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Variation in volatile organic compounds in the breath of normal humans

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Abstract

We studied the variation in volatile organic compounds (VOCs) in the breath of 50 normal humans, using gas chromatography and mass spectroscopy. An average breath sample contained 204.2 VOCs (SD=19.8, range 157–241).The alveolar gradient of each VOC (abundance in breath minus abundance in air) varied with rate of synthesis minus rate of clearance. A total of 3481 different VOCs were observed: 1753 with positive alveolar gradients, 1728 with negative alveolar gradients. Twenty-seven VOCs were observed in all fifty subjects. This study confirmed previous reports of wide inter-individual variations. Two new findings were the comparatively small variation in total number of breath VOCs, and the presence of a 'common core' of breath VOCs in all subjects.

Keywords: Breath tests; Volatile organic compounds

1. Introduction

Alveolar breath is a distinctive gas whose chemical composition differs markedly from inspired air. Volatile organic compounds (VOCs) are either subtracted from inspired air (by degradation and/or excretion in the body) or added to alveolar breath as products of metabolism. Some features of this transformation have been well understood for many years: e.g. oxygen is subtracted and carbon dioxide is added by the oxidative metabolism of glucose [1]. Pauling et al., in 1971, employed cold trapping to concentrate the VOCs in breath and found that normal human breath contained several hundred different VOCs in low concentrations [2]. This observation has been subsequently confirmed in many different laboratories, employing progressively more sophisticated and sensitive assays. More than a thousand different VOCs have been observed in low concentrations in normal human breath [3].

Analysis of VOCs in inspired air and alveolar breath is a useful research tool with potential applications in clinical medicine. Breath analysis opens a non-invasive window on to normal metabolic pathways, and also illustrates how these pathways are altered in disease. For example, breath pentane is a marker of increased oxygen free radical (OFR) activity in several diseases. Pentane is a product of

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OFR-mediated lipid peroxidation of n-6 polyunsaturated fatty acids [4,5] and it is subsequently degraded by cytochrome P450 enzymes [6,7]. Breath pentane is increased in a number of disorders including breast cancer [8], heart transplant rejection [9], acute myocardial infarction [10], schizophrenia [11] and rheumatoid arthritis [12].

Despite numerous studies of pentane and several other breath VOCs, the range of composition of VOCs in normal human breath has not been well defined. Early studies reported substantial quantitative and qualitative differences amongst small groups of normal humans: concentrations of breath VOCs varied widely, and a number of VOCs were detectable in the breath of some subjects but not in others [13,14]. We undertook this study in order to better define the range of inter-individual variation in breath VOCs in normal humans. We employed a portable breath collection apparatus BCA to study a group of normal volunteers; breath VOCs were collected onto sorbent traps which were assayed by gas chromatography–mass spectroscopy (GC–MS) [3].

2. Experimental

2.1. Breath collection apparatus

This device has been described [3]. In summary, the BCA is a portable, microprocessor-controlled device with a heated breath reservoir which prevents condensation of water. Alveolar breath is pumped from the breath reservoir through a sorbent tube which captures the VOCs on activated carbon. In this study, modified sorbent tubes were employed containing 200 mg Carbotrap C (20/40 mesh) and 200 mg Carbopack B (60/80 mesh) (Supelco, Bellefonte, PA, USA) The volume of the breath sample can be varied via a panel-mounted timer and flow meter, and the geometry of the system ensures that the sample comprises alveolar breath virtually uncontaminated by dead-space air.

2.2. Collection of a breath sample

Subjects breathed into the BCA through a disposable mouthpiece. The BCA presented minimal resistance to expiration because the wide-bore breath reservoir (1.0 inch diameter) was open to the air at its far end. Samples could be collected even from elderly or bedridden patients without causing discomfort. The collection period was 2.0 min at 0.5 l/min, and two samples were collected: one of breath, and one of background room air.

2.3. Equipment and procedure

VOCs were desorbed from the sorbent tubes and concentrated in an automated thermal desorber (ATD 400, Perkin Elmer, Norwalk, CT, USA), separated in a gas chromatograph, and identified and quantitated in a mass spectrometer (HP6890 and mass selective detector 5973, Hewlett-Packard, Palo Alto, CA, USA). Sorbent tubes were loaded onto a carousel (capacity 50), checked for leaks, then purged with helium for 1.0 min to remove water vapor and air. An internal standard (0.25 ml 2 ppm 1-bromo-4-fluoro-benzene, Supelco) was added via the ATD 400 standard injection accessory. The sample was desorbed at 300°C onto a 0°C cold trap (low flow ATD 400 air monitoring trap) for 4 min (helium flow 50 ml/min, outsplit flow 2.0 ml/min). The cold trap was then heated rapidly to 300°C and the desorbed sample was flushed through a fused-silica transfer line (0.32 mm I.D., 200°C, helium flow 1.25 ml/min) to the chromatography column (SPB-5 capillary column, 30 m×0.25 mm, 0.25 μm film thickness, Supelco). Column temperatures were ramped as follows: 0°C for 8 min, 4°C/min to 138°C, 0.10 min hold, 10°C/min to 210°C, 0.10 min hold, and 30°C/min to 300°C, 0.25 min hold.

2.4. Data management

Data from each chromatographic peak, comprising retention time, chemical identity (as identified by Wiley 138 library), area under curve (AUC), and quality of fit, were automatically downloaded into a spreadsheet (EXCEL 4.0, Microsoft, Redmond, WA, USA) and consolidated in a relational database (Paradox, Borland, Scotts Valley, CA, USA). The alveolar gradient of each VOC was calculated as:

$$\mathrm{AUC}_{\text{VOC in breath}}/\mathrm{AUC}_{\text{internal standard}} - \mathrm{AUC}_{\text{VOC in air}}/\mathrm{AUC}_{\text{internal standard}}$$

The kinetic determinants of the alveolar gradient are shown in Appendix A and in Figs. 3 and 4.

