Food and Chemical Toxicology 133 (2019) 110727

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Acetoin is a precursor to diacetyl in e-cigarette liquids

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ARTICLE INFO

Keywords:
Acetoin
Diacetyl
Acetylpropionyl
E-cigarettes
E-Liquids

ABSTRACT

Use of the e-liquid flavourings diacetyl and acetyl propionyl has raised concerns that they might cause respiratory diseases amongst vapers. Product surveys show that these compounds, plus a less toxic alternative, acetoin, are widely used in e-liquids.

We have investigated the chemistry of acetoin, acetyl propionyl and diacetyl in e-liquids. They are reactive, with concentrations falling substantially over time. Acetyl propionyl is the most reactive, diacetyl less so, and acetoin significantly more stable. Their reactivity is pH-enhanced when nicotine is present in the e-liquid.

Of major concern, we found that acetoin generates diacetyl in e-liquids. We found diacetyl formation in all acetoin-containing e-liquids, but it is not an acetoin-contaminant. Diacetyl concentrations were proportional to acetoin content, grew over time, and formation was accelerated by nicotine. E-liquids stored for up to 18 months contained significant diacetyl, and reduced acetoin levels, showing that acetoin is a long-term diacetyl source. Other reaction pathways operate, and we advance mechanisms to explain this area of e-liquid chemistry.

Acetoin use in e-liquids is an inevitable source of diacetyl exposure for e-cigarette users. Acetoin, acetyl propionyl and diacetyl are avoidable hazards for vapers, and we recommend e-liquid manufacturers move away from their use in e-liquid formulations.

1. Introduction

Electronic nicotine delivery devices (ENDS), or e-cigarettes have achieved widespread popularity in the decade following their introduction. Most e-cigarettes contain a battery section, electronic control systems, an atomiser (consisting of an electrically heated coil) and a reservoir of liquid ("e-liquid") which forms an aerosol when heated (Margham et al., 2016). The e-liquid is composed of aerosol-formers (usually propylene glycol (PG) and/or glycerol (VG)), water (to regulate viscosity), nicotine and flavourings. E-liquid flavours are viewed as a key factor underpinning growing e-cigarette popularity (Barrington-Trimis et al., 2014). More than 7700 flavoured e-liquids are available to vapers (Zhu et al., 2014), most of which are complex combinations of natural and synthetic flavour ingredients. Recently, regulatory frameworks (EU 2014; FDA 2016) and voluntary national standards in France (AFNOR 2015) and the UK (BSI 2015) have emerged governing the contents and development of e-liquids.

Use of food-grade flavour compounds has been identified as a minimum voluntary standard (AFNOR 2015), although toxicological evaluations used to support ingestion of flavours in foods and beverages do not provide assurance as to their safety when inhaled (NIOSH, 2015). Many flavourants used in e-cigarettes have unknown inhalation toxicities (NIOSH 2015b]. We have reported a risk-assessment approach that can be used to manage uncertainties associated with inhalation of food-grade flavour ingredients (Costigan and Meredith 2015). Perhaps the clearest example of the potential inhalation hazards of food-grade flavours is provided by e-liquids possessing buttery, creamy, sweet and vanilla flavours (Farsalinos 2015). Many e-liquid manufacturers have used at least three related chemicals to achieve these flavour characteristics – diacetyl (2,3-butanedione), acetyl propionyl (2,3-pentanedione), and acetoin (3-hydroxy-2-butanone) (Fig. 1a).

Although these compounds are approved by governmental and industry bodies for ingestion (NIOSH 2011; FEMA 2018), and widely used in food products, occupational diacetyl inhalation causes a decline in human respiratory function through a condition known as bronchiolitis obliterans (Kreiss et al., 2002; Kanwal et al., 2006; Kreiss 2007; Van Rooy et al., 2007; Kreiss 2012; Kreiss et al., 2012; NIOSH 2014). Acetyl propionyl, an α-dicarbonyl homolog of diacetyl, has been used as an alternative and possible supplement (Allen et al., 2016) to diacetyl in both food and e-liquid applications. However, evidence from animal inhalation studies indicates that it may have comparable lung toxicity to diacetyl (Hubbs et al., 2012; Morgan et al., 2012). The flavour

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https://doi.org/10.1016/j.fct.2019.110727
Received 22 January 2019; Received in revised form 27 June 2019; Accepted 26 July 2019
Available online 01 August 2019

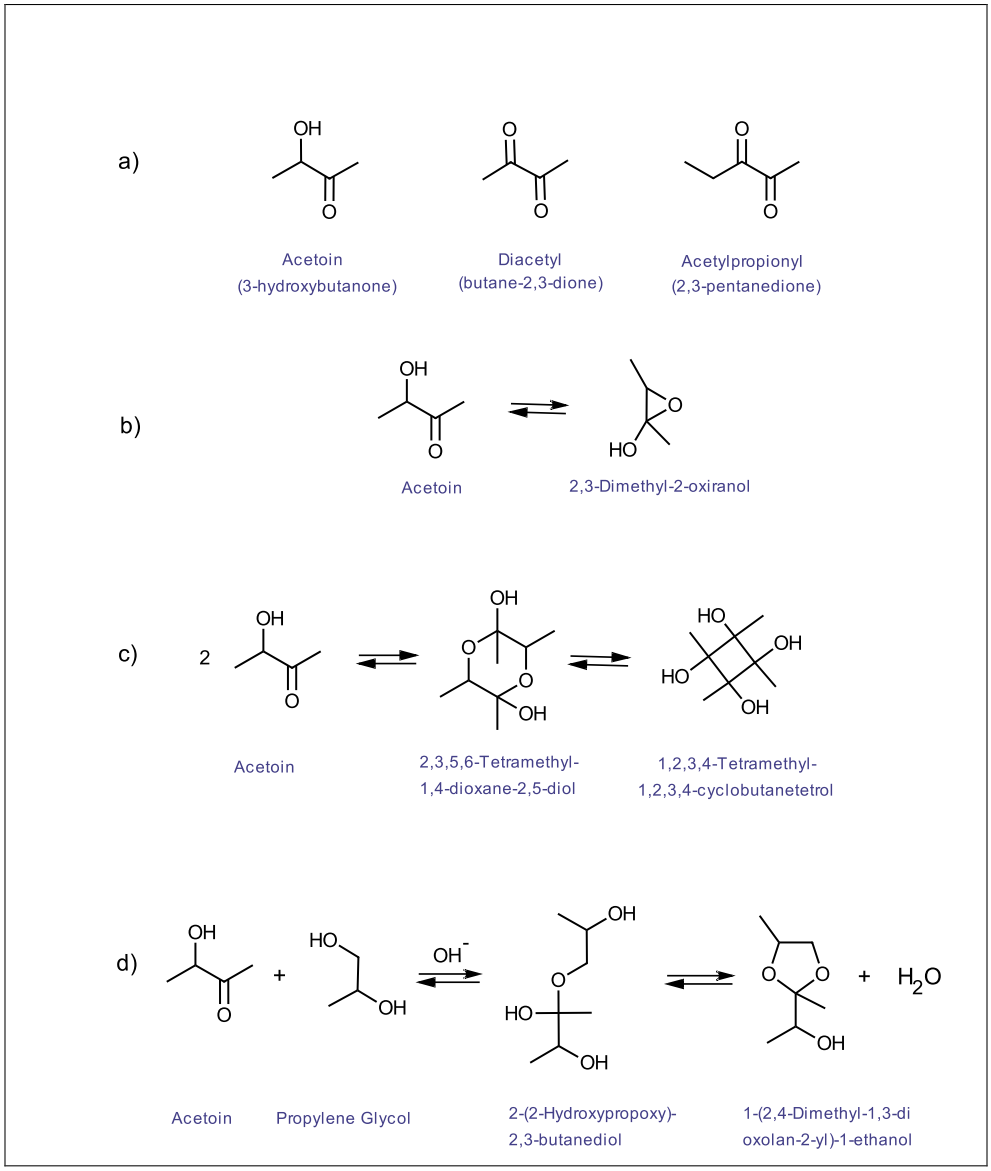

Fig. 1. a) Structures of acetoin, diacetyl and acetylpropionyl b) Acetoin and its isomer 2,3-dimethyl-2-oxiranol c) Dimerisation of acetoin d) Reaction of acetoin and propylene glycol to form the ketal and hemi-ketal.

properties of acetoin are similar to those of diacetyl and acetyl propionyl, but it differs chemically, being a hydroxyl-ketone rather than a di-ketone (Fig. 1a). It also appears to be associated with significantly lower toxicological risk when inhaled (NIOSH 2015b).

Concerns over the potential health implications of diacetyl use in e-liquids were first raised in consumer web forums in 2008 (E-cigarette forum, 2008). However, since then a number of studies covering multiple countries have identified the continued and widespread use of diacetyl, acetyl propionyl and acetoin in sweet, creamy, buttery and vanillic flavoured e-liquids (Farsalinos et al., 2015a, Allen et al., 2016 and Klager et al., 2017). The presence of acetoin in these studies suggests that e-liquid manufacturers may be using it as a less toxic alternative or supplement to diacetyl or acetyl propionyl. However, the continuing presence of both diacetyl and acetyl propionyl is of concern. The health implications of diacetyl and acetyl propionyl exposure during vaping have not been fully established but there is every reason to view these exposures as having the potential to pose health risks to vapers. Investigation of lung exposure amongst workers with flavouring-related lung disease showed the deep lung to be the most severely affected tissue (Akpinar-Elci et al., 2004; Morris and Hubbs, 2009; CDC 2013), an area of the body susceptible to repeated exposure on vaping (Gloede et al., 2011). Farsalinos et al. (2015a) concluded that the presence of diacetyl and acetyl propionyl represented an avoidable risk, and that proper measures should be taken by e-liquid manufacturers to eliminate these hazards from their products.

Acetoin and diacetyl are very similar chemically, with diacetyl an oxidised form of acetoin. Previous studies examining very different systems to e-liquids have established that acetoin can produce diacetyl under certain conditions (O'Meara 1931, Efron and Blom 1947, White and Wainwright 1975, and Pendergrass 2004). Given the correlation between acetoin and diacetyl levels in e-liquids in recent product surveys we investigated the potential for acetoin to act as a source of diacetyl in e-liquids. We examined the stabilities of acetoin, diacetyl and acetyl propionyl in e-liquids, and investigated the development of diacetyl in e-liquid formulations containing acetoin. A series of experiments was designed to assess the effects of formulation including concentrations of acetoin, water, PG, VG and nicotine, effective pH, as well as light exposure on diacetyl formation in e-liquid formulations. Our results demonstrate that acetoin is converted to diacetyl in a wide range of e-liquids. Given the widespread use of acetoin by e-liquid

manufacturers (Allen et al., 2016; Klager et al., 2017) it is clear that its use in e-liquids should be discontinued in order to avoid inadvertent diacetyl generation on storage and consequent exposure to vapers.

