A literature survey of all volatiles from healthy human breath and bodily fluids: the human volatilome

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Abstract

This paper comprises an updated version of the 2014 review which reported 1846 volatile organic compounds (VOCs) identified from healthy humans. In total over 900 additional VOCs have been reported since the 2014 review and the VOCs from Semen have been added. The numbers of VOCs found in breath and the other bodily fluids are: blood 379, breath 1488, faeces 443, milk 290 , saliva 549, semen 196, skin 623 and urine 444. Compounds were assigned CAS registry numbers and named according to a common convention where possible. The compounds have been included in a single table with the source reference(s) for each VOC, an update on our 2014 paper. VOCs have also been grouped into tables according to their chemical class or functionality to permit easy comparison.

Careful use of the database is needed especially as a number of the identified VOCs only have level 2 - putative assignment and only a small fraction of the reported VOCs have been validated by standards. Some clear differences are observed, for instance, a lack of esters in urine with a high number in faeces and breath. However, the lack of compounds from matrices such a semen and milk compared to the breath for example could be due to the techniques used or reflect the intensity of effort e.g. there are few publications on VOCs from milk and semen compared to a large number for breath. The large number of volatiles reported from skin is partly due to the methodologies used, e.g. by collecting skin sebum (with dissolved VOCs and semi VOCs) onto glass beads or cotton pads and then heating to a high temperature to desorb VOCs.

All compounds have been included as reported (unless there was a clear discrepancy between name and chemical structure), but there may be some mistaken assignations arising from the original publications, particularly for isomers. It is the authors' intention that this work will not only be a useful database of VOCs listed in the literature but will stimulate further study of VOCs from healthy individuals. For example although this work lists VOCs reported in the literature more work is required to confirm the identification of these VOCs adhering to the principles outlined in the metabolomics standards initiative. Establishing a list of volatiles emanating from healthy individuals and increased understanding of VOC metabolic pathways is an important step for differentiating between diseases using VOCs.

Keywords

Volatile organic compounds; breath; urine; saliva; blood; milk; skin; faeces; semen.

1. Introduction

Until 2014 there had been no central compendium of volatile organic compounds (VOCs) reported from the human body, this was addressed with a review by de Lacy Costello et al [1]. This review thoroughly updates that compendium and encompasses VOCs from breath, saliva, blood, milk, skin secretions (sweat and follicle fluids), urine, faeces and is extended by the addition of VOCs from semen. In total 906 additional compounds are reported and this opens the question how many more VOCs are yet to be identified? This is very different from other *in vivo* biomolecules e.g. amino acids where it is likely there are no more amino acids to be found. Improving on the 2014 review we include the references that identify each VOC within the table. Therefore, it can now be observed if a particular compound is reported multiple times, which gives more credence to its presence. There is also greater range of sub-tables, based upon the chemical class of the identified VOCs

Only 14 VOCs were found to be reported from all matrices with a further 28 VOCs being common to 7 of the 8 matrices, this is perhaps fewer than anticipated given the large number of total VOCs identified.

The total number of compounds reported has risen since the 2014 review for several reasons. There has been a tendency for larger sample numbers and consequently larger numbers of controls highlighting differences between healthy individuals. There have also been further advances in high throughput devices, automation and pre-concentration methods. This coupled with more sensitive instruments and larger mass spectral databases has further increased the number of "new" VOCs being identified.

To prevent this review from becoming too large and unmanageable general comments will be made about the sources of some compounds, without going into significant detail. The purpose of this review remains to bring together all the reported VOCs from the healthy human body and provide the interested reader with references to the original studies.

2. Compound naming and identification

There is a huge variation in naming conventions used within the source publications. We have kept the compound names as they appear in the original references, so ethanoate and acetate etc. are both used. Frequently, this will be the name as it appears

in the NIST spectral library, but this is not always the case and both common and systematic names appear in the tables. Where different nomenclature has been used between references, the alternatives have been listed. Sometimes structural and positional isomers are not specified in a particular paper, but they are still included as a separate entry, with a comment to this effect. Stereoisomers have generally been grouped together under a single entry, particularly as it is unclear how the specified stereoisomers were identified.

Chemical nomenclature can be challenging to the non-chemist, hence the utility of using CAS numbers, which are intended to aid identification where different naming conventions are used in the original publications. CAS numbers are not infallible though e.g. the mixed (+/-) camphor has a different CAS number from (-) camphor, when they refer to almost identical compounds.

To further aid comparisons subtables have been created based on chemical class. Where a compound contains two different functional groups, it will appear in both of the relevant subtables.

Most of the VOCs reported here were identified using gas chromatography mass spectrometry (GC-MS), with library matching to aid tentative identification of the VOCs. In some earlier studies gas chromatography flame ionisation detection (GC-FID) was undertaken with standards, for measuring breath volatiles of ethanol, methanol [2], isoprene [3], and acetone.

The identification of compounds by GC-MS is often a difficult task. The VOCs reported within this manuscript have often been assigned an identity by spectral library match only, which can sometimes be misleading, particularly for isomers, especially hydrocarbon isomers. More recent work though often incorporates the use of retention indices to increase confidence in the library identification. However, the use of standards to confirm identification remains the gold standard for validating the identity of VOC metabolites.

Improved equipment, for instance two-dimensional gas chromatography combined with high resolution time of flight mass spectrometry (GCxGC-TOF-MS) is able to detect an impressive number of compounds compared to a standard quadrupole GC-MS. It brings in to question the likelihood of co-elution and the possibility of misidentification. Some compounds may not be in the NIST library or other libraries and this can be another reason for misidentification. Other compounds might be from artefacts, such as

contamination, degradation/oxidation, which can result from collection, storage, sample treatment, or measurement.

3. A comparison of the VOC compounds found in breath, saliva, blood, milk, skin secretions, urine, faeces and semen

Table 1 describes 2746 VOCs which have been identified from the healthy human body. This compares to 1840 VOCs identified in a previous 2014 review (de Lacy Costello et al. 2014). The numbers of VOCs found in each bodily fluid and breath are: blood 379 (additional 225 compounds vs 2014), breath 1488 (additional 616 compounds vs 2014), faeces 443 (additional 61 compounds vs 2014), milk 290 (additional 34 compounds vs 2014) , saliva 549 (additional 190 compounds vs 2014), semen 196 (not previously included in 2014), skin 623 (additional 91 compounds Vs 2014) and urine 444 (additional 165 compounds vs 2014). Therefore, there have been increased numbers of VOCS reported for all the sources of VOCs included in the original 2014 review, with marked increases in breath, blood, saliva and urine. We should re-emphasise that these increases probably just reflect the recent research effort in these areas. Likewise, the small number of compounds in semen is likely because, their source is just one publication. The data in Table 1 has been sub-categorised into 12 classes, which were then further sub-divided to help the observation of inter relationships between compounds.

There must be transfer of VOCs throughout the body, from the original source(s) to the final bodily fluid destination. As to whether sufficient chemical transfer occurs for detection, or whether the VOC survives the journey, through the human body is the question. Almost certainly the gut microbiome is the source for many chemicals, and sometimes there is a change of chemistry on route from the gut to e.g. the bladder. Benzoic acid for instance (which naturally occurs in most berries) found in the gut, is derivatised in the liver and excreted as the less volatile hippuric acid (benzoylaminoethanoic acid), the liver can oxidise many compounds e.g. hydrocarbons. Furthermore, esters can be biosynthesised by fatty acid ethyl ester synthases in the liver and pancreas, [4] and there are esterases in the lung etc.

Analysis of the 2014 review table showed there were only 12 compounds found in all the matrices. Three of these benzene, toluene and styrene [5] are common pollutants in the environment and are in cigarette smoke [6]**.** It should be mentioned that 25-40% of

absorbed toluene is exhaled and the remaining amount is metabolised and excreted, by oxidation to benzyl alcohol, which is then metabolised to benzaldehyde [7]. With the increase in numbers of compounds, for this review (and the addition of semen), there are still only 14 chemicals in common: ethyl ethanoate, ethanol, 1-butanol, acetone, 2-butanone, 2-pentanone, 2-heptanone, benzaldehyde, ethanal, hexanal, 3-methylbutanal, ethanoic acid, hexanoic acid and limonene. Ethyl ethanoate and ethanal are the two compounds that were not reported in the previous review. Limonene is likely to originate from the environment, it is a commonly used product in household materials, and is in food stuffs, e.g. potatoes. Benzaldehyde can originate from oxidation of toluene in the human body, toluene, is a common atmospheric pollutant. Ethanol may come from drinking alcohol; however, the gut is also capable of ethanol production, and given the significant amount of ethanoic acid in the gut, this can explain the origins of ethyl ethanoate. Hexanoic acid is likely to have its origins in the gut, although it's not clear why this particular short-chain fatty acid (SCFA) is so prevalent. 2-Ketones are certainly found in the gut [8] e.g. 2-butanone was shown to be in all the faecal samples in one study [9]. Short chain aldehydes such as hexanal can arise from peroxidation of unsaturated fatty acids [10](potentially in many parts of the body including adipose tissue), and also from oxidation of the respective alcohol.

4. Listing of all compounds, with CAS numbers, formulae and origins (Table 1)

Table 1 is an exhaustive table containing every VOC found in the healthy human body to date, across all the different bodily samples (breath, blood, faeces, urine, milk, skin, saliva and semen). Compounds are listed in alphabetical order, and appear with their CAS number assigned by the original authors, where appropriate, and chemical formula. The table rows indicate which particular sample(s) each compound has been found in, and the paper(s) which identified each volatile in each fluid are also noted using reference numbers.

While Table 1 lists all the compounds, Tables 2-12 describe VOCs according to their chemical classes, and they are further split into sub-tables where appropriate. Within the tables, the compounds are described in increasing carbon number. A brief discussion is given for the compounds included in these tables.

4.1. Nitrogen containing VOCs found in the human body (Table 2a-2c)

There are significantly more nitrogen compounds here than in the 2014 review, these compounds have been split into three sub tables: nitrogen-containing (non-heterocyclics, Table 2a), nitrogen-heterocycles (Table 2b) and nitrogen and sulfur containing compounds Table 2c). These 3 sub tables were then further divided into subgroups as follows:

Nitrogen-containing (non-heterocyclics): sundry nitrogen compounds (ammonia, nitric oxide, hydroxylamine, nitric acid), aliphatic monoamine (non-cyclic nitrogen), aliphatic di-, tri, or tetra-amines (non-cyclic nitrogen), anilines, amino acids, amines with carboxylic or sulfonic acids, amines plus other functional groups, hydrazines, azides, azo, nitriles, isonitriles, imines, isocyanates, amides, amide with other functional group, carbamates & carboxamide, ureas, carbamimidate, hydroxylamines, nitroso, oximes and amine oxides.