2.5. Human subjects

Normal volunteers were recruited from the medical and paramedical staff of St. Vincent's Medical Center of Richmond. All subjects had fasted from the previous midnight and samples were collected between 7.00 am and 12.00 noon. The research was approved by the institutional review board of St. Vincent's Medical Center of Richmond.

3. Results

3.1. Human subjects

There were 50 subjects studied, comprising 27 males (mean age 38.8 years, SD=12.8) and 23 females (mean age 38.65 years, SD=11.4). Typical chromatograms of breath and air are shown in Fig. 5, with a subtraction chromatogram of the difference between the two.

3.2. Inter-individual variation in number of VOCs

The number of VOCs detected in each breath sample ranged from 157 to 241 (mean=204.2, SD=19.8, CV=9.7%) (Fig. 1). Different VOCs totaling 3481 were observed at least once, 1753 with positive alveolar gradients and 1728 with negative alveolar gradients, but the majority of those were observed in only one subject. Only 27 VOCs were observed in all subjects (Fig. 2, and listed in Table 1 as observed in 100%).

3.3. Inter-individual variation in frequency and abundance of VOCs

VOCs were ranked by the frequency with which they were observed in different subjects (Table 1) and by their relative abundance in the breath (Table 2).

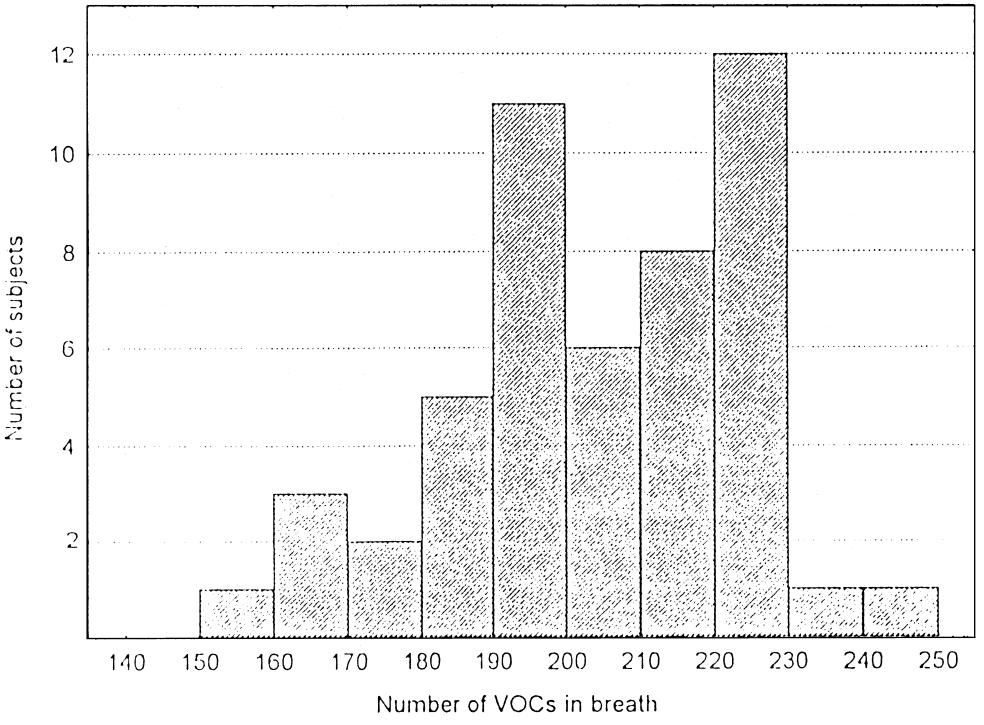

Fig. 1. Inter-individual variation in number of VOCs in breath. Frequency distribution of number of VOCs observed in each breath sample.

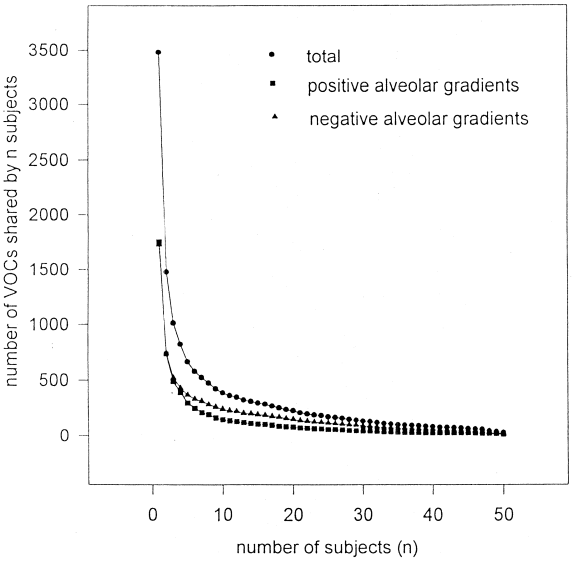

Fig. 2. Variation in number of shared VOCs with sample size: 3481 different VOCs were observed at least once, comprising 1753 VOCs with positive alveolar gradients and 1728 VOCs with negative alveolar gradients. Only 9 VOCs with positive alveolar gradients, and 18 VOCs with negative alveolar gradients were observed in all 50 subjects.

4. Discussion

More than 200 different VOCs were observed in most breath samples, and more than 3000 different VOCs were observed at least once. These numbers probably represent an underestimate of the total number of VOCs in normal human breath, since the assay was limited to C_4 to C_{20} VOCs within the trapping range of the sorbent traps. The majority of these VOCs were observed only once. The number of breath VOCs observed in more than one subject fell rapidly as the size of the group increased, and only a comparatively small number of commonly occurring VOCs were observed consistently in the majority of the population.