2. Methodology

2.1. Reagents and materials

Experimental reagents were sourced as follows. Pharmaceutical-grade glycerol (99.9% purity) was obtained from Sigma Aldrich (Gillingham, UK. Product code 49779, lot number BCBQ6768V); pharmaceutical-grade propylene glycol (> 99% purity) was obtained from Sigma Aldrich, Fluka (code 82281, lot number BCBQ0147V) and pharmaceutical-grade nicotine (99.4% purity by non-aqueous titrimetry) was obtained from Siegfried (Minden, Germany. Lot number 1517/024). The water used in this study was city water connected to a Millipore (Watford, UK) deionised ultra-filter (DIUF) and was purified to a water resistivity value of 18.2 MΩ cm^{-1} at 25 °C. 10 M sodium hydroxide hydroxide solution was obtained from Sigma Aldrich. Acetoin for laboratory studies was sourced from Sigma Aldrich (product code: A17951, lot number MKBQ2240V), with a declared purity of 99.3% by GC. Acetoin used in commercial flavour mixtures was 99% pure and supplied by Hertz flavours (Reinbek, Germany). Diacetyl (a mix of the monomer and dimer) was sourced from Sigma Aldrich (product Code: B85307, lot number BCBM5232V), with a declared purity of 97%. Acetyl propionyl was sourced from Sigma Aldrich (product Code 241962, lot number MKBB7504V), with a declared purity of 97.1%.

Analytical reagents were sourced as follows. Pyridine was sourced from Acros Organics – (Fisher Scientific, Pittsburg, USA. Product code: 41854, lot number B0531131), with a declared purity of 99.9%, Acetonitrile (99.9% purity) was sourced from Fisher Scientific Optima (Product code: A996, lot number 150734) and 2,4-Dinitrophenylhydrazine (DNPH) was sourced from Sigma Aldrich (product code: D199303, lot number BGBC4575V), with a declared purity of 96.7% by HPLC. The internal standard 2,3-butanedione-d_6 was sourced from CDN Isotopes (Quebec, Canada), with a declared purity of 98.5% (lot number I214P13) and the internal standard 2,3-pentanedione-1,1,1,4,4-d_5 was sourced from Medical Isotopes, Inc. (Pelham, USA), with a declared purity of 98.9% (lot number 747). Glass vials were sourced from ThermoFisher Scientific manufactured with superior quality 33 expansion borosilicate (Type 1, Class A for the clear glass vials) and (Type 1, Class B for the amber glass vials) in order to eliminate leaching of ions and to maintain consistent effective pH of the e-liquid sample.

2.2. Analysis of acetoin, acetyl propionyl and diacetyl

All analyses were conducted under contract by Enthalpy Analytical Inc. (Durham, NC, USA). The standard method used in most of the experiments comprising this study was a GC/MS method, but an HPLC method was also used for some experiments, such as those examining the influence of effective pH on acetoin conversion to diacetyl where sodium hydroxide was added to e-liquids. The GC/MS method was not used in these experiments, to avoid the possibility of deuteron exchange in the internal standards (IS) introducing significant errors into the analyte quantification steps. Consequently, these analyses were conducted using an alternative, HPLC/UV approach.. Methods used for each experiment are described in the Supplementary Information File, and as a footnote to each table of results.

2.3. GC/MS

Enthalpy Method SOP ENT225 was used (accredited by A2LA to ISO/IEC 17025:2005). Brief details are given below, and further details are available on request from Enthalpy Analytical Inc.

An internal standard solution was prepared by adding 20 μL of 2,3-butanedione-d_6 and 20 μL of 2,3-pentanedione-1,1,1,4,4-d_5, to a 50-mL clear volumetric grade A flask and brought up to volume with acetonitrile. The diluent was prepared with 95 mL of acetonitrile and 5 mL of the internal standard solution. A measured volume (50 μL) of e-liquid sample was weighed and combined with 1 mL of diluent in an auto sampler vial, which was capped and the contents mixed thoroughly. An aliquot was then analysed quantitatively against a linear calibration curve using an Agilent Technologies Model 6890 N Gas Chromatograph equipped with a 5975 B Mass Selective Detector and an appropriate column for the analyses of interest. Three replicate measurements per time point were measured. Minimum detection limits and limits of quantification are provided in the Supplemental Information. It is noteworthy that the use of an autosampler system in the analyses introduced a temporal uncertainty into the time-course studies; in each experiment the samples were held on the GC autosampler in a batch of samples for a period of between 1 and 30 hours. In reporting results, we therefore quote time intervals as integer values, to reflect this uncertainty.

2.4. HPLC method

The HPLC method was accredited to ISO/IEC 17025:2005. Briefly, an aliquot of 25 μL–50 μL of e-liquid sample was derivatized with 1000 μL of 2,4-dinitrophenylhydrazine (DNPH). After the derivatization reaction was complete, the samples were neutralised with 50 μL of pyridine and analysed using an Agilent Model 1100, High Performance Liquid Chromatograph “Bart” equipped with an appropriate column and an ultraviolet (UV) detector operating at 360 nm. Minimum detection limits and limits of quantification are provided in the Supplemental Information.

2.5. pH measurement

Measurement of effective solution pH were conducted using an InLab Viscous Pro-ISM electrode with integrated temperature probe, designed for viscous samples (Mettler Toledo). A three-point calibration was conducted using technical controlled pH buffers (Mettler Toldeo) of 4.01 (lot 1A212A), 7.00 (lot 1A204A) and 10.01 (lot 1A247A) at 25 °C. pH measurements with non-aqueous e-liquids, or those with low water content, were checked by interpolation of values obtained following serial dilution of the e-liquid with water.

2.6. Individual study designs

The investigation comprised a series of eight experiments designed to examine compound stability and establish how the formation of diacetyl from acetoin may be influenced by various compositional aspects (such as nicotine, water, PG, VG and organic acid contents) and environmental factors (such as light and time). These are described in detail in the Supplementary Information. In each case single large volume liquid samples were sampled to provide three analytical replicates, consistent with the real-world batch production of commercial e-liquids. For analysis of commercial cartomiser samples, five samples were combined into a single sample prior to three analytical samples being taken. Data are provided in the results section as the mean of these three analytical replicates, together with an average coefficient of variation for the repeated analyses.

2.6.1. Choice of flavour concentrations

When conducting controlled laboratory experiments it is desirable to reflect as closely as possible real-world conditions found with commercial e-liquids. However, three challenges faced our experimental design:

i) First, manufacturers do not publicly disclose the exact addition

levels of individual flavour compounds.

ii) Second, commercial e-liquid acetoin concentrations are not available in the literature, and only aerosol concentrations have been published (Allen et al., 2016; Klager et al., 2017). Aerosol concentrations are strongly influenced by both e-liquid concentrations and e-cigarette performance criteria. Unfortunately, insufficient e-cigarette performance data were provided in these publications to allow acetoin e-liquid concentrations to be calculated.

iii) The three published studies in this area sampled products from the market, where product age is an uncontrolled variable. If concentrations change over time it would not be possible to identify the initial formulation from analyses of samples taken from the market.

In this study we therefore examined a number of compound addition levels ranging from 0 to 2500 μg/mL. Our study results showed a great deal of consistency with published values for diacetyl and acetyl propionyl (Farsalinos et al., 2015a).

2.6.2. Stability of acetoin, diacetyl and acetyl propionyl in e-liquids

The stabilities of these compounds were compared by adding 1000 μg/mL of each compound to separate batches of an e-liquid composed of 48.76% VG, 25% PG, 25% water and 1.24% nicotine. These solutions were stored at 22 ± 2 °C for up to 64 days, with analysis by GC/MS. Concentrations of all three compounds were determined in each liquid at the assigned time points.

2.6.3. Effect of acetoin concentration on the rate of conversion to diacetyl

E-liquids consisting of different concentrations of acetoin in an otherwise unflavoured e-liquid base containing VG, PG, water and nicotine were analysed by GC/MS for acetoin and diacetyl immediately after formulating and after 21 and 140 days storage (Supplementary Tables S1 and S2). The effect of light exposure was also examined as part of this study by comparing clear glass to amber glass vials.

2.6.4. Diacetyl production from acetoin in various e-liquids

The loss of acetoin and formation of diacetyl was followed for up to 56 days in eight different e-liquid formulations with analysis by GC/MS (Supplementary Tables S3 and S4). The formulations were designed with different ratios of VG to PG, with (2%) and without nicotine, and with (23–25%) and without water. One sample also included 1% benzoic acid to further investigate effective pH factors.

2.6.5. Systematic evaluation of e-liquid composition on diacetyl formation from acetoin

This series studied the effects of a wider range of concentration of nicotine (0, 1%, 2%, 3%, 4%, and 5% - Supplementary Table S5), water (0, 12.5%, 25%, 37.5% and 50% - Supplementary Table S6), and different proportions of VG and PG (0/85.5% PG/VG, 42.75/42.75% and 85.5/0% PG/VG - Supplementary Table S7). Analysis was performed by HPLC (Supplementary Table S8).

2.6.6. Stability of diacetyl and acetyl propionyl in different e-liquids

E-liquids were prepared with different levels of VG and PG, with and without nicotine, and with a combination of nicotine and acid, to which diacetyl and acetyl propionyl were added at various levels from 0 to 180 μg/mL (Supplementary Tables S9 and S10). Diacetyl and acetyl propionyl concentrations were measured on the day of application and after 18 days by GC/MS (Supplementary Table S11).

2.6.7. Potential reversibility of the conversion of diacetyl to acetoin

This experiment was designed to understand whether the conversion of acetoin to diacetyl was reversible in e-liquids. Given the importance of alkaline pH's in the conversion, an acidic pH solution containing diacetyl was prepared and tested for acetoin content. Measurements were taken on the day of formulation, after 9 days and after 21 days by HPLC (Supplementary Tables S12 and S13).

2.6.8. Impact of effective pH – experiments with sodium hydroxide

Several experiments were conducted where 2% nicotine was replaced with 2% of 10 M sodium hydroxide - Supplementary Table S14. The purpose of these experiments was to establish if pH rather than nicotine effects operated in the e-liquids. The liquids used mirrored those in experiment 4, above. Measurements were made on the day of addition and after 9 and 21 days by HPLC.

2.6.9. Diacetyl production under commercial e-cigarette storage conditions

Ageing experiments were conducted wherein e-liquids containing from 0 to 36 mg/mL nicotine were prepared containing 200 ppm flavour grade acetoin; a control e-liquid with 0 ppm acetoin was also prepared. Samples of the liquid were stored in either sealed commercial e-liquid bottles or placed into empty commercial cartomisers (both cigalike and ego style cartomisers) that were subsequently sealed in commercial-style blister packs. These configurations faithfully represented commercial samples of liquids that would be taken from bottles to use in tank or box-mod e-cigarettes, or used in cartomiser designs respectively. Filled samples were aged in sealed containers in a dark store room at room temperature for a period of 2–18 months prior to analysis; analysis was by GC/MS (Supplementary Table S15).

3. Results

3.1. Stability of acetoin, diacetyl and acetyl propionyl in e-liquids

The relative stabilities of these three compounds in an an e-liquid were investigated by adding 1000 μg/mL of each compound to separate e-liquid samples, i.e. each e-liquid sample tested contained only one of each added compound. Samples were stored at room temperature for periods up to 64 days, and samples taken for analysis at periods throughout this time period. The results of these analyses are shown in Table 1, and in Fig. 2. The data in Table 1 show that control samples were free from detectable levels of the three compounds at the time points tested. Samples to which acetoin, acetyl propionyl and diacetyl were added showed systematic reductions in the concentration of each analyte with storage time, i.e. none of these three compounds were completely stable in the e-liquid at room temperature. The most reactive of the three was acetyl propionyl, whose concentration declined rapidly, halving in the first day after addition to the e-liquid. Diacetyl was less reactive, with a half-life time of approximately eight eight days. Acetoin reacted much more slowly, with a half-life significantly in excess of 64 days.