Nitrogen-heterocycles: azirine/ aziridine [C_2N ring] azete / azetidine [C_3N ring], pyrrolidines, pyrroline/dihydropyrroles [C_4N ring, pyrrole [C_4N ring], pyrazoles and imadazoles and other diazoles [C_3N_2 ring], pyrazoles and imadazoles and other diazoles [C_3N_2 ring], triazoles [C_2N_3 rings], tetraazoles [C_1N_4 rings], piperidines [C_5N ring], pyridines [C_5N ring], piperazines [C_4N_2 ring], diazines (pyrazines and pyrimidenes) [C_4N_2 rings aromatic], indoles, quinolene and hydroquinolines, other multicyclic CN heterocyclics, cyclic amide / lactam, oxazoles, (& oxaline, oxazolidine, isoxazole, isoxazline, isoxazolidine) [C_3NO], other CNO heterocyclics.

Nitrogen and sulfur containing compounds: At least 8 compound groupings containing both sulfur and nitrogen in the functional group were split into thiocyanate and isothiocyanate, thiazole [C_3NS ring], benzothiazoles, thiazolidines [C_3NS ring], thioamide, thiocarbamate & thiourea and others.

Nitrogen-containing (non-heterocyclic) compounds like ammonia, the simplest amine is well known to be linked to breath particularly with high protein intake. Nitric oxide has been found in breath and blood. Human paranasal sinuses and diet can affect production [11]. Hydroxylamine can be synthesised by oxidation of ammonia enzymatically e.g. by ammonia monooxygenase [12]. Interestingly nitric acid has now been reported in breath, it might be considered curious that this strong mineral acid, along with hydrochloric and sulphuric acids can be made by the human body. It is likely that the nitric acid could arise from inhalation of nitrogen dioxide atmospheric pollution, or the oxidation of nitric oxide which can lead to nitric acid synthesis.

The largest molecular weight (MW) nitrogen VOC compound detected so far is N, N-dimethyl-1-octadecanamine/ N, N-dimethyl-1-octadecylamine (20 carbons).

Many amino acids, particularly in breath have now been reported, e.g. glycine, proline, ornithine, arginine, leucine and valine.

Seven hydrazine based compounds have been reported. Hydrazine is a known rocket fuel, however there are rare pointers in the literature for hydrazine synthase enzymes, suggesting conversion from ammonia to hydrazine by bacteria can happen [13]. There are 39 nitrile (cyanide) compounds, the origin of these for instance, alkyl nitrile compounds could arise from diet by ingesting cyanogenic glycosides albeit in small quantities [14]. It has been stated that certain bacteria can make hydrogen cyanide, again confirming that bacteria may be a biosynthetic route.

There are a range of primary, secondary and tertiary amines, presumably at some stage they have been synthesised by alkylation of ammonia, it is beyond the scope of this review to attempt to assess the origins of so many diverse compounds.

There are rarely 3 and 4 membered ring cyclic nitrogen compounds, in contrast to the 21 pyrrole (5-membered) and 18 pyrazines (6-membered di-nitrogen compounds and pyridine), many of which are alkylated. Volatile pyrazines and pyridines can contribute to food flavours [15] and diet is therefore a potential source.

There are 37 nitrogen sulfur compounds, mainly found in breath. Many are thiocyanates being the hydrolysis products of glucosinolates, secondary metabolites characteristic for the family *Brassicaceae* e.g. broccoli. For instance, allyl isothiocyanate is responsible for a significant smell of cooked cauliflower. Moreover, methyl thiocyanate, butyl isothiocyanate, 2-methylbutyl isothiocyanate and other sulphides have been found in Brassica vegetables [16,17].

4.2. Sulfur containing VOCs found in the human body (Table 3)

There were 113 sulfur compounds reported (Table 3), further divided into 13 sub sections: elemental sulfur, thiols, sulphides, sulfoxides, sulfonic acid esters, sulfate esters, thioesters, thietane[C_3S], thiophene, thiolane [C_4S], thiane [C_5S], dithiane [C_4S_2], oxathole [C_3OS] and oxadithiane [C_3OS_2].

For thiocyanates, thiocarbamates, thioureas, sulfonamides and amino thio acids see Nitrogen Table 2c, also for sulfur containing heterocyclics possessing nitrogen atoms.

Many of these compounds probably arise from food and metabolic changes occurring in the body, such as de novo synthesis of glutathione and antioxidative processes in the liver.

4.3. Alcohol containing VOCs found in the human body (Table 4)

The alcohol compounds were divided into 12 sub-groups: straight chain alcohols, branched alcohols, unsaturated alcohols, cycloalkyl alkanols, phenyl alkanols, cyclohexanols, other cycloalkanols, multi-cyclic alkanols, diols, triols, pentols and phenols.

The straight chain primary alcohols were present as a homologous series (present with some gaps). From methanol to 1-eicosanol (20 carbons), there were only 2 gaps, 1-heptadecanol and 1-nonadecanol, comparing all the bodily fluids and breath. It is likely that many of the gaps would be filled by undertaking future studies, for instance 1-heptanol and 1-octanol has not yet been found in breath, but they have been identified in other bodily fluids such as faeces.

Certainly, alcohols can be made in the gut e.g. via the reduction of the respective acid [9], or by carbohydrate fermentation or fermentation of nitrogenous compounds [18]. Moreover, the liver is capable of alcohol synthesis.

Alcohols, from methanol to octanol were derived by oxidation of unsaturated fatty acids, $CH_3(CH_2)_nOH$ from n=0-7 except for propanol (n=2) omitted in the homologous series [10]. This is a likely source of saturated alcohols in all bodily fluids and breath.

4.4. Acid containing VOCs found in the human body (Table 5)

The acids (175 compounds) were divided into 8 sub-groups: aliphatic acids-saturated straight chain, aliphatic acids-branched /cyclic, aliphatic acids-unsaturated, aromatic acids, aliphatic dioc/trioic, acids which also contain an alcohol group, hydroxybenzoic acids, acids containing an aldehyde or ketone group and, acids contacting another unspecified group. Amino acids are given in the nitrogen compound table. Phenols, although very weak acids, have not been included in this group and are tabulated with alcohols.

Of the straight chain carboxylic acids, all the acids from methanoic acid to docosanoic acid have now been detected in one or more of the fluids and breath from the human body. The complete homologous series of acids from ethanoic to docosanoic acid have been found in saliva, apart from decanoic and undecanoic acid and from methanoic to

docosanoic acid in skin secretions, apart from pentanoic acid. The highest MW acid, docosanoic acid possesses 22 carbons. In all the studies, there is a threshold of around 16-22 carbon length for the VOCs reported. As to whether there are real biochemical reasons or it is a limitation of the analytical method, is an open question

As a general comment, SCFAs from methanoic to hexanoic acids have been reported as the most abundant and significant end products of fermentation in the gut. The ratio of compounds found may be dependent on individuals, which have different gastrointestinal transit times (GITT). For instance, a long GITT can have a significant effect on bacteria metabolism, more protein is broken down into amino acids which are in turn broken down into small fatty acids. Branched SCFAs arise from breakdown of branched amino acids, as opposed to straight chain SCFAs which can arise from carbohydrate metabolism (as well as other routes) [19]. A study has also shown that blood in faeces will also affect the ratio of short chain fatty acids due to the breakdown of haemoglobin [20]. Also, carbohydrate availability can affect acid type production in the gut and therefore VOCs in the faeces. Carbon limited fermentation produces more formic acid [21]. Acetic acid the main SCFA produced in the gut is readily absorbed through the colon wall and is transferred to the liver, where it is used to e.g. synthesise cholesterol [22]. It does not appear to have been detected in blood, although it must be present. Other SCFAs are rapidly absorbed into the blood stream, it is considered that only 5-10 % are excreted [22]. It must be noted that butanoic acid and to a lesser extent other SCFAs are used as an important energy source by the gut wall and the amount of these acids reaching the blood stream maybe low.

Acids can also be biosynthesised in the human body from aldehydes. Aldehyde oxidase (AO) is very concentrated in the liver, where it oxidizes multiple aldehydes [23]. AO activity has been indicated as occurring in the epithelial and alveolar cells of the lungs. There have also been indications of AO activity occurring in the kidneys and gastrointestinal tract (both small and large intestine). It should be pointed out that catalysts are not essential, air oxidation can oxidise aliphatic aldehydes into carboxylic acids [24]. A recent report, showed significant, almost 9-fold difference in nonanoic acid abundance between a lung cancer group and control group [25]. Its origins may be due to oxidative stress due to oxidation of unsaturated aldehydes [10].

Of the 32 branched acids found in total, more were found in skin secretions [18]. A commonly found acid in faeces, urine, breath, and skin secretions was 2-ethylhexanoic

acid, a common contaminant derived from plasticisers e.g. plastic tubing, containers for bodily fluids etc.

More unsaturated fatty acids were found in skin than other bodily fluids and breath. The largest chain size was for docosahexaenoic acid (20 carbons), found in breath. Oxidation of unsaturated fatty acids can produce smaller chain unsaturated fatty acids, a list of predicted mono alkene acids expected to be enhanced by oxidative stress is reported in a recent review. The origin of compounds such as 9-decenoic and 10-undecenoic acids (which have been reported from skin) can be satisfactorily explained by such a route [10]. The number of very long chain fatty acids (C-20 plus) found will undoubtedly increase in the future with increased sensitivity of analytical methods. They are present in the human body and have been linked to Refsum's disease and maybe adrenoleukodystrophy. Nervonic acid (C-24) is found in brain tissues, and higher amounts have been correlated to schizophrenia. A note of caution however, as identifying long chain fatty acids accurately can be problematic due their susceptibility to breakdown (particularly in the heat of a GC inlet port). Thus, derivatization or alternate analytical methods might be required for absolute compound identification.

4.5. Ether containing VOCs found in the human body (Table 6a and 6b)

The ethers were split into two sub-tables: non-cyclic ethers (Table 6a) and cyclic ethers (6b).

The non-cyclic ethers were further divided into five sub-classes as follows: (for ethers that contain additional non-hydrocarbon or hydroxyl functional groups see the specific table for that functional group), mono- (34) di- (11), tri- (2) and tetra-ethers (1), non-cyclic hydroxy ethers (27) and peroxides (2).

The cyclic ethers were divided into oxiranes (16), furans (21), benzofurans (3), hydrofurans (13), hydrobenzofurans (1), furanones (see listing under lactones in ester table), furans with other functional groups (22), dioxolanes [C_3O_2] (8), dioxolane with other functional groups (1), pyrans, hydropyrans (4), benzopyrans with other functional groups (for pyranones, see the ester table) (10), dioxanes (1), oxepines and oxepanes (4), cyclooxaoctane/enes (11), crown ethers (1) and multicyclic cyclic ethers (13).