Several of the commonly occurring VOCs were derived from metabolic pathways that have been previously reported e.g. isoprene from the mevalonic acid pathway of cholesterol synthesis [15], acetone from glucose metabolism [16], and alkanes from OFR-mediated lipid peroxidation of fatty acids [4,5]. However, the source of commonly occurring VOCs such as naphthalene and 1-methyl-naphthalene is not yet known; they may be degradation products of steroids, but further studies are required to determine their origin. Also, several highly substituted benzenes and branched chain alkanes were observed in most breath samples. The majority of these VOCs had a negative alveolar gradient (Table 1), so that kinetic analysis (Appendix A) indicates that the rate of clearance of these VOCs was greater than their rate of synthesis. This is consistent with VOCs which were ingested as contaminants of room air then cleared from the body via hepatic and/or renal pathways. Staten Island, where the breath samples were collected, is downwind from Elizabeth (NJ, USA) a highly industrialized area with several oil refineries and an adjacent airport. It is possible that the ambient room air may have been contaminated with VOCs from these sources.

The actual concentration of each VOC in molar or mass units was not determined because this would have required the construction of more than 3000 different standard curves, a very considerable undertaking. Instead, we determined the ratio of the area under curve (AUC) of the chromatographic peak of each VOC to the AUC of the internal standard. This value is a correlate of molar concentration, and it was used to estimate the abundance of each VOC in breath and air. The relative abundance of each VOC was then ranked by its alveolar gradient i.e. abundance in breath minus abundance in room air.

The principle of the alveolar gradient is best understood within the historic context of breath VOC analysis. The earliest and still the most widely used application of breath VOC analysis is the monitoring of blood alcohol concentration. Since ethanol is not normally present in the ambient air, ethanol detected in the breath is reasonably assumed to have originated from within the body. Only the most abundant VOCs, such as ethanol, acetone and isoprene can be detected with assays of unconcentrated breath; however, as assays have become more sensitive, it has become apparent that normal human breath contains a large number of VOCs, and many of them can also be detected in normal room air. Cailleux and Allain observed that concentrations of pentane in room air and breath were approximately the same; as a result they questioned whether pentane is a normal constituent of human breath [17]. Thus, when a breath VOC is detected in both breath and room air, an

Table 1
Breath VOCs ranked by frequency of occurrence

VOC	Mean alveolar gradient	Subjects (%)
50 most frequently occurring VOCs with positive alveolar gradients		
Isoprene	60.34	100
Benzene, (1-methylethenyl)-	4.77	100
Naphthalene	4.07	100
2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)	0.61	100
Naphthalene, 1-methyl-	0.54	100
Butane, 2-methyl-	0.33	100
Tetradecane	0.23	100
Pentodecane	0.1	100
Dodecane	0.02	100
Benzothiazole	0.93	98
1,1′-Biphenyl, 2,2′-diethyl-	0.69	98
Ethane, 1,1,1-trichloro	0.12	98
Tridecane	0.10	98
Styrene	1 .00	96
Benzene, 1-methyl-4-(1-methylethyl)	0.01	96
Ethanone, 1-phenyl	1.49	94
Acetone	27.91	92
Benzenemethanol, α,α-dimethyl	20.39	92
β-Myrcene	0.05	92
Phenol, 2,6-bis(1,1-dimethyiethyl)-4-methyl-	0.34	90
1H-Indene, 2,3-dihydro-1,6-dimethyl-	0.01	84
1,1′-Biphenyl	0.06	78
Ethene, tetrachloro-	7.70	76
2,5-Cyclohexadjene-1,4-dione, 2,5-bis(1,1-dimethylpropyl)	0.24	74
Octane, 2,6-dimethyl-	0.02	74
Benzoic acid, 4-ethoxy-, ethyl ester	0.30	70
Pentane, 3-methylene-	0.28	70
[1,1′-Bicyclopentyl]-2-one	2.63	68
DL-Limonene	1.79	68
Hexane, 2,2,5-trimethyl-	0.36	66
1H-Indene, 2,3-dihydro-4,6-dimethyl-	0.15	64
2-Butene, 2,3-dimethyl-	0.25	64
Naphthalene, 2,7-dimethyl-	0.09	64
Naphthalene, 2-methyl-	0.32	64
Hexadecane, 2,6,10,14-tetramethyl-	0.16	62
2-β-Pinene	0.58	60
Acetic acid	2.63	60
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	0.21	60
l,2-Benzenedicarboxylic acid, diethyl ester	0.06	58
Endobornylacetate	0.42	58
Benzene, (3-methyl-2-butenyl)-	0.13	56
Naphihalene, 1-ethyl-	0.05	56
Naphihalene, 2-ethyl-	0.00	56
Benzene, 1-ethyl-4-(1-methylethyl)-	0.02	54
Benzene, butyl	0.41	54
Cyclohexene	0.05	54
Naphthalene, 1,6-dimethyl-	0.11	54

(continued on next page)

