | | | | | | |
| H60348 | 10 | | 20 | | | | | | | | | | | | |
| H60349 | | | | | | 12 | | | | | | | | | |
| H60351 | | | | | | | 11 | | | | | 11 | | | |
| H60360 | | 9 | | | 9 | | | 14 | | 10 | | | | | |
| H60361 | | | | | | 8 | | | | | 6 | | | | |
| H60363 | | 13 | | | 43 | 11 | | 6 | | | | | 16 | | |
| H60365 | | | 9 | | | | | | | | | | | | |
| H60367 | | | | | | | 6 | | | | | | | | |
| H60369 | | | | | | 29 | | | | | | | | | |
| H60370 | | | | | | 76 | | | | | | | | | |
| H60372 | | | | | | 54 | | | | | | | | | |
| H60376 | | | | | | 39 | | | | | | | | | |
| H60377 | | | | | | | | | 41 | | | 40 | | | |
| H60378 | | | | | 12 | 253 | | | 57 | | 123 | | | 26 | 12 |
| H60379 | | | | 16 | | 11 | | | | | 88 | | | | |
| H60380 | | | | | | | | | | | | 23 | | | |

* All 42 samples were tested, but samples with no detectable levels of solvents are not shown.

4. Discussion

In the absence of therapeutic intention, e-liquids cannot be considered medications, nor are they considered food products in any country. Rather, they are classified either as tobacco products or as consumer products in countries that have a specific regulation [18]. However, it is important to determine the conformity of these products to the maximum concentrations authorized in relevant categories of products: food, pharmaceuticals, flavors and fragrances.

All the products complied with norms for the absence of micro-organisms. Ethylene glycol and diethylene glycol are not authorized as ingredients in food and pharmaceutical products, but maximum residual limits are allowed, as these substances can be found as contaminants in numerous products. None of the liquids showed a concentration of ethylene glycol and diethylene glycol above these limits (1 mg/g according to FDA and 620 µg/g according to the US Pharmacopeial Convention in 2007) [19,20].

Ethanol (beverage alcohol) is a very common compound found in food and other consumer products. The maximum amount found in the tested liquids was 0.4%, which is authorized if mentioned on the label.

High amounts of hydrocarbons were found in several products from *Tasty Vapor*, in particular alpha-pinene in H60376 (4.8 mg/g) and in H60377 (640 µg/g), at levels higher than the limit of 160 µg/g recommended in finished products. Beta-pinene in H60376 (27 mg/g) was also above the 100 µg/g limit recommended for finished products. Gamma-terpinene in H60376 (11 mg/g) exceeded the 40 µg/g limit recommended for finished products, and benzene 1-methyl-4-(1-methylethyl) (para-cymene) in H60376 (5.5 mg/g) was also higher than the 250 µg/g limit recommended for finished products. These compounds were probably present in the flavors added to these liquids by manufacturing processes, perhaps in an attempt to make the flavoring more intense.

Formaldehyde was detected in all the 42 samples. Formaldehyde concentrations between 0.02 and 10.09 mg/L and acetaldehyde concentrations between 0.10 and 15.63 mg/L have already been reported [21]. Formaldehyde is prohibited in food, and it was probably not added on purpose in the e-liquids, but could be a contaminant present in the ingredients, due to the low quality of raw materials. Of note, formaldehyde also occurs naturally in many food products and in beverages, thus the source might be some natural extracts used as flavorings.

Acrolein and crotonaldehyde should be avoided because they are listed as toxic contaminants in most international legislations (food, environment). For other aldehydes (propionaldehyde, butyraldehyde, benzaldehyde, isovaleraldehyde and hexaldehyde), all of which are approved for use as food flavorings, no sample contained levels higher than those recommended for finished products. Although e-liquids are not considered food products (even if they are consumed as oral mists), compounds such as acetone in samples H60348 and H60365, cyclohexane in samples H60351 and H60367, 1-propanol in H60379, and 1-butanol in H60360 and H60363 were found in quantities higher than their authorized maximum limits as residue in food, as required in 1992 already (5 µg/g for acetone, 1 µg/g for cyclohexane, 5 µg/g for 1-propanol and 1 µg/g for 1-butanol) [15]. Again, these substances may result from the contamination of raw materials, possibly through inadequate purification. The same applies to the two products that contained ethylene oxide (H60360 and H60363). Nitrosamines, 1,3-butadiene and 2-methyl-1,3-dioxane are not cited in most regulations of consumer products or medications, but the carcinogenicity of these compounds is well established [22], and they should not be present in e-liquids