Some ethers are used in cosmetics (212), and some food additives (16). Peroxidation of certain polyunsaturated fatty acids, enhanced with oxidative stress, can lead to furan generation [10]. However the confirmation of the ether origins requires more studies.

4.6. Aldehyde containing VOCs found in the human body (Table 7)

The total number of volatile aldehydes found in all bodily fluids and breath was 159, (Table 7), an increase of 56 compounds since 2014 [1]. Aldehydes were further divided into: aliphatic (16), branched aliphatics (13), 2-unsaturated (23), other unsaturated linear compounds (17), unsaturated branched (16), aliphatic cyclic (7), benzaldehyde, phenylalkyl aldehydes (23), aliphatic dialdehydes (2), hydroxyl aldehydes (22), ketone aldehydes (2), ether aldehydes (7), carboxylic acid aldehydes (9) and aldehydes with other various functional groups (7).

A complete homologous series of aliphatic aldehydes was observed, particularly for faeces, from methanal to octadecanal, with the omission of heptadecanal. Perhaps future work will report heptadecanal, or there is no biochemical route to this compound. A recent review of products of oxidative stress (oxidation of unsaturated fatty acids) summarises the origins of straight chain aldehydes from ethanal to decanal, $CH_3(CH_2)_nCHO$ from n=0-8, although there are other potential origins [10].

Of the branched aliphatic aldehydes, five 2-methyl aldehydes were reported, from 2-methylpropanal to 2-methylpentanal, then a gap until 2-methylundecanal and then 2-methylhexadecanal.

A complete homologous series of 2-unsaturated aldehydes was found between 2-propenal and 2-hexadecenal, from one or more of the bodily fluids. This is in contrast to the 2014 review, where the series only reached 2-decenal [1]. As is the case for some of the other chemical classes, more recent papers have filled in some of the previous gaps identified in the homologous series. For example, recently, 2-dodecenal has been reported in breath condensate [26].

The reported aldehydes have a cut off in molecular size around 16-18 carbons: octadecanal (18 carbons), 2-methylhexadecanal (17carbons), 2-hexadecenal (16 carbons), 4-hydroxy-2,6- hexadecadienal (16 carbons) and 4-hydroxy-2-hexadecenal (16 carbons). There are two main reasons for a lack of detection of aldehydes with higher carbon numbers namely a lack of biochemical routes, or the low vapour pressure of these compounds.

Lipid oxidation of monounsaturated and polyunsaturated fatty acids are known to produce 2-alkenals, as well as dienals, such as 2,4-heptadienal, which has been found e.g.

in milk [27]. It has been reported that 23 different aldehydes in milk can be produced by oxidative degradation of oleic, linoleic and linolenic acids [27].

With regard to branched chain saturated aldehydes, a 2020 study of Ratcliffe, *et al* [10] predicted six compounds originating from the oxidation of unsaturated fatty acids: 3-methylbutanal, 3-methylpentanal, 4-methylhexanal, 4-methylpentanal, 5-methylheptanal and 5-methylhexanal, but none were reported in the 2014 review [1]. However, 3-methylbutanal and 3-methylpentanal, have now been reported in the current manuscript. It does suggest that other hypothesised compounds will be found in future studies [10] and does highlight the importance of identifying plausible metabolic routes. for VOCs. It should be observed that 5-methylheptanal is not in the NIST library, so its identification is currently unlikely. This does highlight a potential issue with the identification of compounds which heavily relies on putative identification via current mass spectral library entries. Modern mass spectral libraries contain many thousands of compounds and are constantly updated but still contain only a fraction of the possible organic molecules which could be potential metabolites.

For mono-unsaturated hydroxyl aldehydes, a homologous series of nine 4 hydroxy-2-enals have been detected, whereas conversely in 2014 none had been reported. The lowest MW compound is 4-hydroxy-2-hexenal, then the 4-hydroxy-2-heptenal is "missing", with the last compound being 4-hydroxy-2-hexadecenal. This again provides a potential target for future analytical studies as do all the "gaps" in the homologous series within these tables. Alternatively it might highlight the need for better mechanistic metabolic studies to understand why certain VOCs may be missing. 4-hydroxynonenal in particular has been extensively reported in association with oxidative stress and lipid oxidative breakdown, especially from *n*-6 PUFAs, mainly arachidonic and linoleic acids [26,28]. To further add to the series, 4-hydroxy-2-pentenal has been found in smoker's breath using secondary electrospray ionisation- mass spectrometry (SESI-MS) [29].

The origins of a series of volatile hydroxyl, alkene aldehydes have been listed [10].

A whole series of nine 4 hydroxy-2,6-dienals has now been shown starting from 4-hydroxy-2,6-octadienal to 4-hydroxy-2,6- hexadecadienal.

With regard to aldehyde oxo-acids, a series of 6-oxohexanoic acid, 7-oxo-heptanoic acid, 8-oxooctanoic acid, 9-oxononanoic acid, 10-oxocaproic acid / 10-oxodecanoic acid, 11-oxoundecanoic acid and 12-oxododecanoic acid have been reported herein, four of which have been linked to smoking [29].

As a general comment, aldehydes are capable of oxidation to acids, by oxygen, even without the mediation of a catalyst and these aldehydes could contribute to an increase of concentration of carboxylic acids, and a concomitant decrease in aldehyde concentration.

4.7. Hydrocarbon containing VOCs found in the human body (Table 8a- 8e)

The hydrocarbons were split into five major classes: cyclic hydrocarbons (Table 8a), aromatic compounds (Table 8b), branched chain alkanes (Table 8c), alkenes (Table 8d), and n-alkanes (Table 8e).

The alkenes were split into mono alkenes and non- cyclic, branched alkenes, dienes, tri-enes, tetra-enes, penta-enes and hexa-enes and alkynes.

The cyclic hydrocarbons were split into cyclopropanes, cyclobutane and cyclobutenes, cyclopentane, cyclopentenes, cyclopentadienes, cyclohexanes and cyclohexenes, cyclohexadienes, cyclo- heptane/ heptane/ heptadiene/ heptatriene, cyclo-octane/ octadienes/ octateraenes, cyclic C10, C11, C12, C14, C16, hydronaphthalenes, hydroazulenes, other bicyclo, and other tricycle compounds.

The aromatic compounds were split into several sections: benzyl, phenyl, biphenyl, indane/indene, 1,2,3,4-tetrahydronaphthalenes/ dihydronaphthalenes, 1,2,3,4-tetrahydronaphthalenes, naphthalenes, azulenes, anthracene, and acenaphthalenes.

There is an impressive complete homologous series from methane to tetratriacontane (34 carbons) when taking into account all the bodily fluids and breath. Breath contains the majority of these compounds with the exception of docosane, tricosane, pentacosane, hexacosane and nonacosane.

Alkanes, from methane to octane (at least) can be considered to arise from oxidation of unsaturated fatty acids [10]. It is interesting that many researchers consider that the source of methane in breath is from the gut as 1 in 3 subjects possess gut methanogens [30]. However, lipid oxidation is clearly another potential source. The authors are unaware of any studies undertaken to assess methane lipid origins, in breath, although methane, ethane, propane, butane and pentane have been well described as autoxidation products e.g. from linoleic acid [31]. Straight chain aliphatic hydrocarbons have been considered as non-invasive markers of free-radical induced lipid peroxidation in liver damage, especially breath ethane and pentane, which appear to be better correlated with alcohol induced hepatic injury than to other aetiologies [25].

There were more hydrocarbons reported than any other class of VOCs, 853 in total. The origins have not been extensively considered. As a general consideration, GC-MS spectra of diesel and to a lesser extent petrol, shows the huge numbers of potential compounds present. It is possible that we are observing the human volatilome being significantly affected by the industrial world we live in (the exposome).

The human body in combination with its bacterial hosts are likely to be capable of biotransformations of hydrocarbons to a lesser or greater extent thus producing more VOCs to potentially confuse VOC biomarker discovery. There are also naturally occurring hydrocarbons in food which add to the impressive list here.

Alkenes, from ethene, and propene to decene in a homologous series and their 2-isomers, 2-pentene, 2-hexene, and 2-octene would be expected to occur by oxidation of unsaturated fatty acids [10]**.** As examples in the literature, ethene has been shown in the volatilome of humans and can be formulated from oxidation of omega-3 acids e.g. linolenic acid, by disproportion of ethyl radicals [32] and 1-pentene has been reported to be generated by decomposition of omega-6 unsaturated fatty acid hydroperoxides e.g. from linoleic and arachidonic acid [33]**.**

4.8. Ester-containing VOCs found in the human body (Table 9)

In total 305 esters have been reported. The esters were arranged into sub groups: methanoates, ethanoates, propanoates, butanoates and pentanoates, 2-methylbutanoates, 3-methylbutanoates, hexanoates, heptanoates, hexanoates, nonoates, decanoates, undecanoates, dodecanoates, tridecanoates, tetradecanoates, pentadecanoates, hexadecanoate/heptadecanoate/octadecanoate / docosanoates and tetracosanoates, ene-oates, other-oates, cyclic HC oates and benzoates, salicylates (inc. substituted benzoic acid esters), hydroxy acid esters (except hydroxybenzoic), other mono esters, lactones, delta, pyranones (benzopyranone and dioxanedione), others / uncertain cyclic esters, diesters and triesters (phthalates listed separately) and finally phthalates, carbonates and anhydrides.

Acetate (ethanoate) esters were by far the most abundant esters. This is probably reflected by the fact that acetic acid is the most common gut acid. Acetates found in breath were the major contributor to the overall total. There were many esters reported in breath which were not present in other bodily fluids. Therefore, it is not easy to say that breath ester VOCs arose from other bodily fluids.

Esters are represented from the whole homologous series from methanoate to octadecanoate, when all the bodily fluids and breath are considered. Then there is a big gap in the series to tetracosanoic acid, methyl ester. The largest ester reported, is behenyl behenate (44 carbons), which is likely to originate from its uses in cosmetics.

Bacteria present in faeces have been shown to be capable of ester synthesis [34], and it is very likely that the reaction of alcohols with the respective acid produces many esters in the gut which can then enter the blood stream and circulate throughout the body. Unfortunately for this theory, very few esters have been found in blood, but this is most likely due to the paucity of studies undertaken on VOCs in blood. There are also a variety of esters found in breath which are not found in the gut, this again could be because these esters have not yet been detected in faeces due to analytical imitations or a relative lack of studies. However, it could be that lung based esterases aid ester synthesis and explain in part why more esters have been identified in breath.

The phthalates (phthalate esters) are exclusively endogenous and probably arose from plasticiser exposure, and subsequent metabolism. There is a whole range of long chain fatty acid esters and aromatic esters found in skin, which are mainly missing from other bodily fluids and breath. This could be due to the analytical methodologies used.

If one considers that all the acids and alcohols reported here can undergo esterification it is possible to rationalise the origins of many of the esters described here. One of many interesting observations, is the lack of esters in urine, apart from lactones. Esters have low solubility in water which could explain the lack of esters in urine.

