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Compounds enhanced in a mass spectrometric profile of smokers' exhaled breath versus non-smokers as determined in a pilot study using PTR-MS

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Abstract

A pilot study has been carried out to define typical characteristics of the trace gas compounds in exhaled breath of non-smokers and smokers to assist interpretation of breath analysis data from patients who smoke with respiratory diseases and lung cancer. Exhaled breath was analyzed using proton transfer reaction–mass spectrometry (PTR-MS) for 370 volunteers (81 smokers, 210 non-smokers, 79 ex-smokers). Volatile organic compounds corresponding to product ions at seven mass-to-charge ratios (m/z 28, 42, 69, 79, 93, 97, 123) in the PTR-MS spectra differentiated between smokers and non-smokers. The Youden index (= maximum of sensitivity + specificity − 1, YI) as a measure for differentiation between smokers and non-smokers was YI = 0.43 for ions at the m/z values 28 (tentatively identified as HCN), YI = 0.75 for $m/z = 42$ (tentatively identified as acetonitrile) and YI = 0.53 for $m/z = 79$ (tentatively identified as benzene). No statistically significant difference between smokers and non-smokers was observed for the product ions at $m/z = 31$ and 33 (compounds tentatively identified as formaldehyde and methanol). When interpreting the exhaled breath of lung cancer or COPD patients, who often smoke, compounds appearing at the above-mentioned seven mass-to-charge ratios should be considered with appropriate care to avoid misdiagnosis. Validation studies in larger numbers of patients with more precise delineation of their smoking behavior and using additional analytical techniques such as GC/MS and SIFT-MS should be carried out.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Smoking is the most important preventable cause of morbidity and mortality in many developed countries [1]. Tobacco smoking, mainly cigarette smoking, accounts for approximately 75–90% of the lung cancer risks [2]. There is a consistent association between cigarette smoking and lung cancer as a cause of death [2, 3].

Early recognition of lung cancer remains one of the most crucial goals of modern oncology. In this regard, great hopes are being placed on breath gas analysis. Testing exhaled breath is noninvasive and may therefore be carried out routinely and for screening purposes [4–9]. Recent studies have shown alterations in the profiles of breath trace gas compounds of lung cancer patients, which could be implemented into clinical practice in the future [10–12].

Many patients with lung cancer continue to smoke even after their diagnosis of cancer. Tobacco smoking changes the composition of exhaled air, i.e. the levels of some exhaled volatiles increase either because they are ingested from tobacco smoke or because they are produced in the body as a response to the irritant effects of smoking [13–17].

McKee, Campbell *et al* were the first to show that smokers show acetonitrile in blood, urine and exhaled breath [18] and that cigarette smoke contains considerable amounts of acetonitrile, with about 1 mg in the smoke of one cigarette [13]. In urine, in particular, the lowest concentration of acetonitrile determined for a smoker (>3 cigarettes per day) was 2.2 μg/100 ml of urine, with an average concentration of 11.76 μg/100 ml of urine for the 40 smokers investigated [18]. The non-smokers, in comparison, showed an average concentration for acetonitrile of 0.29 μg/100 ml of urine. Later on, a number of other compounds have been described in tobacco smoke [19] and in the exhaled breath of smokers [14, 20], as, e.g., benzene, 2,5,-dimethylfuran,1,2-butadiene or isoprene.

Cigarette smoking itself is associated with neutrophilic inflammation, which causes the increase of inflammatory markers in the exhaled breath and is involved in pathogenesis of chronic inflammation of respiratory airways [21]. Thus, patients suffering from lung cancer often show co-morbidities as chronic obstructive or non-obstructive pulmonary diseases and emphysema [22]. Therefore, interpretation of breath profile in patients with lung cancer is far from being monosymptomatic, as there are at least three main contributing pathological pathways to which deviations may be attributed:

- previous or current smoking;
- concomitant inflammatory or destructive reactions of airways;
- reactions of malignant cells.

The goal of the present investigation was not the detection of smoking behavior, but to determine the typical concentration patterns in the exhaled breath of smokers in order to differentiate between lung cancer patients, patients with pulmonary disorders and healthy volunteers under the confounding influence of smoking.

2. Methods

2.1. Sample collection

Samples of mixed breath gas were collected in Tedlar bags (SKC Inc., Eighty Four, PA) with parallel collection of ambient air (also in Tedlar bags). Breath gas samples were obtained after a ~5 min rest of a volunteer. Each subject provided one or two breath samples by use of a straw. All samples were processed within 12 h.

A cohort of 370 volunteers was recruited; all individuals gave informed consent for participation in the study. The volunteers completed a questionnaire describing their current smoking status (active smokers, non-smokers) and the time elapsed since their last smoke. The classification as smoker/non-smoker/ex-smoker is based on the self-declaration of the volunteers. The amount of smoking (in pack-years) has not been determined. Ex-smokers have only been considered for illustrative purposes showing joint distributions of concentrations of two compounds, but are not used for comparisons between smokers and non-smokers. Samples of mixed alveolar exhaled breath (including dead space air) were collected in 3 l volume Tedlar bags with parallel collection of ambient air (also in Tedlar bags). The samples were collected at different times of day independent of the time of meals and were processed within 12 h at most. Before measurement, the bags were heated to 40 °C for at least 15 min. For all our samples we measured CO_2 content, sorting our samples with low CO_2 concentration. The study was approved by the local ethics committee.

2.2. PTR-MS instrument used

A high-sensitivity proton transfer reaction mass spectrometer (PTR-MS, 3 turbopumps) with Teflon rings (instead of Viton rings) was used for our measurements. The count rate of primary ions (H_3O^+) was around 10^7 counts per second. Dwell time was 0.5 s for each mass-to-charge ratio measured (m/z = 21–230). Typical compounds used for the determination of transmission coefficients were acetonitrile, acetaldehyde, acetone, DMS, 2-butanone, benzene, toluene, *p*-xylene, benzaldehyde, chlorobenzene, 1,2-dichlorobenzene, 1,2,4-trichlorobenzene. These compounds do not show fragmentation (of their respective protonated form). Concentrations of these compounds were chosen in a range leading to ~10% reduction of primary counts, with subsequent observation of recovery of primary ion counts (measuring at m/z = 21 and the specific mass-to-charge ratio of the respective non-fragmenting compound). The length of the drift tube of our PTR-MS is 9.3 cm, with an applied voltage of 600 V. The usual pressure in the drift tube was ~2.3 mbar (with slight variations). In accordance with the instructions of the manufacturer (Ionicon GesmbH, Innsbruck), we computed concentrations with using only H_3O^+ as primary ion (not considering the first water cluster $H_2O{\cdot}H_3O^+$).

Table 1. Characteristics of the subject groups. Age is quoted as median (range).

		Smokers	Non-smokers	Ex-smokers	Total
Male	Age	37 (23–72)	48.5 (22–83)	65.5 (35–85)	49 (22–85)
	n	35 (21.3%)	95 (57.9%)	34 (20.7%)	164 (100%)
Women	Age	37 (21–79)	57.5 (20–91)	50 (22–85)	49 (20–91)
	n	46 (22.3%)	115 (55.8%)	45 (21.8%)	206 (100%)
All	Age	37 (21–79)	50.5 (20–91)	58 (22–85)	49 (20–91)
	n	81 (21.9%)	210 (56.8%)	79 (21.4%)	370 (100%)

2.3. Mass spectrometric analysis

Proton transfer reaction–mass spectrometry allows on-line monitoring of VOCs with volume mixing ratios as low as a few parts per trillion (pptv) [23, 24]. Chemical ionization, based on proton transfer reactions with H_3O^+ as the primary reactant ion, is a versatile method for identification and quantification of the mixtures of organic molecules. In our study, each sample, including samples of ambient air, was measured three times with mass-to-charge ratios (m/z) ranging from 21 to 230. The median concentrations of these three measurements were used for further statistical analysis. Concentrations of compounds related to some m/z have been calculated based

- either on a 'standard' rate constant for protonation of $k = 2 \times 10^{-9}$ cm^3 s^{-1} recommended by Ionicon (Innsbruck) for compounds which are not identified, the concentration thus being uncalibrated [25–30];
- or on specific thermal equilibrium protonation rate constants for the compounds methanol ($k = 2.7 \times 10^{-9}$ cm^3 s^{-1}), acetonitrile ($k = 5.1 \times 10^{-9}$ cm^3 s^{-1}), isoprene ($k = 2 \times 10^{-9}$ cm^3 s^{-1}) and acetone ($k = 3.9 \times 10^{-9}$ cm^3 s^{-1}). For isoprene, which apart from appearing at m/z 69 fragments to m/z 39 (∼10%) and m/z 41 (∼40%), we used the concentration computed for m/z 69 multiplied by a calibration factor of 2.24.

Identification of compounds is notoriously difficult with PTR-MS. Judging from our GCMS investigations, we know that methanol, acetone and isoprene are present in almost everybody's exhaled breath and that acetonitrile arises in increased concentrations in the breath of smokers. At the respective mass-to-charge ratios (m/z 33, m/z 42, m/z 59, m/z 69) other compounds may be present, even though in low concentrations. Incidentally, protonated isoprene does not only show up at m/z 69, but partly fragments in PTR-MS to m/z 39 (∼10% of protonated isoprene) and m/z 41 (∼40% of protonated isoprene). For formaldehyde and hydrogen cyanide, we cannot presently rely on GCMS measurements. The compounds dimethylsulfoxide, toluene, dimethylfuran and dimethylpyrazole in table 2 are not more than an 'educated guess'.