at any concentration [23]. We did not detect nitrosamines in any of the 42 e-liquids under scrutiny, but our limit of detection was high (1 µg/g). Our results are in agreement with other studies showing that e-cigarette liquids contain nitrosamines in concentrations lower than the µg/mL range found in tobacco products [24,25]. The origin of 1,3-butadiene and 2-methyl-1,3-dioxane is unclear, but may result from the contamination of ingredients (possibly propylene glycol or glycerine). The amount of acetone is often recommended below 8 µg/g in the finished product and the quantities measured in samples H60365 (9 µg/g) and H60348 (20 µg/g) were above this value.

4.1. Acute Oral Toxicity

Although e-liquids are intended to be vaporized and inhaled, the risks associated with ingestion should also be considered. Liquids can be ingested either after deposition of the vapor droplets in the upper aero-digestive tract during normal vaping, or accidentally [26], or intentionally in suicide attempts [27]. Assuming an ingestion of 10 mL of e-liquid, the risk of acute toxicity for components other than nicotine was not significant, because all the estimated concentrations were largely below the known LD50 for various animals (mainly rodents and guinea pigs). Regarding components other than nicotine, the acute oral toxicity of the investigated liquids may not require regulation over and above existing legal requirements or industrial norms. However, it should be mentioned that the proposed scenario of exposure interprets the oral toxicity of detected compounds as ingested compounds that go through the first-pass metabolism, whereas inhaled compounds have direct access to the bloodstream without being metabolized first.

No information concerning the toxicity and maximum thresholds are available concerning cyclohexane 1-methyl-4-(5-methyl-1-methylene-4-hexenyl), 2-methyl-1,3-dioxane, 2-methyl-methylbutyrate, o-tolualdehyde and 2,5-dimethylbenzaldehyde. Therefore, their acute toxicity is not discussed here, but this does not mean that their concentrations found in the liquids are safe.

Thus, the extrapolation of our data to a hypothetical oral ingestion of 10 mL of liquid by an adult (60 kg) should not result in acute toxicity (for compounds other than nicotine), because all the concentrations were at least 480 times below the LD50 for all the compounds under scrutiny. Similarly, the same ingestion by a child (15 kg) should not result in acute toxicity, because all the concentrations were at least 120 times below the LD50 for all the compounds. However, synergistic effects may occur and the acute toxicity of a liquid does not necessarily result from the individual acute toxicity of each compound assessed separately.

4.2. Chronic Oral Toxicity Associated with Intended Use

To assess the chronic toxicity associated with intended use, it was assumed that the composition of liquids does not change after being heated and evaporated during e-cigarette use. However, because we did not analyze the vapor composition, our interpretation was only based on the compounds identified in the liquid refills. Moreover, we assumed that the concentration of chemicals in the aerosol was similar to the concentration in the e-liquid. These hypotheses are not necessarily verified. For instance, the levels many toxicant are dependent on battery voltage [28,29]. However, given the relative dearth of published data on the transformation of e-liquids into aerosols, our assumptions constitute a best-guess scenario that we used for this preliminary evaluation.

For aldehydes, studies have shown that formaldehyde, acetaldehyde, acetone and acrolein are additionally produced during the thermal decomposition of the basic ingredients in e-liquids (propylene glycol and glycerol) [28,30]. Therefore, we did not assess the potential chronic toxicity from oral exposure to these compounds, since this assessment would underestimate the true effects; vapor analysis would be more relevant in the case of aldehydes.

No information concerning the toxicity and maximum thresholds are available concerning cyclohexane 1-methyl-4-(5-methyl-1-methylene-4-hexenyl), 2-methyl-1,3-dioxane, and 2,5-dimethylbenzaldehyde. Therefore, their oral chronic toxicity is not discussed here, but this does not mean that their concentrations found in our sample of e-liquids are safe.

Assuming a chronic exposure of 3 g of e-liquid daily, five terpenic molecules have to be considered: limonene, alpha-pinene and beta-pinene, gamma-terpinene and benzene 1-methyl-4-(1-methylethyl). Terpenic molecules are commonly found in flavors, and they have a relatively low oral toxicity compared to nitrosamines for a same amount. However, the relatively high quantities of these molecules do not guarantee the related products are innocuous.