Table 1. Continued

VOC	Mean alveolar gradient	Subjects (%)
Nonanal	0.32	54
2-Propenoic acid, 2-methyl-, 1,2-ethanediylbis(oxy-2,1-ethanediyl) ester	12.47	52
Octadecane	0.27	52
Octane, 2,5-dimethyl-	0.10	52
Heptadocano	0.12	50
50 most frequently occurring VOCs with negative alveolar gradients		
Benzene	−0.48	100
Benzene, 1-ethyl-2-methyl-	−10.09	100
Benzene, ethyl	−1.73	100
Benzene, methyl	−7.27	100
Benzene, propyl	−1.72	100
Cyclohexane, methyl	−0.75	100
Decane	−0.28	100
Heptane	−1.25	100
Heptane, 2-methyl-	−0.89	100
Heptane, 3-methyl-	−0.83	100
Hexane	−0.79	100
Hexane, 3-methyl-	−1.02	100
Nonane	−0.44	100
Pentane, 2,3,4-trimethyl-	−0.26	100
Pentane, 2-methyl-	−0.43	100
Pentane, 3-methyl-	−0.59	100
Propane, 2-methoxy-2-methyl-	−9.44	100
Undecane	−0.52	100
α-Pinene, (−)-	−0.06	98
Cyclohexane, ethyl	−0.33	98
Cyclopentane, methyl	−1.25	98
Decanal	0.00	98
1-Pentene, 2-methyl-	−0.21	96
Benzene, 1,2,3,5-tetramethyl-	−0.51	96
Pentane, 2,3,3-trimethyl-	−0.10	96
1H-Indene, 2,3-dihydro-4,7-dimethyl-	−0.29	94
Benzaldehyde	−0.31	94
Camphene	−0.20	94
Cyclopentane, 1,3-dimethyl-, *cis*	−0.31	94
Cyclopentane, ethyl	−0.29	94
Cyclopentene	−0.13	94
1H-Indene, 2,3-dihydro-5-methyl-	−0.30	92
Benzene, 1,2,4-trimethyl-	−6.89	92
Benzene, 1,3-dimethyl-	−5.38	92
Benzene, 1-methyl-3-propyl-	−0.21	92
Butane	−0.52	92
Octane, 3-methyl-	−0.26	92
Benzene, 1,2,3,4-tetramethyl-	−0.22	90
Cyclohexane, 1,3-dimethyl-, *cis*	−0.31	90
Hexane, 2-methyl-	−1.48	90
2-Hexene (E)	−0.27	88
Benzene, (1-methylethyl)	−0.76	88
Benzene, 1,4-dimethyl-	−4.95	88

Table 1. Continued

VOC	Mean alveolar gradient	Subjects (%)
Benzene-1-ethyl-2,3-dimethyl-	−0.53	88
Butane, 2,3-dimethyl-	−0.10	88
Benzene, 1,3,5-trimethyl-	−2.44	86
Benzene, 4-ethyl-1,2-dimethyl-	−0.71	86
Heptane, 2,4-dimethyl-	−0.05	86
Heptane, 2,5-dimethyl-	−0.20	84
Hexane, 2,4-dimethyl-	−0.99	82

observer is faced with a dilemma: did that VOC originate from within the body, or was it an artefact of contamination from room air?

Researchers have responded to this dilemma with three different strategies:

(1) Ignore the problem; a number of published studies continue to report VOC concentrations observed in breath alone.

(2) Provide the subject with VOC-free air to breathe prior to collection of the breath sample. This is an apparently logical approach, but it is virtually impossible to achieve in practice. High quality 'pure' breathing air from commercial sources is usually found to contain a large number of VOCs when an assay with picomolar sensitivity is employed.

(3) Correct for the problem by subtracting the background VOCs in room air from the VOCs observed in the breath.

We have elected to follow the third option, by calculating the alveolar gradient as the difference between the concentrations of a VOC in the breath and in the background air [18–20]. There are advantages and disadvantages to this approach:

The main advantage of determining the alveolar gradient is that it provides a logical and consistent method for compensating for the effects of VOCs in room air. In addition, the alveolar gradient provides a valuable insight into the kinetics of a VOC in the body, because it varies with the difference between the rates of synthesis and clearance of the VOC, and its polarity indicates which of the two processes is predominant.

The main disadvantage of determining the alveolar gradient is that it requires an increased investment of time and resources: two samples need to be collected and analyzed every time a patient is studied. Another disadvantage is that kinetic interpretation of the data requires the assumption of an equilibrium between VOCs in the body and in the room air. Also, there is an increase in the standard deviation (SD) of the experimental error of the assay:

$$SD_{\text{alveolar gradient}} = \sqrt{SD^2_{\text{VOC in breath}} + SD^2_{\text{VOC in air}}}$$

Hence, if the experimental errors of the assay of a VOC in breath and air are equal, then the SD of the experimental error of the alveolar gradient is increased by a factor of $\sqrt{2}$ i.e. by approximately 40%.

Fig. 3 demonstrates the pathways which VOCs follow through different compartments of the body. Equilibration is rapid in the pulmonary alveoli, so that the concentration of a VOC in alveolar breath is determined by its concentration in pulmonary arterial blood, while the concentration of a VOC in room air determines its concentration in pulmonary venous blood. The body pool of VOCs is derived from two sources: pulmonary input (from room air) and extrapulmonary input (principally from synthesis in the body, although exogenous sources of VOCs such as foods, drugs, and percutaneous absorption may also contribute). VOCs leave the body pool by two routes: either by pulmonary output (in alveolar breath) or by extrapulmonary output (clearance by metabolism and/or excretion). The kinetics of a VOC in the body may also be modeled by the flow of water into and out of a common pool (Fig. 4).

Kinetic analysis demonstrates that the alveolar gradient of a VOC varies with the difference between the rates at which that VOC enters and leaves the body by extrapulmonary pathways (Appendix A and Fig 4). The polarity of the alveolar gradient indicates which of the two processes is predominant. If the alveolar gradient is positive, the rate of synthesis is greater than the rate of clearance; conversely, if the

