THE VARIABILITY OF VOLATILE ORGANIC COMPOUNDS IN CLINICAL ENVIRONMENTS.

Dahlia Salman[a], Wadah Ibrahim[b,c], Amisha Kanabar[a], Bo Zhao[b,c], Amisha Singapuri[b,c], Michael Wilde[d], Rebecca L Cordell[d], Teresa McNally[b], Dorota Ruszkiewicz[a], Andria Hadjithekli[a], Robert Free[b,c], Neil Greening[b,c], Erol A Gaillard[b], Caroline Beardsmore[b], Paul Monks[d], Chris Brightling[b,c], Salman Siddiqui[b,c], C L Paul Thomas[a]* On behalf of the EMBER consortium

***Corresponding author:** C.L.P.Thomas@lboro.ac.uk

a Department of Chemistry, Loughborough University, Loughborough, LE11 3TU, UK.
b College of Life Sciences, Department of Respiratory Sciences, University of Leicester, University Road, Leicester LE1 7RH
c Leicester NIHR Biomedical Research Centre (Respiratory theme), Glenfield Hospital, Groby Road, Leicester LE3 9QP
d Department of Chemistry, University of Leicester, University Road, Leicester, LE1 7RH

KEYWORDS:

Volatile organic compounds, environmental VOC, indoor air pollution, breath analysis, TD-GC-MS; thermal desorption-gas chromatography-mass spectrometry.

ABSTRACT

The development of clinical breath-analysis is confounded by the variability of background volatile organic compounds (VOC). Reliable interpretation of clinical breath-analysis at individual, and cohort levels requires characterisation of clinical-VOC levels and exposures. Active-sampling with thermal-desorption/gas chromatography-mass spectrometry recorded and evaluated VOC concentrations in 245 samples of indoor air from three sites in a large NHS provider trust in the UK over 27 months.

Data deconvolution, alignment and clustering isolated 7344 features attributable to VOC and mapped the variability (composition and concentration) of respirable clinical VOC. 328 VOC were observed in more than 5% of the samples and 68 VOC appeared in more than 30% of samples. Common VOC were associated with exogenous and endogenous sources and 17 VOC were identified as seasonal differentiators. The presence of metabolites from the anaesthetic sevoflurane, and putative-disease biomarkers in room air, indicated that exhaled VOC were a source of background-pollution in clinical breath-testing activity.

With the exception of solvents, and PPE waxes, exhaled VOC concentrations above 3 μg m^{-3} are unlikely to arise from room air contamination, and in the absence of extensive survey-data, this level could be applied as a threshold for inclusion in studies, removing a potential environmental confounding-factor in developing breath-based diagnostics.

INTRODUCTION

Breathomics, is being developed to stratify patient phenotypes, and monitor metabolic and disease mechanisms[1–3], and biomarker discovery with breathomics is being applied to conditions that include respiratory disease, cancer, infections and pulmonary illnesses[4–6]. Further, breath analysis for assessing occupational exposure to VOC is well-established[7–9]. A challenge in the development of clinical breath-testing is accounting for the heterogeneity of patient responses to variable backgrounds of environmental VOC. Failure to adequately address this factor may confound breathomic biomarker discovery and breath-testing activity [10,11.]

Variability in breath biochemistry derives from: environmental contaminants; genetics; diet and lifestyle; diurnal changes in metabolism; endocrine cycles; medication; emotional/ psychological states; and, disease progression, see Figure 1. Environmental VOC exposure from inhalation, trans-dermal absorption or ingestion may result in elevated exhaled concentrations of VOC, and/or metabolic/catabolic products not originally in the environment[12]. Further, endogenous VOC, and disease markers may also be obscured, consequently, the VOC exposome generates a risk of false-attribution leading to breath-testing outcomes that are difficult to reproduce or translate into clinical practice.

Studies of VOC in hospitals, homes, and workplace settings over a prolonged period have shown exhaled breath VOC and environmental VOC often contain the same VOC. Common VOC have been observed in matched samples from ventilators, blood, breath and the room-air of clinics[13,14.] Widely reported VOC biomarkers for respiratory diseases, such as cyclohexanone for COPD[15] have also been detected in indoor air at concentrations (1.13 µg m^{-3})[16], close to those in exhaled breath (0.4 to 10 µg m^{-3})[17].

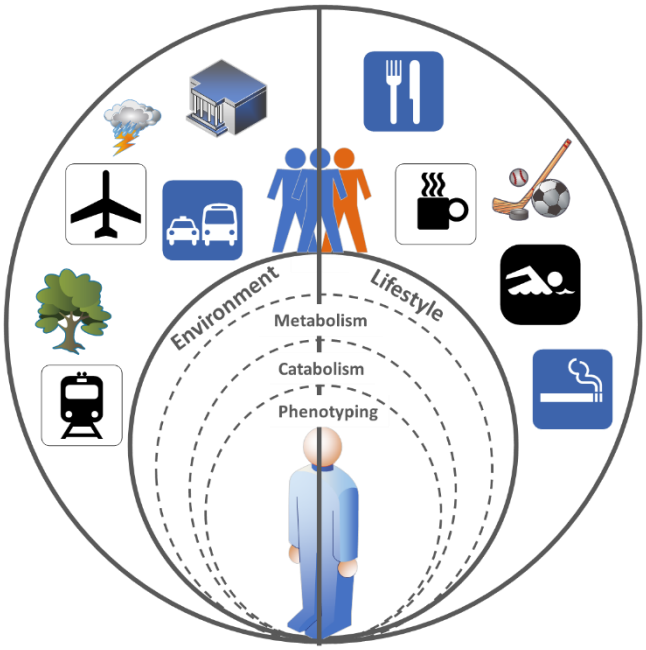


Figure 1 The observed exposome for each individual includes external factors such as environmental contamination and lifestyle, and also internal factors such as metabolism, catabolism and differences in phenotype. These are all factors in exhaled breath VOC.

Spatial and temporal variability of VOC in clinical settings has been observed with acetone, ethanol and propanol concentrations found to vary significantly while other VOC did not. Within the same study exhaled concentrations of acetone, ethanol, acetic acid, ammonia, isoprene and hydrogen cyanide were found to be higher in the breath of 10 clinical staff than in their surrounding environment, and propanol (a disinfectant) was at higher environmental concentrations[18].

The sources and patterns of VOC in the indoor air of dwellings have been studied extensively with 2246 samples monitoring a panel of 61 VOC, enabling seasonal effects to be attributed to 8 proposed VOC patterns and sources [16].

It is also helpful to note that the exogenous VOC toluene and benzene may also arise from degradation of the adsorbent Tenax TA (used in thermal desorption tubes), highlighting the point that analytical systems also create a trace VOC profile that may vary in response to changes in the environmental background (acidity, basicity, ozone and humidity), and that Tenax and multibed thermal desorption sampling tubes should not be assumed to be inert[19].

Exogenous VOC may affect breath marker discovery by: raising exhaled breath concentrations (false-positive); raising the concentration threshold for inclusion of an exhaled VOC as coming from endogenous origin causing endogenous compounds to be excluded from a study (false-negative); give rise to catabolite signals with endogenous features (false-positive); and/or act as a contrast agent that leads to correct identification of a process, mechanism, or breath-biochemical derangement, but in a non-reproducible manner.

This study addressed the environmental VOC breathomics issue, and collected room-air samples from three sites in a large NHS provider trust in the UK – University Hospitals of Leicester. The aim was to characterise the variability of composition and concentration of clinical-VOC, and establish , thresholds, or reference-levels, to inform occupational-exposure and breathomics workflows[20]. The resultant sample set captures real-world operational clinical-VOC exposure across 27 months and reveals the variability and extent of exogenous and endogenous VOC present in clinical room air at trace levels .

MATERIALS AND METHODS

This study and sampling campaign was part of a larger prospective, real-world, observational study, carried out in a tertiary cardio-respiratory centre in Leicester, United Kingdom, and . the study design, and methodology have been described in detail previously[20].

Two hundred and forty five room-air samples were collected, of which 225 samples were collected from Respiratory Medicine and Clinical Decision Units at Glenfield Hospital, Leicester, UK and 20 samples were collected from Paediatric Respiratory Medicine, Leicester Royal Infirmary Hospital, Leicester, UK, from November 2016 to February 2019. These samples were taken during normal clinical operations in the presence of patients participating in respiratory research, and research clinical staff. On occasions, other patients and staff were present, particularly if the samples were collected from a ward-bay.

VOC artefacts and contamination were reduced/eliminated from the materials and components used within this study with appropriate combinations of solvent cleaning, vacuum polishing, and temperature conditioning. All equipment was sealed in aluminium packaging ready for use before shipping to the clinic. Research and clinical staff involved in the study were trained to follow specifically designed sampling standard operating protocols, and were proficiency tested in their use[21]. Sampling protocol checklists were used to verify compliance with the standard operating protocols.

Indoor air was sampled (1000 cm^3) using an Escort ELF pump (Part No. 497702, MSA), at a flow rate of 500 $cm^3\ min^{-1}$ for 120 s onto a Tenax®/Carbotrap 1TD hydrophobic adsorbent tube (Part No. C2-AXXX-5032, Markes International Ltd, Llantrisant, UK, see Figure S1). Samples were sealed and immediately stored at ca. 4°C, before shipping to Loughborough Centre for Analytical Science within approximately 3 days. Environmental samples were dry-purged as soon as possible upon receipt with a 120 cm^3 of purified nitrogen at a flow rate of 60 $cm^3\ min^{-1}$. Toluene-D8 (69 pg) and trichloromethane-d (280 pg) internal standards were loaded during the dry purge onto the sample tube with a six-port valve attached to a permeation tube-based test atmosphere generator (constructed in -house). Dry-purged and internal-standard spiked samples were then sealed and stored at 4°C prior to analysis.

TD-GC-MS operating conditions

Samples were analysed by thermal-desorption/gas-chromatography/mass-spectrometry (TD/GC/qMS). A Unity-2 thermal desorption unit (Markes International, Cardiff, UK) was interfaced to a GC (Agilent, 7890A) coupled to a quadrupole mass spectrometer (Agilent MS 5977A). The VOC collected during sampling were recovered and concentrated into a hydrophobic cold trap yielding a 10,000-fold enrichment, the instrumentation parameters are summarised in Table S1.

Statistical Process Control

Instrumentation performance was continuously monitored by analysing 0.2 µl of a reference mixture containing 20 standards (Table S2) daily before analysis. Instrument performance was evaluated by monitoring the Z-scores of: retention time, peak area, height, width, and symmetry for the 20 standards in the reference mixture. Analysis was undertaken when instruments were within $Z = \pm 3$ for more than 80% of the 100 quality control parameters[22]. The two internal standards were also monitored to track the combined stability of the TD-GC-MS analysis and dry purging system.