In the e-liquid to which acetyl propionyl was added there appeared to be low (~0.5%) levels of diacetyl impurity, which declined with a similar half-life as diacetyl in the e-liquid to which it was added at 1000 μg/mL. Diacetyl was also found in the acetoin e-liquid, at levels that increased over time from an initial 0.2%–4.6% of the initial acetoin concentration. The data in Fig. 2 (inset graph) confirm clearly that acetoin produces diacetyl in e-liquids.

3.2. Effect of acetoin concentration on the rate of conversion to diacetyl

Acetoin and diacetyl concentrations were measured in an e-liquid sample to which varying concentrations of acetoin had been added; in addition, “control”, acetoin-free samples (the row containing the cell labelled ‘0’ in the ‘[Acetoin] added’ column of Table 2) were measured for the same analytes. Analyses were performed on the day of acetoin addition and after storage for 21 and 140 days at room temperature. These analyses show that acetoin and diacetyl were not detected in the control e-liquid sample at any time-point (Table 2).

Analysis of the other e-liquid samples on the day of acetoin addition showed a strong ($p < 0.001$) linear correlation ($[acetoin]_1 = 8.58 + 0.9849[acetoin]_0$, $r^2 = 99.97\%$) between the day 1 measured ($[acetoin]_1$) and initially added concentrations ($[acetoin]_0$) of acetoin in the e-liquid (Table 2, Supplementary Fig. S1), with measured acetoin

Table 1
Comparative stabilities of acetoin, diacetyl and acetyl propionyl in an e-liquid.

Time	Added Acetyl Propionyl			Added Acetoin			Added Diacetyl		
	DA	AP	Acetoin	DA	AP	Acetoin	DA	AP	Acetoin
(days)	Mean (μg/mL)	Mean (μg/mL)	Mean (μg/mL)	Mean (μg/mL)	Mean (μg/mL)	Mean (μg/mL)	Mean (μg/mL)	Mean (μg/mL)	Mean (μg/mL)
0	–	1000	–	–	–	1000	1000	–	–
1	4.38	521	1.87	2.36	1.07	1169	1114	1.07	1.87
3	3.49	135	1.87	13.7	1.07	1054	–	–	–
6	2.94	92.3	1.87	17.1	1.07	1033	603	1.07	1.87
9	1.40	64.2	0.75	19.6	0.47	963	–	–	–
12	1.26	60.5	0.75	21.2	0.47	963	348	0.47	0.75
15	0.87	55.0	0.75	–	–	–	–	–	–
17	–	–	–	26.5	0.47	1059	–	–	–
18	0.86	70.3	0.75	29.3	0.47	981	366	0.47	0.75
21	0.58	60.5	0.75	29.6	0.47	905	–	–	–
24	–	–	–	–	–	–	240	1.07	1.87
30	–	–	–	–	–	–	190	1.07	1.87
36	1.50	50.4	1.87	39.4	1.07	933	164	1.07	1.87
64	0.98	38.5	2.08	46.4	0.47	760	–	–	–

Analysis conducted by GC/MS. Average CoVs for replicate analyses were 1.7% for added AC, 2.1% for DA formed from AC; 1.6% for added DA; 2.6% for added AP and 5.1% for DA initially present in AP.

levels on average 99.4% of those added. Analysis for diacetyl amongst the liquids with added acetoin on the day of addition (Storage time = 1 in Table 2) showed the presence of diacetyl in the two liquids with highest acetoin concentrations (> 2000 μg/mL - Fig. 3); however, the levels were below the limit of quantification of the method (i.e. < 11.3 μg/mL, Supplementary Table S2). Diacetyl was not detected (LOD: 1.13 μg/mL) in any of the other acetoin-containing liquids at this time point. On average, diacetyl levels could be estimated at 0.2% of the added acetoin at this time-point.

After 21 days storage at ambient conditions, there were again strong ($p < 0.001$) linear correlations ($[acetoin]_{21} = 12.55 + 1.044[acetoin]_0$, $r^2 = 99.99\%$), between the amounts added and measured (Supplementary Fig. S1) with acetoin concentrations on average 102% of the added levels. Quantifiable levels of diacetyl were found in all solutions containing acetoin; corresponding on average to conversion of 2.3% (range = 1.7–2.9%) of the added acetoin. The relationship (Fig. 3) between initial added acetoin concentration ($[acetoin]_0$) and diacetyl produced after 21 days ($[diacetyl_{21}]$) was well-defined ($p < 0.001$) but apparently non-linear with lower % conversion at higher initial acetoin contents. A quadratic function of the form $[diacetyl_{21}] = -0.2059 + 0.03239\ [acetoin]_0 - 0.000006[acetoin]_0^2$, ($r^2 = 99.77\%$) provided the best regression fit for the data.

After 140 days storage at ambient conditions, the measured acetoin concentrations ($[acetoin]_{140}$) had fallen on average to 95% of the initially added levels, although there were still strong ($p < 0.001$) linear correlations ($[acetoin]_{140} = 7.47 + 0.9829[acetoin]_0$, $r^2 = 99.65\%$), between the amounts added and measured (Supplementary Fig. S1). Partnering these reductions in acetoin levels were increased levels of diacetyl, at an average 6% (range = 3.7–11.9%) of the mass of added acetoin. There was again a clearly defined ($p < 0.001$) quasilinear relationship between the level of added acetoin and the amount of diacetyl formed (Fig. 3), also with apparent lower rate of conversion at the highest acetoin concentration. A quadratic fit of the form $[diacetyl_{21}] = 5.671 + 0.07669[acetoin]_0 - 0.000018[acetoin]_0^2$, ($r^2 = 97.25\%$) provided the best simple regression for the data at this time point.

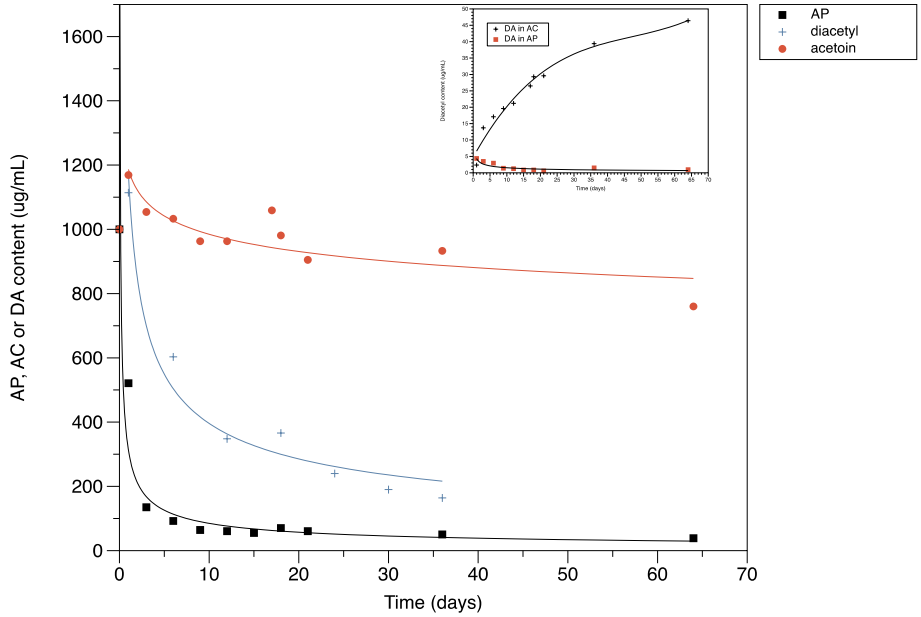

Fig. 2. Comparison of the stabilities of acetoin, acetyl propionyl and diacetyl in an e-liquid.
Note: Lines fitted through data points are empirical fits provided to guide the viewers eye.

Table 2
Effect of initial acetoin concentration on the presence of diacetyl in e-liquid, on the day of acetoin addition, 21 and 140 days afterwards.

[Acetoin] added	Vial Vessel	Storage Time	Diacetyl Content	Acetoin Content	Storage Time	Diacetyl Content	Acetoin Content	Storage Time	Diacetyl Content	Acetoin Content
[ug/mL]		(Days)	(μg/mL)	(μg/mL)	(Days)	(μg/mL)	(μg/mL)	(Days)	(μg/mL)	(μg/mL)
0	Clear	1	< LOD	< LOD	21	< LOD	< LOD	140	< LOD	< LOD
405	Clear	1	< LOD	398.3	21	11.4	397.7	140	39.5	333
607	Clear	1	< LOD	639	21	16.8	611	140	48.9	589
1010	Clear	1	< LOD	997.3	21	28	1052	140	65.9	1012
1512	Clear	1	< LOD	1483.3	21	34.6	1573.7	140	79	1572
2012	Clear	1	< LOQ	1995	21	40.5	2076	140	80.1	1909
2509	Clear	1	< LOQ	2480.7	21	44	2614.7	140	90.5	2450
2509	Amber	1	< LOQ	2433.7	21	49.8	2562.3	140	116	2358

Analysis conducted by GC/MS. Average CoVs for replicate analyses were 2.9% for added AC, 1.6% for DA formed from AC. LOD values were 1.87 μg/mL for acetoin and 1.13 μg/mL for diacetyl; the corresponding LOQ values were 18.7 μg/mL and 11.3 μg/mL respectively.

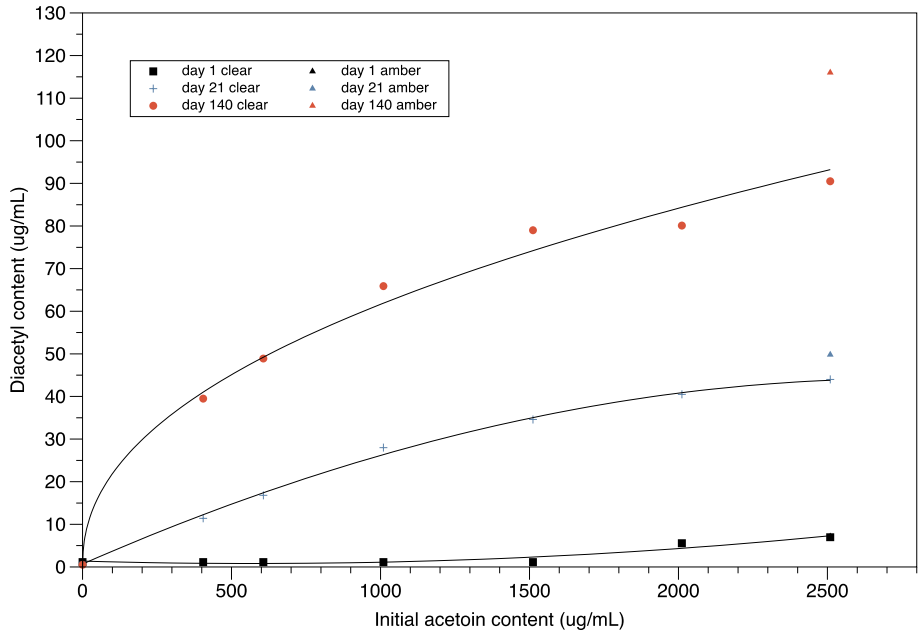

Fig. 3. Influence of acetoin content on the development of diacetyl in e-liquids:
Note: Lines fitted through data points are empirical fits provided to guide the viewers eye.