4.9. Ketone containing VOCs found in the human body (Table 10a, 10b)

The ketone table (Table 10a) was divided into a range of sub groups: aliphatic, straight chain ketones, straight chain alkene ketones, aliphatic diones, branched aliphatic ketones, alkyl phenyl ketones, alkyl cyclohexyl ketones, other aliphatic and aromatic ketones, hydroxy ketones, phenol ketones, acid ketones, and ketones with other functional groups. Table 10b presents cycloketones.

A homologous series of 2-ketones from acetone (propan-2-one) to 2-nonadecyl ketone (19 carbons) was reported herein. In contrast, the 2014 review described a homologous series which went from acetone to nona-2-one [1].

Acetone was found to be one of the most reported volatiles from the human body and is well known to be produced by fatty acid breakdown whereas 2-butanone derives from

carbohydrate metabolism. Methyl ketones are produced by many species of bacteria and can also be produced by fungi.

The carbonyl group in ketones was found in different positions, in 2, 3, 4, 6 and 8. This is quite selective when compared with the options available. Substitution in the 2 position was by far the most common class of ketone.

4.10. Halogenated containing VOCs found in the human body (Table 11)

All the halogenated compounds were separated into 6 sub-sections: fluorinated compounds (16), chlorinated compounds (35), alkenyl & benzyl chloro-compounds (19), bromo-compounds (8), iodinated compounds (6), mixed halogen and halogen plus other hetero compounds (17), chlorinated biphenyls and chlorinated and brominated phenol compounds (43).

Most of the fluorinated compounds were discovered in breath. Sevoflurane was listed: this is a sweet-smelling, non-flammable, highly-fluorinated methyl isopropyl ether is used as an inhalational anaesthetic, and its occurrence in breath of healthy humans is presumably because of the clinical environment where the breath was collected. 1,1,2-trichloro-1,2,2-trifluoroethane / Freon 113 is used as an electrical cleaning agent and is likely to have come from the environment.

With regard to chlorinated compounds, some are solvents. Vinyl chloride originates from PVC and some can arise from chlorinating water.

Dibromomethane occurs naturally in small amounts in the ocean where it is formed, most likely by algae and kelp. This and similar brominated compounds can enter the food chain and hence reach humans via the diet. It may also still be used for the fumigation of stored grains, fruits, and vegetables [35].

Volatile iodine compounds, such as methyl iodide, ethyl iodide, chloroiodomethane, diiodomethane (CH_2I_2) and bromoiodomethane are widely detected over oceans, where the biogenic activity of phytoplankton and macroalgae are likely to be an important source of these VOCs. Presumably, these types of compounds can also enter the human food chain [36].

Many chlorinated fluorinated compounds (CFCs) have been used, especially in the past as refrigerants, propellants in aerosols and solvents. As these are being phased out in consumer products, they and their degradation products must be originating from the

environment. Dibromochloromethane and bromodichloromethane also have environmental origins [37].

A large number of chlorinated biphenyls and chlorinated and brominated phenol compounds were found such as 4-hydroxy-2,2',3,4',5'-pentachlorobiphenyl which was found in blood and no other bodily fluid.

4.11. VOCs found in the human body not categorised previously (Table 12)

Table 12 shows compounds not categorised in Tables 2 to 11, encompassing carbon dioxide, carbon monoxide, hydrogen, hydrogen peroxide dimethylselenide and tetramethyl-germane all reported in breath.

5. DISCUSSION

Discussion of the VOCs reported in breath, saliva, blood, milk, skin secretions (sweat and follicle fluids), urine, faeces and semen.

5.1. Volatile organic compounds in breath

Exhaled breath contains many different volatile compounds. It has been stated previously that a total of more than 1000 VOCs can be observed, even though they are not present in each person studied [38]. Our literature search revealed 1488 named volatile compounds as being related to exhaled breath. More than half of the screened papers used gas chromatography mass spectrometry (GC-MS) to quantify VOCs in breath, confirming this instrument as the gold standard technique for the analysis of this biological matrix. In most of the papers, GC-MS is typically used in combination with thermal desorption (TD) sorbent tubes to collect and analyse breath.

The most used direct sampling techniques are proton transfer reaction mass spectrometry (PTR-MS) and selected ion flow tube mass spectrometry (SIFT-MS) used in 25 % of the screened papers. The absence of chromatographic separation in direct sampling techniques however can only tentatively identify the VOC molecular structure, and generally those assignments are confirmed with GC-MS [39] or in the case of breath condensate, with UPLC-MS [40–43].

However, in the last five years, a new direct sampling technology, named secondary electrospray ionization (SESI) has been increasingly applied in breath research and is opening new avenues in the field. Since it is based on electrospray ionization of the VOCs,

589 it is able to ionise previously difficult to detect compounds, by covering higher molecular
590 weight, less volatile and more polar species which are not easily analysed with GC
591 approaches [29,41,42,44–47]. While it lacks chromatographic separation and often forms
592 ion adducts (e.g. $M+Na^+$) due to the electrospray ionization, the use of high resolution
593 mass spectrometers with multi-stage (MS^n) capabilities partially counterbalances the
594 aforementioned limitations [48].
595 Many of the volatile compounds related to exhaled breath are not endogenously
596 produced, and some compounds appeared only in a few individuals. The list reported in
597 our table of VOCs is considered as a list for discussion, and we do not consider it
598 comprehensive.
599 Water, oxygen, nitrogen, argon and other rare gases are not listed in this table. For many
600 of these compounds it is unknown if they are produced endogenously. Among the
601 compounds which are listed as appearing in exhaled breath (Table 1), many are related
602 to smoking e.g. 29 dienes, 27 alkenes and 3 alkynes are mentioned as smoking-related
603 [1]. If you smoke it has been stated that your breath contains 2,5-dimethylfuran. A team
604 of Catalan researchers have proved that the presence of this chemical compound
605 indicates that a person has smoked in the last three days and they state that this
606 substance does not appear in the breath of non-smokers, unless they have been in direct
607 contact with tobacco smoke for a long time [49].
608 More recent work of exhaled breath from healthy volunteers, divided into three groups
609 (non-smokers, ex-smokers and smokers) showed that nonanal concentration was
610 dependent on smoking, but was independent of the amount of tobacco consumed, age
611 and gender [50]. A targeted analyses studying healthy smokers showed that acetonitrile
612 is readily detected by SIFT-MS in the breath and urinary headspace of smokers at levels
613 dependent on the cigarette consumption, but is practically absent from the breath and
614 urine headspace of non-smokers, see some further references re breath and smoking
615 [51–57], which also describe various furans. This is not to say that these compounds arise
616 only in smokers, but that they show higher concentrations in them.
617 Quite a number of volatile compounds may be related to food consumption, medication
618 (or effects of) or professional exposure [58–61]. Some of the compounds in breath are
619 produced by bacteria in the mouth [62] and by bacteria in the gut, such as hydrogen [63]
620 and methane [64] and undoubtedly many more. It could very well be the case that

volatiles from oral anaerobes in the mouth confound breath biomarker discovery and this has been studied [9].

The most prominent volatile compounds in breath are isoprene [65–68] and acetone [68–70]. Isoprene, identified and quantified in more than half of the papers analysed for this review, is a by-product of the mevalonate pathway, but also produced (or at least stored) in the periphery of the human body [71,72]. Acetone can be formed from acetoacetate by acetoacetate-decarboxylase. Isoprene is 'the' paradigmatic example for a compound whose concentration in exhaled breath changes enormously during exertion of an effort [71,73–75]. If, for example, a volunteer starts to pedal on a stationary bicycle with 75W, the isoprene concentration increases by a factor 3–4 in end tidal breath. Originally, it was thought that this increase is just due to an increase of cardiac output [76]. But the pioneering work of King *et al* [71–73,75] demonstrated that the increase in cardiac output alone would not be able to lead to the observed pronounced increase in isoprene. For the isoprene concentration in exhaled breath to increase, it is not even necessary to exert an effort. A few leg contractions or arm contractions suffice to increase the isoprene concentration in exhaled breath [71–75]. Apart from isoprene, also other compounds increase during exertion. Among these compounds are methyl acetate, dimethylsulfide and 2-pentanone [74]. This is in contrast to the prediction of Farhi's equation [77], which would predict a decrease in concentration during effort. An example of a compound which follows Farhi's equation is butane [74].

The big advantage of exhaled breath, in comparison to blood, is the fact that it can be sampled as often as is desirable. Breath can even be sampled and analysed in real time, down to breath-to-breath resolution. Breath analysis during sleep illustrates this most convincingly [78]. In measurements during sleep, isoprene and acetone display very different concentration characteristics. Both show (often) increasing concentrations during the night. The isoprene concentration displays a very pronounced peak structure, which is due to movements of the body or changes in sleep stage. Acetone does not show such a peak structure but just a smooth increase.

In contrast to GC-MS and SESI-MS, a more limited number of volatile compounds in exhaled breath have been investigated with PTR-MS [79-83] and SIFT-MS [84-86]. These techniques are inherently quantitative, without the need of external calibration which greatly expands their real-time measurement capabilities. More recently they have been coupled with thermal desorption units, to enable sample collection and later analysis for

large-scale studies [87]. In the future, real-time measurements should be performed for all VOCs, giving rise to the possibility of modelling their production and metabolism within the human body. Also their connection to food consumption, smoking habits or medication would be very interesting. A particular interest is in therapeutic monitoring of drugs and their metabolites. As an example, consider valproate which is administered to avoid seizures in epileptic patients or in persons suffering from propionic acidemia [58] and is metabolized to 3-heptanone which can be observed in exhaled breath [58]. Since the concentration of 3-heptanone in normal healthy volunteers is <1 ppb, virtually all the 3-heptanone in exhaled breath can be attributed to metabolized valproate. Such metabolic changes inducing the release of specific VOCs may allow therapeutic monitoring of different drugs in the future.

Many of VOCs in breath may have exogeneous sources [88–92], be produced through medication [58,93] or be released by bacteria in the airways [94,95], the oral cavity [93,96–101] or in the gut [30,63]. The concentrations of volatile compounds in exhaled breath may depend on the sampling method [102–105] and on the specific Henry's constant between blood and breath [106–108] which depends on haematocrit (blood cell volume) and other parameters.

5.2. Volatile organic compounds found in saliva

The profile of VOCs in saliva can give information about the oral health and oral microbiome. Saliva has many advantages over breath in terms of sampling, shipping and storage of samples. Moreover, saliva is considered as an equivalent of blood which does not require invasive collection, because there is an equilibrium of the dissolved metabolites between the blood capillaries and the membranes of the salivary glands [109]. On the other hand, the problem with saliva is the possibility of contamination during sampling and the problem with optimal sampling time, with some people being less capable of saliva production.