Data processing

The TD-GC-MS data were deconvolved and an average of 120 VOC features per sample (AnalyzerPro Spectral Works, UK) were extracted. The deconvolution method was optimised to minimise over-deconvolution, (AnalyzerPro software method parameters were: minimum peak area value, = 200, S/N= 3, width of peak = 0.01 and smoothing factor=3). The extracted features were aligned using Kovats retention indexing (AnalyzerPro Spectral Works, UK) [23], and clustered using the VOCCluster algorithm[24] that assigned a unique identifier in the form of (ERI- m/z1 - m/z2 - m/z3 - m/z4 - m/z5) to each VOC isolated and grouped from the 245 samples; ERI indicated the retention index for the VOC environmental-feature and m/z1… m/z_n were the nominal masses of the compound's ion fragments in decreasing order of abundance needed to uniquely define the deconvolved VOC features within the data-cube. The resultant sorted, and grouped features were consolidated into an environmental-VOC-data matrix that contained the extracted peak areas for each of the features isolated from each room-air sample.

Ubiquitous siloxanes arising from analytical artefacts were also removed from the Environmental-VOC-Matrix, and 7 samples were discarded as they did not meet the quality control measures.

Multivariate analysis for seasonality effects

Once compounds that occurred in less than 30 % of samples had been excluded, the matrix features were $\log_{10}$ transformed and Pareto scaled before multivariate analysis was used to determine if there were any seasonal effects in the study [25,26]. The data was classified into two groups; September-February, n=99 samples; and, March-August, n=139 samples. Orthogonal partial least squares-discriminant analysis (OPLS-DA) was initially used to identify seasonally invariant VOC, and these were removed from the analysis leaving 44 compounds for unsupervised principle components analysis (PCA). This approach followed a multivariate statistical processing workflow using SIMCA-P+ software with integrated 7-fold cross-validation to protect against overfitting (Version 16.1, Umetrics, UK)[25]. Discriminating compounds were putatively assigned a Level-2 identification in accordance with the Metabolomics Standards Initiative[27], based on retention-index and NIST mass-spectral library matches.

RESULTS

Two hundred and forty five clinical room air samples yielded 7344 VOC features, with 328 compounds that occurred more than 12 times (5%) that were selected for further assessment, see Figure 2. Sixty eight compounds appeared in more than 30% of the total samples. Thirty nine of these have been assigned a putative identity (Class 2 identification level[27]) and a further 11 compounds were classified as hydrocarbons (Class 3 identification level[27]), see Table 1. These data were used for multivariate analysis. (The identities of eighteen of these compounds have yet to be elucidated.)

Seasonality

Seasonal variations in concentration, were identified from multi-variate analysis with 17 compounds found to be present at higher concentrations in samples obtained between September and February, Figure S2 and Table S2. In 12 of the 17 compounds the differences in concentration were statistically significantly different (normalised to the internal standard).

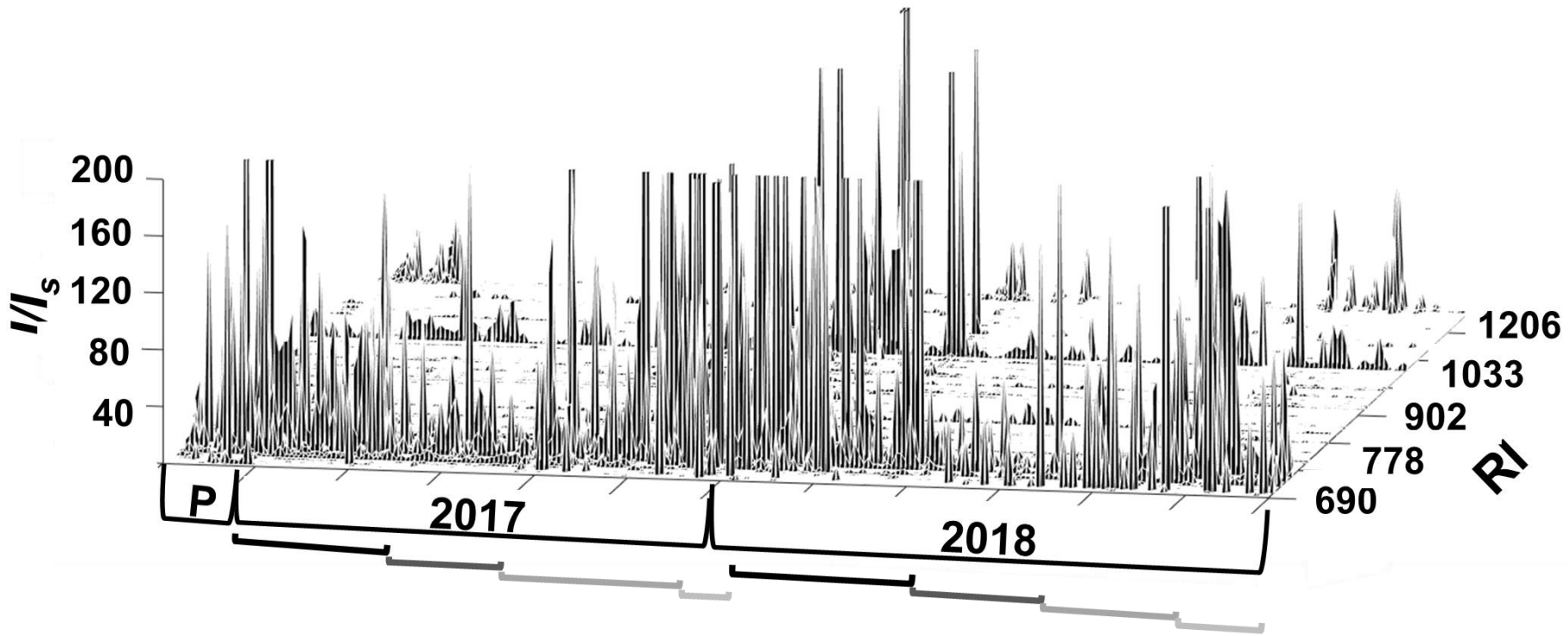


Figure 2: Common indoor VOC (> 5% of samples) found in air samples from adult and the paediatrics clinics taken during November 2016 to February 2019. The VOC are plotted along the sampling timeline against their retention index (RI) values with their intensity normalised to 280 pg of $C(^2H)Cl_3$. P represents the data from the children's clinic. The rest of the data were obtained from the adults' wards with seasons of each year (spring, summer, autumn and winter) indicated by the grey shaded brackets found underneath each year. Note that 7016 VOC isolated from less than 5% of samples are not included.

Table 1 50 VOC that have been putatively identified that appeared in more than 30% of the samples. ERI code (showing the retention index and quantifier m/z 1 and the qualifier ions m/z 2-5), NIST-library matched identification, CAS number, mean intensity ratio to C(^{2}H)Cl^3 internal standard ($\bar{I}/I_{IS}$), median intensity ratio to C(^{2}H)Cl$_3$ internal standard ($\tilde{I}/I_{IS}$), standard deviation (S) and frequency of observation (F)

VOC no.	ERI Code generated by VOCCluster [24]	Putative compound identification	CAS	$\bar{I}/I_{IS}$	$\tilde{I}/I_{IS}$	S	F
1	ERI-670-101-151-103-85-153	•1,1,2-trichloro-1,2,2-trifluoroethane-	76-13-1	0.64	0.72	0.32	91
2	ERI-683-45-46-43-42-44	[b]ethanol	64-17-5	21.9	11.37	39.72	234
3	ERI-685-45-43-58-42-0	2-propanol	67-63-0	104.1	38.95	218.3	169
4	ERI-688-67-53-68-51-50	[b]•2-methyl-1,3-butadiene	78-79-5	0.64	0.44	0.84	144
5	ERI-700-43-45-60-42-41	[b] ethanoic acid	64-19-7	2.77	1.72	3.41	184
6	ERI-712-78-77-51-50-52	[b] benzene	71-43-2	1.76	1.44	2.68	220
7	ERI-778-91-92-65-63-51	[b] toluene	108-88-3	1.71	0.97	2.71	204
8	ERI-787-41-86-45-68-68	2-methyl-2-propenoic acid,	79-41-4	0.42	0.33	0.26	80
9	ERI-791-50-46-66-66-82	N-containing compound		0.46	0.52	0.19	83
10	ERI-801-43-41-56-43-57	[b] hexanal	66-25-1	0.40	0.29	0.30	157
11	ERI-814-43-56-41-73-61	butyl ethanoate	123-86-4	0.50	0.38	0.48	113
12	ERI-854-43-72-57-42-58	[b] 2-butanone	78-93-3	0.39	0.28	0.27	93
13	ERI-861-91-106-51-65-65	[b] ethyl benzene	100-41-4	0.50	0.30	0.51	161
14	ERI-869-91-106-105-77-51	[b] 1,4-dimethylbenzene	106-42-3	1.62	0.52	5.27	134
15	ERI-893-55-42-98-98-69	[b] cyclohexanone	108-94-1	0.61	0.49	0.71	94
16	ERI-895-91-106-105-77-51	[b] 1,2-dimethylbenzene,	95-47-6	0.64	0.40	1.50	170
17	ERI-907-57-45-41-87-56	[b]2-butoxy-ethanol,	111-76-2	0.55	0.37	0.71	80
18	ERI-935-93-91-92-77-79	(1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	7785-70-8	0.57	0.47	0.51	157
19	ERI-960-77-105-106-51-50	[b]benzaldehyde	100-52-7	1.05	0.63	2.44	231
20	ERI-983-94-66-65-63-55	[b]phenol	13127-88-3	1.09	0.64	2.86	228
21	ERI-985-103-76-50-75-75	•trans-cinnamic acid ((2E)-3-Phenyl-2-propenoate)	140-10-3	0.37	0.25	0.31	144
22	ERI-987-43-41-55-69-108	[b]6-methyl-5-hepten-2-one,	110-93-0	0.48	0.39	0.37	195
23	ERI-995-105-120-91-103-51	[b]1-methylethylbenzene	98-82-8	0.50	0.34	0.97	80
24	ERI-997-105-120-77-91-119	[b]1,2,3-trimethylbenzene,	526-73-8	0.54	0.49	0.42	77
25	ERI-1003-41-43-57-56-0	[b]octanal	124-13-0	0.39	0.29	0.27	110
26	ERI-1026-119-134-134-117-77	1-methyl-4-(1-methylethyl) benzene	99-87-6	0.52	0.36	0.60	101
27	ERI-1029-57-41-43-55-56	[b]2-ethyl 1-hexanol-	104-76-7	1.21	0.68	1.86	222
28	ERI-1030-68-67-93-79-53	[b]1-Methyl-4-methylethenylcyclohexene	138-86-3	2.15	0.57	13.46	212
29	ERI-1035-79-108-77-107-51	[b]phenylmethanol	100-51-6	0.46	0.38	0.31	100
30	ERI-1068-105-77-51-120-50	[b]1-phenylethanone	98-86-2	0.50	0.34	0.46	172
31	ERI-1073-59-43-55-41-67	2,6-dimethyl -7-octen-2-ol	18479-58-8	0.76	0.38	1.80	111