Table 2
Breath VOCs ranked by abundance

VOC	Mean alveolar gradient	Subjects (%)
50 VOCs with highest mean positive alveolar gradients		
4,5-Dimorpholino-2-methoxy-6-phenylpyrimidine	655.61	4
(4,4-D2)-15,16-Dimethoxyerythrinan-7,8-dion-enol	162.20	2
Cyclohexanol, 5-methyl-2-(1-methylethyl)-	75.83	4
Isoprene	60.34	100
Methanol	28.90	2
Acetone	27.91	92
Benzenemethanol, α, α-dimethyl	20.39	92
L-Menthalone	13.64	20
Eicosane, 9-octyl-	12.60	2
2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl)	12.49	18
2-Propenoic acid, 2-methyl-, 1,2-ethanediylbis(oxy-2,1-ethanediyl) ester	12.47	52
Menthol	7.82	2
Ethene, tetrachloro-	7.70	76
(+)-Menthylacetate	6.92	2
1,8-Cineole	6.39	14
Oxetane, 2-ethyl-3-methyl-	5.02	6
Benzene, (1-methylethenyl)	4.77	100
Pyrazine, 2-ethyl-3-methyl-	4.11	2
Naphthalene	4.07	100
1H-1,2,4-Triazol-3-amine	3.79	18
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	3.72	2
Cyclopropane, (1-methylethyl)	3.59	2
Methane, trichlorofluoro	3.45	40
2-Methyl-5-propylpyrazine	3.18	2
Benzene, (2-methyl-1-methylenepropyl)	3.11	4
Cyclopentanone	3.07	34
Cyclohexane, methoxy	2.81	4
[1,1-Bicyclopentyl]-2-one	2.63	68
Acetic acid	2.63	60
Butanoic acid, butyl ester	2.44	2
2-Propenoic acid, 2-methyl-, 1,2-ethanediyl ester	2.39	48
Acetic acid, {bis[(trimethylsilyl)oxy]phosphinyl}-, trimethylsilyl ester	2.28	40
Thiourea	2.16	4
Cyclopentane, (1-methylethyl)	2.15	2
1,3,7-Octatriene, 3,7-dimethyl-	2.13	2
2-(1-Methylpropyl)pyrazine	2.09	2
Hexadecanoic acid, 1-methylethyl ester	2.08	4
Linalool	2.06	12
Furan, 2-butyltetrahydro-	1.95	6
Cyclohexanol, 2-amino-, *cis*	1.93	6
1,3-Propanediol, 2-methyl-2-propyl-	1.91	36
1,*E*-11,*Z*-13-Hexadecatriene	1.85	2
9-Homonoradamant-9-ene	1.85	2
Peroxydihydrocostunolide	1.83	4
Heneicosane	1.80	8
DL-Limonene	1.79	68
Pyrazine, 2,3-dimethyl-	1.77	2
1-Propene, 1-(methylthio)-, (*E*)	1.75	16

Table 2. Continued

VOC	Mean alveolar gradient	Subjects (%)
1-Propanol, 2,2-dimethyl-	1.73	2
Bicyclo[4.1.0]heptane, 3,7,7-trimethyl-	1.71	2
50 VOCs with highest mean negative alveolar gradients		
2-Propanol	−61.41	28
1-Propene	−27.15	2
Benzene, 1-ethyl-2-methyl-	−10.09	100
Propane, 2-methoxy-2-methyl-	−9.44	100
Octane, 3,4-dimethyl-	−8.91	2
Benzene, methyl-	−7.27	100
Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (*R*)	−7.17	58
Benzene, 1,2,4-trimethyl-	−6.89	92
4-Penten-2-ol	−6.42	2
Benzene, 1,3-dimethyl-	−5.38	92
3-Butenoic acid	−5.34	2
Benzene, 1,4-dimethyl-	−4.95	88
2-Chloro-4-(4-methoxyphenyl)-6-(4-nitrophenyl)pyrimidine	−4.30	18
Pentane	−3.95	44
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1α,2β,5α)-(±)-	−3.30	2
Hexanol-4-D2	−2.85	4
1-Butene, 2-methyl-	−2.72	78
Oxirane, trimethyl	−2.68	2
Benzene, 1,3,5-trimethyl-	−2.44	86
Ethanone, 1-(3-ethylcyclobutyl)-	−2.31	2
Pyrrolidine	−2.24	6
Xylene	−2.14	32
Octane	−2.02	74
3,4-Dihydropyran	−1.82	2
Undecane, 3,5-dimethyl-	−1.75	6
Benzene, 1-methyl-2-propyl-	−1.75	76
α-Ylangene	−1.73	2
Benzene, ethyl	−1.73	100
Benzene, propyl	−1.72	100
Methane, dichloro-	−1.71	10
Butene, 2,3-dimethyl-	−1.69	48
1,2-Pentadiene	−1.65	2
Benzene, 1-methyl-4-propyl-	−1.60	10
Phosphonic acid, diphenyl ester	−1.60	2
Heptadecane, 9-octyl-	−1.58	4
1-Octadecene	−1.54	4
Bicyclo[3.2.1]oct-2-ene, 3-methyl-4-methylene-	−1.51	2
Pentane, 2,2,3,4-tetramethyl-	−1.51	28
4-Heptanone, 3-methyl-	−1.48	4
Hexane, 2-methyl-	−1.48	90
3-Iodothiophene-2-carboxamide	−1.47	2
1*R*-Methyl-2T-phenylcyclopropane	−1.41	6
Benzene, 1,2,3-trimethyl-	−1.39	56
Palmitic acid, 2-(trimethylsiloxy)ethyl ester	−1.34	2
β-Ocimene-x	−1.32	8

(continued on next page)

Table 2. Continued

VOC	Mean alveolar gradient	Subjects (%)
4-Hydroxy-2-isopropyl-4,7-dimethyl-1 (4H)-naphthalenone	−1.31	2
7-Azabicyclo[4.1.0]heptane, 3-methyl-	−1.31	2
4,7-Diphenyl-6-hydroxymethyl-1,2,5-oxadiazolol[3,4-c]pyridine	−1.27	2
Benzene, 2-ethyl-1,3-dimethyl-	−1.26	54
2-Butanol, 3-methyl-	−1.26	2

alveolar gradient is negative, then the rate of clearance is greater than the rate of synthesis. As an example, the mean alveolar gradient of the long-chain *n*-alkane tetradecane was positive, demonstrating that in vivo synthesis predominated over clearance. Conversely, the mean alveolar gradient of methylbenzene was negative, demonstrating that clearance was greater than in vivo synthesis. This was consistent with ingestion of methylbenzene as a pollutant of room air which was then cleared from