The most comprehensive profile of VOCs is saliva was provided by al Kateb *et al*. in 2013 [110] and this has not changed since the previously published review [1]. After 2014, the biggest contribution to the saliva volatilome was made by Monedeiro *et al*. [111] who reported the presence of 162 VOCs in healthy subjects using headspace solid phase microextraction (HS-SPME)-GC-MS methodology. The total number of VOCs reported in saliva in this review is 549, which represents an increase of 96 compounds since 2014.

These additional VOCs were sourced from papers studying differences between diseased subjects and controls (which were hopefully healthy [111–118]). All of these compounds had previously been identified in other body fluids [1]. Most of the studies used SPME fibres with different modifications as a sampling technique. As SPME is based on absorption, the number of compounds detected is limited by the sorption properties of the coating material. Improvements to the SPME method, using materials with larger surface of absorption like coupons, blades and thin-films can significantly improve the absorption capabilities, resulting in the detection of less abundant compounds, impossible to detect with conventional SPME fibres.

The application of other, non-absorptive techniques, such as solvent extraction [119] may allow for the extraction of a wider range of metabolites, and the detection of a higher number of salivary metabolites in the future.

According to the recent database (Table1), the dominant chemical class in saliva is alcohols, comprising approx. 16 % of all VOCs, followed by ketones (14 %) and cyclic hydrocarbons (12 %). Taking into account all the types of hydrocarbons, they make up 34 % of all VOCs in saliva. The difference between the percentage composition of saliva reported previously [1] is mainly due to the work of Monedeiro *et al*. [111] who reported that alcohols and ketones are the dominant groups in saliva.

Aside from the studies aimed at profiling bodily fluids, some articles reported attempts to apply saliva characterization for diagnostic purposes. The VOCs in subjects with oral diseases of a possible bacterial origin, such as submandibular abscesses and halitosis were compared to the saliva profiles of healthy individuals [111]. The authors reported the presence of 23 and 41 VOCs specific for halitosis and submandibular abscess, respectively. Halitosis resulted in a larger number of sulfur compounds, while submandibular abscesses, which is an inflammatory disease, was characterized by a greater abundance of inflammation-associated alcohols, aldehydes, and hydrocarbons. The comparison of saliva VOCs between healthy children and children with celiac disease showed that the abundance of some VOCs, such as ethyl acetate, nonanal, and 2-hexanone is different in children with celiac disease treated with a gluten-free diet, compared to healthy children [113].

Moreover, saliva analysis has raised interest in the forensic science area. The SPME-GC-MS analysis of different bodily fluids showed that despite the similarities within a fluid, there is a large number of quantitative differences in each specimen, characteristic for

the individual person, with a low occurrence of matching errors [112]. It was found that saliva and hand odour were the most efficient for differentiation of subjects, providing sufficient stability and variability for differentiation.

SPME in thin-film geometry (TF-SPME) was used for the retrospective analysis of the intake of 49 prohibited substances and steroids by measuring their metabolites in saliva [114]. As the authors underlined, saliva is a good specimen for doping control as it contains mostly non-conjugated, biologically active forms of drugs. GC-MS analysis allowed for the detection of 26 VOCs in saliva, without derivatisation.

5.3. Volatile organic compounds in blood

Blood directly reflects the internal environment of the body, including nutritional, metabolic, and immune status [120]**.** Thus, the analysis of plasma-derived VOCs in blood has been an active area of research. However, obtaining blood samples is not trivial requiring trained phlebotomists. It is not well tolerated by patients in comparison to producing a breath or urine sample, and blood samples usually require pre-treatment which is costly and time consuming.

379 VOCs have been identified from blood, which is relatively few compared to the number found in breath [106]. However, this is a large increase compared to the previous review in 2014 where only 154 VOCS were reported. There certainly is not a lack of studies reporting the analysis of volatile compounds in blood. However, these studies tend to be focused on the monitoring of exposure to environmental pollutants [121], the quantification of blood alcohol [122] and other inhalants derived from solvents [123], and storage and aging of blood for forensic applications [124–127].

However, there have been relatively few studies which compared the volatile profiles above blood in healthy volunteers versus a diseased group. Zlatkis *et al* [128] studied the sera of seemingly healthy individuals versus virus infected patients using capillary GC. Although example chromatograms were presented showing a large number of peaks for both groups, the identification of compounds was limited. It was found that virally infected patients had a wider range of VOCs associated with their samples [129]. Recently there have been two studies which measured the blood volatiles of patients with liver [130] and lung cancer [131] versus healthy individuals. Horvath *et al* [132] described the results of a study where trained dogs could discriminate between blood samples from ovarian cancer patients and blood samples taken from patients with other gynaecological

cancers or from healthy control subjects. A paper by Wang *et al* [133] used SPME-GC-MS to differentiate blood samples of 20 healthy volunteers from colorectal cancer patients. Only the few compounds which were significantly higher in the healthy group were reported.

A few papers exist looking solely at the VOC profiles of healthy volunteer blood without a disease group for comparison [106,118,134]**.** Mochalski *et al* [106] and Ross *et al* [134] compared the volatiles appearing in blood to those found in breath, and Kusano *et* al used hand odour, oral fluid, breath, blood, and urine to differentiate between individuals.

Much of the work relating to environmental exposure to pollutants centres around the National Health and Nutrition Examination Surveys (NHANES) which have been undertaken in the US [135]. These studies have aimed to quantify a range of common environmental pollutants in the blood of over 1000 volunteers. There have been a number of publications relating to the methods used and the results of these studies [136–139]**.** The studies tended to use purge and trap analysis combined with GC-MS [137] but more recently they have adopted SPME based methods coupled to GC-MS [136]. The data from NHANES is used to set expected limits for a range of VOCs in blood (usually in the ppb/ppt range) for non-occupationally exposed individuals [135]. Most recently this data has been used comparatively in measuring the blood VOC levels of people living on the gulf coastline of the US who have been exposed to VOCs derived from the Deepwater Horizon oil spill [140]. There are commercial tests available which give a measure of the volatile solvent profile in blood versus the NHANES data [135].

The high level of alcohol consumption in the US and Europe means that blood alcohol analysis is one of the most common clinical analyses performed. Headspace GC is commonly used to determine blood alcohol levels. This method is convenient as it can be automated and biological products that can cause interference are not directly injected into the GC. A dedicated range of columns have been developed specifically for blood alcohol analysis and the analysis can be completed in 2 min [141]**.** Blood gas analysis usually involves the measurement of methanol, ethanol, isopropyl alcohol, 1-propanol, acetaldehyde, and acetone. The analysis usually includes the use of an internal standard for example t-butyl alcohol (internal standard for the European blood alcohol analysis). However, many forensic laboratories are also interested in the measurement and quantification of an extended number of VOCs which may be derived from inhaling and ingesting dangerous and controlled substances [123]. Volatiles such as diethyl ether,

786 butane, ethyl acetate, hexane, toluene, xylene, and some halogenated hydrocarbons are
787 common VOCs with the potential for abuse via sniffing [142]**.** It may be particularly
788 important to measure these compounds in blood samples taken at autopsy, if the death
789 is suspicious [143]. These additional VOCs also have the potential to interfere with the
790 blood alcohol analysis, so their separation and measurement is important [141]**.**
791 The measurement of ammonia in blood is also an established clinical test [144]. Many of
792 the procedures for ammonia determination involve two general steps: the release of
793 ammonia gas or capture of ammonium ions from the sample and the quantitation of the
794 liberated gas or captured ions [145]. Detection is typically via colourimetric/fluorimetric
795 methods [146], gas sensitive electrode [147] or enzymatic methods [148,149]. Elevated
796 levels of ammonia in blood is considered a strong indicator of an abnormality in nitrogen
797 homeostasis, the most common reason is related to liver dysfunction. Hyperammonemia
798 arises from excessive production by colonic bacteria and the small intestine. At high
799 levels ammonia is a potent toxin of the central nervous system and has been linked to
800 hepatic encephalopathy (HE). However, breath ammonia determination is not currently
801 accepted as a reliable marker of HE, although a large amount of data supports the role of
802 hyperammonaemia in the direct and indirect alterations of brain function underlying HE.
803 A relatively recent paper [150] describes the measurement of capillary blood (an
804 equivalent to arterial blood) following an oral glutamine challenge. This method was
805 more successful at identifying minimal HE than the use of capillary blood measurements
806 alone.
807 Since our previous 2014 review [1], there have been a handful of forensic science papers
808 on how storage and aging of blood impacts its VOC profile [124–127], as this has
809 implications for sniffer dog training. Dubois *et al.* used variable energy electron impact
810 ionization TD-GC-GC-TOF-MS and found it was able to monitor subtle changes in blood
811 VOCs within the first week of aging. Whilst these publications have yielded a great of deal
812 of data, and found new compounds previously unidentified in blood, only the data from
813 fresh blood which hasn't aged or decomposed could be included in this review.
814
815 ***5.4. Volatile organic compounds in milk***
816 This review has identified 290 compounds in human milk. This represents only a small
817 increase vs the 2014 review where 256 compounds had been identified. There are many
818 papers on the nutritional composition of human milk (as an example see the review by

819 Jenness [151] and also on the presence of environmental chemicals (as an example see
820 the review by LaKind [152]), but there is relatively little specifically relating to the
821 volatile components. Most GC-MS analytical studies appear to be directed at identifying
822 the presence of a specific pollutant, medicinal substance, or group of environmental
823 compounds, to support research on chemical exposure to the nursing infant or using milk
824 as a geographical pollutant indicator. A literature search revealed numerous papers on
825 organochlorine pesticides, brominated diphenyl ethers, dioxins, polychlorinated
826 biphenyls, parabens, triclosan, polycyclic musk fragrances, flavonoids, and many others.
827 However, not all these compounds can be considered as volatiles at body temperatures.
828 Others studies looked for compounds transferring to breast milk from mothers taking
829 specific dietary supplements, such as the search for odorous components from fish oil
830 [153] or 1,8-cineole metabolites after taking 1,8-cineole capsules [154]. Studies looking
831 for specific compounds after exposure to environmental contamination, medication, or
832 dietary supplementation have not been included in the tables. The most extensive list of
833 likely volatiles was given by Pellazari *et al* [155] who identified 156 'purgeable'
834 compounds from maternal milk, in a study to evaluate the utility of using milk in pollutant
835 studies. A wide range of classes of compounds was identified by GC-MS from passing
836 helium gas through warm milk and trapping vapours on a Tenax cartridge. Similar classes
837 of compounds were reported by Shimoda *et al* [156] using a diethyl ether distillation-
838 extraction. Other studies have looked for specific organic compounds in the headspace
839 above milk using SPME with GC-MS (four VOCs [157], monocyclic aromatic amines [158],
840 phthalate esters [159], benzene and alkylbenzenes [5,160]. A broader study, also using
841 the SPME method, attempted to quantify 36 different VOCs [161] and identified 10
842 compounds whose median concentration across 12 samples was above the 'lowest
843 recordable level'. Buettner *et al* has analysed the volatiles from milk and in one study
844 identified 45 odour-active constituents, using olfactory GC in combination with GC-MS
845 [162].
846 A study from 2009 [163] made a comparison between mother milk and formulas,
847 underling in these, the presence of different volatiles related to the heat treatment of milk,
848 such as methional, 2-furfural, and sulphides. On the other hand, the GC-MS analyses
849 revealed a higher variation in the volatiles from milk compositions for the mother's milk,
850 exposing the infant to more diverse flavour, including a higher variety of terpenes
851 probably originating from the maternal diet. Another study regarding the quality of