32	ERI-1104-57-41-43-55-56	[b]nonanal	124-19-6	0.79	0.64	0.69	215
33	ERI-1145-55-41-97-70-57	•6-methyl-1-octanol	110453-78-6	0.46	0.38	0.43	84
34	ERI-1169-105-77-122-51-50	N-containing compound	N/A	0.39	0.31	0.27	82
35	ERI-1176-71-81-95-41-55	(1R,2R,5R)-2-Isopropyl-5-methylcyclohexanol	491-02-1	0.95	0.45	3.32	89
36	ERI-1206-41-43-57-55-44	[b]decanal	112-31-2	0.43	0.32	0.32	155
37	ERI-1377-71-43-56-89-41	3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	74367-34-3	0.59	0.33	0.93	92
38	ERI-1606-71-43-41-56-55	2,4,4-trimethyl-1,3-pentanediyl bis (2-methyl propanoate)	74381-40-1	2.02	0.53	3.25	112
39	ERI-2333-102-260-232-231-76	2,5-diphenyl-2,5-cyclohexadiene-1,4-dione,-	844-51-9	3.10	0.58	8.77	74
40	ERI-679-44-43-41-42-56			6.75	2.55	21.55	219
41	ERI-690-43-57-41-56-42			0.76	0.41	1.41	119
42	ERI-722-43-57-56-58-86			0.42	0.32	0.26	98
43	ERI-991-57-56-41-43-55			0.72	0.39	1.39	161
44	ERI-1000-43-57-41-71-85			0.61	0.34	2.21	173
45	ERI-1100-57-43-41-71-55	hydrocarbon		0.65	0.40	1.67	86
46	ERI-1200-57-43-41-71-85			0.42	0.30	0.37	192
47	ERI-1303-57-43-41-67-85			0.46	0.38	0.35	86
48	ERI-1400-57-43-71-41-85			0.45	0.34	0.38	159
49	ERI-1605-57-43-71-41-85			0.62	0.40	0.85	96
50	ERI-2342-57-43-71-85-41			14.58	0.94	64.72	74

•: qualified retention index match,

[b]: previously observed in breath ,

Cyclohexanone (1.5-fold increase, t- statistic = 1.79, one-tailed critical t-value = 1.66, p = 0.038 with 62 degrees of freedom) has been previously reported as a marker for COPD (sensitivity = 60% and specificity = 91%)[28]. Benzaldehyde (2.2-fold increase t-statistic = 2.27, one-tailed critical t-value = 1.66, p=0.012 with 100 degrees of freedom) is involved in fatty-acid and tryptophan metabolism as well as glycolysis/gluconeogenesis[29]. Exhaled benzaldehyde has also been reported: in breath samples from participants with severe pulmonary arterial hypertension, as well as healthy individuals[30]; possibly generated from bacterial degradation of common amino acids such as phenylalanine, tryptophan or tyrosine[30]; lung cancer cell lines (n=6) discriminating from healthy control cell lines (n=1) [31]; and, has also been proposed to originate from exposure to tobacco smoke, radiation or air pollution with peroxidative properties capable of damaging enzymes and DNA [32]. Phenol (2.1-fold increase, t-value statistic = 1.84, one-tailed critical t-value = 1.66, p=0.034 with 95 degrees of freedom.) is associated with petroleum products as well as tobacco smoke[33]. Phenol is also associated with oesophageal or gastric adenocarcinoma, and has been observed to be significantly higher in cancer patients (n=81) compared to healthy individuals (n=129) ($P<0.05$)[34], Figure 3.

Ethanol (2-fold increase, t- statistic = 2.63, one-tailed critical t-value = 1.66, p = 0.0048 with 113 degrees of freedom) and ethanal (2-fold increase but not statistically significant) may be attributed to ethanol consumption and metabolism with the observed increases in abundances of due to higher seasonal alcohol consumption. (Note that the ethanal mass spectrum did not fall completely in the mass spectrometric scan range and verification with derivatisation was not undertaken.)

Cleaning product/disinfectants such as 2-propanol (2-fold increase, t- statistic = 2.18, one-tailed critical t-value = 1.66, p = 0.016 with 85 degrees of freedom) were also higher in winter

Variation between different clinical settings was discernible with 3 compounds unique to the paediatric setting that were related to the paediatric anaesthetic sevoflurane [35,36]. Sevoflurane and its two metabolites 1,1,1,3,3,3-hexafluoro-2-propanone, and 1,1,1,3,3,3-hexafluoro-2-propanol were putatively identified based on NIST-mass-spectral library matches, see Figure S3. Sevoflurane and its metabolite 2-propanol, 1,1,1,3,3,3-hexafluoro have been measured previously in human breath (n=6), and used to build a three-compartment pharmacokinetics model to study environmental contaminants and breath data[37], The presence of these VOC may well have arisen from a ventilation circuit shared with a surgical theatre on a different floor of the building.

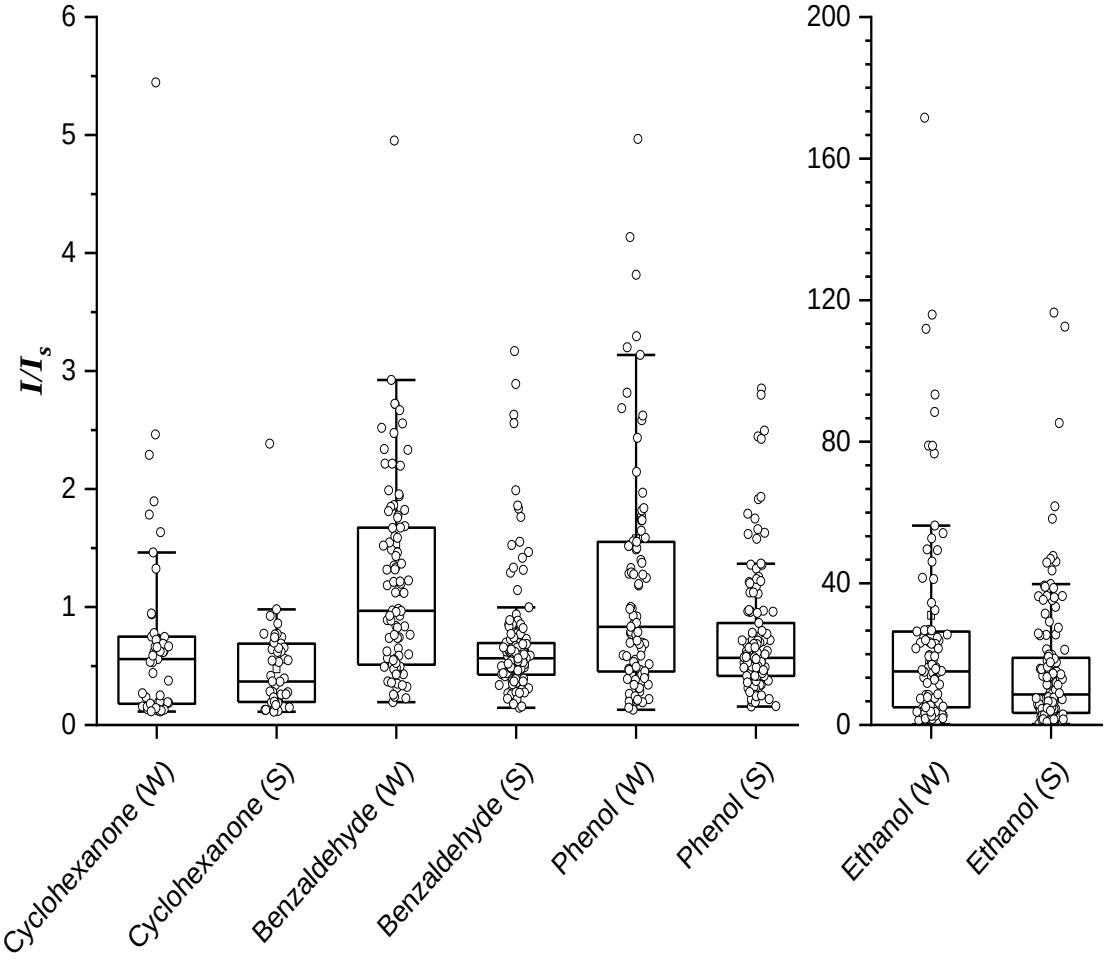


Figure 3 Box whisker plots for cyclohexanone, benzaldehyde, phenol, and ethanol contrasting the differences in profiles between Winter (W), September-February, n=99 samples and Summer (s), March-August, n=139 samples. Note that winter outliers for benzaldehyde and phenol with I/I_s values of 36.6 and 42.6

respectively are not shown. I/I_s denotes signal intensity relative to the internal standard C(^{2}H)Cl$_3$ signal.

As well as cyclohexanone and benzaldehyde other disease markers were found in the room air of clinics and Figure 4 also includes the distributions of the inflammatory biomarkers nonanal, hexanal and decanal across the sampling campaign.

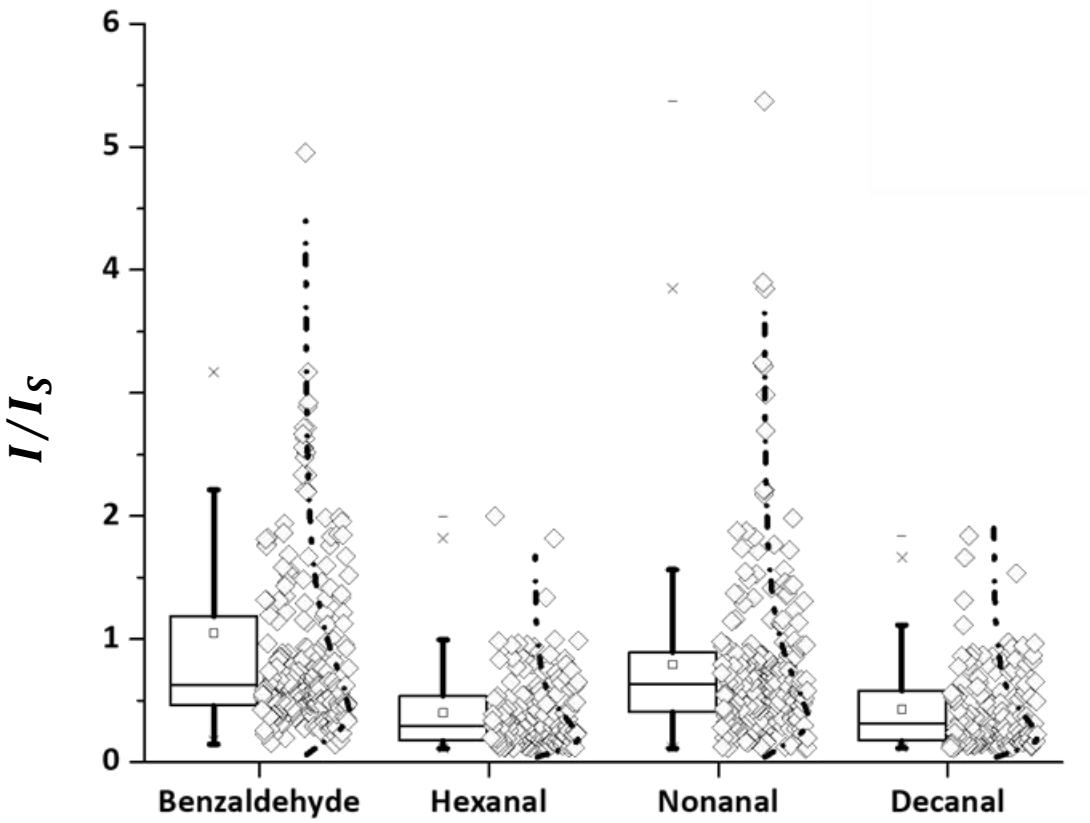


Figure 4 Box plots and lognormal distribution curves for benzaldehyde, hexanal, nonanal and 2-propanol, decanal, showing the prevalence of these often-reported breath biomarkers in clinical room air samples.