The concentrations relating to product ions at m/z 31 (tentatively identified as protonated formaldehyde) have been corrected for isotope effects from m/z 30 (= NO^+ which contributes $^{15}NO + N^{17}O = 0.37\% + 0.04\% = 0.41\%$ to m/z 31). On mass-to-charge ratio $m/z = 31$ one may also observe fragments from reaction products of ethanol and O_2^+ or of methanol and O_2^+. Accurate absolute values for formaldehyde concentrations can only be achieved with PTR-MS by appropriate calibration measurements. The ions at m/z 43 (tentatively identified as originating from isopropanol) may partly originate from compounds other than isopropanol. It can be both $C_3H_7^+$ (as from propanol) or CH_3CO^+ as sometimes occurs from the reactions of aldehydes, ketones and carboxylic acids [7, 8]. Ions at m/z 31 might also be fragments from reaction products of ethanol or methanol with O_2^+.

The age effect of exhaled breath samples is negligible (apart from water, which quickly diffuses through the walls of Tedlar bags). Acetonitrile seems to diffuse quickest through bag walls, with an exponential decay constant $\tau \sim 31$ h.[8]

2.4. Statistical analysis

Concentrations of compounds are expected to be log-normally distributed, since the contributing physiological factors act multiplicatively and not additively. If the concentrations of compounds are log-normally distributed, the logarithms of the concentrations are normally distributed. This was tested with Lilliefors test (with level of significance at 5%). Histograms of distributions of concentrations are therefore shown using a logarithmic concentration scale.

Since the data are expected to be log-normally distributed, the concentrations are expressed by giving medians of concentrations and geometric standard deviation (GSD), instead of mean and standard deviation (which would be appropriate parameters for normally distributed concentrations). Repeated measure analysis of variance (ANOVA where Lilliefors test confirmed log-normal distribution, Kruskal–Wallis otherwise) was used to compare the *logarithmic* concentrations of the different groups (smokers versus non-smokers) [31, 32]. Statistical results were considered to be significant if $p < 0.01$. Receiver-operator-characteristics (ROC) curves [33–35] were applied to determine the thresholds for the concentrations of compounds that yielded the highest combined accuracy for distinguishing patients with the high and low concentration of definite substances. Sensitivity, specificity as well as positive and negative predictive values were determined for these thresholds. The Youden index was determined, which is defined to be the maximum of (sensitivity + sensitivity − 1). We may illustrate Youden index with some examples:

[8] Herbig J, personal communication.

Table 2. Concentrations of compounds in the breath of smokers versus non-smokers in parts per billion, ppb, indicated according to marker ions at the mass-to-charge ratios, m/z (see the text). We used the standard rate constant for protonation $k = 2 \times 10^{-9}$ cm^3 s^{-1}, apart from some tentatively identified compounds where the rate constants are known: methanol (m/z 33, $k = 2.7 \times 10^{-9}$ cm^3 s^{-1}), acetonitrile (m/z 42, $k = 5.1 \times 10^{-9}$ cm^3 s^{-1}) and isoprene ($k = 2 \times 10^{-9}$ cm^3 s^{-1}). For isoprene, which apart from appearing at m/z 69 does fragment to m/z 39 (∼10%) and m/z 41 (∼40%), we used the concentration computed for m/z 69 multiplied by a calibration factor of 2.24. The concentrations of the m/z values 31 and 33 (tentatively formaldehyde and methanol) are *not* significantly different in the exhaled breath of this cohort of smokers and non-smokers. Classification as smoker/non-smoker/ex-smoker was based on self-declaration of volunteers. GSD: geometric standard deviation. The concentrations of hydrogen cyanide and formaldehyde are underestimated by PTR-MS measurements (due to their low proton affinity).

		Smokers		Non-smokers		
m/z	Tentative identification of VOCs	Median of concentration* (ppb)	GSD	Median of concentration* (ppb)	GSD	p-Value
	Using filtered data (*ANOVA, **Kruskal–Wallis)					
28	Hydrogen cyanide	1.6	1.5	1.0	1.5	$<1 \times 10^{-8}$**
31	Formaldehyde	9.9	1.8	10.4	1.6	n.s.*
33	Methanol	208.0	1.7	193.3	1.6	n.s.**
42	Acetonitrile	35.2	2.4	7.6	2.1	<1 x10^{-15}**
69	Isoprene	137.2	1.6	100.9	1.9	<0.004**
79	Benzene Dimethylsulfoxide	2.3	1.9	0.9	1.7	$<1\times10^{-15}$**
93	Toluene	5.2	1.6	3.2	1.6	$<1\times10^{-9}$**
97	Dimethylfuran, Dimethylpyrazole	3.2	2.0	1.8	1.9	$<1\times10^{-6}$**
123	*N*,*N*-dimethyl-pyridineamine Methoxymethyl-benzene	0.8	1.8	0.6	1.8	<0.003*
	Without filtering the data (*ANOVA, **Kruskal–Wallis)					
28	Hydrogen cyanide	1.6	1.5	1.1	1.5	$<1\times10^{-8}$**
31	Formaldehyde	4.5	2.2	5.2	2.0	n.s.**
33	Methanol	208.1	1.7	196.1	1.6	n.s.**
42	Acetonitrile	33.1	2.3	7.6	2.1	<1 x10^{-10}**
69	Isoprene	137.2	1.6	100.9	1.9	<0.004**
79	Benzene Dimethylsulfoxide	2.4	1.9	1.0	1.8	$<1\times10^{-15}$**
93	Toluene	5.5	1.6	3.5	1.7	$<1\times10^{-7}$**
97	Dimethylfuran, Dimethylpyrazole	3.4	2.0	2.0	2.0	$<1\times10^{-4}$**
123	*N*,*N*-dimethyl-pyridineamine Methoxymethyl-benzene	0.9	1.9	0.6	2.0	<0.001*

- if sensitivity = 0.8 and specificity = 0.9, Youden index = 0.8 + 0.9 − 1 = 0.7,
- if sensitivity = 0.5 and specificity = 0.9, Youden index = 0.5 + 0.9 − 1 = 0.4.

We consider sensitivity, specificity and ROC curves much more instructive than p-values: ROC curves do not depend too much on the numbers n_1 and n_2 of volunteers in the two groups considered, whereas p-values are very sensitive to n_1 and n_2.

2.5. Selection of data

In certain situations, the inhaled air shows a higher concentration of some compounds than the exhaled air. In such situations the corresponding concentrations of the compound in exhaled air may *not* reflect the blood concentrations of this compound (if blood concentrations are involved at all, which is not the case for a compound like, e.g., nitric oxide, which is produced in the lungs and the sinuses [36–38]). A similar caveat holds if the concentration of a compound in inhaled air is just below the concentration in exhaled air. We therefore not only considered the raw concentrations of compounds in exhaled breath, but also applied a *filter* to these raw concentrations as described in the following.

Filtering data. A value for the expiratory concentration is considered if and only if

$$(\text{inspiratory concentration})_i \leqslant 0.5 \times (\text{expiratory concentration})_i. \quad (1)$$

Hence, the filter discards all those expiratory concentrations which are less than double the respective inspiratory concentration. For the compounds, where the concentration in exhaled air is expected to be higher (i.e., endogenous compounds from the human body), this filter works well with the factor 0.5—if this factor is increased, more samples are added, if this factor is decreased less samples are taken into account.

For compounds, where the influence depends less on the human body (e.g. but on cigarette smoke), there

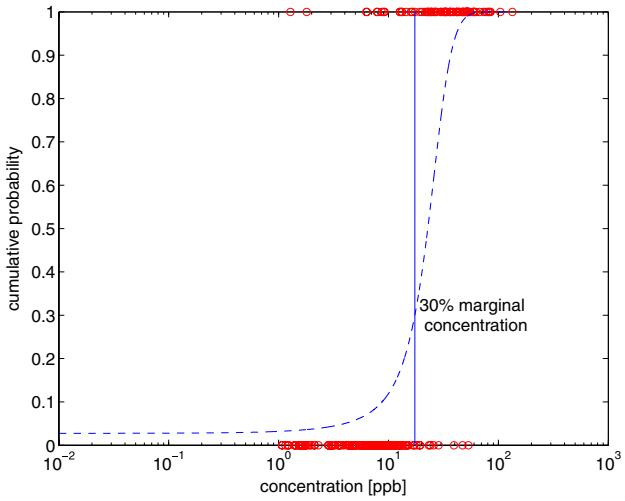


Figure 1. The example of the logistic regression curve and 30% marginal concentration (*not filtered*) for acetonitrile as calculated from the ion count rate at $m/z = 42$ using $k = 5.1\times10^{-9}$ cm^3 s^{-1}.

have to be *exceptions* to this filter condition (1) for very low expiratory concentrations; if we compare, say non-smokers with smokers, the expiratory concentrations of some compounds in non-smokers are often so small that the indoor air concentrations (inhaled) and the expiratory concentrations are in the same range. If these expiratory concentrations are filtered out, almost all data are 'lost'. Therefore, we do *not* filter out these expiratory concentrations, but have to concede that these expiratory concentrations are only *upper bounds* for the 'real' expiratory concentrations (which would appear if the indoor air would be absolutely clean and free of any contamination).