breast milk has been published in 2010 [164], using high-resolution gas chromatography–olfactometry (HRGC-O) to investigate the reasons behind the formation of the typical fish-like and metallic off odour during the storage of human milk, not to be found in the cow milk under the same conditions. In this case, the studies underlined the presence of oxidation products from long-chain (poly)unsaturated fatty acids such as (Z)-1,5-octadien-3-one, trans-4,5-epoxy-(E)-2-decenal, 1-octen-3-one and (Z)-3-hexenal. Fatty acid degradation products have also been found to be responsible for changes in milk flavour [165,166] using two-dimensional high-resolution gas chromatography-mass spectrometry (TD-HRGC-MS) and GC-MS analyses. These studies investigated the modifications occurring in the metabolite profile when breast milk is subjected to different treatments. Analogously, Garrido *et al* [167], showed how high-pressure thermal (HPT) treatments can modify the volatile profile, increasing the abundance of different chemical groups (aldehydes, ketones, furan, pyrans, alcohols), and decreasing, on the other hand, the content of aliphatic hydrocarbons present in the non-treated human milk samples. Also in these cases, the changes in the VOC profile can be attributed to the negative odours sometimes attributed to human milk. As much as the storage and ambient conditions, also the mother's diet, both in the phases of pregnancy and nursing, was found to have a direct connection with the breast milk volatiles profile [168]. On the same issue, Ramsons (a plant with garlic like odour) consumption was found to affect milk aroma, as pointed out by Scheffler *et al* [169], who identified volatile ramson-derived metabolites in human milk, applying gas chromatography-mass spectrometry/olfactometry (GC-MS/O). An analogous study was also conducted regarding garlic consumption [170].

Hartmann *et al* [171] employed GC-MS to investigate the presence of 5-α-androst-16-en-3-one in human breast milk, underling the issues and the procedures needed when it is necessary to underline a specific compound in the milk matrix. Another research group also focused on a specific compound [154,172], 1,8-cineole, again investigated by GC-MS. These studies also point out how the analysis of the volatiles in human milk are promising for health monitoring since metabolite profiles in milk might be substantially different from those in the commonly analysed body fluids of blood and urine, due to the high lipid content.

5.5. Volatile organic compounds from skin secretions

885 The number of different compounds identified from human skin secretions is very large.
886 Our literature search revealed 623 named VOCs analysed from skin secretions (an
887 increase compared to the 532 found in the previous version in 2014 [1]. Odour can be
888 particular to an individual and distinguishable both by people and by canines [173]. Also
889 skin is not homogeneous and the distribution of the different types of glands and
890 microbiota across the body can be expected to lead to different VOC profiles. Even the
891 odours of a single individual varies; with diet, emotional state, menstrual cycle, age, and
892 many others factors [174,175]. Studies of the secretions from the skin are particularly
893 susceptible to interference from personal care products. Although experimental
894 procedures attempt to minimize the presence of exogeneous compounds by asking
895 subjects to refrain from use of such products apart from a designated soap for a time
896 period before testing, some identified compounds are highly likely to come from
897 exogeneous sources [176,177]. Bernier *et al* [178] reported hundreds of compounds
898 spanning a wide range of classes, in a study attempting to identify candidate mosquito
899 attracting compounds. Samples were collected from the hands using glass beads and
900 analysed by GC-MS. Many of the compounds were relatively high MW species and it could
901 be argued that some would be expected to have limited volatility at body temperature.
902 The papers of Zeng *et al* [179,180] list a number of C-6 to C-11 acids and in particular E-
903 3-methylhex-2-enoic acid, as responsible for characteristic axillary (armpit) odours along
904 with a large n-dodecanoic acid peak, lactones and alcohols found in solvent extraction of
905 worn absorbant pads. Other studies also look specifically for odiferous axillary
906 compounds. Kuhn and Natsch found a genetic contribution to odorant carboxylic acids
907 [177] and Hasegawa *et al* [181] found a difference between 'spicy' and 'sour' axillary
908 odour and identified sulfanyl alcohols. Another study analysed compounds on the
909 forearm [176] by using ethanol and hexane extraction. However, relatively few
910 compounds are common to these or other papers.
911 The difficulty of identifying a set of VOCs characteristic of human sweat is exemplified in
912 the paper of Penn *et al* [182] looking at 'fingerprints' in human odour. They used
913 polydimethylsiloxane coated stirrer bars to collect axillary samples from 194 individuals
914 over 10 weeks; 4941 separate GC-MS peaks were found of which only 373 were
915 consistent over time within an individual (118 were chemically identified). They report
916 very few of the peaks as common to all samples. Only 38 compounds were found to be
917 present in at least half the samples. There are a few studies that attempt to collect the

compounds that are volatile at body temperatures rather than by volatilization of collected skin secretions. Gallagher *et al* [176] lists a set of volatile compounds from the forearm, when collected using SPME fibres held above the arm compared with solvent extraction. Haze *et al* [183] identified straight chain hydrocarbons, alcohols, acids and aldehydes from headspace analysis of cloth worn on the back and found a link with 2-nonenal and ageing. Zhang *et al* [184] identified 35 compounds predominantly alcohols, alkanes and aldehydes using SPME fibres to collect volatiles from the hand and forearm and found differences between the hot humid spring and cold dry winter.

SPME-GC-MS has also been used to study axillary odour [185] para-axillary and areola volatile compounds for possible mother–infant recognition chemicals [186,187] report aldehydes (e.g. 3-methyl-2-butenal, benzaldehyde, octanal, nonanal, decanal) and ketones (e.g. 6-methyl-5-en-2-one). In these papers, there are very few named compounds that are common between studies. As an example, nonanal occurs in twelve of the publications under examination, decanal (11 times), octanal and 6-methyl-5-epten-2-one (10 times each) and finally octanoic acid and acetic acid (7 times each). This was also observed by Prada e*t al* [188], using SPME-GC-MS. Dormont *et al* [189] pointed out the great importance of sampling when the sample collection occurs outdoors. The authors compared four methods for sampling skin odours: solvent extraction, headspace SPME, and two new techniques not previously used for the study of mammal volatiles, contact SPME and dynamic headspace with a chromatoprobe design (miniaturized trapping tubes that are directly inserted into the GC injector for thermal desorption). The same study underlined the prevalensce of aldehydes in the volatile profile, in particular nonanal and decanal. The same research group in 2013 [190] pointed out the complexity, in terms of the number of compounds, featuring in the chemical profile of skin volatiles. This work underlined, that the compounds found in human skin vary widely depending on the part of the body where the samples are collected and the sampling methods employed. For example, the axillae region is characterised by apocrine, eccrine and sebaceous glands, which in addition to the microbiota bring about a specific volatile profile. This profile features mostly alkane and C_6-C_{11} carboxylic acids. Different VOCs were found in the hand, primarily aldehydes and ketones (nonanal, decanal, undecanal, 6-methyl-5-hepten-2-on and geranylacetone). This was also confirmed by Mochalski in 2018 [191], where the use of ion mobility spectrometer coupled with gas chromatography (GC-IMS) was found to present considerable potential for the detection

951 of VOCs. At the same time it presented some drawbacks, like the fact that some interesting
952 classes of VOCs such as alkanes cannot be measured using that IMS instrument. The
953 ionisation source determines the range of compounds that may be detected, e.g. a beta
954 emitter such as nickel 63 does not detect alkanes, while a photo ionisation source in
955 conjunction with an IMS detects alkanes sensitively.
956 An IMS coupled with a short multi-capillary column (MCC) was instead employed by
957 Ruzsanyi et al [187] for near real-time monitoring of human skin emissions, who pointed
958 out that octanal, nonanal and decanal may originate from the skin. Curran *et al* [192]
959 presented 24 different compounds employing SPME-GC-MS to measure human scent, and
960 utilize it to identify and distinguish between individuals.
961 Another interesting avenue for VOCs from the skin is finding a correlation between them
962 and the compounds found in blood. From the study of the literature, families of VOCs have
963 been found to be present in both blood and skin. Namely: aromatic compounds (16
964 compounds in common), aldehydes (15), acyclic alkanes, alcohols (14), ketones (13),
965 nitrogen-containing compounds (8), esters (7), acyclic alkenes, acids (6) non-aromatic
966 cyclic hydrocarbon, sulfur-containing compounds and ethers (3 each) and halogenated
967 compounds.
968
969 ***5.6. Volatile organic compounds from urine***
970 The recent review revealed 444 VOCs associated with urine [196–198] compared to 279
971 reported in the previous version. The largest number of compounds identified in urine
972 belong to the ketone group. Ketones in urine are likely to at least partially arise from
973 bacterial action in the gut, maybe by decarboxylation from the corresponding oxo-acids,
974 since ketones were found at much lower concentrations in the urine of 'germ free' rats
975 [193]. Levels of the key ketone bodies, propanone (acetone) and acetoacetate have been
976 found to vary between 1.16–14 mol L^{-1} and 1.3–15 mmol L^{-1} respectively in urine [199].
977 The ketone bodies (acetoacetate, hydroxybutyrate and propanone) are produced in the
978 liver during periods of rapid fat oxidation, when the rate of fat breakdown exceeds the
979 capacity of the Krebs cycle to process the resulting acetyl CoA [200,201].
980 Several significant studies of VOCs in urine have been undertaken e.g. [193] **,**[194]. Nine
981 compounds were present in all studies: propanone, 2-butanone, 2-pentanone, 2-
982 heptanone, 3-hexanone, 4-heptanone, 2, 5-dimethylfuran, 2-ethyl-5-methylfuran and
983 toluene, so can be present with a very high degree of certainty. A study [195] of 4-

heptanone in urine strongly suggest its presence originates at least in part from *in vivo* oxidation of the plasticizer component, 2-ethylhexanoic acid.

Propanone, 2-butanone, 2-pentanone and 2-heptanone were also found ubiquitously in the headspace of faecal samples from healthy individuals [9]. Propanone can be produced by the non-enzymatic decarboxylation of acetoacetate and may sometimes be smelt on the urine and breath in acute diabetes.