Another commonly reported breath biomarker, octanal, was also present, and provides a useful case-study into the variability of the concentrations encountered and the frequency of occurrence. Octanal was isolated from 101 environmental samples, a frequency of occurrence of 42%, the highest concentration observed was estimated to be 96 ng m^{-3} (expressed as a C(^{2}H)Cl$_3$ equivalent) with a minimum observed concentration of 1.04 ng m^{-3}, and the median observed octanal concentration was 24.32 ng m^{-3}. Tests for normality and log-normality (Shapiro-Wilk) indicated a non-normal distribution and assessment of the time

series data did not reveal any seasonal pattern, see Figure 5. Widening such an assessment to all of the 328 most frequently observed VOC revealed that most were present at concentrations below a threshold of 3 µg m^{-3} (expressed as a $C(^2H)Cl_3$ equivalent) with 14 compounds present at higher concentrations in the range 10 to 100 µg m^{-3}, see Figure 6. The compounds present at the highest concentrations appear to be associated with solvent and disinfection formulations (2-propanol for example), or, higher molecular weight waxes associated with the use of nitrile protective gloves. The more volatile the contaminant the more frequently it was observed.

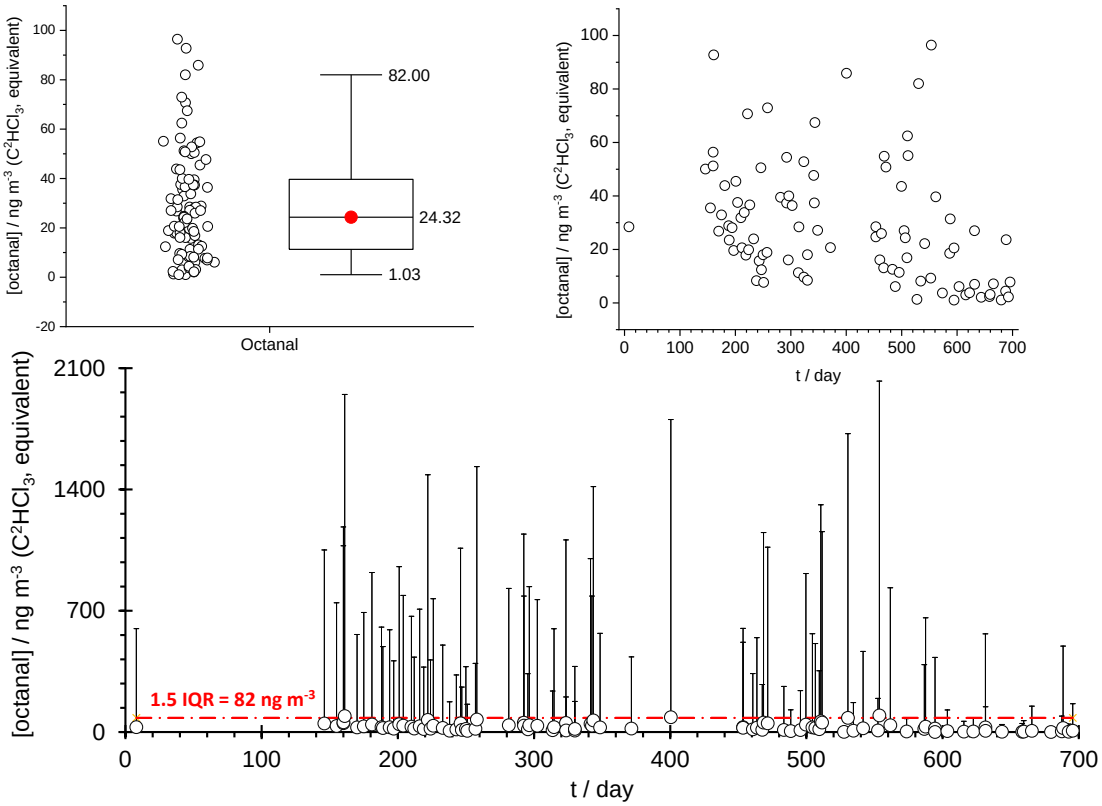
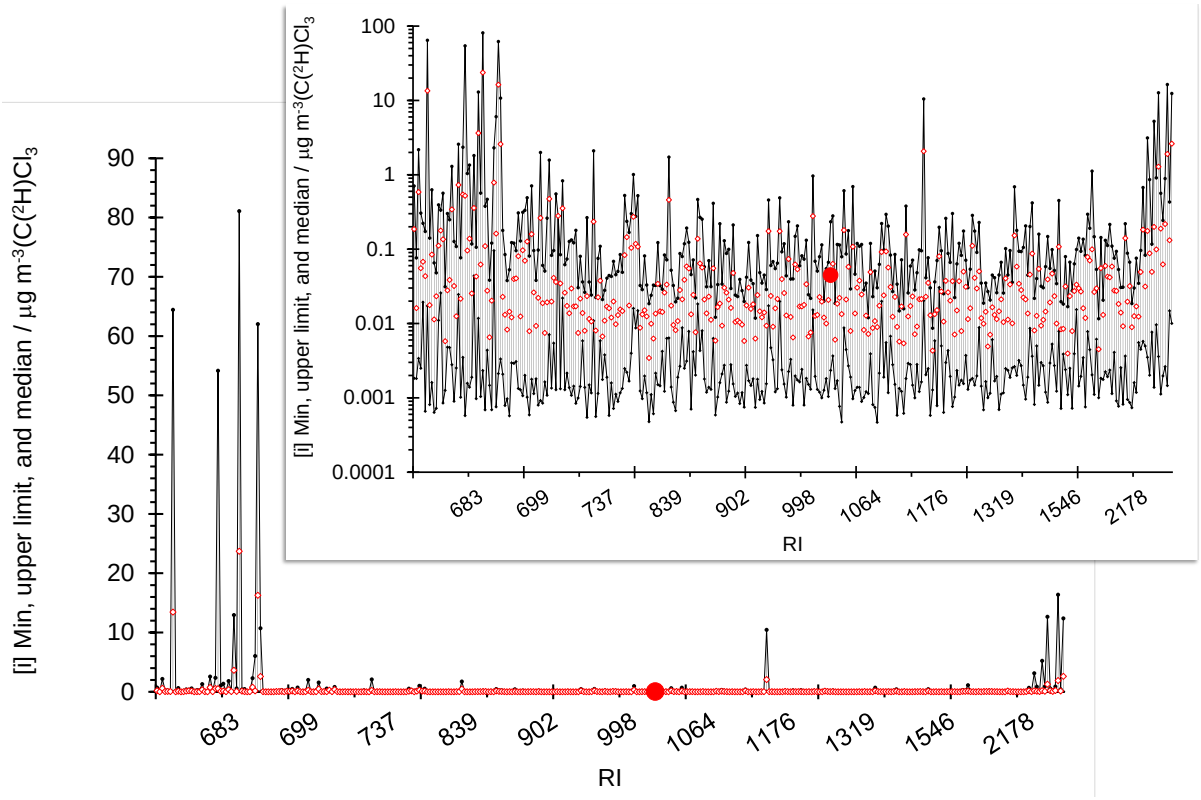


Figure 5 A summary of the environmental concentrations of octanal observed over the study. Top left, the distribution of octanal concentration; top right, the time series concentration data of background octanal concentration; and, bottom the octanal levels indicated by circles with the dashed line showing the upper limit from the distribution in the top left figure. The bottom figure's whiskers indicate the 20-fold threshold widely applied for the inclusion/exclusion threshold for admitting a VOC into a breath biomarker discovery and validation data set. The non-reproducibility and variability of such an approach may be discerned.



267

268 Figure 6 The minimum, median (red diamonds) and upper limit of observed
269 concentrations across the 328 most frequently observed (> 5%) VOC over the
270 duration of the study. The octanal entry is denoted by the solid red circle. The
271 concentrations of these compounds spanned a range of 2 to 3 orders of
272 magnitude and were typically below 3 µg m^{-3} (expressed as a $C(^2H)Cl_3$ equivalent)
273 with the exception of volatile solvents (RI less than 690) and heavier waxes
274 associated with PPE production (RI greater than 2180).

275 ## DISCUSSION

276 This study describes the complexity of the contamination profiles and concentration
277 distributions of VOC in the indoor air of hospitals and highlights seasonal and clinical
278 variations. Ninety five percent of the compounds observed occurred in in less than 5 % of
279 samples, creating a highly variable and non-reproducible VOC profile. Such variability may
280 be attributed to the constantly changing demographic of the occupants of a busy clinical
281 facility, combined with the range of therapies and therapeutics being administered. A less
282 variable constituency of the background contained a mixture of ubiquitous exogenous VOC.
283 Further, evidence that exhaled VOC may also be considered as VOC source in a clinical
284 environment, and that seasonal factors were also present, was noted.

Exhaled VOC

Changes in the VOC composition of room air from exhaled breath have been described with on-line proton transfer mass spectrometric studies reporting the presence of low molecular weight exhaled VOC, with estimated exemplar office air concentrations for acetone, ethanol, and isoprene of 52, 32, and 13 μg m^{-3} respectively[38], while studies with cinema audiences have monitored, and coded, VOC levels in room air to the emotional states of the scenes being viewed[39]. Other VOC associated with skin volatiles and the gastric tract were also reported as constituents of the cinema auditorium's air. At a significantly larger scale, changes in VOC levels in stadium air have been reported at sports events with isoprene, acetone and ethanol increasing to 8.5, 9.7 and 580 μg m^{-3} respectively during a football match[40]. Such studies suggested that the observation of exhaled volatiles in clinical room air was to be anticipated, and that the 10^4 sample enrichment obtained through two stage thermal desorption, combined with temperature programming, would enable concentrations lower than 1 μg m^{-3} to be monitored.