To formulate the *exceptions* to our filter condition (1) in a precise quantitative way, we consider a logistic regression (setting non-smoker $= 0$, smoker $= 1$; see figure 1) and choose the *marginal concentration* as being that particular concentration for which the logistic regression curve takes a value of 0.3 ($= 30\%$). All expiratory concentrations below the marginal concentration are taken into account (both for smokers and non-smokers).

If this value (here 0.3) is too low, one risks that most samples of non-smokers are filtered, especially those with higher concentration. This results in a false decrease of the overall concentration of non-smokers and the statistics would show a larger difference than in reality exists. If this factor is too high, samples with low concentration of smokers, which were filtered out by the filter rule (1), are taken into account and the overall concentration of smokers would decrease. Moreover, more samples of non-smokers with higher concentrations would pass the filter and would enlarge the overall concentration of non-smokers. Therefore, the statistic would show a smaller difference than in reality exists.

Nevertheless, it should be noted that these values (below 30% of the logistic regression) do not necessarily represent exhaled breath concentrations of some systemic compound in the blood of non-smokers, but possibly indoor air concentrations, only (of compounds which are just inhaled and exhaled).

We consider raw concentrations *and* filtered data. The filtering is a kind of cross-check, hinting at problems with high indoor air concentrations. For compounds with roughly equal concentrations in smokers and non-smokers (e.g., formaldehyde or methanol), the second part of the filtering process (taking into account the expiratory concentrations below the marginal concentration) is not effective, and therefore the filtered concentrations may be unacceptably high.

We *never* use differences (expired concentration − inspired concentration) and consequently never use 'negative concentrations'. Whenever a VOC behaves like carbon dioxide, differences do not make sense: the concentration of carbon dioxide in exhaled air is ∼4%, independent of the CO_2 concentration in inhaled air (0%, 1% or 2% in indoor air). The differences (expired concentration − inspired concentration) in concentration of CO_2 would nevertheless be very different (namely 4%, 3% and 2%) without any physiological reason for this in the body.

2.6. Receiver-operator characteristics (ROC curves)

To differentiate between smokers and non-smokers, a threshold concentration c_0 can be chosen, non-smokers being expected to show lower concentration than c_0 and smokers being expected to show higher concentrations than c_0. Such a threshold concentration c_0 gives rise to a corresponding sensitivity and specificity (for detection of smokers). Sensitivity is defined as the number of true positives [i.e., smoker $\geqslant$ threshold] divided by the number of all smokers. Specificity is defined as the number of true negatives [i.e., non-smoker $<$ threshold] divided by the number of all non-smokers. If many different candidates for threshold concentrations c_0 are chosen, the corresponding sensitivities may be plotted versus the corresponding (1 − specificity): this is called an ROC curve [33, 34, 39]. The Youden index is the maximum of (sensitivity + specificity − 1). If the sensitivity and the specificity are at 70%, the Youden index is 0.4. If the sensitivity and the specificity are at 90%, the Youden index is 0.8.

3. Results

Demographic data of patients are presented in table 1.

Ions at seven mass-to-charge ratios (m/z 28, 42, 69, 79, 93, 97, 123) were selected out of the mass spectrometric profile (m/z 21–230) for the exhaled breath of smokers versus non-smokers using discriminant analysis (table 2).

Figure 2 shows the derived concentrations of compounds tentatively identified as hydrogen cyanide (m/z 28),

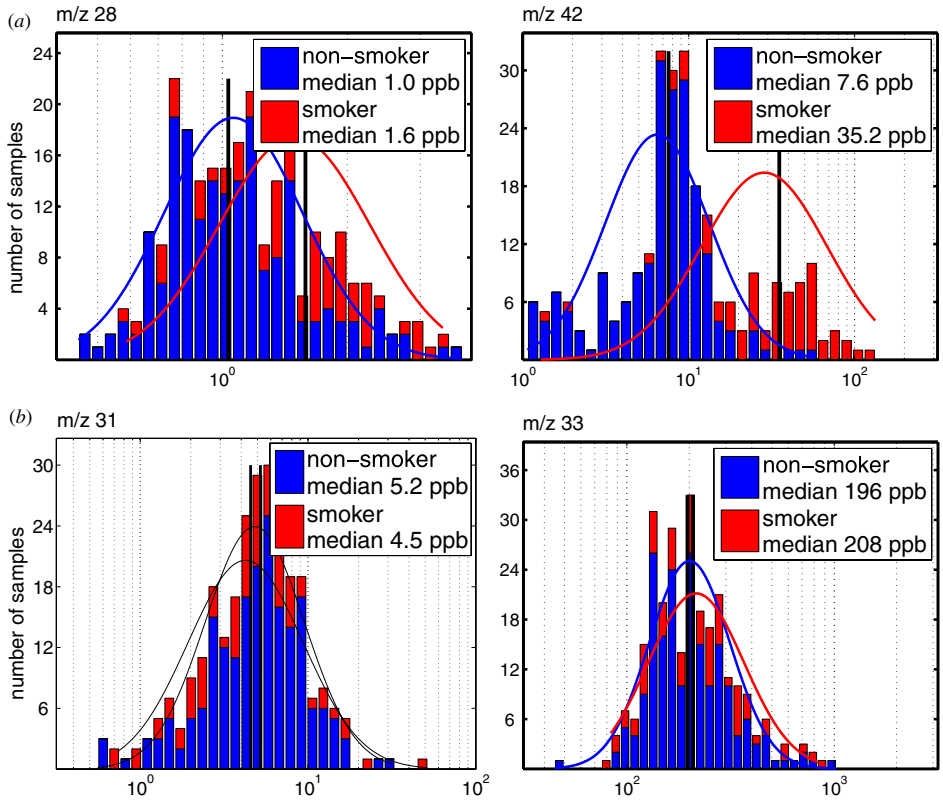


Figure 2. (*a*) *Examples*: histograms of *filtered* concentrations of hydrogen cyanide (m/z 28) and acetonitrile (m/z 42) present in the breath gas of smokers and non-smokers (significantly higher concentrations ($p < 0.01$)). The curves show the estimated log-normal distribution and the vertical black lines show the median values. (*b*) *Counter-examples*: histograms of concentrations (*without filtering*) of formaldehyde (m/z 31) and methanol (m/z 33) present in the breath gas of smokers and non-smokers. These do *not* show a significant difference in concentrations. The curves show the estimated normal distribution and the vertical black lines show the median values. Filtering would not make sense for m/z 31 and m/z 33, since there are no differences in concentration between smokers and non-smokers, and therefore all very low concentrations would be eliminated (see section 2).

acetonitrile (m/z 42), formaldehyde (m/z 31) and methanol (m/z 33) presented as histograms on a ppb log scale separately for the groups of smokers and controls (non-smokers). It can be seen that the distributions are essentially log-normal (as are those for several common breath metabolites studied using SIFT-MS [40–43]) and the concentrations of hydrogen cyanide and acetonitrile are significantly higher in the breath of smokers in comparison with non-smokers.

Threshold concentrations that yielded highest combined sensitivity and specificity were determined using ROC curves to distinguish smokers from non-smokers (table 3). The Youden index (= maximum of sensitivity + specificity − 1, YI) as a measure for differentiation between smokers and non-smokers was YI = 0.43 for ions at the m/z values 28 (tentatively identified as HCN), YI = 0.75 for m/z = 42 (tentatively identified as acetonitrile) and YI = 0.53 for m/z = 79 (tentatively identified as benzene). An example of an ROC curve for the m/z 79 ion is shown in figure 3. For the ions at m/z 31 (tentatively identified as formaldehyde) and m/z 33 (tentatively identified as methanol) we did *not* observe differences in concentrations between smokers and non-smokers.