Limonene was found in five products, but following our chronic toxicity scenario (3 g/day consumption), only H60376 from *Tasty Vapor* (153 mg) was above the MSDI USA limit (13 mg/pers/day), by more than 10 times. [31,32]. The other products contained limonene in quantities below or in the range of MSDI USA. The level of alpha-pinene present in H60376 (14 mg for 3 g ingested) was 6 times above the MSDI-EU limit of 2.2 mg/pers/day cited by EFSA, calculated for a person of 60 kg [32]. Beta-pinene was detected in H60376 at levels corresponding to a daily intake more than 100 times above the MSDI USA limit of 760 µg/pers/day (MSDI EU of 1.3 mg/pers/day) [32], indicating a potential risk from oral chronic exposure. Gamma-terpinene was also found in H60376 at levels leading to a total daily intake more than 100 times above the MSDI USA level of 321 µg/pers/day [32]. The intake of benzene 1-methyl-4-(1-methylethyl) from H60376 represented almost 35 times the MSDI USA limit of 470 µg/pers/day [32]. Consequently, the sample H60376 (*Amaretto Stone Sour* from *Tasty Vapor*) revealed a potential for oral toxicity from chronic daily exposure. For 2-methyl, methylpropanoate, the MSDI-EU limit is 20 µg/pers/day, and this limit would be exceeded from daily exposure to 3 g of sample H60378 by *Tasty Vapor* (36 µg); however, that sample contained levels lower than the maximum recommended in finished product (200 µg/g). 2,3-butanedione (diacetyl) is a diketone associated with the development of respiratory disease; it was present in three samples, with one of them (H60363, *Red Oak Tennessee Cured* by *Johnson Creek*) containing higher than safety levels calculated based on NIOSH-recommended safety limits [33]. For 1,3 butadiene, the non-carcinogen Tolerable Daily Intake (TDI) values for inhalation is 0.57µg/kg/day [34], thus, sample H60348 by *Ecigexpress* would result in marginally safe daily oral exposure (30 µg/day compared to the acceptable level of 34 µg/day in a 60 kg person). No data on safety limits or ADI and TDI values were found for cyclohexane and 2-methyl-1,3-dioxane, while ethyl acetate is of low oral toxicity and all samples were within the recommended maximum values in finished products. For the rest of the hydrocarbons and solvents, the levels of daily exposure were lower than the MSDI values.

For aldehydes, daily exposure to benzaldehyde in all samples was much lower than the MSDI-EU value of 7900 µg/pers/day. The same applies to o-tolualdehyde (MSDI-EU: 1 µg/pers/day; MSDI-USA: 9100 µg/pers/day), m-tolualdehyde (MSDI-EU = 0.85 µg/pers/day) and p-tolualdehyde (MSDI-EU: 160 µg/pers/day; MSDI-USA = 9100 µg/pers/day). Although formaldehyde contents were below the

TDI of 150 $\mu\text{g}/\text{kg}$ body weight/day defined by WHO for drinking water [35], it was previously mentioned that formaldehyde is a product of thermal degradation and is thus formed during heating and evaporation of the liquid. The same applies for acetaldehyde (which is approved for use in food), and for acrolein and crotonaldehyde [36,37]. No data on MSDI, TDI and EHE exist for propionaldehyde, butyraldehyde, isovaleraldehyde and hexaldehyde. However, these are structural class I chemicals, and for this class, the human exposure threshold for concern is 1800 $\mu\text{g}/\text{person}/\text{day}$; none of the samples exceeded this level. For butyraldehyde, the ADI is defined at 0.1 $\text{mg}/\text{kg}/\text{day}$ [38], and none of the samples resulted in exposure to such levels. For hexaldehyde, the estimated limits for intake in USA and EU are 260 and 781 $\mu\text{g}/\text{pers}/\text{day}$ respectively [39]; again none of the samples would approach the estimated intake limits when consumed at 3 g/day .

It should be emphasized that the exposure will depend on several factors in addition to the liquids themselves, including the e-cigarette model (power, temperature, technical characteristics) and the behavior of the user (duration of use, volume and depth of inhalation, number of puffs). The emission of compounds related to thermal degradation (such as aldehydes) should also be taken into consideration. Therefore, this study represents a preliminary, exploratory approach based on the current knowledge. Clearly, the oral chronic toxicity and the cytotoxicity of e-liquids and e-vapors should be further investigated [40,41].