Exhaled VOC at trace levels were found in the room air of the clinics studied, most notably the two metabolites of sevoflurane, for it is difficult to conceive of an alternative source. The aldehydes in Figure 4, noted as oxidative stress biomarkers associated with respiratory disease, have also been associated with aging and outgassing from linoleum flooring[16], and decanal is also an oxidative product of skin lipids. It seems plausible to propose that the observed airborne concentrations of aldehydes were derived from a combination of constant background emissions due to outgassing from flooring and building materials, overlaid with concentration transients from exhaled breath combined with changes in room occupancy and associated ventilation.

Seasonal VOC.

Previous studies on VOC domestic air contaminants[16] have considered seasonal factors in indoor contamination profiles and levels. Factors such as seasonal changes in ventilation

311 and indoor-based activities were identified. Further, the effect of solar-radiation on building
312 materials and outgassing was highlighted. The VOC in Table S2 that differentiate between
313 the summer and winter months may be attributed to sources that include: cleaning and
314 disinfection materials (propan-2-ol, (1R,2S,5R)-2-isopropyl-5-methylcyclohexanol
315 [dimenthol], (6S)-2,6-Dimethyl-7-octen-2-ol, (6S)-2,6-Dimethyl-7-octen-2-ol and 2-
316 butoxyethanol); outgassing from plasticiser/polymer components (2-ethyl-1-hexanol and
317 2,5-Cyclohexadiene-1,4-dione-2,5-diphenyl, phenol, 1-methylethylbenzene
318 [isopropylbenzene] and cyclohexanone); pollutants associated with fugitive emissions from
319 vehicles and their exhausts (toluene, ethylbenzene, benzene, 1,4-dimethylbenzene and 1,2-
320 dimethylbenzene, 1-methylethylbenzene); and exhaled volatiles (cyclohexanone, ethanol
321 and ethanal). Such seasonal changes are consistent with changes in ventilation, noted
322 previously[16], and increased usage of cleaning products associated with higher bed
323 occupancy rates during the winter season. Increased concentrations of ethanol and its
324 catabolite, ethanal are consistent with winter patterns of alcohol consumption, combined
325 with changes in ventilation.

326 Breath-biochemistry research and biomarker discovery and monitoring studies manage
327 room air contamination compounds in different ways. Some studies invoke an “alveolar
328 gradient” concept[41] and others remove compounds from the data-processing workflow
329 where the environmental background is greater or equal to 5% of the exhaled concentration.
330 An alveolar gradient approach is problematic because: the data presented in this paper
331 indicate that important disease markers are routinely also present in room air; and in a
332 clinical environment a participant’s exposure to a specific VOC is unknown, as is the
333 subsequent rate of the compound’s elimination prior to the breath sample being taken.
334 Another approach that excludes breath data if it is detected in the environment[42] is also
335 unsatisfactory for the first reason given above.

Alveolar gradient and environmental exclusion approaches are unlikely to resolve the confounding factor of variable environmental backgrounds, participant exposures, and participants' catabolisms. Further, such approaches also create the possibility of uncontrolled, and variable, site and time dependent factors determining whether or not participant data is admitted into a study. Figure 5 shows a range of inclusion concentrations (20 times the environmental background that might be applied over the 245 breath data sets acquired) for octanal with a range from ca. 20 ng m^{-3} to 2 µg m^{-3} (expressed as a $C(^2H)Cl_3$ equivalent). Adopting a concentration gradient approach means that the inclusion criteria for breath data varies between participants in an apparently arbitrary manner with little or no evidence to relate the studies threshold concentration (for that particular sample) to exposure or washout.

With the exception of a few solvents (formulation compounds) and some mould release agents, associated with disposable PPE, most volatiles, when present, were below a threshold, see Figure 6. In the case of octanal this was 82 ng m^{-3} (expressed as a $C(^2H)Cl_3$ equivalent), and an exhaled breath concentration above this level was highly unlikely to be due to environmental exposure. A study of the 328 common pollutants indicates that a default level of 3 µg m^{-3} (expressed as a $C(^2H)Cl_3$ equivalent) is a threshold concentration above which most clinical room air VOC do not rise. In the absence of reliable site-specific environmental survey data applying such a threshold is a more reliable, reproducible and systematic approach than adopting the variable levels acquired by spot sampling. Infrequent VOC contamination transients would be identified as outliers and rare events in the exhaled breath profiles and be excluded from the data pipeline in the event they occurred.

Seasonal factors mean that studies and clinical breath testing need to account for possible bias. More robust discovery designs will randomise recruitment of participants over the seasons of the year and avoid overweighting those months with higher occupancy and

higher clinical VOC levels. Such attention to scheduling might also be usefully applied to sampling sessions in clinic to identify other possible synchronisation of environmental exposure with sampling activities; cleaning, cooking and medication for example.

VOC that are frequently present within a facility at elevated concentrations may introduce a "contrast agent" into breath studies, in that differentiation between classes of participants may be based on differences in how the contaminant is metabolised, arising from catabolic changes due to disease or treatment. Such a situation may result in fortuitous discovery, or non-reproducible results. It will be helpful to note and report background contaminants routinely detected at elevated concentration (i.e., above 3 μg m^{-3}) and whether they may be modelled as part of breath data or not.

Finally, adopting a concentration threshold approach does not remove the necessity for obtaining reliable environmental background data as part of breath testing operations, such samples and analysis should still an integral part of a quality assurance and control protocol.

ACKNOWLEDGMENTS

This research was funded by the Medical Research Council (MRC), Engineering and Physical Sciences Research Council (EPSRC) Stratified Medicine Grant for Molecular Pathology Nodes (Grant No. MR/N005880/1), Midlands Asthma and Allergy Research Association (MAARA) And British Lung Foundation (Grant No. BLFPHD17-1). The work was carried out at the University Hospitals of Leicester NHS Trust, University of Leicester and Loughborough University, supported by the NIHR Leicester Biomedical Research Centre and the NIHR Leicester Clinical Research Facility. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care. The authors would like to acknowledge the invaluable efforts of the research nurses responsible for the in-clinic sample collection as well as the input from the wider East Midlands

Breathomics Pathology Node consortium (members list can be found at: https://ember.le.ac.uk/web).

The authors would like to thank Spectralworks for support with data processing and their AnalyzerPro software that we used for data deconvolution and alignment.

Ethics approval. The study obtained full ethical approval from the London South East Research ethics Committee, REC reference 16/LO/1747.

REFERENCES

(1) Lourenço, C.; Turner, C. Breath Analysis in Disease Diagnosis: Methodological Considerations and Applications. *Metabolites* **2014**, *4* (2), 465–498. https://doi.org/10.3390/metabo4020465.

(2) Wheelock, C. E.; Goss, V. M.; Balgoma, D.; Nicholas, B.; Brandsma, J.; Skipp, P. J.; Snowden, S.; Burg, D.; D'Amico, A.; Horvath, I.; Chaiboonchoe, A.; Ahmed, H.; Ballereau, S.; Rossios, C.; Chung, K. F.; Montuschi, P.; Fowler, S. J.; Adcock, I. M.; Postle, A. D.; Dahlén, S.-E.; Rowe, A.; Sterk, P. J.; Auffray, C.; Djukanović, R.; Group, the U.-B. S. Application of 'omics Technologies to Biomarker Discovery in Inflammatory Lung Diseases. *Eur. Respir. J.* **2013**, *42* (3), 802–825. https://doi.org/10.1183/09031936.00078812.

(3) *Breathborne Biomarkers and the Human Volatilome*, 2nd ed.; Jonathan Beauchamp; Cristina Davis; Joachim Pleil, Ed.; Elsevier B.V., 2020. https://doi.org/10.1016/c2018-0-04980-4.

(4) Pereira, J.; Porto-Figueira, P.; Cavaco, C.; Taunk, K.; Rapole, S.; Dhakne, R.; Nagarajaram, H.; Câmara, J. S. Breath Analysis as a Potential and Non-Invasive Frontier in Disease Diagnosis: An Overview. *Metabolites* **2015**, *5* (1), 3–55. https://doi.org/10.3390/metabo5010003.

(5) Boots, A. W.; van Berkel, J. J. B. N.; Dallinga, J. W.; Smolinska, A.; Wouters, E. F.; van Schooten, F. J. The Versatile Use of Exhaled Volatile Organic Compounds in Human Health and Disease. *J. Breath Res.* **2012**, *6* (2), 027108. https://doi.org/10.1088/1752-7155/6/2/027108.

(6) Boots, A. W.; Bos, L. D.; van der Schee, M. P.; van Schooten, F.-J.; Sterk, P. J. Exhaled Molecular Fingerprinting in Diagnosis and Monitoring: Validating Volatile Promises. *Trends Mol. Med.* **2015**, *21* (10), 633–644. https://doi.org/10.1016/j.molmed.2015.08.001.

(7) Amorim, L. C. A.; de L. Cardeal, Z. Breath Air Analysis and Its Use as a Biomarker in Biological Monitoring of Occupational and Environmental Exposure to Chemical Agents. *Journal of Chromatography B: Analytical Technologies in the Biomedical and*

Life Sciences. Elsevier June 15, 2007, pp 1–9. https://doi.org/10.1016/j.jchromb.2007.03.023.

(8) Droz, P. O.; Guillemin, M. P. Occupational Exposure Monitoring Using Breath Analysis. *J. Occup. Med.* **1986**, *28* (8), 593–602. https://doi.org/10.1097/00043764-198608000-00014.

(9) Sanchez, J. M. Air and Breath Analysis for the Assessment of Exposure to Solvent Emissions in University Chemistry Laboratories. *Atmos. Pollut. Res.* **2019**, *10* (6), 1795–1802. https://doi.org/10.1016/j.apr.2019.07.011.

(10) Pleil, J. D.; Stiegel, M. A.; Risby, T. H. Clinical Breath Analysis: Discriminating between Human Endogenous Compounds and Exogenous (Environmental) Chemical Confounders. *J. Breath Res.* **2013**, *7* (1), 017107. https://doi.org/10.1088/1752-7155/7/1/017107.

(11) Blanchet, L.; Smolinska, A.; Baranska, A.; Tigchelaar, E.; Swertz, M.; Zhernakova, A.; Dallinga, J. W.; Wijmenga, C.; van Schooten, F. J. Factors That Influence the Volatile Organic Compound Content in Human Breath. *J. Breath Res.* **2017**, *11* (1), 016013. https://doi.org/10.1088/1752-7163/aa5cc5.

(12) Lan, J.; Gisler, A.; Bruderer, T.; Sinues, P.; Zenobi, R. Monitoring Peppermint Washout in the Breath Metabolome by Secondary Electrospray Ionization-High Resolution Mass Spectrometry. *J. Breath Res.* **2020**. https://doi.org/10.1088/1752-7163/ab9f8a.

(13) Hüppe, T.; Lorenz, D.; Wachowiak, M.; Maurer, F.; Meiser, A.; Groesdonk, H.; Fink, T.; Sessler, D. I.; Kreuer, S. Volatile Organic Compounds in Ventilated Critical Care Patients: A Systematic Evaluation of Cofactors. *BMC Pulm. Med.* **2017**, *17* (1), 116. https://doi.org/10.1186/s12890-017-0460-0.