Table 3. Classification value, sensitivity, specificity for maximal Youden index [39] for the discriminating ions at the m/z values relating to breath compounds of smoking origin *for filtered data*. Classification as smoker/non-smoker/ex-smoker was based on self-declaration of volunteers. For all possible values, the value to classify between the groups is taken for which the Youden index is at its maximum.

m/z	Classification value (ppb)	Sensitivity (%)	Specificity (%)	Max. Youden index
28	1.3	74.2	68.7	0.43
42	13.1	91.3	83.8	0.75
69	131.2	68.0	54.8	0.23
79	1.45	81.5	71.7	0.53
93	4.3	77.1	68.2	0.45
97	2.46	72.2	64.0	0.36
123	0.67	59.1	73.4	0.33

The correlation coefficient R for the concentrations of acetonitrile and benzene is $R = 0.53$, for the concentrations of acetonitrile and hydrogen cyanide $R = 0.35$, and for the concentrations of acetonitrile and hydrogen cyanide $R = 0.35$

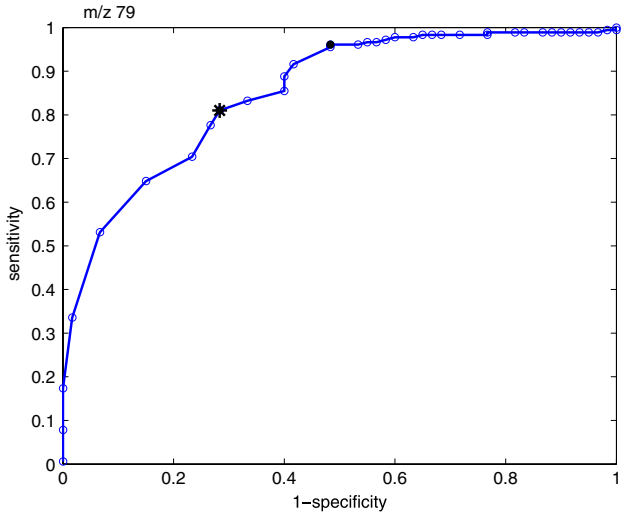


Figure 3. Receiver-operating characteristic (ROC) curve for *filtered* concentrations of m/z 79. This plot demonstrates the ROC curve of prediction of the breath test for the m/z 79 in view of the continuum of sensitivity and specificity (the star marks the point of maximum Youden index, and the dot the point of maximal accuracy).

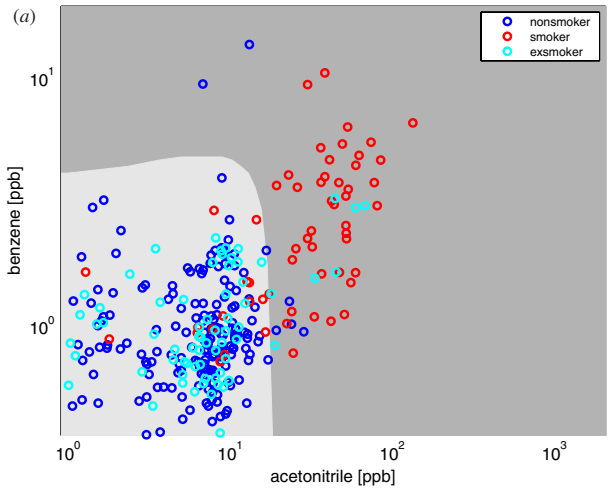
(see figure 5). Due to these correlations, the joint analysis of the concentrations of two different compounds does not give rise to a substantial increase in differentiation between smokers and non-smokers.

Significant but small differences for m/z 54, m/z 105 and m/z 109 occurred between smokers and non-smokers. Since the concentration levels were quite low, perhaps influenced by the zero counts of PTR-MS and the differences perhaps questionable, we did not consider these mass-to-charge ratios in tables 2 and 3. Our results indicate (see table 4) that there are no other m/z which show higher concentrations for smokers in comparison with non-smoking healthy volunteers (apart from m/z which are isotopes of the m/z's mentioned above, and not taking into consideration water clusters and m/z for the compounds released by Tedlar bags).

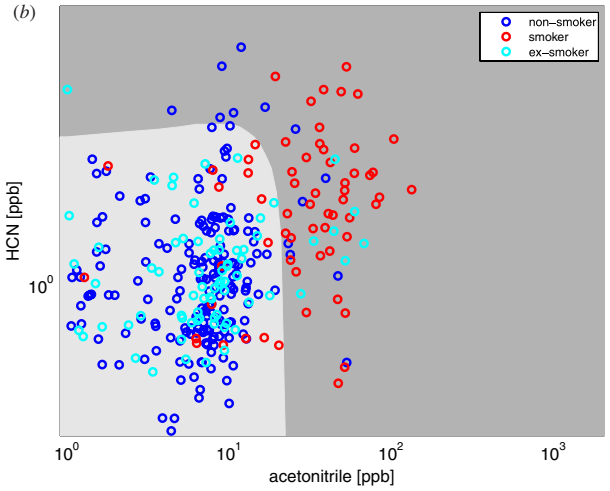
In addition to seven mass-to-charge ratios with higher concentrations for smokers as compared with the concentrations in non-smoking volunteers we found three mass-to-charge ratios (m/z 40, m/z 59, m/z 74), where the smokers show *lower* concentrations than non-smokers (with isotopic effects at m/z 41 and m/z 60, respectively, see table 4). By introducing table 4 and by excluding water clusters, primary ions and isotopic effects we try to be more precise than Moser *et al* [44] who just stated that 'significant differences in exhaled breath composition could be found between smokers and non-smokers in 32 out of 179 masses'.

4. Discussion

The main result of the present study is the identification of distinctive characteristics of smokers' exhaled air (breath) profiles and the delineation of reference concentrations for the volatile biomarkers of smoking using PTR-MS. The



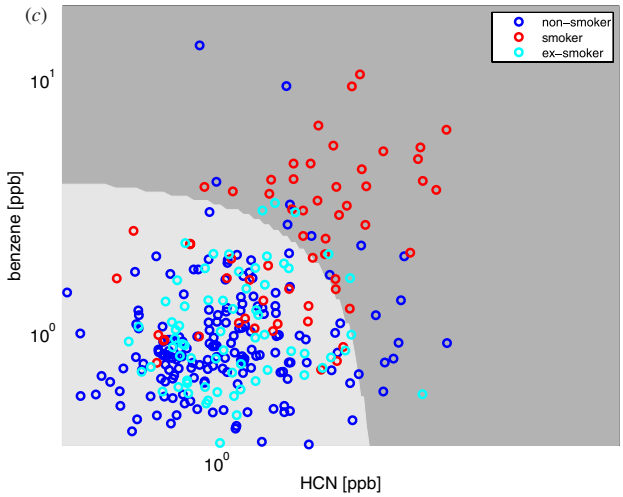
Sensitivity %	Specificity %	Youden-Index	Correlation coefficient R
73.7	95.5	0.69	0.53



Sensitivity %	Specificity %	Youden-Index	Correlation coefficient R
74.2	93.1	0.67	0.35

Figure 4. *Filtered* concentrations (parts per billion, ppb) of compounds in breath derived from product ions at m/z 42 and m/z 79 (*a*) identified as acetonitrile and benzene, respectively, and those derived from product ions at m/z 28 and m/z 42, identified as hydrogen cyanide and acetonitrile (*b*), and hydrogen cyanide and benzene (*c*) in smokers, non-smokers and ex-smokers. Between all the three pairs there is a significant correlation. In these pictures we can see a classification based on two substances, the area where smokers are classified is dark gray, the one for non-smoker light gray. The classification was computed with a quadratic discriminant analysis (MATLAB® command classify.m with quadratic boundaries between groups) based on *filtered* data. The sensitivity, specificity and the Youden index are shown in the tables besides the plots.

screening of human exhaled breath for VOCs characteristic of certain diseases is gaining increasing attention in the recent



Sensitivity %	Specificity %	Youden-Index	Correlation coefficient R
55.9	90.6	0.46	0.35

Figure 4. (Continued.)

literature [4, 5]. Yet many pathological conditions that may be diagnosed by breath analysis (e.g. lung cancer) commonly coexist with a variety of morbidities and/or are related to substance abuse, e.g., tobacco smoking, drug, alcohol, etc. Therefore, the results of diagnostic breath testing may be distorted by volatiles having an exogenous origin.

Besides the mentioned seven m/z, significant but small differences for m/z 54, m/z 105 and m/z 109 occurred between smokers and non-smokers. Since the concentration levels were quite low, perhaps influenced by the zero counts of PTR-MS and the differences perhaps questionable, we did not consider these mass-to-charge ratios in section 3.

PTR-MS is now an established tool for the rapid determination of exhaled breath profiles of volatile gases either in real time or using breath samples collected into bags or onto traps [12, 45–50]. The results of a gas chromatography mass spectrometric (GC/MS) study of the profiles of exhaled breath in a healthy population have been reported [51] and several selected ion flow tube mass spectrometer (SIFT-MS) studies of the distributions of the common breath metabolites have been carried out [40–43], including a study of acetonitrile in the exhaled breath and urine headspace of smokers [52]. However, none of the known investigations has provided a comprehensive overlook of smoking-related VOCs in human breath, being mainly focused on the quantification of a single or a few chemicals of smoking origin [14, 15, 17, 19, 23, 52–58]. The present pilot investigation is the attempt to circumscribe the specific characteristics of exhaled air profiles in smokers that can be determined using PTR-MS.

We compared PTR-MS with GCMS-SPME measurements of exhaled breath, using the most up-to-date quadrupole GCMS instrument of Agilent (gas chromatograph 7890A with 5975C inert XL mass spectral detector). Our PTR-MS measurements (e.g., for acetonitrile) are by a factor of ∼20 more sensitive than GCMS measurements. Furthermore, the intra-sample variability in GCMS-SPME measurements was higher than the intra-sample variability of PTR-MS measurements. The reason for this may be the preconcentration method which is necessary for GCMS measurements in order to improve sensitivity. PTR-MS measurements, on the other hand, can be quickly done without preconcentration procedures being necessary. We would like to stress that we used a high-sensitivity PTR-MS with Teflon rings (instead of Viton rings). With the old type of instrument (with Viton rings), contamination effects can arise which may need several days purging with clean air to be eliminated. In particular, contamination between successive measurements of exhaled breath samples is possible.