4.3. Strengths and Limitations of This Study

The strengths of our study included the analysis of a large number of some of the most popular brands of e-liquids, and the analysis of two batches of each model for the microbiological tests. One limitation is that some popular brands were not included, which makes our convenience sample of e-liquids not representative of the market in any country. We purchased only commercial liquids, even though home-mixed liquids and random recipes can be of major toxicological concern. Another limitation is that, for cost reasons, we tested only one batch per model for the chemical tests, and therefore could not assess inter-batch variability. Moreover, the data is limited due to a lack of reproduction for outlying data points.

This study was initiated in 2013 based on popular brand data that was collected earlier. With the rapidly changing marketplace, the products analyzed may not represent the brands that currently dominate the market in the USA and Europe.

Although our list of analyzed substances is longer than in most previous reports, analyses of other substances are necessary. These include flavors and fragrances, aroma transporters (propylene glycol, glyceryl mono-, di- and triacetate), food dyes, phthalates and plasticizers (that can migrate from the container during heating and vaporization), metal particles that can detach from imperfect soldering or from the resistance coils [42], allergens and other infectious agents. Moreover, oral toxicity was evaluated based on currently established norms; it is important that direct toxicological assessment is performed, by cytotoxicity experiments on relevant cell cultures, in animals and in clinical studies.

It should be also mentioned that the proposed scenario of exposure interprets the oral toxicity of detected compounds as ingested compounds subjected to metabolism, but inhaled compounds are not metabolized and are potentially more toxic than their metabolites. Therefore, different levels of safety based on route of administration should be considered in the interpretation of such data.

We analyzed refill liquids only, but future studies should analyze the vapors as well, because new substances may be created during the heating and vaporization processes. Tests for delivered dose uniformity and aerodynamic particle size distribution should also be performed, because these tests are mandatory for medications intended to be inhaled.

5. Conclusions

None of the products under scrutiny were totally exempt of potentially toxic compounds. As this new market has developed largely outside an appropriate regulatory framework, some manufacturers and vendors apparently lack the adequate know-how about safety.

Acknowledgments

The authors thank Hall Analytical Labs in Manchester and Helvic laboratories in Stoke-on-Trent, who performed the analytical work. The analyses were paid for by a grant from the Swiss Tobacco Prevention Fund (at the Swiss Federal Office of Public Health), grant 12.000189.

Author Contributions

Vincent Varlet: Wrote manuscript, performed the toxicological interpretations.

Konstantinos Farsalinos: Wrote manuscript, performed the toxicological interpretations.

Jean-François Etter: Wrote manuscript, initiated and designed the research, collected e-liquid samples.

Aurélien Thomas and Marc Augsburger: Participated in the elaboration and revision of the manuscript.

Conflicts of Interest

Jean-François Etter was reimbursed by a manufacturer of e-liquids for traveling to London and to China in 2013, but he received no honoraria for these meetings aimed at mutual information.

Some of the other studies performed by Konstantinos Farsalinos used unrestricted funds provided to the Onassis Cardiac Surgery Center by e-cigarette companies.

Vincent Varlet, Marc Augsburger, and Aurélien Thomas declare no conflict of interest.

References

1. Brown, J.; West, R.; Beard, E.; Michie, S.; Shahab, L.; McNeill, A. Prevalence and characteristics of e-cigarette users in Great Britain: Findings from a general population survey of smokers. *Addict. Behav.* **2014**, *39*, 1120–1125.
2. Hajek, P.; Etter, J.F.; Benowitz, N.; Eissenberg, T.; McRobbie, H. Electronic cigarettes: Review of use, content, safety, effects on smokers and potential for harm and benefits. *Addiction* **2014**, *109*, 1801–1810.
3. Etter, J.F.; Bullen, C.; Flouris, A.D.; Laugesen, M.; Eissenberg, T. Electronic nicotine delivery systems: A research agenda. *Tob. Control* **2011**, *20*, 243–248.
4. Regan, A.K.; Promoff, G.; Dube, S.R.; Arrazola, R. Electronic nicotine delivery systems: Adult use and awareness of the “e-cigarette” in the USA. *Tob. Control* **2011**, *22*, 19–23.