In summary, the VOCs in urine cover a range of chemical classes: e.g. acids, alcohols, ketones, aldehydes, amines, N-heterocycles, O-heterocycles, sulfur compounds and hydrocarbons (Table 1). When comparing the VOCS from urine and faeces a notable difference is the number of esters. The relative levels have not altered since 2014 with additional esters idemntified in faeces (10) and urine (7). However, there were no new straight chain hydrocarbons identified in urine thus a notable difference remains, making alkanes the smallest group for urine volatiles. Although previously identified, Cozzolino *et al* [202] again detected hexane in their study of healthy children using SPME-GC-MS.

Cozzolino *et al* [196] pre-treated samples under both acidic and alkaline conditions, followed by analysis with SPME GC-MS, identifying a total of 162 urine compounds, 42 of which were previously undetected. The combination of salting, pH change and solvent extraction by Cozzolino *et al* has shown many hundreds of compounds can be readily detected by a typical benchtop quadruple GC-MS..

A large number of terpenes are described and are considered to be derived from food [193]. Little data exists on quantitative measurements of VOCs in urine. Concentrations of phenol (typically 10 mg day^{-1} excreted in urine) and p-cresol (typically 52 mg day^{-1} excreted in urine) have been reported to increase in urine with increasing protein intake. Their formation is considered to be due to gut microbiota acting on tyrosine; anaerobic bacteria in the left colon producing phenol and aerobic bacteria in the ileum/cecum producing p-cresol. The relationship is complicated by fibre intake. High fibre intake with high protein resulted in a smaller increase in concentration due to decreased transit time [203]. This study was motivated by phenols being implicated in bladder and colon cancer, which no longer is considered to be the case.

Normal alcohol emission ranges reported are 0-46 mg/24 h for ethanol, 0–300 μg/24 h for n-propanol and 0–18 μg/24 h for n-butanol; these levels approximately mirror blood serum levels [183]. Trimethylamine and 4- heptanone, were quantified as 0.5 -20 μg ml^{-1} and 40–800 ng ml^{-1} respectively in urine [204].

It has been suggested that methylamine and other short chain aliphatic amines may play a significant role in central nervous system disturbances observed during hepatic and renal disease [205]. To this end a quantitative method was developed for methylamine determination in the gas phase from urine. The average output was 11 mg day^{-1} with a range of 1.7– 62 mg day^{-1}, with diet having a small effect. The source was considered to be mainly endogenous. Gut bacteria are likely to be implicated in the production of methylamine (probably from creatinine) as rats with no gut bacteria produced less than half the output [205]. The average daily output for dimethylamine was about 17 mg with values for the majority of the population lying within the 0.68–35.72 mg range [206]. Healthy young adults excrete about 1 mg of trimethylamine and 40 mg of trimethylamine N-oxide daily, although these levels are markedly influenced by diet, particularly when it contains marine fish. When marine fish is a dietary component, several hundred mg of trimethylamine N-oxide may be excreted [207].

New, alternative, and combined approaches have been employed to enhance how urine volatiles are detected. The volatiles in urine have recently been evaluated by combined odour and GC-MS chemical analysis. For the first time a comprehensive description of the smell of the individual components has been described [208]. This work also involved enzymatic (glucuronidase) pre-treatment followed by solvent extraction. Recently, Zou *et al* [197] developed a novel ultrasonic nebulization extraction proton transfer reaction mass spectrometry (UNE-PTR-MS) technique to rapidly detect selected compounds within a urine sample. Encouragingly, only 0.66 mL of urine is required for a full scan, which delivers a response in 34 s. The authors state this method overcomes lengthy pre-concentration processes, extended sampling procedures, and prevents alteration to the urine whilst in storage. Although no new urine compounds were detected, the technique showed promising results for common urine VOCs: methanol, acetaldehyde, and acetone, yielding relative recoveries of between 88.39 % and 94.54 %. However, the results stem from just one urine sample, therefore, further analysis would be needed to determine whether this new method is sufficient in detecting larger numbers and more specific VOCs in urine, perhaps identifying new compounds that may aid in disease diagnosis as suggested by the authors.

Benign prostatic hypertrophy (BPH), the medical term for an enlarged prostate, is so common in older men, it could be considered normal. About half of all men between ages

51 and 60 have BPH and up to 90% of men over age 80 have it. This could affect urine volatiles but has not been investigated in any detail.

5.7. Volatile organic compounds from faeces

The first report of gas analysis from faeces was in 1861 when Rüge reported that human rectal gas contained hydrogen, carbon dioxide, and methane, in addition to other unidentified gases [209]. Flatus is considered to be a mixture of hydrogen (0–50 %), nitrogen (5–90 %), oxygen (0–10 %), carbon dioxide (10–30 %), and methane (0–10 %). Methane production occurs in about 50 % of the healthy population, some members producing higher levels than others; methane production is correlated with methanogenic bacteria. Similarly, sulfate-reducing bacteria are responsible for the generation of pungent sulfides [210]. In the original compendium 381 compounds were reported in faeces, since then a further 62 compounds not stated in the original compendium have been found. Of these, 24 compounds had been reported from other fluids and have now been identified in faecal samples (Table 1). This now means that in total 443 compounds have been assigned an identity from faecal samples. These additional 62 compounds came from just 5 papers; this is indicative that while compounds have been added to the compendium it is very likely that there are more to be found. The 443 compound value still falls far short of the number of compounds found in breath, which is likely to be a function of a smaller number of studies carrying out qualitative analysis on faecal samples when compared to breath.

Significant concentrations of a range of volatile fatty acids [211], indoles [212] and phenols [213] have been observed in faeces. Fermentation of carbohydrates in the gut produces ethanoic, propionic, butanoic, pentanoic, and hexanoic acids, particularly by *Bacteroides* [214]. *In vitro* studies [215] have provided evidence that proteinacious foods also produce SCFAs via the action of bacteria such as *Clostridia spp*.; BCFAs, such as 2-methylbutanoic acid and methylpropionic acids, are principally produced by gut microbial action on proteins via the respective branched amino acid.

Gould *et al* [216], conducted a study in which ^{13}C labelled compounds were used as internal standards in faecal samples to quantify 15 compounds. This study is unique as it is the only work, we have identified in which many compounds were quantified based on what is in the faeces and not just the headspace. This work also turned the faeces alkaline by the addition of sodium hydroxide to quantify trimethylamine, which is the first-time

this has been reported from faeces [216]. This paper contributed 12 new compounds to the previous compendium [1], including 4-isopropyl benzaldehyde (cuminaldehyde), and 2,4-dithiapentane which are associated with cumin and truffle fungus, respectively. Long chain fatty acids (LCFAs) were quantified in work by Song *et al* [217]. Nine of these compounds were previously reported as being found in skin and/or saliva (Table 11).

Both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are compounds that were not found in the original compendium. EPA and DHA are omega 3 fatty acids found in cold water fish, these compounds are also used as dietary supplements as they are the fatty acids that form cellular walls in the brain and eyes [218]. A recent mechanistic study in how unsaturated long chain fatty acids are oxidized in the body to form many smaller metabolites is described [10].

Volatiles such as methanethiol and ammonia are considered to be derivable from methionine by the action of bacteria such as *Clostridium sporogenes* [219]. Hydrogen sulfide and methanethiol can be damaging to the large intestinal epithelium and are also generated from sulfur-containing substances in the diet [220]. Similarly, fermentation of tyrosine and tryptophan in faeces has been shown to produce the VOCs phenol and indole, respectively [219]. Phenol and *p*-cresol are considered to be produced by aerobic intestinal microbiota acting on tyrosine and the latter by anaerobic organisms [211].

Of the 58 compounds new to faecal samples 13 of those were previously found in saliva. There is newly emerging evidence that the oral microbiome might have an impact on the gut microbiome [221]. Olsen and Yamazaki present work in which patients with chronic periodontitis the bacteria *Prophyromonas gingivalis* creates dysbiosis which in turn cause dysregulation of the gut microbiota [221].

Two earlier studies stated that a total of 297 and 135 different VOCs have been identified respectively by Garner *et al* [9] and De Preter *et al* [222] in the headspace of faeces from healthy individuals on an *ad libitum* diet. These two studies showed that typically, for each donor the number of VOCs ranged from 78 to 125 (median = 101). Interestingly, 44 compounds were stated to be common to 80 % of the cohort samples [9]. Dixon *et al* [223] hypothesized that the varied functionality of the metabolites in the headspace of faeces, dictated the use of several diverse SPME fibre coatings for more comprehensive metabolomic coverage. They evaluated eight different commercially available SPME fibres in combination with GC-FID and GC-MS. This approach appears very promising; 267 peaks were found with GC-FID though the authors have yet to

identify all the compounds. SPME can suffer from competitive absorption, the length of equilibration time of the sample, and length of time the SPME fibre is exposed can all effect what compounds are absorbed onto the fibre. This means that not all the compounds from a matrix, particularly one as complex as faeces, are absorbed.

Alcohols were thought uncommon in adult faeces [224]. However, the studies reported in this review reported 52 different alcohols to be present. Ethanol is very commonly observed. It is likely that gut bacteria can reduce acids to alcohols. Esters were found to represent the largest group of compounds identified. An interesting readily observed feature of the esters in stool is the similarity of the higher MW compounds, they either possess a long-chain acid and short-chain alcohol or a short-chain acid and long-chain alcohol. This suggests that the number of esters identified is not a true picture of what is present in the faeces but a limit on the method i.e. the volatility of the esters. It is very likely that a more sensitive method or better pre-concentration will significantly increase the compounds observed.

A diverse range of aromatic compounds (Table 1) including mono-, di-, tri- and tetra-substituted benzenoids, mono- and di-substituted furans, and nitrogen containing derivatives of pyridine, pyrrole, and indole have been reported. Most of these have only been recently reported in faeces, although it has been established that phenolic and indole compounds arise from the metabolism of aromatic amino acids by gut bacteria [215]. There are many publications which have observed that alkyl furans are produced by fungi. In contrast there is a paucity of publications relating to furan biosynthesis by bacteria. Fungi are well known to be commensal organisms in the gut, which could explain the origins of furans, possibly from the metabolism of fructose. Furans are now considered to be also synthesisable from the oxidation of polyunsaturated fatty acids *in vivo* [10]**.** Some benzenoid compounds such as dimethylbenzenes, ethylbenzene, and toluene (constituents of petrol) probably arise from air pollution.