The influence of light was examined at one acetoin concentration level only (2509 μg/mL). The data in Table 2 show that there was a minor but significant ($p = 0.02$ 2-tailed t-test) role of ambient light in the conversion of acetoin to diacetyl, at both 21 and 140 days storage, with up to 30% higher levels of diacetyl in the samples stored in the amber vials.

3.3. Diacetyl production from acetoin in different e-liquids

In order to establish whether generation of diacetyl from acetoin in e-liquids was influenced by the composition of the e-liquid, a 56-day time-course experiment was conducted under controlled temperature conditions. Both nicotine-containing (2%) and nicotine-free e-liquids were included in this study to better examine possible pH factors. Data are presented in Table 3, and in Supplementary Fig. S2a (acetoin contents) and S2b (diacetyl yields).

Analysis of the control unspiked e-liquids (without acetoin or diacetyl) over this time showed no detectable levels of acetoin nor diacetyl at any time-point. Measurements of acetoin loss did not provide any clear trends over the 56-day time-course of the experiment (Supplementary Fig. S2a). Although the nicotine-free e-liquids gave acetoin levels close to the administered level, the e-liquids containing both PG and nicotine gave higher than expected acetoin contents, with significant scatter in the data. The chromatograms were examined for possible changes in peak areas of the internal standards that might indicate deuteron exchange and lead to apparent increases in acetoin concentrations. Some small effects were observed but they did not exceed the laboratory quality control limits and were insufficient to explain the high acetoin levels seen in these experiments. The anomalous acetoin analyses were therefore attributed to a possible unspecified matrix effect, or to acetoin formation from dimers, as discussed below.

The nicotine-free e-liquids to which acetoin had been added did not show diacetyl levels above the limit of quantification at any time point. Inspection of diacetyl peaks in the chromatograms 0 identified a minor and slow increase in the peak corresponding to diacetyl, with estimated levels reaching a maximum of about half the limit of quantification after about 56 days of storage (Supplementary Fig. S2b).

In contrast, the equivalent nicotine-containing e-liquids (2% w/w nicotine) showed significant (Table 3, two sample t-test, $p < 0.05$) and sustained increases in the levels of diacetyl over the time-course of the study. The highest diacetyl level observed in these experiments was 87.5 μg/mL, which represents about 8% of the initial acetoin content. This was obtained in a formulation with 2% nicotine, 25% water and 36.5% each of VG and PG as shown in Supplementary Fig. S2b. The concentration of diacetyl in this formulation was more than halved by adding 1% benzoic acid (day 7, 8.2 (with benzoic acid)) vs 17.9 μg/mL

Table 3
Acetoin conversion to diacetyl in different e-liquids.

VG	PG	Water	Nicotine	Benzoic Acid	Time	Acetoin	Diacetyl	Time	Acetoin	Diacetyl	Time	Acetoin	Diacetyl	Time	Acetoin	Diacetyl	Time	Acetoin	Diacetyl
(%w/w)	(%w/w)	(%w/w)	(%w/w)	(%w/w)	days	(μg/mL)	(μg/mL)	days	(μg/mL)	(μg/mL)	days	(μg/mL)	(μg/mL)	days	(μg/mL)	(μg/mL)	days	(μg/mL)	(μg/mL)
100	0	0	0	0	1	937	< LOQ	7	861	< LOD	14	942	< LOQ	21	1004	< LOQ	35	1048	< LOQ	56	996	< LOQ
75	0	25	0	0	1	1062	< LOQ	7	887	< LOD	14	984	< LOQ	21	962	< LOQ	35	1052	< LOQ	56	902	< LOQ
75	0	23	2	0	1	1144	< LOQ	7	997	13.4*	14	1043	19.5*	21	1073	23.5*	35	1056	32.0*	56	1043	40.9*
0	100	0	0	0	1	1102	< LOQ	7	913	< LOQ	14	1064	< LOQ	21	976	< LOQ	35	1106	< LOQ	56	933	< LOQ
0	75	25	0	0	1	1113	< LOQ	7	907	< LOQ	14	1073	< LOQ	21	1032	< LOQ	35	1086	< LOQ	56	959	< LOQ
0	75	23	2	0	1	1278	< LOQ	7	1059	< LOQ	14	1478	12.4*	21	1142	< LOQ*	35	1609	18.8*	56	1188	18.1
36.5	36.5	25	2	0	1	1328	< LOQ	7	1104	17.9*	14	1546	42.1*	21	1129	43.7	35	1514	87.5*	56	1090	76.0*
36	36	25	2	1	1	1311	< LOQ	7	1161	< LOQ	14	1602	18.1*	21	1216	17.7	35	1675	41.0*	56	980	38.7

Analysis by GC/MS. Average COV of measurement replicates: 2.3% for added AC, 4.7% for DA formed from AC. LOD for diacetyl was 1.13 μg/mL and the corresponding LOQ was 11.3 μg/mL.
* indicates concentration of diacetyl for this sample is significantly different to the preceding time-point value for this e-liquid (two sample *t*-test. $p < 0.05$; values < LOQ were imputed, using a value half of the LOQ, with SDs calculated from the average COV for the sample set).

(without benzoic acid); day 14, 18.1 vs 42.1 μg/mL; day 21, 17.7 vs 43.7 μg/mL; day 35, 41 vs 87.5 μg/mL; and day 56, 38.7 vs 76 μg/mL) effective solution pH decreased from 9.3 to 7.66 on addition of acid. This indicates that nicotine may be enhancing the formation of diacetyl by increasing the effective pH of the solution. The role of pH is examined further below.

Despite the relatively low concentrations of diacetyl formed compared with the initial concentration of acetoin ($[\text{diacetyl}]_{max}$ which reached a level < 8% of $[\text{acetoin}]_0$), the rate of diacetyl formation appeared to slow significantly after 30–40 days reaching a plateau concentration by the end of the experiment (Supplementary Fig. S2b). This was observed for all the nicotine-containing formulations examined in the time-course study.

Significant differences were observed in the rate of diacetyl production between nicotine-containing liquids of differing PG/VG composition. The relative rates of diacetyl production followed the order 36.5%VG/36.5%PG/25% water > 75% VG/23% water > 75%PG/23% water (all 2% nicotine).

3.4. Systematic evaluation of e-liquid composition on diacetyl production from acetoin

To further characterise factors influencing the conversion of acetoin to diacetyl a series of experiments was conducted to investigate the effects of nicotine concentration, water content and relative levels of VG and PG in the liquid. It should be noted that in some cases the formulations used in these experiments went beyond those found with normal e-liquids but were employed in order to shed more light on the mechanistic processes involved. For example, commercial e-liquids containing nicotine at 5% are relatively rare (although examples can be found outside of the European Union) and we examined a wider range of water contents than generally found in commercial e-liquids.

3.4.1. Nicotine concentration

Liquids were formulated with 0, 1%, 2%, 3%, 4% or 5% nicotine and 12.5% water (Supplementary Table S5). The remainder of the liquid consisted of equal proportions of VG and PG. This liquid was treated with 1000 μg/mL of acetoin and analysed for acetoin, diacetyl and acetyl propionyl at three time periods: on the day of acetoin addition, after 9 days and after 21 days. The results of these experiments are shown in Table 4. Acetyl propionyl was not quantifiable (< MDL or < LOQ μg/mL) in any of the samples.

The effects of nicotine concentration on diacetyl formation and acetoin depletion are shown in Fig. 4 (diacetyl concentrations) and Supplementary Fig. S3 (acetoin concentrations). Rather than decrease with time, acetoin levels increased by up to 25% over the time of the experiment for all samples, but the increases were smaller at higher nicotine levels (Supplementary Fig. S3 inset graph). Examining acetoin contents at each time point showed apparent lower levels of acetoin in higher nicotine solutions. Thisis is not significant ($p = 0.44$) on the day of addition, or after 9 days ($p = 0.076$),but reaches significance after 21 days ($p < 0.005$) and is well described by a negative linear regression ($R^2 = 93.7\%$, $[\text{acetoin}]_{21} = 1138–16.23[\text{nicotine}]_0$).

Levels of diacetyl also increase with time (Fig. 4). On the day of addition, the levels of diacetyl were all below the minimum detection limit. After both 9 and 21 days there were either no detectable or unquantifiably low levels of diacetyl in the nicotine-free liquid, but when nicotine was present in the e-liquid the levels of diacetyl were quantifiable and higher, at 1.5–3.3% of the inital acetoin content. The diacetyl content appeared to approach or reach steady-state concentrations that did not change between 1 and 5% nicotine content. Diacetyl levels were 40–70% higher after 21 days than after 9. Diacetyl yields are also plotted against effective pH in Fig. 4 (inset graph), and these data suggest that pH is an important factor in accelerating diacetyl production.

Table 4
Effect of nicotine concentration on the conversion of acetoin to diacetyl.

VG	PG	Water	Nicotine	Acetoin (μg/mL)			Diacetyl (μg/mL)			Acetyl Propionyl (μg/mL)			pH (base solution)
(%)	(%)	(%)	(%)	1 day	9 days	21 days	1 day	9 days	21 days	1 day	9 days	21 days	
50	50	0	0	1121	1181	1270	< MDL	< MDL	< LOQ	< MDL	< MDL	< LOQ	7.61
43.75	43.75	12.5	0	1075	1147	1141	< MDL	< MDL	< LOQ	< MDL	< MDL	< LOQ	7.66
43.25	43.25	12.5	1	1000	1006	1116	< MDL	15.4	25.6	< MDL	< MDL	< LOQ	9.15
42.75	42.75	12.5	2	1086	1099	1117	< MDL	18	28.5	< MDL	< MDL	< LOQ	9.24
42.25	42.25	12.5	3	1014	991	1079	< MDL	19.5	28.2	< MDL	< MDL	< LOQ	9.34
41.75	41.75	12.5	4	1045	989	1071	< MDL	20	28.4	< MDL	< MDL	< LOQ	9.42
41.25	41.25	12.5	5	1010	976	1062	< MDL	17.4	27	< MDL	< MDL	< LOQ	9.88

Analysis by HPLC. Average CoV for replicate analysis in Tables 4–6: 1.0% for added AC, 3.3% for DA formed from AC. MDLs (minimum detection limits) for acetyl propionyl and diacetyl were 0.76 and 0.54 μg/mL respectively; LOQs for acetyl propionyl and diacetyl were both 3.7 μg/mL (Supplementary Table S8).

3.4.2. Water content

In solutions of differing water content, a number of the measured acetoin levels were significantly higher than the applied amount (Supplementary Table S6 and Supplementary Fig. S4a). These increased concentrations were seen most clearly in the lower water content solutions (up to 25% higher in water-free solutions) and the increases were smaller (~10%) at higher water contents (Supplementary Fig. S4a) and later time-points. Examining the effect of water showed significant reductions in acetoin content as the e-liquid water concentration increased on day 9 ($p < 0.005$, $[acetoin]_9 = 1112–2.12[water]_0$, $R^2 = 96.3\%$) and day 21 ($p = 0.005$, $[acetoin]_{21} = 1213–5.73[water]_0$, $R^2 = 95.0\%$).