5. Pearson, J.L.; Richardson, A.; Niaura, R.S.; Vallone, D.M.; Abrams, D.B. e-Cigarette awareness, use, and harm perceptions in US adults. *Am. J. Public Health* **2012**, *102*, 1758–1766.
6. King, B.A.; Alam, S.; Promoff, G.; Arrazola, R.; Dube, S.R. Awareness and ever use of electronic cigarettes among U.S. adults, 2010–2011. *Nicotine Tob. Res.* **2013**, *15*, 1623–1627.
7. Adkison, S.E.; O'Connor, R.J.; Bansal-Travers, M.; Hyland, A.; Borland, R.; Yong, H.H.; Cummings, K.M.; McNeill, A.; Thrasher, J.F.; Hammond, D.; *et al.* Electronic nicotine delivery systems: International tobacco control four-country survey. *Am. J. Prev. Med.* **2013**, *44*, 207–215.
8. Etter, J.F.; Zather, E.; Svensson, S. Analysis of refill liquids for electronic cigarettes. *Addiction* **2013**, *108*, 1671–1679.
9. Vakkalanka, J.P.; Hardison, L.S., Jr.; Holstege, C.P. Epidemiological trends in electronic cigarette exposures reported to U.S. Poison Centers. *Clin. Toxicol. (Phila)* **2014**, *52*, 542–548.
10. Martinez, R.E.; Dhawan, S.; Sumner, W.; Williams, B.J. On-line chemical composition analysis of refillable electronic cigarette aerosol-measurement of nicotine and nicotine. *Nicotine Tob. Res.* **2014**, doi:10.1093/ntr/ntu334.
11. Etter, J.F. Electronic cigarettes: A survey of users. *BMC Public Health* **2010**, doi:10.1186/1471-2458-10-231.
12. Etter, J.F.; Bullen, C. Electronic cigarette: Users profile, utilization, satisfaction and perceived efficacy. *Addiction* **2011**, *106*, 2017–2028.
13. EDQM. *European Pharmacopeia 7.0*; European Directorate for the Quality of Medicines and Healthcare: Strasbourg, France, 2012.
14. ICH. ICH Topic Q 3 B (R2) Impurities in New Drug Products. In Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Step 4 Version, Geneva, Switzerland, 2006; pp. 1–12.
15. EU Commission. Report EUR 14482 EN Food Science and techniques. In *Reports of the Scientific Committee for Food*, Twenty-Ninth Series; EU Commission: Brussels, Belgium, 1992; pp.1–37.
16. Good Scents Company. The Good Scents Company Information Database. Available online: <http://www.thegoodscentcompany.com/#> (accessed on 27 November 2014).
17. Farsalinos, K.E.; Romagna, G.; Tsiapras, D.; Kyrzopoulos, S.; Voudris, V. Characteristics, perceived side effects and benefits of electronic cigarette use: A worldwide survey of more than 19,000 consumers. *Int. J. Environ. Res. Public Health* **2014**, *11*, 4356–4373.
18. WHO. Electronic Nicotine Delivery Systems, Including Electronic Cigarettes. Report by the Convention Secretariat. In Proceedings of the Conference of the Parties to the WHO Framework Convention on Tobacco Control, Geneva, Switzerland, 18 June 2012.
19. FDA. Guidance for industry. In *Testing of Glycerin for Diethylene Glycol*; US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER): Rockville MD, USA, 2007.
20. US Pharmacopeial Convention. (467) Residual solvents. In *United States Pharmacopeia 30*; United States Pharmacopeial Convention: Rockville, MD, USA, 2007.
21. Lim, H.; Shin, H. Measurement of aldehydes in replacement liquids of electronic cigarettes by headspace gas chromatography-mass spectrometry. *Korean Chem. Soc.* **2013**, 2691–2696.
22. IARC. N'-nitrosornicotine and 4-(methylnitrosamino)-1-(3pyridyl)-1-butanone. *Iarc Monogr. Eval. Carcinog. Risks Hum.* **2012**, *100*, 319–331.