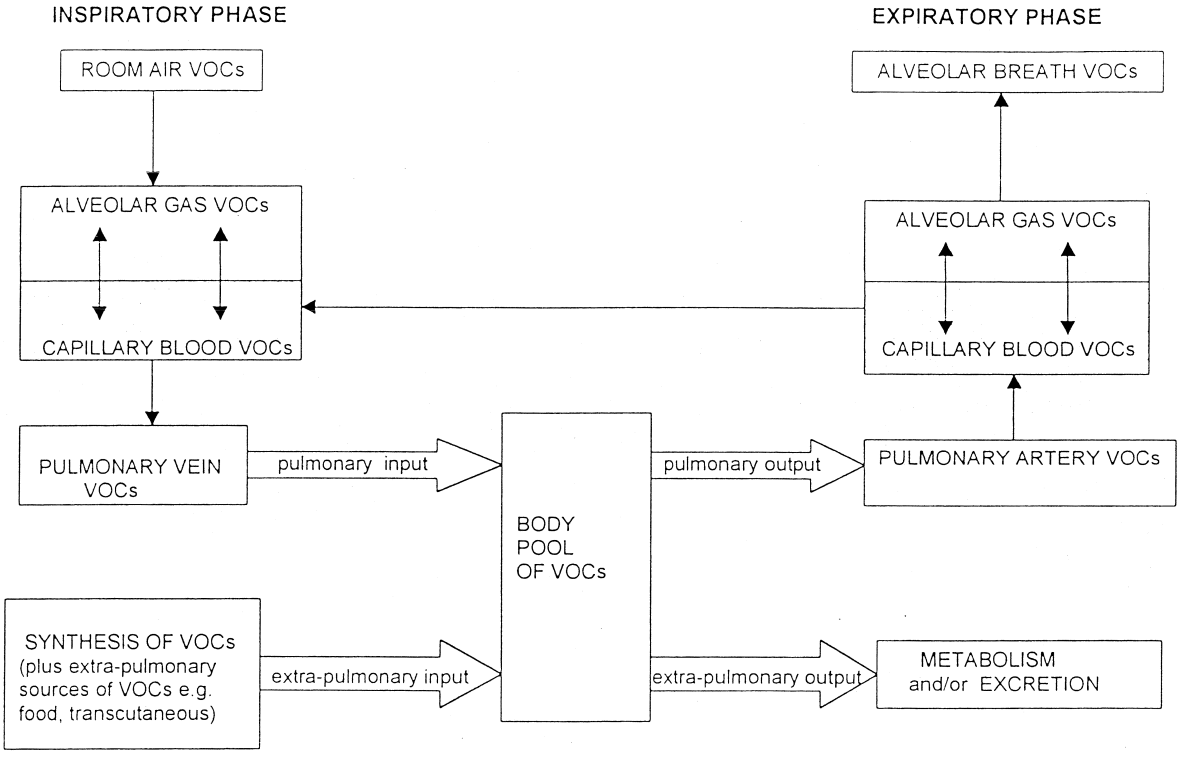

Fig. 3. Pathways of VOCs through body compartments. Gaseous and capillary VOCs equilibrate rapidly in the pulmonary alveoli, and the dominant process varies with the phase of respiration. During the inspiratory phase, room air VOCs equilibrate with pulmonary venous blood, while during the expiratory phase, pulmonary arterial blood equilibrates with VOCs in alveolar breath. Extra-pulmonary input of VOCs is primarily from endogenous synthesis, and extra-pulmonary output of VOCs is predominantly by metabolism in the liver and excretion in the kidneys.