Ionic species at seven m/z values, selected by discriminant analysis, and hence the corresponding compounds in the breath of smokers, can be tentatively attributed to the substances given in table 2 (where the attributions to dimethylsulfoxide, toluene, dimethylfuran and dimethylpyrazole are not more than an 'educated guess'). The occurrence of benzene, acetonitrile and 2,5-dimethylfuran in the exhaled breath of smokers is well established [14, 17, 57–63].

This is in concordance with the present results, which also show median concentrations of these compounds in smokers' breath within the same range. The present study also shows that the concentrations of acetonitrile and benzene are correlated and that this is also the case for the combination of benzene with hydrogen cyanide and for the combination of acetonitrile with hydrogen cyanide (see figure 4). Due to this correlation, the combined use of two different marker compounds does not necessarily increase the quality of differentiation between smokers and non-smokers.

Such volatiles as hydrogen cyanide, acetonitrile and benzene (tentatively attributed to the m/z 28, 42 and 79) are well-known toxic components of the cigarette smoke [13, 64–66]. Hence, their presence in the exhaled air of smokers is not surprising. We should mention that the concentrations of the compounds indicated by the ions at m/z values 31 and 33 (tentatively identified as formaldehyde and methanol, respectively) are not significantly different in the exhaled breath of this cohort of smokers and non-smokers, despite the fact that formaldehyde and methanol have also been found in the mainstream cigarette smoke [65, 66].

Finally, some limitations of the present study should be discussed. Identification of compounds measured by PTR-MS is always tentative. In particular, overlap of different protonated compounds having the same m/z values may occur. For example, protonated 1,3-butadiene, which is expected to appear at $m/z = 55$, has been reported as one of the markers of smoking behavior at the level of 360 μg m^{-3}, corresponding to a few ppb, but this compound cannot be detected using PTR-MS, since the water cluster ion $(H_2O)_2H_3O^+$ also appears at m/z 55 in the PTR-MS spectrum. Also, the quantification of compounds with proton affinities close to that of water (such as hydrogen cyanide and formaldhehyde) gives rise to concentrations which are lower than the actual ones: for these

Table 4. All mass-to-charge ratios between 28 and 230 were checked for differences in concentration between smokers and non-smokers. This table gives additional information on the reasons why certain mass-to-charge ratios were not considered as showing different concentrations for a particular volatile compound between smokers and non-smokers. Typically, mass-to-charge ratios for water clusters and *N*,*N*-dimethyl-acetamide and phenol (released from Tedlar bags and arising at $m/z = 88$ and $m/z = 95$) were not considered. Also mass-to-charge ratios which are expected to be only isotope effects (e.g. $m/z = 70$ can be expected to be an isotope effect from isoprene $m/z = 69$) were not considered. Finally, only seven mass-to-charge ratios show an effect of smoking on the respective concentration. This might contrast with the result of Moser *et al* [44] that 'Significant differences in exhaled breath composition could be found between smokers and non-smokers in 32 out of 179 masses'. Notation: ***bold, italic*** = *significantly higher in smokers as compared to non-smokers*; ***italic***: significantly lower in smokers than in non-smokers; **bold** = could be considered, but $p > 0.01$ is possible or concentrations are lower than 1 ppb. For certain compounds (like 2-propanol) we added underlined comments based on calibration measurements of dry samples of the respective pure compound: as an example, for isoprene we observed that 88.7% of the transmission-corrected counts observed at m/z 69 are observed at $m/z = 41$. In this particular case, this is due to expulsion of neutral ethene from protonated isoprene. Similarly, 22.6% of the transmission-corrected counts observed at m/z 69 are observed at $m/z = 39$ due to expulsion of neutral ethane from protonated isoprene.

m/z	Possible substances	After filtering	Before filtering
28	***Hydrogen cyanide***	***Significant***	***Significant***
29		Not significant; concentration smoker < concentration non-smoker	Not significant; concentration smoker < concentration non-smoker
30		NO^+ from the ion source	NO^+ from the ion source
31	Formaldehyde CH_2NH_2	Not significant; concentration smoker < concentration non-smoker	Not significant; concentration smoker < concentration non-smoker
32		O_2^+ from the ion source	O_2^+ from the ion source
33	Methanol (main fragment)	Not significant	Not significant
34	Methanol (isotope of main fragment, 1.2% of m/z 33)	Not significant	Not significant
35	Hydrogen sulfide	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
36		Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
37	Water cluster	Water cluster	Water cluster
38		Isotope of water cluster	Isotope of water cluster
39	Isoprene (22.6% of m/z 69)	Not significant	Not significant; concentration smoker < concentration non-smoker
40		*Significant; concentration smoker < concentration non-smoker*	*Significant; concentration smoker < concentration non-smoker*
41	2-Propanol (34.7% of m/z 43) 1-Propanol (37.1% of m/z 43) Isoprene (88.7% of m/z 69)	Isotope effect of m/z 40	Isotope effect of m/z 40
42	***Acetonitrile***	***Significant***	***Significant***
43	Acetaldehyde (1.5% of m/z 45, possibly by reaction with parasitic ion NO^+) 2-Propanol (main fragment) 1-Propanol (main fragment)	Not significant; concentration smoker < concentration non-smoker	Not significant; concentration smoker < concentration non-smoker
44	*2-Propanol (isotope of main fragment, 3.5% of m/z 43)* *1-Propanol (isotope of main fragment, 3.4% of m/z 43)* *Isocyanic acid* *CH_2CHO* *n-Methyl methanimine* *Acetaldimine* *Ethenamine* *Ethylenimine*	Not significant; concentration smoker < concentration non-smoker	*Significant (but $p > 0.01$ possible); concentration smoker < concentration non-smoker*
45	Acetaldehyde (main fragment) CARBON DIOXIDE (CARBON DIOXIDE HAS A SMALLER PROTON AFFINITY THAN WATER, AND IS ALMOST NOT PROTONATED; IT APPEARS AT M/Z 45 DUE TO ITS VERY HIGH CONCENTRATION IN EXHALED BREATH AND DUE TO NON-EQUILIBRIUM PHENOMENA IN THE DRIFT CHAMBER) Ethylene oxide Carbon monosulfide	Protonated carbon dioxide (CO_2H^+) disturbs measurements on m/z 45	Protonated carbon dioxide (CO_2H^+) disturbs measurements on m/z 45

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
46	*Acetone (1.4% of m/z 59)* *Acetaldehyde (isotope of main fragment, 2.5% of m/z 45)* *CH_2CH_2OH* *Formamide* *Ethylamine* *N-methyl-methanamine*	*Significant; concentration smoker < concentration non-smoker*	*Significant (but $p > 0.01$ possible); concentration smoker < concentration non-smoker*
47	Formic acid Thioformaldehyde Ethanol Dimethyl ether Methyl-hydrazine	Not significant	Not significant
48	*o*-Methyl-hydroxylamine	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
49	Methanethiol	Not significant; concentration < 1 ppb; concentration smoker < concentration non-smoker	Not significant; concentration < 1 ppb; concentration smoker < concentration non-smoker
50		Not significant; concentration $\ll$ 1 ppb	Not significant; concentration smoker < concentration non-smoker concentration $\ll$ 1 ppb
51	Water cluster of methanol (0.7% of m/z 33) 1,3-Butadiyne Difluoromethylene	Already difference in inhaled air, concentration < 1 ppb	Already difference in inhaled air, concentration < 1 ppb
52	Propiolonitrile	Isotope effect of m/z 51; concentration $\ll$ 1 ppb	Isotope effect of m/z 51; concentration $\ll$ 1 ppb
53		Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
54	**2-Propenenitrile**	**Significant** (concentrations < 1 ppb)	**Significant** (concentrations < 1 ppb)
55	Fragment of 3-heptanone (10.4% of m/z 115) Water cluster	Water cluster	Water cluster
56	Propanenitrile Isocyano-ethane 1-Azabicyclo[1.1.0]butane Propargylamine Vinylimine	Isotope of m/z 55	Isotope of m/z 55
57	2-Butene 2-Propenal 2-Methyl-1-propene $NCCH_2NH_2$ Methylketene C_2S	Tedlar-bag-related m/z	Tedlar-bag related m/z
58	Isocyanato-methane CH_2COCH_3 Methyl azide Cyclopropylamine 2-Propen-1-amine 2-Methyl-aziridine 1-Methyl-aziridine 2-Propanimine 1-Methylethenylamine Azetidine	Not significant	Not significant
59	*Acetone (main fragment)* *Propanal* *Propylene oxide* *Thioketene* *Methoxy-ethene* *(e)-Dimethyldiazene* *Dimethyl-diazene* *$CH_3C(=NH)NH_2$*	*Significant; concentration smoker < concentration non-smoker*	*Significant; concentration smoker < concentration non-smoker*
60	Acetone (isotope of main fragment, 3.4% of m/z 59) $CH_2CH_2CH_2OH$	Isotope effect of m/z 59	Isotope effect of m/z 59