A range of aldehydes have been reported [9] in the faeces of individuals. A complete homologous series has been reported from ethanal to octadecanal. Ethanal is of particular interest due to its abundance and is considered to promote mutagenesis [225–227] and be associated with bowel cancer. The toxic effects of higher aldehydes have received much less attention. The origins of some aldehydes may be dietary. For instance, 2-methylpropanal, 3-methylpropanal, hexanal, nonanal, decanal, and benzaldehyde are found in potato tubers and hexanal in carrots. However, it is doubtful that these

compounds would remain unchanged through the digestive system and biosynthesis by microorganisms in the gut and oxidation of unsaturated fatty acids appears more likely. Acetone and butan-2-one were reported in 100 % of faecal samples from a longitudinal cohort study [9], which probably arise from fatty acid and carbohydrate metabolism [228]. Methylketones can be produced by many species of bacteria and can also be produced by fungi from the respective alkanoic acid and undoubtedly other ketonic compounds can also be synthesized by bacteria. The universal presence of 2,3-butanedione is interesting in faeces [9] since it may have health implications by impacting on the growth of some bacteria and yeasts [229]. This group of compounds, and indeed other groups, are not normally the end products of metabolism by microorganisms therefore their concentrations would be expected to be continually changing in the gut. Methane is a product of bacterial reduction of carbon dioxide, or from acetic acid, and potentially from oxidation of some unsaturated fatty acids *in vivo*.

Numerous hydrocarbons have now been discovered in faeces although the longer chain species have been found in small numbers [9]. Isoprene has been extracted from faeces [230]. Isoprene in the gut may be the result of cholesterol biosynthesis [231] and it is considered to be the most common hydrocarbon in the human body and therefore would be expected to be found in faeces.

Many alkenes/terpenoid compounds found are well documented as naturally occurring plant products [232]. Limonene has been reported as the most abundant of the terpenoid compounds and occurs in high concentration in citrus fruits. Most of the terpenes identified [9] are found in vegetable food stuffs and do not originate from animal products. For instance the following volatiles are present in carrots: pinene, limonene, terpinene (1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene), p-cymene, terpinolene caryophyllene, and humulene [233]. Copaene is found in potato extracts [234].

Many ether compounds have been reported in the headspace of faeces. Commonly, 2-ethoxyethanol occurs in manufactured products like soaps and cosmetics [235] and 1,3-dimethoxybenzene is a registered food additive in Europe [9]. Similarly, it is very unlikely that chlorinated compounds found are of biological origin. Consumption of contaminated food or water is the likely source of these compounds. Chloroform may arise as a faeces VOC component from several sources, it is an air contaminant and has been detected in foodstuffs [236]. Chlorination for disinfection of drinking water is another source resulting in the production of chloroform and halogenated methanes [237].

Many nitrogen compounds have been reported (Tables 2a-2c) and are likely to arise from the diet; for instance, methylpyrazine, pyridine, and pyrrole are constituents of coffee. However, pyrrole readily polymerizes with acid and, therefore, its presence is unlikely to be dietary, as it would be unlikely to survive transit through the stomach. Ammonia results from microorganism activity. In addition, increasing the amount of protein in the diet from 63 g to 136 g/day was found to increase the amount of faecal ammonia from 15 to 30 mmol l^{-1}. Interestingly, increasing the amount of fibre to the high protein diet was reported to not alter the ammonia concentration [203]. In a study of nitrogen containing compounds in the faeces of 30 healthy individuals indole was the only compound found ubiquitously [9], followed by 3-methylindole, in 73 % of individuals, these compounds are well known to be produced by microbial degradation of l-tryptophan in the gut. Many compounds are present in a minority of volunteers. Allyl isothiocyanate was found to be present in 23 % of cases; this compound is of particular interest due to its suspected anti-cancer properties. Its occurrence would be expected to be determined by a number of factors such as diet (cruciferous vegetables e.g. broccoli, cauliflower, and cabbage), the cooking of these vegetables, and the ability of the host's bacteria to break down sinigrin, the main glucosinolate of Brussel sprouts.

A diverse range of sulfur compounds has been reported. For instance, methanethiol and dimethylsulfide have been commonly observed; the former is, at least in part, considered to be produced from methionine by *Clostridia* in the gut [219]. Methanethiol has a toxicity approaching cyanide and the factors controlling its concentration and biosynthesis might warrant further investigation. Methanethiol and dimethylsulfide may also be produced by methylation of hydrogen sulfide as a detoxification mechanism by mucosal thiol *S*-methyltransferase [238]. Dimethyldisulfide and dimethyltrisulfide have both been commonly reported in faeces [9,239,240]. Hydrogen sulphide is probably most likely to occur due to the metabolism of sulphate by sulphate-reducing bacteria [239]. Sulphate, which is poorly absorbed in the small bowel, is naturally present in cruciferous vegetables and nuts and as an additive in bread and beer [239]. The main sulfur-containing flatus components in healthy individuals have been quantified: hydrogen sulphide (1.06 μmol l^{-1}), followed by methanethiol (0.21 μmol l^{-1}) and dimethyl sulphide (0.08 μmol l^{-1} [239]. The authors were concerned about the social aspect of pungent flatus and found in their study that hydrogen sulphide and methanethiol appeared to be principally responsible and not indole-based compounds as previously thought.

5.8. Volatile organic compounds from semen

In semen, 196 compounds have been reported. To date, it appears only one research group has published on VOC profiles in semen, using an investigation of healthy subjects, using SPME in the headspace above the semen combined with GC-MS detection [241].

Semen assessment is the key test for infertility problems with a seminogram being the gold standard. Recently, metabolomics research was proposed as a method supporting male fecundity. Changes in the pattern of metabolites in semen may reflect the metabolic status of the sperm cells and the composition of the seminal fluid, which could affect the reproduction capacity. Most of the metabolomic studies on semen have been conducted using NMR and LC-MS, focusing on the secondary metabolites [242–244]. On the other hand, the volatile pattern of semen which could contribute to the fast detection of fertility problems remains hardly explored [241]. The authors detected the presence of 196 VOCs in semen samples collected from 69 men. The number of VOCs in semen, from each man, ranged from 3-28 VOCs. Curiously, no compound was present in all samples and 126 compounds was observed only once. Also, interestingly, 98 of the reported compounds were detected for the first time in biological fluids. The dominant group of compounds in semen were nitrogen-containing volatiles, comprising more than 30 % of all the compounds identified. The tetramine, spermine, a compound found in semen at about 3.3 mg/g and responsible for the characteristic odour of semen [245] was not reported in the study of Longo *et al* [241].

It is worthwhile to underline that the majority of the compounds were detected only in one of the analysed samples, and only 70 VOCs were detected at least twice. The most frequently observed compounds were pyrrole, ethanol and 2-methylbutanal. The majority of the compounds had an exogenous origin according to the Human Metabolome Database [246], with 57 compounds that could have both exogenous and endogenous origin. The authors found there was an association between the VOCs profile and the sperm motility. There surely are more volatile compounds to be discovered in semen, considering the number of VOCs reported from other bodily fluids. It is suggested that further research in this area to establish a better base of VOC composition in semen from healthy men, could be beneficial to aid diagnoses of certain urological diseases.

6. Conclusion

A study of VOCs from healthy humans is presented for a variety of reasons. There are many more papers than ever before now comparing ill patients with controls, These publications more often than not, have a favourable conclusion, that there are promising differences in the VOC profiles between the diseased patients and the non-diseased volunteers. Furthermore, there are many published studies where presence and absence of VOCs is considered for correlations with disease and controls (some researchers, now avoid the term VOC biomarkers). The present review now shows many of these "absences" are being found in healthy subjects, which neutralizes to a degree, their use in disease diagnoses. Presence and absence is no longer good enough, concentration is key. Absence could be that the compound really is not there, such as in the case of detecting a microbial toxin, where a bacterium does or does not produce a toxin. It is appreciated there may still be a case for comparison if exactly the same conditions and equipment sensitivity is applied. Diet from weeks, months ago could affect breath volatiles. It is simply very hopeful to design methods for clean air breathing with the belief that this will permit standardised results. An important reason for justifying this, is expanded on. Diet from weeks/months ago affects the lipid composition of the body, our MUFAs and PUFAs are determined by genetics and diet. These lipids are continuously being oxidized, producing a wide range of VOCs. such as alcohols, alkenes, alcohols and carboxylic acids [10], which can then be further metabolized into daughter compounds, e.g. by further oxidation in the liver etc., also concomitantly there are many new compounds being reported. There is a huge difference, almost 1000 compounds, between the numbers reported in 2014 and in 2020. There is then more scope, considering the huge variety of compounds, for finding correlations for disease diagnoses.

Limited studies have been undertaken on exercise/movement and VOCs in breath etc. One such study has shown isoprene for instance does fluctuate with exercise in healthy humans. This might simply be considered as a simple, interesting observation, however if this phenomenon occurs for isoprene, what about the thousands of VOCs now listed in this review, which have not been studied, maybe the same phenomenon occurs for many of these. It could very well be the case that ill people may be less active, they may even be horizontal in a hospital. If a range of VOCs are being used for disease diagnoses it may be somewhat compromised by this situation.

When the 2014 review was published the tables showed there were many gaps in the sub tables i.e. there would be a homologous series with compounds missing here and there

i.e. “gaps), such as in the first years of the periodic table being constructed. The absence of a certain compound could be considered to be due to a lack of a metabolic route, or due to the inability of the detection equipment, or some other reason. Many of these “gaps” have been filled in this current review compared to 2014 highlighting that further studies are required to identify the extent of the human volatilome. Another important consideration is the lack of validation of the current reported compounds from the human volatilome with a small % validated by standards. Therefore, effort should also focus on proper validation of the already reported compounds adhering to the principles of identification outlined in the metabolomic standards initiative.

This review, unlike the earlier 2014 review shows within the tables the publications where each compound was originally reported, this can add confidence to the data especially where several research groups have identified the same compounds.

For discussion, one might think the healthy controls would have many similarities, although this review shows only 14 compounds were common to all the bodily fluids and breath. One might not have expected this, and it would be preferable for disease diagnoses if there was a greater core number of compounds that differ in concentration between disease states. As an example, a recent study, described herein, found 4941 GC-MS peaks in the sweat of a group of healthy humans and found very few peaks common to all samples.

In an attempt to have more control over the jungle of compounds, one might consider controlling diet, between patients and volunteers however then there is the difficulty that there are different type and concentrations of bacteria, in our bodies. Gut transit time in healthy humans, varies between individuals. and this is known to affect gut chemistry. Then there are the VOCs in the environment – “the human exposome” which is highly individual, and furthermore these compounds can often be converted to other compounds in our bodies. The control group and patients are unlikely to individually be exposed to the same compound types at the same concentration levels.

We are therefore assured that there will be a wide range of differences in the human volatilome, each of us could very well be unique, hopefully though with enough similarity so that quality correlations between control and disease states, will occur.

This review now summarises many classes and sub-classes of compounds and hopefully now that they are easily visible will assist in deciding whether to target particular classes or sub-classes or combinations thereof, to aid disease diagnoses, and also to decide which

is the appropriate bodily fluid or breath, which is the goal for many researchers in the VOC field.

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Conflict of Interest

Authors declare no conflict of interest

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