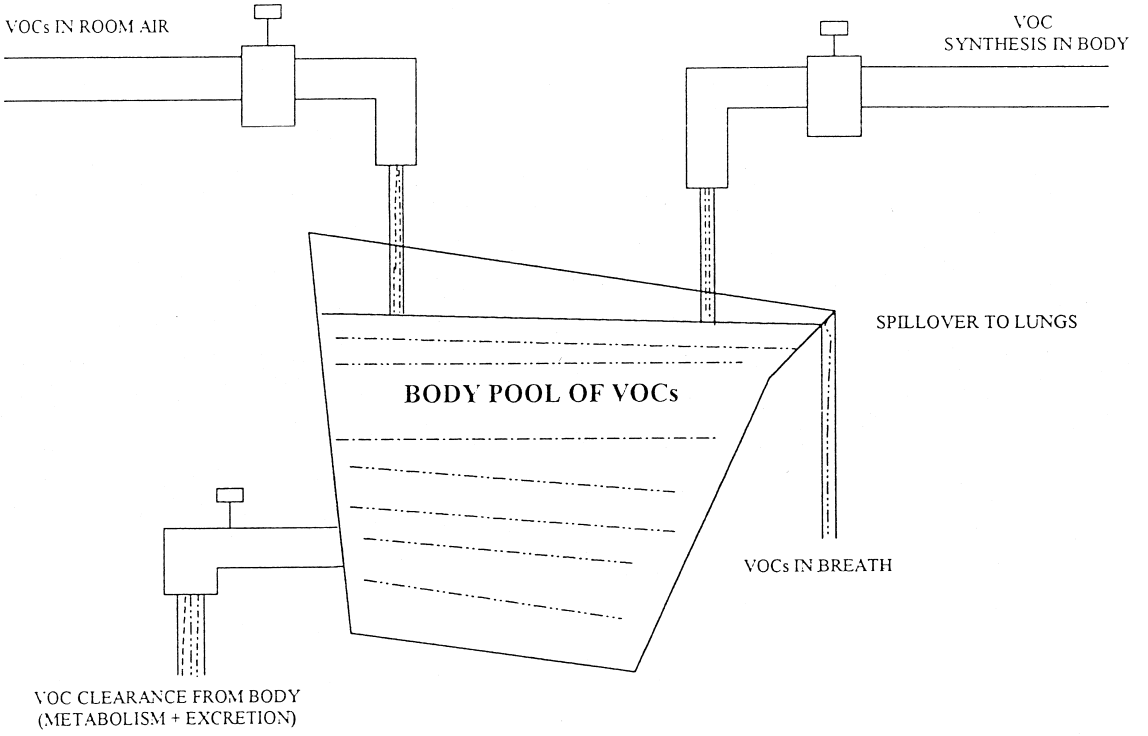

Fig. 4. Water flow analogy of VOC kinetics: a VOC enters the body pool either from the inspired air or from synthesis in the body (ignoring minor inputs such as VOCs in foodstuffs). The VOC leaves the body pool either by clearance (metabolism and/or excretion) or else in the breath. If the VOC is neither synthesized nor cleared from the body, then the amount leaving in the breath must equal the amount entering from inspired air, and the alveolar gradient (amount in breath minus amount in air) will be zero. If the VOC is synthesized in the body but not cleared, more leaves in the breath than is inspired from the air, and the alveolar gradient becomes positive. Conversely, if the VOC is cleared from the body but not synthesized, less leaves in the breath than is inspired from the air, and the alveolar gradient becomes negative. Hence, if a VOC is both synthesized and cleared in the body, the alveolar gradient will vary with their combined effect: positive if synthesis is greater than clearance, and negative if clearance is greater than synthesis.

the body by metabolism and excretion. An example is shown in Fig. 5 of the chromatograms of breath and air observed in a normal subject. In the subtraction chromatogram of breath minus air, positive peaks demonstrate VOCs with positive alveolar gradients, and negative peaks demonstrate VOCs with negative alveolar gradients. Fig. 6 demonstrates mass spectrographic identification of a VOC with a negative alveolar gradient, 1-ethyl-2-methylbenzene.

We observed several VOCs with negative alveolar gradients such as benzene and polymethylated benzenes which may have originated from industrial sources. Since a number of these VOCs are known to be toxic, further studies are required to quantitate human exposure to polluted air, and to determine whether this exerts adverse effects on health. The results of this study accorded with previous reports that normal humans differ widely from one another in the composition of their breath VOCs, both qualitatively and quantitatively. However, it also demonstrated two points of similarity between individuals which have not been previously reported: first, the total number of breath VOCs did not vary widely within a fairly narrow range; second, despite the large total number of different VOCs observed, there was a comparatively small ‘common core’ of

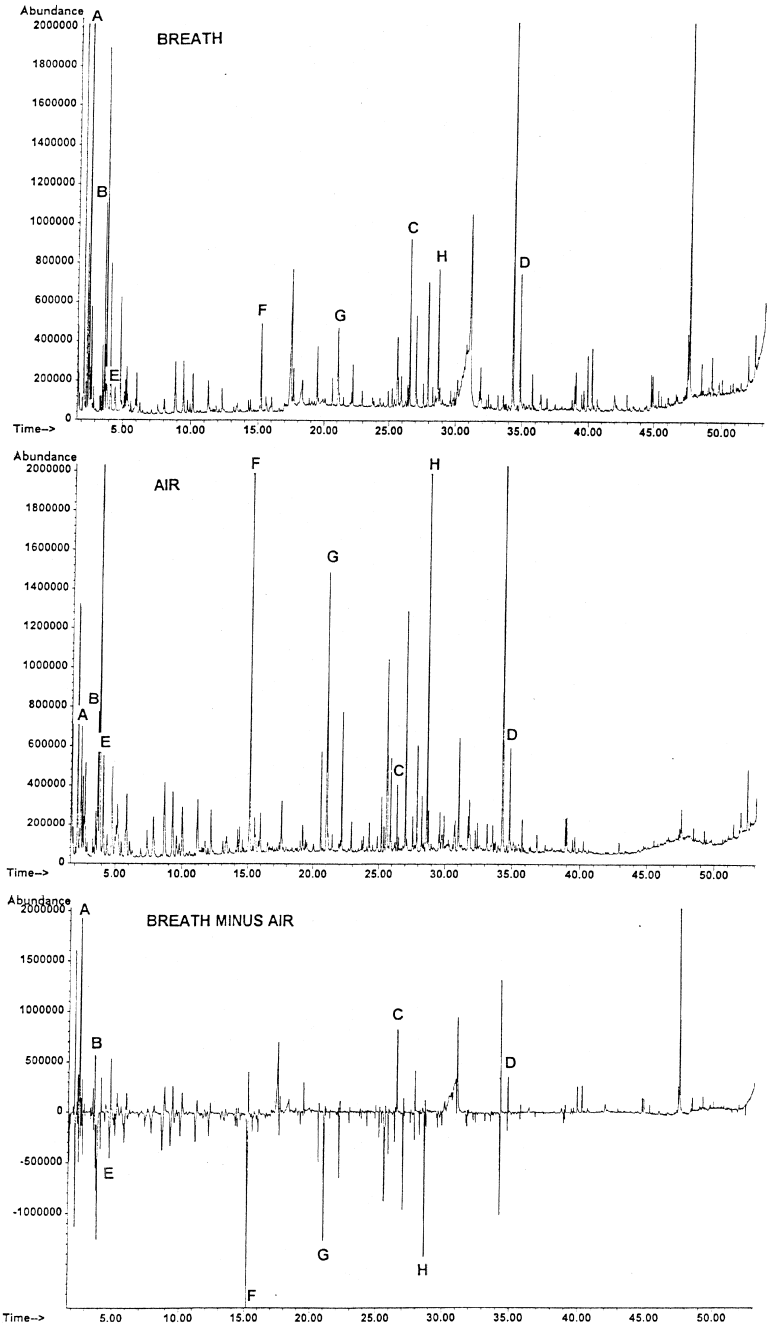

Fig. 5. Chromatograms of breath and air. These results were obtained from a study of a 31-year-old female. The top and middle panels display chromatograms of breath and air, respectively. The bottom panel is a subtraction chromatogram of breath minus air, so that peaks above the line represent VOCs with positive alveolar gradients, and peaks below the line represent VOCs with negative alveolar gradients. The peaks were tentatively identified as A = isoprene, B = 2-methylpentane, C = 1-methylethenylbenzene, D = azulene, E = hexane, F = methylbenzene, G = 1,3-dimethylbenzene, and H = 1-methyl-4-(1-methylethenyl)-cyclohexene.