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	N-methyl-formamide Acetamide 1-Propanamine 2-Propanamine *N*-methyl-ethanamine Trimethylamine		
61	Acetaldehyde (3.7% of m/z 45) Methoxy-ethane 1-Dimethyl-hydrazine 1 Ethylenediamine Acetic acid Methyl formate 1-Propanol (but this fragments mostly to m/z 43 by loss of water) 2-Propanol (but this fragments mostly to m/z 43 by loss of water)	Not significant	Not significant
62		Not significant; concentration smoker < concentration non-smoker	Not significant
63	Dimethyl-sulfide Ethanethiol H_2N–NO_2 CH_2=S=O 1,2-Ethanediol	Not significant	Not significant
64	Nitric acid 2-Fluoro-ethylamine	Not significant; concentration < 1 ppb; concentration smoker < concentration non-smoker	Not significant; concentration < 1 ppb; concentration smoker < concentration non-smoker
65	2-Fluoro-ethanol 1,1-Difluoro-ethene	Difference in inhaled air concentration < 1 ppb	Difference in inhaled air concentration < 1 ppb
66		Concentration $\ll$ 1 ppb	Concentration $\ll$ 1 ppb
67	Isoprene (3.8% of m/z 69, possibly by reaction of isoprene with parasitic ion NO^+) Malononitrile Chlorofluoromethylene 1,3-Cyclopentadiene	Fragment of m/z 69	Fragment of m/z 69
68	Cyanoketene Cyclopropanecarbonitrile HNCCCO Pyrrole	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
69	***Isoprene (main fragment)*** ***Cyclopentene*** ***Furan*** ***2-Pentyne*** ***Ethenylcyclopropane*** ***3-Methyl-1-butyne*** ***2-Methyl-1,3-butadiene*** ***1,3-pentadiene*** ***1-Methyl-cyclobutene*** ***3,3-Dimethyl-cyclopropene*** ***1H-Pyrazole*** ***C_3S*** ***1H-Imidazole***	***Significant***	***Significant***
70	Isoprene (isotope of main fragment, 5.9% of m/z 69) CH_3COCN Butanenitrile 2-Methyl-propanenitrile Isoxazole 1-Isocyano-propane Oxazole 1,2,3-Triazole-1H 1,2,4-Triazole-1H	Isotope of m/z 69	Isotope of m/z 69
71	Cyclobutanone 2-Methyl-2-propenal 2-Methyl-2-butene	Not significant	Not significant

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	2,5-Dihydro-furan 2-Butenal Methyl vinyl ketone Dimethyl-cyanamide CH_3NHCH_2CN $H_2NCH_2CH_2CN$ 2,3-Dihydro-furan		
72	*Methoxyacetonitrile* *2-Azetidinone* *Acrylamide* *Ethyl azide* *N-ethyl-azetidine* *2-Methyl-2-propen-1-amine* *N-thylidene-ethanamine* *Pyrrolidine* *$(CH_3)_2NCH{=}CH_2$*	*Significant (but $p > 0.01$ possible); concentration smoker < concentration non-smoker*	*Significant; concentration smoker < concentration non-smoker*
73	Water cluster appears (but in relatively low concentrations) Butanal 2-Methyl-propanal Tetrahydro-furan 2-Butanone Ethoxy-ethene 2-Methoxy-1-propene Iron monoxide 2-Silaisobutene	Effected by water cluster	Effected by water cluster
74	*Thiocyanic acid methyl ester* *Isothiocyanato-methane* *N,N-dimethyl-formamide* *N-methyl-acetamide* *2-Methyl-1-propanamine* *1-Butanamine* *2-Butanamine* *2-Methyl-2-propanamine* *N-methyl-2-propanamine* *N-ethyl-ethanamine* *N,N-dimethyl-ethanamine*	*Significant; concentration smoker < concentration non-smoker*	*Significant; concentration smoker < concentration non-smoker*
75	1-Butanol 2-Methyl-1-propanol Propanoic acid Formic acid ethyl ester 1,1-Dimethyl-ethanol 2-Butanol Methyl propyl ether Acetic acid methyl ester 2-Methoxy-propane Ethoxy ethane Thietane Methyl-thiirane Methyl vinyl sulfide 1,3-Propanediamine	Not significant	Not significant
76	Chloro-acetonitrile Nitro-ethane Nitrous acid ethyl ester *N*-hydroxy acetamide Ethanethioamide Glycine 2-Methoxy-ethanamine 3-Amino-1-propanol *N*-oxide-*N*,*N*-dimethyl-methanamine	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
77	2-Methoxy-ethanol 1-Propanethiol 1-Fluoro-2-propanone 2-Propanethiol Benzyne	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	(Methylthio)-ethane 1,3-Propanediol Thiourea Trimethyl-phosphine		
78	Methyl nitrate 1,5-Hexadiyn-3-yl radical $FCH_2CH_2CH_2NH_2$	Not significant; concentration $<$ 1 ppb	Not significant; concentration $<$ 1 ppb
79	***Benzene*** ***Fluoro-acetic acid*** ***Dimethyl sulfoxide***	***Significant***	***Significant***
80	Pyridine	Isotope of m/z 79	Isotope of m/z 79
81	**2-Chloro-ethanol** **1,4-Cyclohexadiene** **1,3-Cyclohexadiene** **Pyrazine** **1-Methyl-3-methylenecyclobutene** **1,3-Diazine** **Pyridazine**	**Significant (but $p > 0.01$ possible)**	**Significant (but $p > 0.01$ possible)**
82	NCC(CH_3)CO 1,3,5-Triazine 2,2-Difluoro-ethylamine CH_3NCCCO	Isotope of m/z 81 ($p > 0.01$ possible)	Not significant
83	**CF_2HCH_2OH** **Cyclohexene** **1-Methyl-cyclopentene** **H_3PO_3** **Methylene-cyclopentane** **2,3-Dimethyl-1,3-butadiene** **1,2-Dimethylcyclobutene** **3-Methyl-furan** **$CH_3CH{=}C(CH_3)CH{=}CH_2$** **1-Ethenyl-1-methyl-cyclopropane** **dichloromethylene** **2-Methyl-1,3-pentadiene** **2-Methyl-Furan** **(1-Methylethenyl)-cyclopropane** **1,3,3-Trimethylcyclopropene** **3(5)-Methylpyrazole** **4-Methylpyrazole** **1-Methylpyrazole** **4-Methylimidazole** **1-Methyl-1H-imidazole** **2-Methyl-1H-imidazole**	**Significant (but $p > 0.01$ possible)**	**Significant (but $p > 0.01$ possible)**
84	Pentanenitrile 2,2-Dimethyl-propanenitrile *tert*-Butyl isocyanide 4-NH_2-pyrazole 3(5)-Aminopyrazole *N*,*N*-dimethyl-2-propyn-1-amine	Isotope of m/z 83, concentration $<$ 1 ppb	Isotope of m/z 83, concentration $<$ 1 ppb
85	2-Methyl-2-pentene $CH_3CH{=}C(CH_3)C_2H_5$ 2,3-Dimethyl-2-butene Thiophene Cyclopentanone 2-Pentenal 3-Methyl-3-buten-2-one 2-Methyl-2-butenal 2-Methyl-2-butenal 1-Cyclopropyl-ethanone 3-Methyl-2-butenal 3-Penten-2-one 3,4-Dihydro-2H-pyran 4-Methyl-2,3-dihydrofuran (Dimethylamino)-acetonitrile 2,3-Dihydro-5-methyl-furan	Not significant	Not significant

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	1,4,5,6-Tetrahydropyrimidine		
86	Carbonocyanic acid methyl ester CH_3COOCN Methacrylamide 2-Methyl-2H-azetidin-2-one 2-Butenamide Thiazole Piperidine *N*,*N*-dimethylallyl amine $(CH_3)_2C{=}NC_2H_5$ 1-Methyl-pyrrolidine $CH_3CH{=}CHN(CH_3)_2$ *N*-(2-propylidene)ethanamine	Not significant; concentration $<$ 1 ppb	Not significant; concentration $<$ 1 ppb; concentration smoker $<$ concentration non-smoker
87	**Pentanal** **2,3-Butanedione** **Acetic acid ethenyl ester** **2-Methyl-2-propenoic acid** **Crotonic acid** **Tetrahydro-2H-pyran** **Cyclopropanecarboxylic acid** **2,3-Dihydro-1,4-dioxin** **2-Propenoic acid methyl ester** **Isocrotonic acid** **$C_2H_5OCH_2CH{=}CH_2$** **3-Methyl-2-butanone** **3-Pentanone** **γ-Butyrolactone** **Tetrahydro-2-methyl-furan** **4-Fluoropyrazole** **$C_2H_5OCH{=}CHCH_3$** ***trans*-$CH_3CH{=}CH\text{-}OC_2H_5$** ***c*-$C(CH_3)(C_2H_5)NHNH$** **Piperazine** **$CH_3NHCH_2CH_2NHCH_3$** **$(CH_3)_2N{-}CH{=}N{-}CH_3$**	**Significant (but $p > 0.01$ possible)**	**Significant (but $p > 0.01$ possible)**
88	CH_3SCH_2CN 1,4-Dioxyl radical *N*-C_3H_7NHCHO *N*-ethyl-acetamide	Not significant; concentration smoker $<$ concentration non-smoker	Not significant; concentration smoker $<$ concentration non-smoker
	N,*N*-dimethyl-acetamide *N*-methyl-propanamide 1-Pentanamine Morpholine Neopentylamine 2-Methyl-2-butanamine $(C_2H_5)(i\text{-}C_3H_7)NH$ $(CH_3)_2$ $(n\text{-}C_3H_7)N$ *N*-ethyl-*N*-methyl-ethanamine *N*,*N*-dimethyl-2-propanamine	*N*,*N*-dimethylacetamide is released by Tedlar bags	*N*,*N*-dimethylacetamide is released by Tedlar bags
89	2,2-Dimethyl-1-propanol 1,4-Dioxane Formic acid propyl ester Formic acid 1-methylethyl ester Ethylene carbonate 1-Methoxy-butane 1,3-Dioxane Propanoic acid methyl ester Ethyl acetate 2-Methoxy-2-methyl-propane 2-Ethoxy-propane Tetrahydro-thiophene $CH_2{=}C(CH_3){-}SCH_3$ Tetramethylhydrazine 1,1-Dimethoxy-ethene 1,4-Butanediamine	Possibly influenced by isotope of m/z 88 (*N*,*N*-dimethyl acetamide released by Tedlar bags)	Possibly influenced by isotope of m/z 88 (*N*,*N*-dimethyl acetamide released by Tedlar bags)