(14) Schubert, J. K.; Miekisch, W.; Birken, T.; Geiger, K.; Nöldge-Schomburg, G. F. E. Impact of Inspired Substance Concentrations on the Results of Breath Analysis in Mechanically Ventilated Patients. *Biomarkers* **2005**, *10* (2–3), 138–152. https://doi.org/10.1080/13547500500050259.

(15) van Velzen, P.; Brinkman, P.; Knobel, H. H.; van den Berg, J. W. K.; Jonkers, R. E.; Loijmans, R. J.; Prins, J. M.; Sterk, P. J. Exhaled Breath Profiles Before, During and After Exacerbation of COPD: A Prospective Follow-Up Study. *COPD J. Chronic Obstr. Pulm. Dis.* **2019**, *16* (5–6), 330–337. https://doi.org/10.1080/15412555.2019.1669550.

(16) Rösch, C.; Kohajda, T.; Röder, S.; von Bergen, M.; Schlink, U. Relationship between Sources and Patterns of VOCs in Indoor Air. *Atmos. Pollut. Res.* **2014**, *5* (1), 129–137. https://doi.org/10.5094/APR.2014.016.

(17) Koureas, M.; Kirgou, P.; Amoutzias, G.; Hadjichristodoulou, C.; Gourgoulianis, K.; Tsakalof, A. Target Analysis of Volatile Organic Compounds in Exhaled Breath for Lung Cancer Discrimination from Other Pulmonary Diseases and Healthy Persons.

Metabolites **2020**, *10* (8), 317. https://doi.org/10.3390/metabo10080317.

(18) Boshier, P. R.; Cushnir, J. R.; Priest, O. H.; Marczin, N.; Hanna, G. B. Variation in the Levels of Volatile Trace Gases within Three Hospital Environments: Implications for Clinical Breath Testing. *J. Breath Res.* **2010**, *4* (3), 031001. https://doi.org/10.1088/1752-7155/4/3/031001.

(19) Axel Clausen, P.; Wolkoff, P. Degradation Products of Tenax TA Formed during Sampling and Thermal Desorption Analysis: Indicators of Reactive Species Indoors. *Atmos. Environ.* **1997**, *31* (5), 715–725. https://doi.org/10.1016/S1352-2310(96)00230-0.

(20) Ibrahim, W.; Wilde, M.; Cordell, R.; Salman, D.; Ruszkiewicz, D. M.; Brynt, L.; Richardson, M.; Free, R.; Zhao, B. Assessment of Breath Volatile Organic Compounds in Acute Cardio-Respiratory Breathlessness: A Protocol Describing a Prospective Real World Observational Study. *BMJ Open* **2019**.

(21) Ruszkiewicz, D.; Henderson, B.; Wilkinson, M.; Beauchamp, J.; Holz, O.; Salman, D.; Francesco, F. Di; Langejürgen, J.; Koppen, G.; Pedrotti, M.; Moreno, S.; Slingers, G.; Sinues, P.; Wilde, M.; Zenobi, R.; Zanella, D.; Lomonaco, T.; Franchina, F. A.; Grassin-Delyle, S.; Focant, J.-F.; Stefanuto, P.-H.; Cristescu, S.; Fowler, S.; Thomas, C. L. P. A Benchmarking Protocol for Breath Analysis: The Peppermint Breath Experiment. *J. Breath Res. Res.*

(22) Broadhurst, D.; Goodacre, R.; Reinke, S. N.; Kuligowski, J.; Wilson, I. D.; Lewis, M. R.; Dunn, W. B. Guidelines and Considerations for the Use of System Suitability and Quality Control Samples in Mass Spectrometry Assays Applied in Untargeted Clinical Metabolomic Studies. *Metabolomics* **2018**, *14* (6), 72. https://doi.org/10.1007/s11306-018-1367-3.

(23) Hübschmann, H.-J. *Handbook of GC/MS : Fundamentals and Applications*; Wiley-VCH, 2009.

(24) Alkhalifah, Y.; Phillips, I.; Soltoggio, A.; Darnley, K.; Nailon, W. H.; McLaren, D.; Eddleston, M.; Thomas, C. L. P.; Salman, D. VOCCluster: Untargeted Metabolomics Feature Clustering Approach for Clinical Breath Gas Chromatography - Mass Spectrometry Data. *Anal. Chem.* **2019**, acs.analchem.9b03084. https://doi.org/10.1021/acs.analchem.9b03084.

(25) L. Eriksson, E. Johansson, N. Kettaneh-Wold, J.Trygg, C. Wikström, and S. W. *Multi- and Megavariate Data Analysis Part I: Basic Principles and Applications*; 2006. https://doi.org/10.1002/cem.713.

(26) Eriksson, L.; Byrne, T.; Johansson, E.; Trygg, J.; Wikström, C. *Multi- and Megavariate Data Analysis Basic Principles and Applications – Umetrics*, 3rd ed.; MKS Umetrics AB: Malmo Sweden, 2013.

(27) Salek, R. M.; Steinbeck, C.; Viant, M. R.; Goodacre, R.; Dunn, W. B. The Role of

Reporting Standards for Metabolite Annotation and Identification in Metabolomic Studies. *Gigascience* **2013**, *2* (1), 13. https://doi.org/10.1186/2047-217X-2-13.

(28) Westhoff, M.; Litterst, P.; Maddula, S.; Bödeker, B.; Rahmann, S.; Davies, A. N.; Baumbach, J. I. Differentiation of Chronic Obstructive Pulmonary Disease (COPD) Including Lung Cancer from Healthy Control Group by Breath Analysis Using Ion Mobility Spectrometry. *Int. J. Ion Mobil. Spectrom.* **2010**, *13* (3–4), 131–139. https://doi.org/10.1007/s12127-010-0049-2.

(29) Zimmermann, D.; Hartmann, M.; Moyer, M. P.; Nolte, J.; Baumbach, J. I. Determination of Volatile Products of Human Colon Cell Line Metabolism by GC/MS Analysis. *Metabolomics* **2007**, *3* (1), 13–17. https://doi.org/10.1007/s11306-006-0038-y.

(30) Mansoor, J. K.; Schelegle, E. S.; Davis, C. E.; Walby, W. F.; Zhao, W.; Aksenov, A. A.; Pasamontes, A.; Figueroa, J.; Allen, R. Analysis of Volatile Compounds in Exhaled Breath Condensate in Patients with Severe Pulmonary Arterial Hypertension. *PLoS One* **2014**, *9* (4), e95331. https://doi.org/10.1371/journal.pone.0095331.

(31) Jia, Z.; Zhang, H.; Ong, C. N.; Patra, A.; Lu, Y.; Lim, C. T.; Venkatesan, T. Detection of Lung Cancer: Concomitant Volatile Organic Compounds and Metabolomic Profiling of Six Cancer Cell Lines of Different Histological Origins. *ACS omega* **2018**, *3* (5), 5131–5140. https://doi.org/10.1021/acsomega.7b02035.

(32) Nakhleh, M. K.; Haick, H.; Humbert, M.; Cohen-Kaminsky, S. Volatolomics of Breath as an Emerging Frontier in Pulmonary Arterial Hypertension. *Eur. Respir. J.* **2017**, *49* (2). https://doi.org/10.1183/13993003.01897-2016.

(33) Weisel, C.; Yu, R.; Roy, A.; Georgopoulos, P. Biomarkers of Environmental Benzene Exposure. *Environ. Health Perspect.* **1996**, *104 Suppl* (Suppl 6), 1141–1146. https://doi.org/10.1289/ehp.961041141.

(34) Kumar, S.; Huang, J.; Abbassi-Ghadi, N.; Mackenzie, H. A.; Veselkov, K. A.; Hoare, J. M.; Lovat, L. B.; Španěl, P.; Smith, D.; Hanna, G. B. Mass Spectrometric Analysis of Exhaled Breath for the Identification of Volatile Organic Compound Biomarkers in Esophageal and Gastric Adenocarcinoma. *Ann. Surg.* **2015**, *262* (6), 981–990. https://doi.org/10.1097/SLA.0000000000001101.

(35) Ruszkai, Z.; Bokrétás, G. P.; Bartha, P. T. Sevoflurane Therapy for Life-Threatening Acute Severe Asthma: A Case Report. *Can. J. Anaesth.* **2014**, *61* (10), 943–950. https://doi.org/10.1007/s12630-014-0213-y.

(36) Schutte, D.; Zwitserloot, A.; Hoog, M. d.; Houmes, R.; Draaisma, J.; Lemson, J. Sevoflurane Therapy for Life Threatening Asthma in Children. *Arch. Dis. Child.* **2012**, *97* (Suppl 2), A123–A124. https://doi.org/10.1136/archdischild-2012-302724.0419.

(37) Ghimenti, S.; Di Francesco, F.; Onor, M.; Stiegel, M. A.; Trivella, M. G.; Comite, C.; Catania, N.; Fuoco, R.; Pleil, J. D. Post-Operative Elimination of Sevoflurane Anesthetic

and Hexafluoroisopropanol Metabolite in Exhaled Breath: Pharmacokinetic Models for Assessing Liver Function. *J. Breath Res.* **2013**, *7* (3), 036001. https://doi.org/10.1088/1752-7155/7/3/036001.

(38) Fenske, J. D.; Paulson, S. E.; Fenske, P. Human Breath Emissions of VOCs. *J. Air Waste Manage. Assoc.* **1999**, *49* (5), 594–598. https://doi.org/10.1080/10473289.1999.10463831.

(39) Williams, J.; Stönner, C.; Wicker, J.; Krauter, N.; Derstroff, B.; Bourtsoukidis, E.; Klüpfel, T.; Kramer, S. Cinema Audiences Reproducibly Vary the Chemical Composition of Air during Films, by Broadcasting Scene Specific Emissions on Breath. *Sci. Rep.* **2016**, *6* (0000), 4–7. https://doi.org/10.1038/srep25464.

(40) Veres, P. R.; Faber, P.; Drewnick, F.; Lelieveld, J.; Williams, J. Anthropogenic Sources of VOC in a Football Stadium: Assessing Human Emissions in the Atmosphere. *Atmos. Environ.* **2013**, *77*, 1052–1059. https://doi.org/10.1016/j.atmosenv.2013.05.076.

(41) Phillips, M.; Greenberg, J.; Awad, J. Metabolic and Environmental Origins of Volatile Organic Compounds in Breath. *J. Clin. Pathol.* **1994**, *47* (11), 1052–1053. https://doi.org/10.1136/jcp.47.11.1052.

(42) Martin, H. J.; Turner, M. A.; Bandelow, S.; Edwards, L.; Riazanskaia, S.; Thomas, C. L. P. Volatile Organic Compound Markers of Psychological Stress in Skin: A Pilot Study. *J. Breath Res.* **2016**, *10* (4), 046012. https://doi.org/10.1088/1752-7155/10/4/046012.

555 SUPPLEMENTARY DATA

556

557 # THE VARIABILITY OF VOLATILE ORGANIC COMPOUNDS IN CLINICAL ENVIRONMENTS.