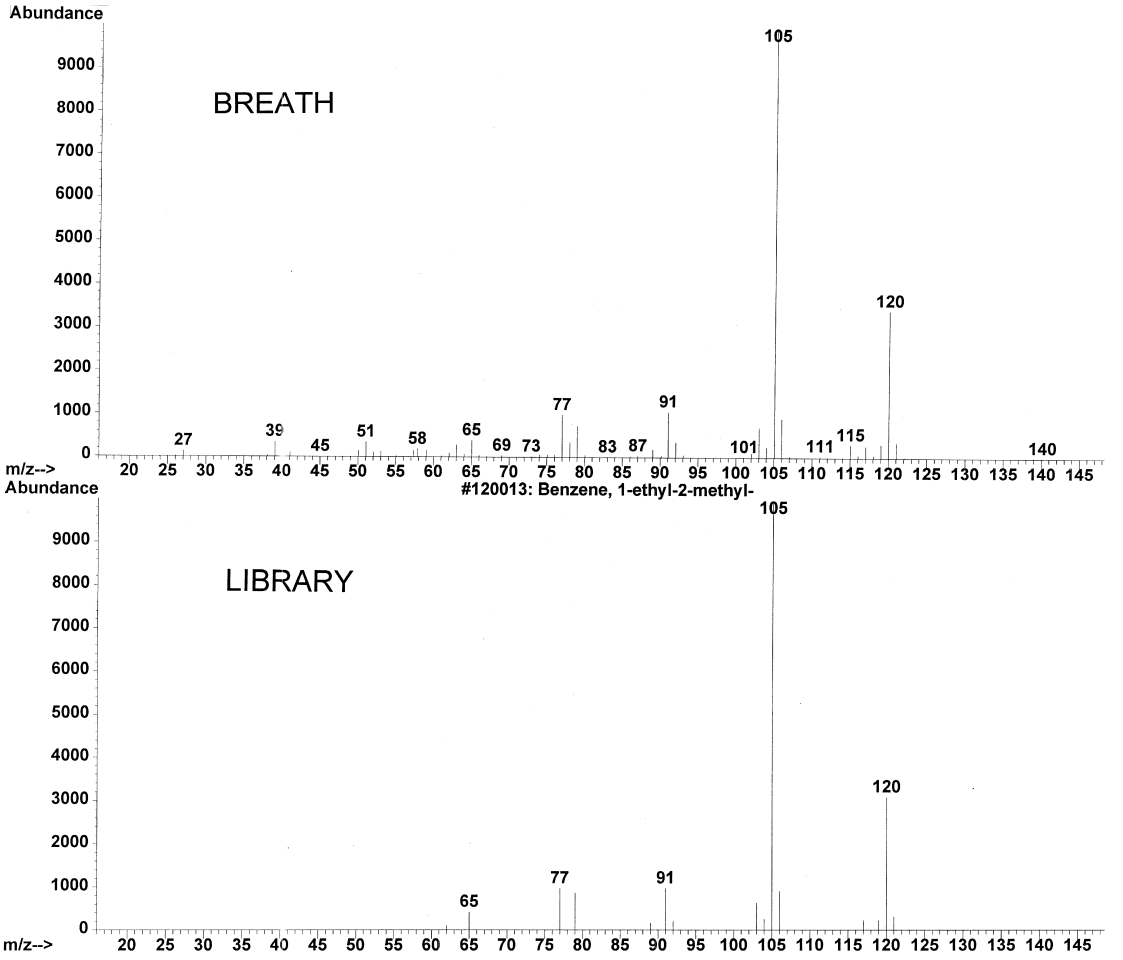

Fig. 6. Mass spectrum of a chromatographic peak. The upper panel shows the mass spectrum of a peak in a chromatogram of normal human breath, which eluted at 25.58 min with a negative alveolar gradient. The lower panel shows the mass spectrum of 1-ethyl-2-methylbenzene, the best fit encountered in an automated library search. The quality of the match was 95%.

breath VOCs which was present in all subjects, and which was probably produced by metabolic processes common to most humans.

Appendix A. Kinetic basis of the alveolar gradient

This compartmental analysis is based upon VOC movements shown in Fig. 3.

R = rate of movement of VOC (mol/min)

C = concentration of VOC (mol/l)

RMV = respiratory minute volume (l/min)

At equilibrium: $R_{\text{into body}} = R_{\text{out of body}}$

$R_{\text{pulmonary input}} + R_{\text{extra-pulmonary input}} = R_{\text{pulmonary output}} + R_{\text{clearance}}$

$R_{\text{extra-pulmonary input}} - R_{\text{clearance}} = R_{\text{pulmonary output}} - R_{\text{pulmonary input}}$

$= (C_{\text{alveolar breath}} - C_{\text{room air}}) \times \text{RMV}$

i.e. alveolar gradient $= C_{\text{alveolar breath}} - C_{\text{room air}}$

$$= \frac{(R_{\text{extra-pulmonary input}} - R_{\text{clearance}})}{\text{RMV}}$$

For a VOC synthesized in body and not ingested from extra-pulmonary sources:

$$\text{alveolar gradient} = \frac{(R_{\text{synthesis}} - R_{\text{clearance}})}{\text{RMV}}$$

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