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
90	$Cl(CH_2)_2CN$ *iso*-Propyl nitrite *N*-Hydroxy-*N*-methyl acetamide *N*-Methoxy acetamide *N*,*N*-dimethyl-methanethioamide $NH_2(CH_2)_4OH$	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
91	1-Butanethiol 2-Methyl-1-propanethiol 2-Butanethiol 2-Methyl-2-propanethiol *N*-Methyl-*N*-nitro-methanamine Ethanethioic acid *S*-methyl ester Carbonic acid dimethyl ester $CH_3C(=S)OCH_3$ Diethyl sulfide 1,2-Dimethoxy-ethane 1,4-Butanediol	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
92		Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
93	***$FCO_2C_2H_5$*** ***Toluene*** ***2,5-Norbornadiene*** ***1,2,3-Propanetriol*** ***Trimethylphosphine oxide***	***Significant***	***Significant***
94	Aniline *N*-2-propynyl-2-propyn-1-amine 3-Methyl-pyridine 4-Methyl-pyridine 2-Methyl-pyridine	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
95	CFH_2COCFH_2 Chloro-acetic acid 3,3′-Oxybis-1-propyne Dimethyldisulfide	Not significant	Not significant
	Phenol 2-Norbornene 3-Pyridinamine 2-Pyridinamine 4-Pyridinamine	Phenol is released by Tedlar bags	Phenol is released by Tedlar bags
96	2,5-Dimethyl-1H-pyrrole 1-Oxidepyridine	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
97	***Fluoro-benzene*** ***Methanesulfonic acid*** ***Phosphabenzene*** ***1,2-Dimethyl-cyclopentene*** ***1-Methyl-cyclohexene*** ***3-Heptanone (2.7% of m/z 115)*** ***7-oxa-bicyclo[2.2.1]hept-2-ene*** ***2,5-Dimethyl-furan*** ***3,4-Dimethylfuran*** ***2(1H)-Pyrimidinone*** ***$(CH_3)_2C{=}CHC(CH_3){=}CH_2$*** ***2,4-Dimethylfuran*** ***trans-Dimethylamino acrylonitrile*** ***3(5),4-Dimethylpyrazole*** ***1,4-Dimethylpyrazole*** ***1,3-Dimethylpyrazole*** ***1,5-Dimethylpyrazole*** ***3,5-Dimethyl-1H-pyrazole*** ***1,4-Dimethylimidazole*** ***1,5-Dimethylimidazole*** ***1,2-Dimethyl-1H-imidazole***	***Significant***	***Significant***
98	4-NO_2-pyrazole 2-Fluoropyridine *N*′-cyano-*N*,*N*-dimethyl formamidine 3-F-pyridine	Isotope of m/z 97	Isotope of m/z 97

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	4-F-pyridine 1-Methyl-3-aminopyrazole 1-Methyl-5-aminopyrazole *N*-2-propenyl-2-propen-1-amine		
99	2,4-Dimethyl-2-pentene $(CH_2{=}CHCH_2)_2O$ $(CH_3)_2NCOCN$ Cyclohexanone 7-Oxabicyclo[2.2.1]heptane Cyclohexene oxide 2-Methyl-thiophene 3-Hexen-2-one(E) 3-Methyl-3-penten-2-one 4-Methyl-3-Penten-2-one 4,4-Dimethyl-2-imidazoline $(CH_3)_2N$-CH=N-(2-propenyl)	Not significant	Not significant; concentration smoker < concentration non-smoker
100	Trifluoronitrosomethane $NCCOOC_2H_5$ 3-Ethoxy pentanenitrile 2,2,2-Trifluoroethylamine *N*,*N*-dimethyl 2-propenamide 1-Methyl-2-pyrrolidinone 2-Methylthiazole Cyclohexanamine *N*-butylidene-ethanamine *N*,*N*-2-trimethyl-1-propen-1-amine 1-Methyl-piperidine $(CH_3)_2NC(CH_3){=}CHCH_3$	Not significant; concentration < 1 ppb; concentration smoker < concentration non-smoker	Not significant; concentration < 1 ppb; concentration smoker < concentration non-smoker
101	CF_3OCH_3 Cyclobutane carboxylic acid 2-Methyl-2-butenoic acid 3-Methyl-2-butenoic acid Eta-penteneoic acid *trans*-alpha 2-Propenoic acid 2-methyl-methyl ester Oxepane Ethenyltrimethyl-silane 3,3-Dimethyl-2-butanone 3-Hexanone Cyclopropanecarboxylic acid methyl ester 2,2-Dimethyltetrahydrofuran 2-Butenoic acid methyl Acetylacetone 2-Aminothiazole 1,2-Dimethyl-pyrazolidine $(CH_3)_2N$–CH=N–C_2H_5 $(CH_3)_2N$–$C(CH_3){=}NCH_3$	Not significant	Not significant; concentration smoker < concentration non-smoker
102	1-Hexanamine *N*-propyl-1-propanamine *N*,*N*-dimethyl isobutylamine *N*,*N*-dimethyl-1-butanamine *N*-(1-methylethyl)-2-propanamine (*sec*-C_4H_9)$(CH_3)_2N$ *N*,*N*-2-trimethyl-2-propanamine Triethylamine	Already difference in inhaled air; significant; concentration smoker < concentration non-smoker	Already difference in inhaled air; significant (but $p > 0.01$ possible); concentration smoker < concentration non-smoker
103	Formic acid butyl ester 1-Methoxy-2,2-dimethyl-propane Phenylacetylene Butanoic acid methyl ester Acetic acid 1-methylethyl ester 2-Methyl-propanoic acid methyl ester *n*-Propyl acetate di-*n*-Propyl ether	Not significant	Not significant

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	Tetrahydro-2H-thiopyran Diisopropyl ether 2-Ethoxy-2-methyl-propane 4-Cl-pyrazole *cis*-1,2-Cyclopentanediol 2-Imidazolidinethione $(CH_3)_2N–CH=N–OCH_3$ *N*,*N*-*N*′,*N*′-tetramethyl-methanediamine 1,5-Diaminopentane *N*,*N*-dimethyl-1,3-propanediamine		
104	Benzonitrile $(CH_3)_3CONO$ Isocyano-benzene $(CH_3)_22NCOOCH_3$ $CH_3NHCOOC2H_5$ Dimethyl thioacetamide *N*-(2-aminoethyl)-1,2-ethanediamine	Not significant; concentration $<$ 1 ppb; concentration smoker $<$ concentration non-smoker	Not significant; concentration $<$ 1 ppb; concentration smoker $<$ concentration non-smoker
105	**2,2-Dimethyl-1-propanethiol** **Styrene** **$C_2H_5OCOOCH_3$** **Thioacetic acid *o*-ethyl ester** **2-Pyridinecarbonitrile** **3-Pyridinecarbonitrile** **4-Pyridinecarbonitrile** ***o*-Xylylene** **1,3-Dimethoxy-propane** **3,6-bis(Methylene)-1,4-cyclohexadiene** ***N*,*N*′-dimethyl-thiourea**	**Significant** **(concentrations $<$ 1 ppb)**	**$0.01 < p < 0.05$;** **(concentrations $<$ 1 ppb)**
106	$C_6H_5CH=NH$ 4-Ethenyl-pyridine 2,3-Cyclobutenopyridine 3,4-Cyclobutenopyridine Diethanolamine	Not significant; concentration $<$1 ppb	Not significant; concentration $<$1 ppb; concentration smoker $<$ concentration non-smoker
107	Cyanogen bromide Ethylbenzene *p*-Xylene 1,2-Dimethyl-benzene 1,3-Dimethyl-benzene Benzaldehyde Methyl dithioacetate $HOCH_2CH(OH)CH_2CH_2OH$ 2,4,6-Cycloheptatrien-1-one 4-Methylene-2,5-cyclohexadiene-1-one	Already difference in inhaled air; significant (but $p > 0.01$ possible); concentration smoker $<$ concentration non-smoker	Not significant
108	$ClCON(CH_3)_2$ Nitroso-benzene 2-Me-phenoxy 3-Me-phenoxy 2-OH-benzyl 4-Me-phenoxy 3-OH-benzyl 2-Methyl-benzenamine 3-Methyl-benzenamine 4-OH-benzyl *p*-Toluidine 4-Pyridinecarboxaldehyde Benzylamine *N*-methyl-aniline $(iso\text{-}C_5H_{11})_3N$ 2,5-Dimethyl-pyridine 2,3-Dimethyl-pyridine 3-(C_2H_5)-pyridine 2,4-Dimethyl-pyridine	Not significant	Not significant