There were no detectable levels of diacetyl in any of the samples after 1 day (Supplementary Table S6, Supplementary Fig. S4b). After 9 and 21 days diacetyl levels were higher than at day 1, but there wereas no significant influence of water content on diacetyl concentrations ($p > 0.05$).

3.4.3. VG and PG content

Measured acetoin contents were also higher than those applied in solutions with different PG/VG ratios (Supplementary Table S7, Supplementary Fig. S5). Acetoin levels increased over the 21 day experiment for all of the solutions other than 42.75%VG/42.75%PG/12.5Water/2%nicotine. There was also a consistent trend for higher acetoin levels in the higher PG-content solutions; in contrast, highest diacetyl production was found with the 42.75%VG/42.75%PG solution/12.5%water/2%nicotine solution.

3.5. Stability of diacetyl and acetyl propionyl in different e-liquids

Given the chemical similarity between acetoin, diacetyl and acetyl propionyl (Fig. 1a), the reactivity of each compound in e-liquids (Results, Section 2) and the sensitivity of acetoin stability to e-liquid composition, experiments were conducted specifically to examinee the stability of diacetyl and acetyl propionyl in different e-liquids. Different e-liquid formulations were used together with an 18-day storage period.

Regression analysis of the data in Supplementary Table S9 and Supplementary Fig. S6 (diacetyl) and S7 (acetyl propionyl) show greater/faster losses of acetyl propionyl than diacetyl ($p < 0.05$) in the same solutions (other than the 50VG/50PG solution at day 1, $p = 0.422$) and over the same timescales. For example, in nicotinic solutions, at day 18 acetyl propionyl losses were 93% in comparison to 49% losses of diacetyl; losses of acetyl propionyl were also much greater than losses of diacetyl in non-nicotinic solutions. The presence of nicotine significantly ($p < 0.001$) accelerated losses of both compounds. For example comparing losses of acetyl propionyl between e-liquids without nicotine to those with nicotine showed very substantial differences (average losses on day 1 of 1% vs 73%, and average losses on day 18 of 28% vs 93%). Similar effects were seen with diacetyl. Addition of citric acid to the e-liquid stabilised both compounds to the

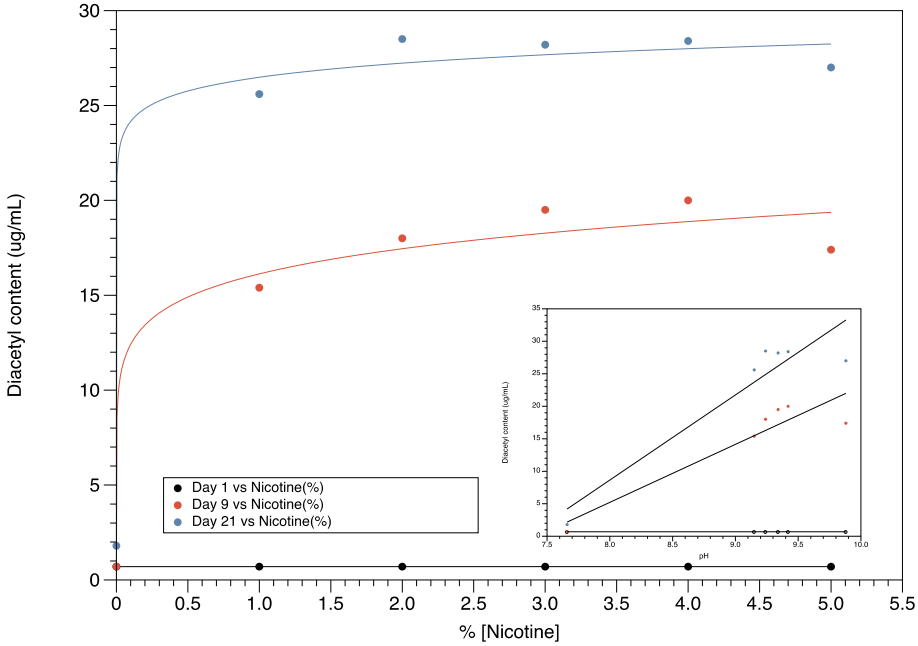

Fig. 4. Changes in diacetyl content 1, 9 and 21 days after addition of acetoin to e-liquids containing nicotine concentrations between 0 and 5%.
Note: Lines fitted through data points are empirical fits provided to guide the viewers eye.

levels seen with zero-nicotine e-liquids.

There was no clear contribution of water to on the rate of diacetyl or acetyl propionyl loss. Relative levels of VG and PG did not appear to affect diacetyl stability, but losses of acetyl propionyl were greater with high VG liquids at day 1 ($p < 0.05$) than with high PG liquids; data at day 18 was not significantly different as nearly all of the AP had been consumed.

An interesting observation was that the nicotine-free e-liquids (and the nicotinic solution containing citric acid) showed initial diacetyl levels greater than added on day 1 ($p < 0.05$) for solutions above 40.5 μg/mL, as was reported above with acetoin in most experiments where acetoin was added to e-liquids. This was not observed with acetyl propionyl, where initial measured levels were consistent with the quantities added.

3.6. Examining the potential for reverse production of acetoin from diacetyl

In the experiments above, acetoin was observed in only one diacetyl solution, (73% VG, 25% water, 2% nicotine) at one time point. The level detected, 5.1 μg/mL, i.e. just above the LOQ (Supplementary Table S9), was independent of the added diacetyl concentration. While this is likely to be an experimental artefact, an experiment was conducted to test for the potential formation of acetoin from diacetyl. As the conversion of acetoin to diacetyl occurs faster in alkaline pHs, and we saw no evidence for acetoin formation from diacetyl in alkaline solutions, we used an acidic nicotine-free solution to which 1000 μg/mL diacetyl was added. The data are shown in Supplementary Table S13. Although there appeared to be a slow, small decline (~10%) in diacetyl content over the timescale of the experiment no acetoin was observed with an experimental sensitivity of around 0.5% conversion. From these data we conclude that diacetyl is not a source of acetoin in e-liquids.

3.7. Impact of effective pH – sodium hydroxide experiments

In these pH experiments, nicotine was replaced in the liquid formulation by an equivalent quantity of 10 M sodium hydroxide (NaOH) solution. The aims were to distinguish between a pH effect as opposed to a nicotine-specific response in the diacetyl formation reaction, and also to determine if these reactions were accelerated further in higher effective pH solutions. The NaOH containing solutions had higher pH's than the nicotine solutions, raising effective pH values from 8.32 to 11.36–12.66. As these solutions are not directly relevant to commercial e-liquids we have provided the data in Supplementary Table S14 and Supplementary Figs. S8a–c (acetoin) and S9a-c (diacetyl) and briefly summarise the findings, but do not report the data here. Addition of sodium hydroxide accelerated the loss of acetoin, and increased the level of diacetyl at a faster rate than seen with nicotine solutions. Very similar trends were observed regarding the influence of water, VG and PG on diacetyl production to those seen in comparable experiments with nicotine-containing solutions.

3.8. Diacetyl production under commercial e-cigarette storage conditions

To establish the relevance of these controlled experiments to real-world e-liquid environmental factors, experimental nicotine-containing e-liquids were developed containing flavour-grade acetoin at 200 ppm. Samples of the liquid were stored in either standard sealed commercial e-liquid bottles, or injected into two types of empty commercial cartomisers, a cigalike cartomiser and an ego-style cartomiser, and analysed after storage in a dark room at room temperature for periods between 2 and 18 months.

Table 5 presents the results of these analyses. Acetoin-free e-liquid samples were found to be free from diacetyl on manufacture and after 7 months storage. In the bottled e-liquids measurable diacetyl content, and lower than applied acetoin content, was found after 2 months (17.1 μg/mL diacetyl, 162 μg/mL acetoin), after 15 months (2–67 μg/mL diacetyl and 61–131 μg/mL acetoin) and after 17 months (61.9 μg/mL diacetyl and 48.1 μg/mL acetoin). Loss of acetoin and production of diacetyl were enhanced by the presence of nicotine in the e-liquids. The concentration of diacetyl was 3–4 times higher after 17 months than after 2 months in nicotinic e-liquids. The short-fall in the combined masses of diacetyl and acetoin compared to the mass of added acetoin was greater after 17 months than after 2 months, implying the operation of additional progressive chemical reactions of these compounds.

After 2 months storage in cartomisers the acetoin-bearing e-liquid also developed levels of diacetyl whilst losing acetoin (38–43 μg/mL diacetyl, 119–120 μg/mL acetoin). After 15 months there were greater acetoin losses and diacetyl production when nicotine was present ($p < 0.05$). Conversion of acetoin to diacetyl was consistent for the two cartomisers, despite differences in [nicotine], but more pronounced than found in bottles at the same storage time. This may reflect greater atmospheric oxidation as the cartomisers were not as air-tight as bottled liquids.

4. Discussion

4.1. Stability of acetoin, diacetyl and acetyl propionyl in e-liquids

All three compounds were found to be reactive in e-liquids, particularly those containing nicotine. The relatively slow reactivity of the hydroxyl-ketone acetoin in these e-liquid solutions contrasted with the greater reactivities of the dicarbonyls diacetyl and acetyl propionyl; diacetyl was approximately an order of magnitude more reactive than acetoin, and acetyl propionyl approximately 7 times more reactive than its smaller homolog diacetyl. These observations suggest greater reactivity of carbonyl groups than hydroxy groups in these molecules.

The significantly greater reactivity of acetyl propionyl compared with diacetyl suggests that the additional CH_2- group is an important contributor to reactions of this compound either directly or possibly through a more favourable cyclic intermediate state in the reaction. The product data sheet provided with the high purity acetyl propionyl sample used in these studies identifies acetyl propionyl as air and light sensitive and is supplied under argon to stabilise it. Despite these precautions the compound is provided with a shelf-life by the manufacturer. Our observations of acetyl propionyl reactivity in e-liquids is consistent with these intrinsic properties of the compound.

The stability of these compounds appeared to be strongly influenced by e-liquid composition. The most important factor for the stability of all three compounds was effective solution pH, with reactivity increasing as nicotine level increased, or effective pH was raised. Increasing the water concentrations of basic e-liquids caused increasing losses of acetoin at all sampling times over a 21-day period, but the stability of the dicarbonyls was not impacted significantly by changes in water content from 0 to 25%. Diacetyl showed little sensitivity to VG or PG levels except at high effective pH levels when increasing PG levels were found to accelerate its removal. A similar effect was found with acetoin at high effective pHs, but in nicotinic solutions lower levels of acetoin were found in higher VG solutions, an effect related to initial excess acetoin levels discussed in Section 3, below. Acetyl propionyl stability also appeared lower in high VG nicotine containing solutions.

These changes in acetoin, acetyl propionyl and diacetyl stability in e-liquids of differing composition are complex and can be explained through the operation of several mechanistic factors. First, reaction rates may be influenced by the viscosities of the e-liquids. At 300 K the absolute viscosities of VG, PG and water are 950 cP, 42 cP and 0.89 cP respectively (Engineering Toolbox). Most diffusion-controlled reactions are slowed by increasing viscosity. Alternatively, compound stabilities may be affected by the different solvation activities of the glycols and water, an effect likely to be found particularly in high glycerol/low water systems. Water activity measurements (data not shown) do tend to show a suppression of water activity in high glycerol/low water formulations. Third, chemical reactions between acetoin, acetyl

Table 5
Acetoin and diacetyl analyses of e-liquids stored under commercial conditions for 2–17 months.