23. Stepanov, I.; Jensen, J.; Hatsukami, D.; Hecht, S.S. Tobacco-specific nitrosamines in new tobacco products. *Nicotine Tob. Res.* **2006**, *8*, 309–313.
24. Kim, H.J.; Shin, H.S. Determination of tobacco-specific nitrosamines in replacement liquids of electronic cigarettes by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2013**, *1291*, 48–55.
25. Farsalinos, K.E.; Polosa, R. Safety evaluation and risk assessment of electronic cigarettes as tobacco cigarette substitutes: A systematic review. *Ther. Adv. Drug Saf.* **2014**, *5*, 67–86.
26. Schuster, B. AAPCC and Poison Centers Issue Warning about Electronic Cigarette Devices and Liquid Nicotine American Association of Poison Control Centers. Available online: <http://www.aapcc.org/press/29/> (accessed on 27 November 2014).
27. Christensen, L.B.; van't Veen, T.; Bang, J. XXXIII International Congress of the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) 28–31 May 2013, Copenhagen, Denmark (85. Three cases of attempted suicide by ingestion of nicotine liquid used in e-cigarettes.). *Clin. Toxicol.* **2013**, *51*, 252–378.
28. Kosmider, L.; Sobczak, A.; Fik, M.; Knysak, J.; Zaciera, M.; Kurek, J.; Goniewicz, M.L. Carbonyl compounds in electronic cigarette vapors: Effects of nicotine solvent and battery output voltage. *Nicotine Tob. Res.* **2014**, *16*, 1319–1326.
29. Jensen, R.P.; Luo, W.; Pankow, J.F.; Strongin, R.M.; Peyton, D.H. Hidden formaldehyde in e-cigarette aerosols. *N. Engl. J. Med.* **2015**, *372*, 392–394.
30. Goniewicz, M.L.; Knysak, J.; Gawron, M.; Kosmider, L.; Sobczak, A.; Kurek, J.; Prokopowicz, A.; Jablonska-Czapla, M.; Rosik-Dulewska, C.; Havel, C.; *et al.* Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob. Control* **2014**, *23*, 133–139.
31. EFSA. Scientific Opinion on Flavouring Group Evaluation 20, Revision 2 (FGE.20Rev2): Benzyl alcohols, benzaldehydes, a related acetal, benzoic acids, and related esters from chemical groups 23 and 30. *EFSA J.* **2009**, *8*, 1405.
32. EFSA. EFSA Scientific Opinion on Flavouring Group Evaluation 78, Revision 1 (FGE.78Rev1). Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2. *EFSA J.* **2011**, *9*, doi:10.2903/j.efsa.2011.2178.
33. Farsalinos, K.E.; Kistler, K.A.; Gillman, G.; Voudris, V. Evaluation of electronic cigarette liquids and aerosol for the presence of selected inhalation toxins. *Nicotine Tob. Res.* **2014**, *17*, 168–174.
34. EPA. Non-Carcinogen Tolerable Daily Intake (TDI) Values from US EPA. Available online: http://www-esdworldbankorg/popstoolkit/POPsToolkit/POPSTOOLKIT_COM/TOOLS/HHRA/TDI_USEPAHTM (accessed on 27 November 2014).
35. EFSA. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the Commission related to use of formaldehyde as a preservative during the manufacture and preparation of food additives. *EFSA J.* **2006**, *415*, 1–10.
36. Van Andel, I.; Sleijffers, A.; Schenk, E. *Adverse Health Effects of Cigarette Smoke: Aldehydes. RIVM Report 340603002*; The Netherlands National Institute for Public Health and the Environment (RIVM): Bilthoven, The Netherlands, 2006; pp. 1–65.
37. EPA. *Ambient Water Quality Criteria for Acrolein*; US Environmental Protection Agency EPA: Washington, DC, USA, 1980; pp. 1–102.

38. Council of Europe. *Flavouring Substances and Natural Sources of Flavourings*, 3rd ed.; Council of Europe: Strasbourg, France, 1981
39. WHO. Safety evaluation of certain food additives and contaminants. In *International Programme on Chemical Safety*; World Health Organization: Geneva, Switzerland, 1998.
40. Bahl, V.; Lin, S.; Xu, N.; Davis, B.; Wang, Y.H.; Talbot, P. Comparison of electronic cigarette refill fluid cytotoxicity using embryonic and adult models. *Reprod. Toxicol.* **2012**, *34*, 529–537.
41. Etter, J.; Bullen, C. Cotinine levels in users of electronic cigarettes. *Eur. Respir. J.* **2011**, *38*, 1219–1220.
42. Williams, M.; Villarreal, A.; Bozhilov, K.; Lin, S.; Talbot, P. Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. *PLoS One* **2013**, *8*, doi:10.1371/journal.pone.0057987.

© 2015 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).