558 Dahlia Salman[a], Wadah Ibrahim[b,c], Amisha Kanabar[a], Bo Zhao[b,c], Amisha Singapuri[b,c], Michael
559 Wilde[d], Rebecca L Cordell[d], Teresa McNally[b], Dorota Ruszkiewicz[a], Andria Hadjithekli[a], Robert Free[b,c],
560 Neil Greening[b,c], Erol A Gaillard[b], Caroline Beardsmore[b], Paul Monks[d], Chris Brightling[b,c], Salman
561 Siddiqui[b,c], C L Paul Thomas[a]* On behalf of the EMBER consortium

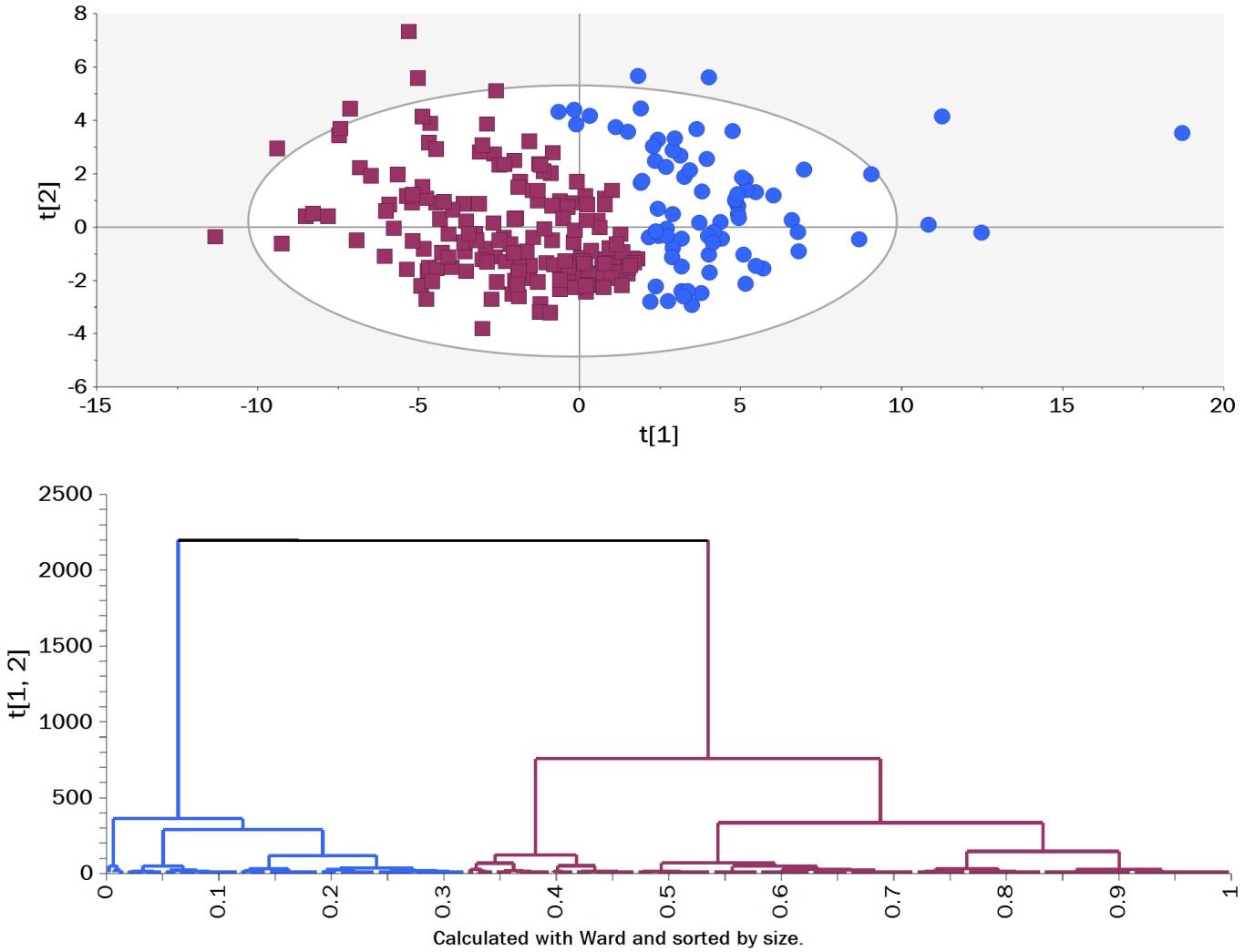
562 ***Corresponding author:** C.L.P.Thomas@lboro.ac.uk

563 a Department of Chemistry, Loughborough University, Loughborough, LE11 3TU, UK.
564 b College of Life Sciences, Department of Respiratory Sciences, University of Leicester,
565 University Road, Leicester LE1 7RH
566 c Leicester NIHR Biomedical Research Centre (Respiratory theme), Glenfield Hospital, Groby
567 Road, Leicester LE3 9QP
568 d Department of Chemistry, University of Leicester, University Road, Leicester, LE1 7RH
569

570

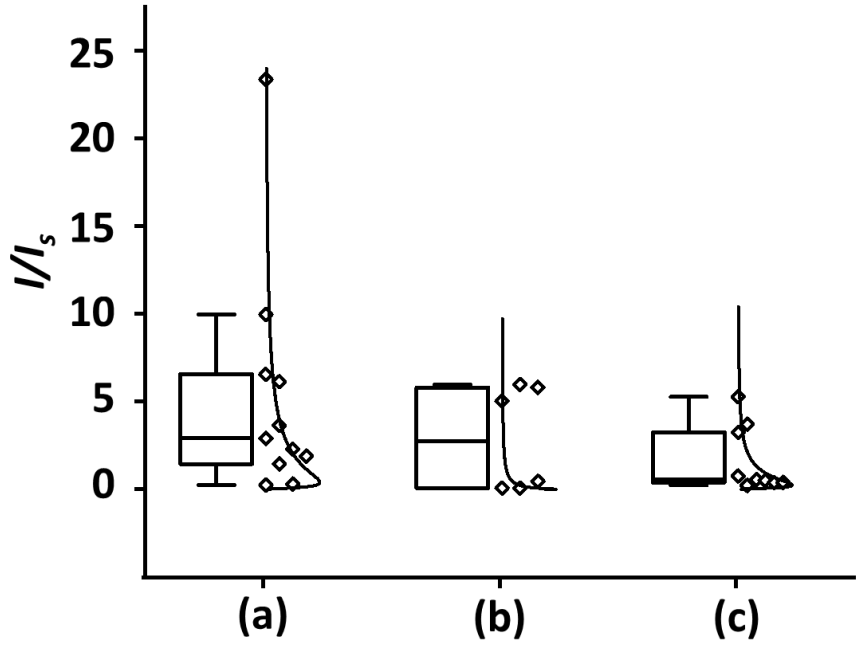
FIGURES

Figure S7: The Escort Elf pump attached to a Tenax®/Carbotrap 1TD hydrophobic adsorbent tube to continuously sample (500 cm3 min-1) the indoor air of the clinic/ward for 120 s.



578

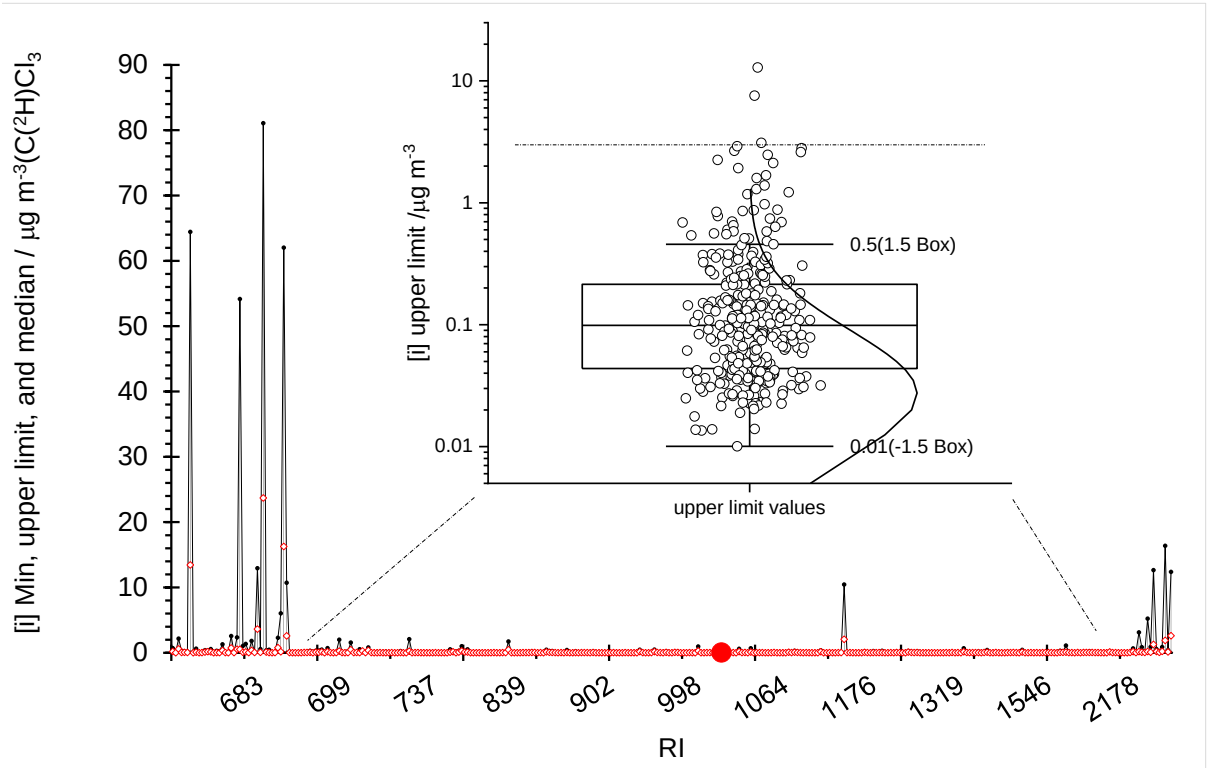
579 **Figure S8:** PCA (top) and its associate dendrogram (bottom) to examine the seasonality effects on the environmental VOCs profile in the
580 two clinics. Winter and summer groups are coloured with blue and red, respectively.