Sample Details	Initial acetoin content of e-liquid (μg/mL)	[Nicotine] (mg/mL)	Storage time (Months)	Acetoin content (μg/mL)	Diacetyl content (μg/mL)	Total Acetoin and Diacetyl content (μg/mL)
Bottled e-liquid with no added acetoin	0	12	7	ND	ND	ND
Bottled e-liquid	200	12	2	162	17.1	179
Bottled e-liquid	200	0	15 ± 1	131	2.03	133
Bottled e-liquid	200	6		61	52.6	113.6
Bottled e-liquid	200	12		75.5	66.8	142
Bottled e-liquid	200	12	17	48.1	61.9	110
Ego cartomiser	200	12	2	120	38.0	158
Ego cartomiser	200	0	15 ± 3	160	4.91	165
Ego cartomiser	200	6		65.2	42.6	108
Ego cartomiser	200	12		28.8	54.5	83.3
Cigalike cartomiser	200	36	2	119	42.7	162
Cigalike cartomiser	200	36	18	< 2.1	17.2	> 17.2, < 19.3

Acetyl propionyl was also analysed for in these experiments but was not detected in any sample.

propionyl, diacetyl and the glycols are possible, leading to the formation of ketals. It is challenging to understand the relevance of these possible mechanisms with the existing database, and further investigation of these mechanistic factors is warranted.

4.2. Formation of diacetyl from acetoin in e-liquids

Our results have shown clearly that acetoin is a source of diacetyl in e-liquids during storage; this was a continuous process observed both in controlled experimental studies as well as in experiments reproducing commercial e-liquid storage conditions for 18 months. These findings demonstrate that the presence of diacetyl would be a long-term consequence of including acetoin in e-liquids. Our results ruled out the possibility that the observed diacetyl was a contaminant in the flavour ingredients, with strong, positive, time-dependent correlations between initial acetoin concentration and diacetyl formation. Our study also demonstrated that the reaction is not reversible under the conditions examined. Focusing on the production of diacetyl from acetoin, we identified formulation factors (effective pH, water concentration and relative levels of VG and PG) that affected the formation of diacetyl, as well as a physical factor, light. These are discussed in turn below.

4.2.1. Formulation factors

Comparatively low levels of diacetyl were observed in nicotine-free (and NaOH-free) solutions, but it is notable that it was still produced from acetoin. Incorporating nicotine increased the rate of diacetyl formation significantly, which was significantly faster with NaOH (effective pH's around 12) than solutions containing nicotine (effective pH's around 9), which were in turn significantly faster than solutions containing no basic component (effective pH's 7–8). Adding organic acids to nicotine solutions decreased the rate of production. Changes in nicotine content from 1 to 5% had relatively minor impact on both levels of diacetyl production and solution pH. From these results we conclude that it is increasing effective pH rather than nicotine itself that accelerates the production of diacetyl from acetoin. Slight decreases in diacetyl content at the highest nicotine contents may point to similar pH mediated reactions of diacetyl in these liquids.

Increasing the water concentrations of nicotine-containing e-liquids caused modest increases in diacetyl concentration after 1 and 9 days as water increased, but there were losses of diacetyl with increasing water after 21 days. In NaOH-containing solutions there was also little clear impact of water level. Two sets of experiments showed higher diacetyl levels in nicotinic-solutions containing equivalent PG/VG ratios than solutions containing either VG/water/nicotine or PG/water/nicotine. Effects of PG/VG ratio were also observed in the NaOH experiments. The reason for the significant differences in diacetyl production rate as PG/VG is changed is not immediately apparent, but may point to the operations of some possible reactions of PG and/or VG with these compounds; this is discussed further below.

These observations are consistent with the observations above, of factors impacting the individual stabilities of acetoin, acetyl propionyl and diacetyl. A major mechanistic factor in these base-accelerated reactions is the relative reactivities of acetoin and diacetyl in e-liquids leading to the development of an apparent steady state concentration of diacetyl in some of the experiments, consistent with the operation of consecutive reactions of these species. The combination of slower reactivity but higher concentrations of acetoin being balanced in these experiments by the higher reactivities but lower concentrations of diacetyl.

4.2.2. Light

Light had a small but significant effect on diacetyl formation from acetoin. When adsorbed onto silica gel, acetoin and diacetyl have been reported to be degraded by sunlight. For example, Simmons & Hendricks found that exposure to 3 hours of direct sunlight resulted in losses of more than 30% of acetoin and 90% of diacetyl [Simmons and Hendricks 2008). They also determined their stabilities in solution by storing diacetyl and acetoin extracts in amber or clear glass vials on an auto-extractor. Acetoin concentrations were stable over a period of 9 days in both the clear and amber vials. Diacetyl was also stable over 9 days in the amber vials but there was a 40% drop in concentration after storage in the clear glass vials. These findings are consistent with our experimental results, in which exposure to light resulted in ca 20% lower diacetyl levels in a clear vial compared to an amber vial.

4.2.3. Literature reports of diacetyl generation by acetoin

Formation of diacetyl from acetoin has been observed previously in other systems, and is the basis of the Voges-Proskauer reaction used to estimate acetoin in bacterial cultures [O'Meara 1931). In its simplest form this reaction involves the oxidation of acetoin, produced from glucose in the bacterial culture, by atmospheric oxygen in the presence of a strong base (typically potassium hydroxide) to form diacetyl. The presence of diacetyl is indicated by its reaction with a chromophore to produce a coloured product.

Diacetyl formation has also been reported during steam distillation of acetoin in the presence of air (White and Wainwright, 1975), and during vapour pressure measurements on acetoin under air or even under nitrogen or carbon dioxide (Efron and Blom 1947). Pendergrass (2004) found that samples of acetoin dissolved in acetone:methanol (99:1) were gradually oxidised to diacetyl during storage in the dark at

room temperature. After 7 days stored in the dark at ambient temperatures 6.2% of the acetoin was lost and diacetyl levels increased from 0 to 6.5%. Further storage for up to 30 days resulted in 16.4% loss of acetoin and diacetyl levels had increased to 17%, although by that time the acetoin losses appeared to be levelling off. The author attributed this to oxidation by atmospheric oxygen.

In the present study, the storage conditions of the e-liquids are similar to those reported for the standard solutions of acetoin studied by Pendergrass (2004). We also found that the oxidation of acetoin is accelerated by addition of alkali, which is required for the Voges-Proskauer reaction. Hence it seems probable that a similar reaction is occurring in the e-liquids containing acetoin. Under this hypothesis acetoin undergoes a keto-enol tautomerism at high effective pH and is then subject to oxidation. Experiments comparing diacetyl generation in cartomisers to those in sealed e-liquid bottles showed higher levels of diacetyl in the cartomisers, consistent with greater availability of atmospheric oxygen.

4.3. Other reactions involving acetoin

4.3.1. Acetoin's chemical forms

Acetoin, with a melting point of 15 °C, is a colourless to pale yellow liquid at room temperature and is miscible with propylene glycol, water and ethanol (Haynes 2011; Food Chemicals 2010). Acetoin has two structural isomers. The predominant isomer is the keto form although it can also exist in a cyclic configuration as 2,3-dimethyl-2-oxiranol (Fig. 1b). Acetoin also forms solid dimers, predominantly 1,4-dioxane-2,5-diol, 2,3,5,6-tetramethyl- (Fig. 1c), which is a white, odourless powder with a M. Pt. of 90 °C (Stivers and Washabaugh 1993). The dimer is soluble in hot propylene glycol and slightly soluble in weak alkali (Food Chemicals 2010). Monomeric acetoin, when left standing, slowly converts to the dimer, and commercially pure acetoin is usually a mixture of the monomer and dimer. The dimer has also been reported (Food Chemicals 2010) to exist as the isomer, 1,2,3,4-tetramethyl-1,2,3,4-cyclobutanetetrol (Fig. 1c).

4.3.2. Initial increases in acetoin and diacetyl contents

There were also surprising increases in acetoin content (over and above the amount added) post-formulation. The level of addition of acetoin in most experiments was approximately 1000 ppm. We would therefore expect acetoin analyses of about 1000 ppm at the start of the experiments followed by a gradual reduction as diacetyl or other reaction products are formed. In studies with e-liquids containing NaOH this behaviour was observed (Supplementary Fig. S8a). Surprisingly, however with both nicotine-free and nicotine containing e-liquids, where acetoin removal was slower, initial increases in acetoin were found in a majority of experiments over the same time period (Supplementary Figs. S2a, S3, S4a and S5). Greater levels of excess acetoin were found in formulations containing equivalent levels of PG and VG, with low/zero water contents. Some of these formulations are those in which formation of diacetyl was slowest.

An apparent lack of change in acetoin concentration over time may in part be due to the low extent of oxidation of acetoin to diacetyl compared with the experimental uncertainty involved in acetoin analysis. However, the apparent increases in acetoin concentration, in some cases by 30–60%, are harder to account for.

As described in the methodology and results sections there were small losses of the internal standards, 2,3-butanedione-d_6 and pentanedione-d_5 in some experiments, but insufficient to explain the increases in acetoin. Moreover, the observed increases in acetoin content were progressive with time, with some formulations showing increases even up to 140 days (Tables 1–3), whereas the internal standard was introduced to the e-liquid matrix only on each day of analysis and should show consistent time-based errors if this mechanism operated. Also, deuteron exchange occurs most easily in alkaline solutions, whereas some of the biggest increases were seen in solutions containing no basic constituents and, in contrast, during experiments with NaOH-containing liquids the initial acetoin increases were small. For this reason, e-liquids were also analysed by HPLC, using no internal standard. Increases in acetoin content beyond the level added were also observed in these experiments (Table 4, Supplementary Tables S6, S7 and S14). These observations show that internal standard loss is not a significant source of the observed increased acetoin content.

A third possibility could be the slow degradation of acetoin dimers in solution. As noted above, commercially available pure acetoin is usually a mixture of monomers and dimers, and the presence of dimers increases on standing. The ratio of monomer to dimer in the acetoin used in these experiments is unknown. Although the dimer dissociates to the monomer when in solution the rate of this dissociation is not clear. Some authors have claimed that dimer solutions must be boiled for more than 25 minutes (Langlykke and Peterson 1937) to completely dissociate the dimer. Other studies state that dissociation is only complete in aqueous solvents after 24 hours. If dimer exists in the extract when the first analyses are performed (at time 0) then the monomer concentration will be under-reported and will apparently increase with time as more dimer dissociates, consistent with these experimental observations. Similar arguments can be made for the conversion of the cyclic configuration of acetoin, 2,3-dimethyl-2-oxiranol (Fig. 1), to acetoin if such a conversion is compatible with the chemical environments of an e-liquid.

In the diacetyl stability experiments higher than added diacetyl levels were observed. The data-sheet for the high purity diacetyl used in this study described it as a mixture of monomer and dimer, consistent with the proposed mechanism of monomer formation from dimer in these experiments. In contrast, the acetyl propionyl used in these experiments was supplied as monomer and did not show any evidence for excess content in these experiments. We therefore conclude that in e-liquids dimeric acetoin and diacetyl convert to monomeric forms of these compounds.

4.3.3. Alternative reactions of acetoin

The mass-balances for the combined acetoin + diacetyl levels in the liquids show significant levels of unaccounted-for acetoin. We found that even in the early part of the reaction where diacetyl increases linearly (i.e. further reactions of diacetyl are slow), the mass losses of acetoin did not balance with the increase in diacetyl, implying other reaction pathways for acetoin in e-liquids. Acetoin as well as diacetyl and acetyl propionyl have ketone groups that can potentially undergo reversible and irreversible addition reactions with VG and/or PG to form hemiketals and ketals. Acetoin for example forms a dioxolane with propylene glycol (Fig. 1d). These reactions are acid catalysed, and favoured at low pH, but may well contribute to loss of acetoin in non-acidic e-liquids over the relatively long time periods of these experiments.

There was no evidence from experiments investigating the stabilities of diacetyl and acetyl propionyl that acetoin was formed from these species, either in the acidified, neutral, or base-treated e-liquids. These observations rule-out the possibility that the formation of diacetyl from acetoin is reversible.

4.4. Other reactions involving acetyl propionyl and diacetyl

After diacetyl was added to basic e-liquids its concentration declined rapidly, this behaviour was also seen for acetyl propionyl when it was added to basic e-liquids. Moreover, when diacetyl was formed by the oxidation of acetoin, it was seen to undergo subsequent reactions which result in concentrations plateauing and then dropping over time.

Probably the greatest contributor to diacetyl and acetyl propionyl losses is dimer formation. Diacetyl and acetyl propionyl rapidly form dimers in aqueous alkaline solutions via an aldol condensation. These dimers can rearrange to form various stable molecules and, in the case of diacetyl, reported products have included 2,4-dihydroxy-2,4-

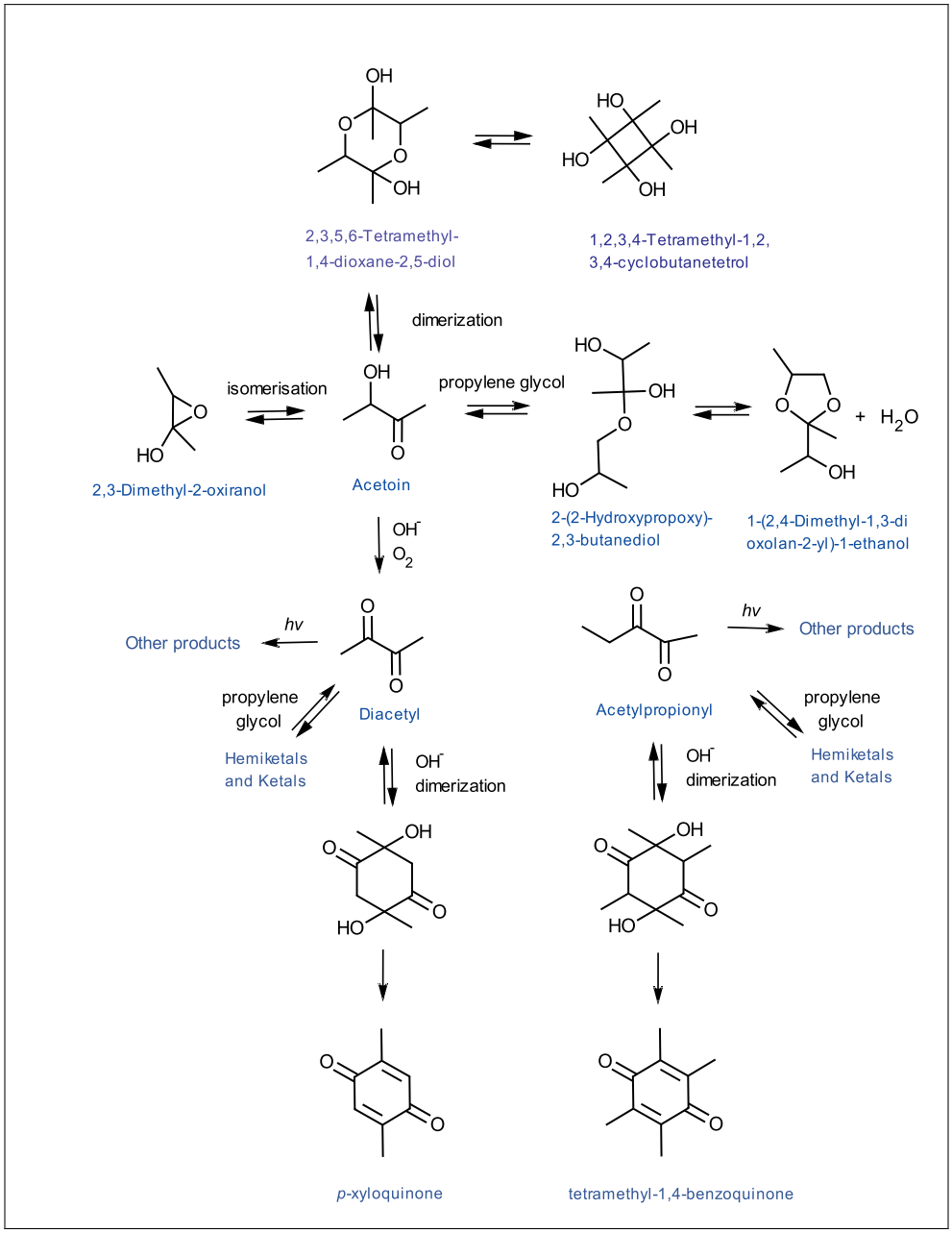

Fig. 5. Proposed reaction mechanisms for acetoin, acetyl propionyl and diacetyl in e-liquids.

dimethyl-5-oxohexanoic acid, 2-butene-2,3-diolacetic acid, acetic acid and p-xyloquinone (O'Daniel and Parsons 1943; Birch and Moye 1957; Machell 1960). This is also supported by the observation that acetoin, diacetyl and acetyl propionyl are all more stable in less alkaline solutions. Finally, as noted above diacetyl and acetyl propionyl are light sensitive and may react to other products.

Integrating all of these observations leads to the proposed global reaction mechanism for acetoin, diacetyl and acetyl propionyl chemistry in e-liquids shown in Fig. 5.

4.5. Acetoin, acetyl propionyl and diacetyl contents of commercial e-liquids

The only literature study that specifically measured levels of diacetyl in commercial e-liquids was that of Farsalinos et al., (2015a), who conducted a seven-country study in 2014 examining 159 "sweet" flavoured e-liquids sampled from 36 manufacturers and retailers for levels of diacetyl and acetyl propionyl. Farsalinos et al. reported a median level of 29 μg/mL found for 110 diacetyl-containing e-liquids with an IQR (inter-quartile range) of 10–170 μg/mLL (Farsalinos et al., 2015a). The authors also found acetyl propionyl in 53 samples. In comparison, in our studies on model e-liquids under laboratory conditions the average levels of diacetyl arising from acetoin were 20 μg/mLL with an IQR of 4–34 μg/mL. Therefore, despite the very wide range of experimental conditions examined in our study, our diacetyl data are consistent with the measured real-world contents reported by Farsalinos.

Two subsequent studies have examined aerosol concentrations emitted by e-cigarettes. Allen et al., (2016) sampled 51 e-liquids from the USA, and detected diacetyl in the aerosols of 39, acetyl propionyl in 23 and acetoin in 46. Allen et al., (2016) showed that many diacetyl containing samples (range of diacetyl levels from LOQ to 239 μg/e-cigarette) also contained acetoin (LOQ-529 μg/e-cigarette). Only 3 of the 51 flavours had detectable levels of diacetyl in the absence of acetoin. With the exception of one flavour (Peach Schnapps with high levels of both diacetyl and acetoin) the levels of diacetyl in the

remaining 47 flavours are consistent with the formation of diacetyl from acetoin. On average, the levels of diacetyl found in these represented about 7% of the acetoin levels, similar to levels observed in this work.

More recently, in a study of aerosols generated from 24 e-cigarette flavours (Klager et al., 2017), diacetyl and acetoin were the most prevalent aerosol flavouring chemicals identified, with at least one of these two flavours found in more than 80% of the samples, and acetyl propionyl was found in 20% of the aerosols. A significant correlation between the measured levels of acetoin and diacetyl led the authors to conclude that they are used widely as a flavour combination. However, examination of their data shows that in every sample in which both acetoin and diacetyl were present the acetoin levels were substantially larger than the diacetyl levels – consistent with the findings of the current study.

Hence, for almost all of the e-liquids measured in these two recent studies, it is entirely possible that in the majority of cases diacetyl was not added to the e-liquids but was formed in situ from acetoin. This would also explain Allen et al.'s comment that "two companies explicitly stated that their products do not contain diacetyl in written communication, yet in our testing we did find diacetyl in their products".

Acetyl propionyl was identified in the aerosols of 23 of the 51 e-liquids measured by Allen et al. Only 19 out of 51 aerosols had both acetoin and acetyl propionyl compared with 34 that had both acetoin and diacetyl. These results are also consistent with our findings that acetoin is not a precursor to acetyl propionyl. In the study of Klager et al. (2017) acetyl propionyl was found in five e-liquids, all of which also contained acetoin. None of the three studies disclose the chemical composition of the formulations investigated. In interpreting the presence of acetyl propionyl in these commercial liquids in the light of our findings as to its reactivity in e-liquids, we note that in our experiments, acetyl propionyl was most stable in high glycerol, zero or low nicotine liquids, which may reflect the compositions of the commercial e-liquids in which acetyl propionyl was found.

Our observations of sustained diacetyl production from acetoin in e-liquids over an 18-month time period is relevant to the findings of these product surveys. Most commercial e-liquids tend to have a designed shelf-life of between 12 and 24 months. After 18-months aging diacetyl was still present in the e-liquid and is therefore likely to be present throughout the standard product shelf-life times of e-liquids. Hence, these recent product surveys, combined with our findings, point to a sustained hazard to vapers arising not only from use of diacetyl and acetyl propionyl as flavour compounds in e-liquids, but also from acetoin. Contrary to expectations arising from the low intrinsic toxicity of acetoin, consideration also needs to be given to its potential for diacetyl generation in e-liquids post-manufacture.

4.6. Toxicity of diacetyl, acetyl propionyl and acetoin

Inhalation of diacetyl in occupational settings has been shown to cause a decline in human respiratory function (Kreiss et al., 2002; Kanwal et al., 2006; Kreiss 2007; Van Rooy et al., 2007; Kreiss et al., 2012; NIOSH 2014). Prolonged exposures to levels of diacetyl as low as 0.2 ppm were associated with development of a condition known as bronchiolitis obliterans (an irreversible obstructive lung disease), more commonly known as "popcorn lung disease". This disease was first observed in workers employed at popcorn factories, where diacetyl was used as a flavour ingredient (Kreiss et al., 2002; Akpinar-Elci et al., 2004; Kullman et al., 2005; Boylstein et al., 2006; NIOSH 2011), but it has also been identified in workers at flavour production factories (California Department of Public Health 2012; Kreiss et al., 2012; CDC 2007, 2013). Rodent studies have confirmed the toxicity of diacetyl to airway linings, with short-term exposure to 200 ppm of diacetyl being sufficient to produce severe nasal, tracheal, and bronchial injury (Hubbs et al., 2002; Hubbs et al., 2008; Morgan et al., 2008, Larsen et al., 2009; Palmer et al., 2011; Morgan et al., 2012; Goravanahally et al., 2013; Zaccone et al., 2013). NIOSH recommends keeping exposure to diacetyl below a concentration of 5 ppb as a time weighted average (TWA) during a 40-h (8 h/day) work week (NIOSH 2016). Recently evidence has emerged that the highly reactive α-dicarbonyl group is responsible for the protein damage associated with diacetyl inhalation (Miller and Gerrard, 2005, Hubbs et al., 2016). Consistent with this, evidence from animal inhalation studies has shown that acetyl propionyl, may be as toxic to the lungs as diacetyl (Hubbs et al., 2012; Morgan et al., 2012). NIOSH has established a recommended exposure limit (REL) of 9.3 ppb for acetyl propionyl on a TWA, 40-h work week basis (NIOSH 2016). This concentration was regarded as the lowest level that can be reliably quantified.