581

582 **Figure S9** Box plots and lognormal distribution curves for (a) Sevoflurane (m/z 131,
583 retention index 661, mean 3.09, sd 5.51) and its two metabolites (b) 2-
584 Propanone, 1,1,1,3,3,3-hexafluoro (m/z 69, retention index 655, mean 0.92,
585 sd 2.04) and (c) and 2-Propanol, 1,1,1,3,3,3-hexafluoro (m/z 51, retention
586 index 670, mean 0.79, sd 1.48)

587



588

589 **Figure S10** Further detail on Figure 6 comparing the minimum, median (red diamonds)
590 and upper limit of observed concentrations across the 328 most frequently
591 observed (frequency > 5%) VOC over the duration of the study. The octanal
592 entry is denoted by the solid red circle. The concentrations of these
593 compounds spanned a range of 2 to 3 orders of magnitude and the Q3+1.5
594 IQR was 0.5) The inset box whisper plot consolidates the upper limits of the
595 328 VOC. A working threshold of 3 µg m^{-3} (expressed as a $C(^2H)Cl_3$ equivalent
596 and indicated by the dotted line) may be reliably applied across the RI range
597 of RI 700 to RI 1900. This excludes volatile solvents (RI less than 700) and
598 heavier waxes associated with PPE production (RI greater than 1900),

599

600

TABLES

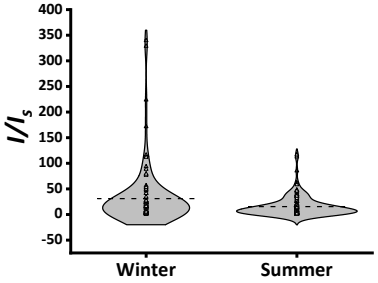
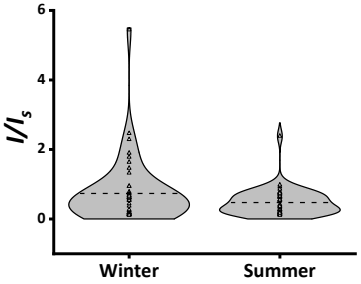
Table S2: TD-GC-MS operational conditions

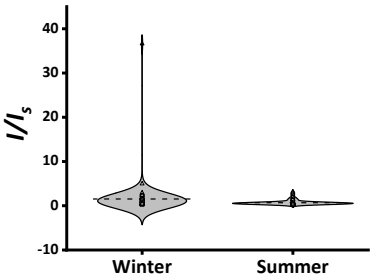
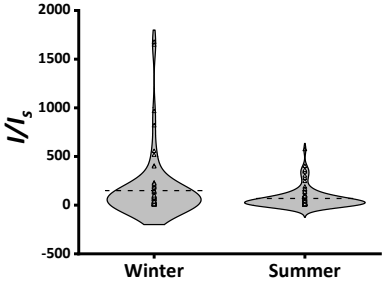
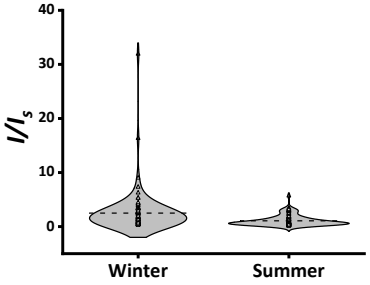
Thermal desorption		**Gas chromatography**		**Mass spectrometer**	
Parameters	Setting	Parameters	Setting	Parameters	Setting
t Primary desorption	1 min	F He carrier gas	2 cm^3 min^{-1}	Scan type	Full scan (+ve)
F Primary desorption	40 cm^3 min^{-1}	T Initial	40°C	Mass range	40 to 550 m/z
T Primary desorption	300°C	t Initial hold	0 min	Ionisation type	EI
T Secondary desorption	5 min	T program	5°C min^{-1} to 300°C	ν scan	3 Hz
F Secondary desorption	50 cm^3 min^{-1}	T End	300 °C	T line temperature	300 °C
T Secondary desorption	300°C	t End hold	0 min	T Quadrupole	150 °C
F Cold trap	20 cm^3 min^{-1}	t Total run	60 min	T Manifold	230 °C
T Cold trap	-10°C	T Post run	45 °C	t Solvent delay	5 min
$\left(\frac{\delta T}{\delta t}\right)_{trap}$	Max °C min^{-1}	t Post run	0 min		
T Trap high	300°C				
t Trap hold	5 min				
T Flow path	200 °C				
Mode	Spitless				

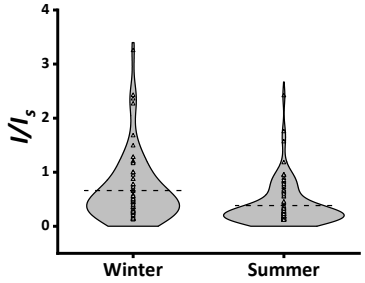
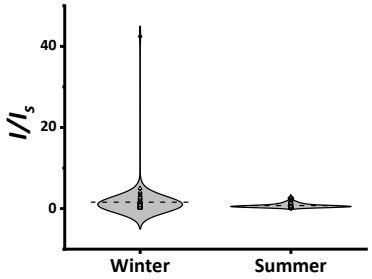
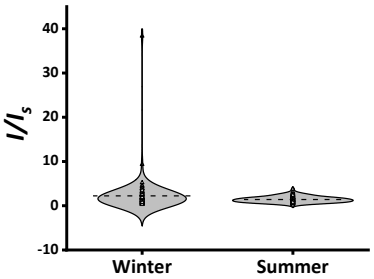
Note: **t**, time; **F**, flow; **T**, temperature; and **ν**, frequency.

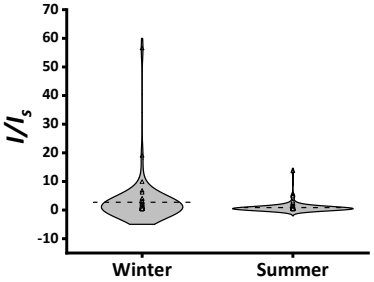
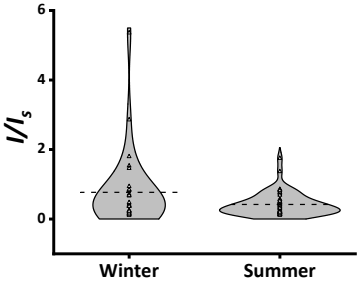
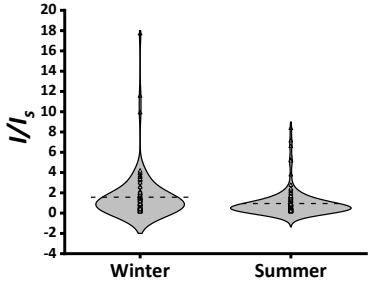
Table S3: A list of volatile organic compounds that were detected in the environmental air of both hospitals and were found to be higher in the winter seasons than summer.* is for compounds that were higher in winter by 1.5-foldhowever not statistically different following a one-tail t-test. ‡Identification of ethanol is qualified in that the full mass spectrum was not captured within the scan range of the method.

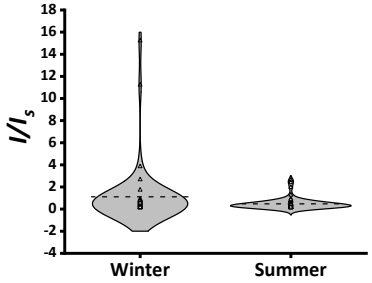
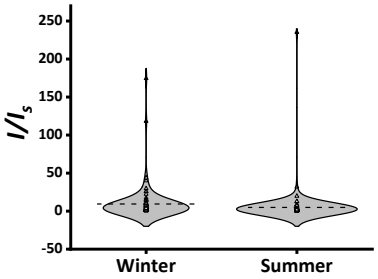
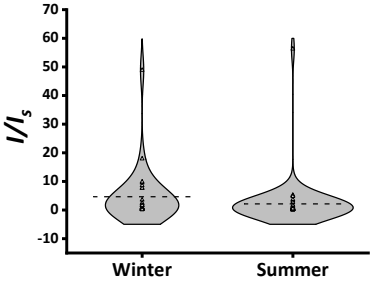
$\overline{I_W}/I_{IS}$: mean intensity ratio to C(^{2}H)Cl$_3$ internal standard in winter; S_W standard deviation (winter); $\overline{I_S}/I_{IS}$: mean intensity ratio to C(^{2}H)Cl$_3$ internal standard in summer; S_S standard deviation (summer); $\overline{I_W}/\overline{I_S}$ fold change in abundance between winter and summer; DoF: degrees of freedom

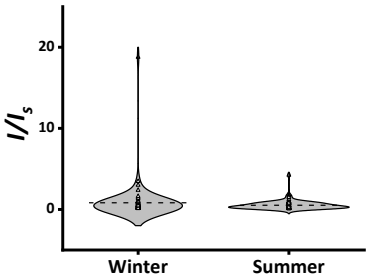
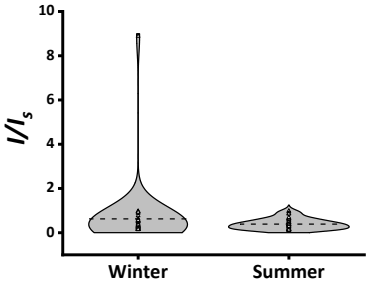
Compound	Winter		Summer		$\overline{I_W}/\overline{I_S}$	t Stat	t_{crit} 1-tail	$P \leq t$ 1-tail	DoF	Violin plots
	$\overline{I_W}/I_{IS}$	S_W	$\overline{I_S}/I_{IS}$	S_S						
Ethanol	30.98	55.89	15.44	19.06	2.01	2.63	1.65	0.004	113	
Cyclohexanone	0.73	0.90	0.47	0.38	1.55	1.79	1.67	0.038	62	

Benzaldehyde	1.54	3.65	0.69	0.51	2.23	2.27	1.66	0.010	100	
Propan-2-ol	149.49	304.00	68.82	100.02	2.17	2.17	1.66	0.016	85	
Toluene	2.49	3.79	1.07	0.93	2.33	3.48	1.66	0.0004	99	

Ethyl benzene	0.66	0.60	0.38	0.39	1.72	3.35	1.66	0.0006	111	
Phenol	1.58	4.36	0.74	0.53	2.13	1.84	1.66	0.034	95	
Benzene	2.23	4.00	1.40	0.68	1.59	1.96	1.66	0.0260	96	

1,4-dimethylbenzene	2.73	7.93	0.88	1.65	3.11	1.67	1.67	0.0499	56	
2-butoxyethanol	0.77	1.04	0.42	0.32	1.83	1.76	1.69	0.0441	32	
2-ethyl 1-hexanol-	1.57	2.38	0.94	1.29	1.67	2.30	1.66	0.0113	133	

(6S)-2,6-Dimethyl-7-octen-2-ol	1.11	2.61	0.47	0.48	2.36	1.68	1.68	0.0491	51	
Ethanal *‡	9.36	22.37	4.88	20.75	1.92	1.49	1.65	0.068	185	
2,5-Cyclohexadiene-1,4-dione, 2,5-diphenyl- *	4.65	9.47	2.16	8.17	2.15	1.13	1.68	0.130	50	

1,2-dimethylbenzene *	0.82	2.21	0.51	0.52	1.60	1.14	1.66	0.1280	77	
Benzene, (1-methylethyl)- *	0.63	1.42	0.39	0.23	1.61	0.97	1.68	0.1669	37	
(1R,2S,5R)-2-isopropyl-5-methylcyclohexanol *	1.45	5.01	0.59	0.42	2.44	1.04	1.69	0.1517	37