In contrast, acetoin appears to be associated with significantly lower toxicological risk when inhaled (NIOSH 2015b). First, it is a hydroxylketone rather than an α-dicarbonyl compound. Second, short-term (13 week) inhalation of acetoin at 800 ppm by rodents did not show significant respiratory tract changes, in contrast to the significant changes observed with much lower doses (25 ppm) of diacetyl under similar conditions. NIOSH has not established a safety limit for exposure to acetoin. However, beyond any primary end-points associated with acetoin exposure, our findings also point to the need to consider indirect risks i.e. the development of diacetyl in the e-liquid and subsequent exposure during vaping. Our results show that diacetyl concentrations in e-liquids can rise over time to a significant percentage of the initial acetoin content and, given that there are few constraints on the amount of acetoin that manufacturers can add to e-liquids, its use stands as an additional and avoidable hazard to vapers.

In terms of quantitative risk assessment, Farsalinos et al., (2015a) estimated the daily exposure to diacetyl and acetyl propionyl for vapers of various e-liquids. Using published average respiratory rates and tidal volumes, Farsalinos et al. calculated that daily amounts of inhaled diacetyl and acetyl propionyl that corresponded to the respective NIOSH limits (5 ppb, 18 μg/m^3 for diacetyl and 9.3 ppb 38 μg/m^3 for acetyl propionyl) was 65 μg for diacetyl and 137 μg for acetyl propionyl. Use of median diacetyl levels of 29 μg/mL (10–170 μg/mL) and median acetyl propionyl levels of 44 μg/mLL (7–172 μg/mL) in the liquids they analysed, assuming 3 mL e-liquid consumption per day, 100% transfer of diacetyl or acetyl propionyl in the e-liquid to the vapour, and allowing for dilution of liquid concentrates where appropriate, they estimated median exposures of 56 μg/day (IQR: 26–278 μg/day) for diacetyl and 91 μg/day (IQR: 20–432 μg/day). While the median exposures were below the NIOSH limits, a substantial proportion of the individual liquids investigated exceeded the NIOSH limits.

The liquids investigated in the present study were experimental, and therefore caution should be exercised in extrapolating the diacetyl levels found to estimates of exposure for commercial e-liquids. However, it is important to understand whether the production of diacetyl from acetoin-containing e-liquids may pose a risk to users. In considering these potential risks, it is necessary to first estimate likely daily diacetyl exposure, which is the product of daily e-liquid consumption and diacetyl concentration in the e-liquid, and then compare the exposures to establish limits for human exposure to diacetyl.

There is currently little clarity in the scientific literature on quantities of e-liquid consumed by vapers. Farsalinos et al. (2014) reported a survey of 1900 e-cigarette consumers, where a median (interquartile range) of 3 (1–5) mL/day was identified. Anecdotal evidence from internet discussion groups suggests that e-liquid consumption of 10 mL or more has become common amongst users of sub-ohm devices (Korzun et al., 2018; Vaping Underground, 2016, E-cigarette forum, 2017). Alsoso of note, in the EU, Article 20 of the EU Tobacco Product directive (2014) stipulates an upper limit to the volume of e-liquid bottles of 10 mL, and 2 mL for the liquid capacity of e-cigarette cartridges. Daily consumption behaviours of nicotine products have been seen to follow the size of the sales unit (Digard et al., 20092009), with consumption patterns that follow either integer multiples of the sales unit or half

Table 6
Estimates of daily diacetyl exposure arising from use of acetoin-containing e-liquids stored under commercial conditions (data from the current study).

[Diacetyl] in e-liquids		E-liquid consumption		
		1 mL/day	3 mL/day	10 mL/day
	μg/mL	Estimated daily diacetyl exposure (μg/day)		
Q1	17.1	17.1	51.3	171[b]
Median	42.6	42.6	128[b]	426[b]
Q3	54.5	54.5	164[b]	545[a,b]
Maximum	66.8	66.8	200[b]	668[a,b]

[a] Estimated exposure exceeds SCOEL 8-h occupational exposure limit for diacetyl.
[b] Estimated exposure exceeds NIOSH 8-h occupational exposure limit for diacetyl.

sales unit. Given the uncertainty in consumption patterns, in developing calculations of likely diacetyl exposure from use of acetoin-containing e-liquids, we therefore used three consumption parameters to encompass likely variation in daily consumption:

a) A low-end value of 1 mL/day corresponding to the first quartile value for e-liquid consumption reported by Farsalinos et al., (2014), and also to use of half of an EU-compliant cartomiser;
b) A mid-range estimate of 3 mL, to the median value for daily e-liquid consumption reported by Farsalinos et al. (2014);
c) A high-end estimate of 10 mL/day, corresponding to self-reporting data on internet discussion groups, the volume of one EU-compliant e-liquid bottle, and an upper limit value used by Varlet et al. (2015).

To estimate diacetyl contents of commercial acetoin-containing e-liquids we used the data in Table 5, corresponding to liquid samples stored under commercially relevant conditions for the likely shelf-life of e-cigarette products. We calculated lower quartile, median and upper quartile diacetyl concentrations for these liquids and used these values to create a matrix of possible diacetyl exposures, as shown in Table 6.

In comparing the values in Table 6 to the SCOEL and NIOSH occupational exposure limits, we used a standard breathing volume is $20m^3$ over 24 h, corresponding to an 8-h breathing volume of 6.7 m^3. The SCOEL recommended occupational exposure 8-h Time Weighted Average limit is 0.02 ppm or 0.07 mg/m^3 (SCOEL 2014). Over 8 h this limit corresponds to a maximum supportable chronic exposure to diacetyl of 469 μg/day. NIOSH limits (NIOSH 2011) are lower (5 ppb, 18 $\mu g/m^3$), and an 8-h exposure period corresponds to 121 μg diacetyl/day.

Examination of the estimated daily exposures presented in Table 6 suggests that e-liquids of the kind examined in this study, stored under commercial conditions for up to 18 months may potentially lead to daily diacetyl exposures in excess of the NIOSH and SCOEL occupational exposure limits. Median or greater diacetyl concentrations of the acetoin-containing e-liquids, together with median daily consumption values led to exposures in excess of the NIOSH limit. Higher content e-liquids combined with consumption patterns being reported for higher yield e-cigarettes may exceed SCOEL limits.

In this exercise we did not compare diacetyl doses from acetoin-containing e-liquids to short term exposure limits, as this would require knowledge of diacetyl exposure during a vaping session, e.g. numbers of puffs taken per session and per-puff diacetyl emissions from the e-cigarette. These parameters are not widely available and are highly device dependent. In particular, they are likely to differ markedly between different e-cigarettes, where differences in aerosol emissions can reach an order of magnitude or more depending upon the puffing conditions and device characteristics (McAdam et al., 2019).

The use of NIOSH limits to establish safe maximum levels for vaping has been criticised from a number of aspects, mainly with concerns that these limits do not adequately represent the exposure risks associated with the inhalation patterns and extended daily timescales that occur with vaping. For example, Hubbs et al. (2015) noted that vaping inhalation patterns are very different to occupational breathing patterns; diacetyl vapor absorption models suggest that mouth inhalation of diacetyl (as occurs during vaping) would increase the dose to the deep lung when compared with nose inhalation (the predominant mode during occupational exposure) (Gloede et al., 2011). As noted above, this is the area of the lung most severely affected by diacetyl inhalation (Akpinar-Elci et al., 2004). Farsalinos et al., (2015b) and Allen et al., (2016) have pointed out that NIOSH limits are developed for occupational exposures of 8-h rather than recreational exposures with undefined time limits. Allen et al. have also noted that NIOSH limits are for healthy, adult workers, and may not be appropriate for adolescents.

A counterview to these concerns has recently been expressed by Public Health England (PHE) (McNeill et al., 2018), who noted that levels of diacetyl in e-cigarette aerosols are hundreds of times lower than found in cigarette smoke (Margham et al., 2016, Fujioka and Shibamoto, 2006). PHE also noted that cigarette smoking is not a major risk factor for bronchiolitis obliterans, and therefore concluded that the diacetyl content of e-liquid flavourings is unlikely to pose much risk to vapers.

Despite these differing viewpoints on risk, levels of diacetyl in e-liquids arising from use of acetoin is an avoidable hazard to vapers. It is fully possible for e-liquid manufacturers to formulate commercially successful products without use of diacetyl, acetoin, or acetyl propionyl, and we therefore advise e-liquids manufacturers to move away from use of these compounds.

5. Conclusion

Acetoin has been shown to be a precursor to diacetyl in e-liquids. Formation of diacetyl is accelerated by the presence of nicotine, which acts by raising the e-liquid effective pH. Other compositional and environmental factors, such as the relative levels of VG, PG and water, and the influence of light, also influence the levels of diacetyl produced, but to a lesser degree than nicotine/pH. Both acetoin and diacetyl, as well as the related di-carbonyl acetyl propionyl are reactive in e-liquids and their levels evolve over time. The chemistry of these species in e-liquids is surprisingly complex, and we have proposed a reaction mechanism to account for our experimental observations. In general, the reactivity of flavour compounds in e-liquids has received relatively little attention to date. Our study findings point to the potential for complex chemistry to operate with some flavour compounds in e-liquids, and further studies are warranted in this area.

It is increasingly clear that the use of diacetyl and acetyl propionyl in e-liquids poses an avoidable hazard for vapers. Our study shows that acetoin, whose intrinsic toxicity appears to give rise to little concern, also poses a hazard to vapers through generation of diacetyl post-manufacture. Our findings of diacetyl in liquids stored for 18 months shows that use of acetoin will lead to diacetyl production in e-liquids throughout their normal shelf lives. Consequently, use of acetoin should be viewed with the same concern as use of diacetyl and acetyl propionyl. As a result of these studies we have added acetoin to the compounds (such as diacetyl and acetyl propionyl) that we do not use in our e-liquid formulations, and we advise that other e-liquid manufactures and flavouring suppliers move away from use of acetoin as ingredient in e-liquids.

Conflict of interest disclosure

The study was funded by British American Tobacco. CV and KM were employees of BAT at the time of the study. AP is a paid contractor to BAT.

Declaration of interest

The study was funded by British American Tobacco (BAT). The authors were employed by BAT (Carl Vas and Kevin McAdam) during the course of the study. While writing the manuscript Kevin McAdam and Andrew Porter were paid Consultants to BAT.

Acknowledgements

We thank Montserrat Sanchez Pena, Gregorio Naredo, Sandra Costigan and Gareth Waters for helpful discussions and input during the course of these studies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110727.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.110727.

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