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	4-(C_2H_5)-pyridine 2-Ethyl-pyridine 3,5-Dimethyl-pyridine 2,6-Dimethyl-pyridine		
109	**Carbonochloridic acid ethyl ester** **Benzyl alcohol** ***p*-Benzoquinone** **Bicyclo[2.2.1]hept-2-en-7-one** **Methoxy-benzene** **2-Methyl-bicyclo[2.2.1]hept-2-ene** **Bicyclo[2.2.1]hept-2-en-5-one** **2-Methylenebicyclo[2.2.1]-heptane** **1,2-Benzenediamine** **1,1′-Ethenylidenebis-cyclopropane** **1,4-Benzenediamine** **1,3-Benzenediamine**	**Significant (concentrations $\sim$ 1 ppb)**	**Significant (concentrations $\sim$ 1 ppb)**
110	Cyclohexanecarbonitrile 3-Fluorobenzyl radical 3-Amino-phenol 2-Amino-phenol 1-Methyl-2(1H)-pyridinone 2-Methoxy-pyridine 1-Oxide 3-methyl-pyridine 3-Methoxy-pyridine 1-Azabicyclo[2.2.2]oct-2-ene 4-Methoxy-pyridine	Not significant; concentration $<$ 1 ppb	Not significant; concentration $<$ 1 ppb
111	1-Fluoro-4-methyl-benzene 1-Fluoro-2-methyl-benzene 1-Fluoro-3-methyl-benzene Norbornan-7-one 2-Norbornanone $(CH_3)_2C{=}C(CH_3)C(CH_3){=}CH_2$ 1-Carbonitrile-piperidine Dicyclopropyl-methanone Phosphonic acid dimethyl ester 4-Cyanopiperidine 3,4,5-Trimethylpyrazole 1,3,5-Trimethylpyrazole $(CH_3)_2N$–CH=N-(2-propynyl)	Not significant	Not significant; concentration smoker $<$ concentration non-smoker
112	3-Fluoro-benzenamine *p*-Fluoroaniline exo-2-Aminonorbornane endo-2-Aminonorbornane $(CH_3)_2N$–CH=N–CH_2CN 4-Amino-2(1H)-pyrimidinone Histamine	Not significant; concentration $<$ 1 ppb	Not significant; concentration $<$ 1 ppb
113	1,1,1-Trifluoro-2-propanone Chloro-benzene 1,4-Cyclohexanedione 4-Methyl-cyclohexanone Cycloheptanone *c*-Hexane-1,2-dione 1,3-Cyclohexanedione Triethylenediamine Tetrahydro-1H5H-pyrazolo [12-a]pyrazole $(CH_3)_2N$–CH=N–(c-propyl)	Not significant	Not significant
114	1,1,1-Trifluorotrimethylamine 3(5)-Nitropyrazole $CF_3CH_2NHCH_3$ 3,3,3-Trifluoro-propylamine 3-Fluoro-pyridine-1-oxide 3-Chloro-pyridine 2-Chloro-pyridine	Not significant; concentration $<$ 1 ppb; concentration smoker $<$ concentration non-smoker	Not significant; concentration $<$ 1 ppb; concentration smoker $<$ concentration non-smoker

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	N,*N*,2-trimethyl-2-propenamide 4-Chloropyridine 1-Methyl-2-piperidinone *c*-$C_6H_{11}CH_2NH_2$ Acetylpyrrolidine *N*,*N*-dimethyl-butenamide $(CH_3)_2NC(C_2H_5)$=$CHCH_3$		
115	3-Heptanone (main fragment) Trifluoro-acetic acid 1,4-Difluoro-benzene 1,2-Difluoro-benzene 2,2,2-Trifluoroethyl methyl ether 1,3-Difluoro-benzene Carbonothioic dichloride Cyclohexanemethanol Cyclopentane carboxylic acic 1-Methoxycyclohexane 4-Heptanone 2,4-Dimethyl-3-pentanone $CH_3COCH_2CH_2COCH_3$ 1,3-Dimethyl-2-imidazolidinone Hexahydro-1,2-dimethyl-pyridazine $(CH_3)_2N$–CH=N-(*n*-propyl) $(CH_3)_2N$–CH=N–(1-methylethyl) $(CH_3)_2N$–$C(CH_3)$=NC_2H_5	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
116	3-Heptanone (7.8% of m/z 115) *N*,*N*-Dimethylbutyramide 1-Heptanamine *N*,*N*-diethyl-acetamide *c*-$C_5H_{10}N(2\text{-}OCH_3)$ $(CH_3)_3CCH_2N(CH_3)_2$ *N*,*N*-Diethyl-1-propanamine (*t*-C_5H_{11})$(CH_3)_2N$ (*i*-C_3H_7)$N(C_2H_5)_2$ *N*,*N*,*N*′,*N*′-tetramethyl- methanehydrazonamide	Already difference in inhaled air; significant (but $p > 0.01$ possible); concentration < 1 ppb; concentration smoker < concentration non-smoker	Already difference in inhaled air; significant; concentration < 1 ppb; concentration smoker < concentration non-smoker
117	4-Hydroxy-4-methylpentan-2-one *trans*-1,3-Cyclohexanol 3-Methylphenylacetylene 2,2-Dimethyl-propanoic acid methyl ester Indene 1-Ethynyl-4-methyl-benzene 2-Methyl-2-(1-methylethoxy)- propane *cis*-1,3-Cyclohexandiol Tetramethyl-urea *N*,*N*′-diethyl-*N*, *N*′-dimethylhydrazine Propyltrimethylhydrazine $(CH_2)_5PCH_3$ 1,6-Hexanediamine *N*,*N*,*N*′,*N*′-tetramethyl- 1,2-ethanediamine	Not significant; concentration smoker < concentration non- smoker	Not significant; concentration smoker < concentration non-smoker
118	Benzeneacetonitrile $(CH_3)_2NCOOC_2H_5$ 4-H_2–C_6H_4–CCH Indole $NH_2(CH_2)_6OH$ $(CH_3)_3SiN(CH_3)_2$	Not significant; concentration < 1 ppb	Not significant; concentration smoker < concentration non- smoker; concentration < 1 ppb
119	1-Propenyl-(e)-benzene Cyclopropyl-benzene 1-Phenylpropene 3-Amino-benzonitrile 1-Ethenyl-3-methyl-benzene 1-Ethenyl-2-methyl-benzene	Not significant; concentration smoker < concentration non- smoker; concentration < 1 ppb	Already difference in inhaled air; significant; concentration smoker < concentration non-smoker; concentration < 1 ppb

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	1-Ethenyl-4-methyl-benzene 1,1′-Thiobis-propane Methylstyrene Diisopropyl sulfide 1H-indazole $CH_3O(CH_2)_4OCH_3$ 1H-pyrrolo[2,3-b]pyridine 1H-benzimidazole Imidazo[1,2-a]pyridine Triethyl-phosphine		
120	Azido-benzene 2-Phenyl-2-propyl radical $C_6H_5(CHC_2H_5)$ radical Benzoxazole $CH_3OC(S)N(CH_3)_2$ 1-Phenyl-aziridine 6,7-Dihydro-5H-1-pyrindine 6,7-Dihydro-5H-2-pyrindine 2,3-Dihydro-1H-indole	Not significant; concentration $<$ 1 ppb	Not significant; concentration smoker $<$ concentration non-smoker; concentration $<$ 1 ppb
121	Propyl-benzene (1-Methylethyl)-benzene 2,6,7-Trioxa-1-phosphabicyclo[2.2.1]heptane 3-FC_6H_4CCH 4-FC_6H_4CCH $C_2H_5S(OCH_3)CO$ 1,3,5-Trimethyl-benzene 3-$CH_3C_6H_4CHO$ 4-Methyl-benzaldehyde Acetophenone 1-Oxide 4-pyridinecarbonitrile 1-Oxide 3-pyridinecarbonitrile 9H-purine 1-(Dimethylthio)ethene	Not significant; concentration smoker $<$ concentration non-smoker	Not significant; concentration smoker $<$ concentration non-smoker
122	Benzamide 3-$C_2H_5C_6H_4NH_2$ 2,6-Dimethyl-benzenamine 4-Aminobenzenecarbonal 1-(4-pyridinyl)-ethanone 1-(3-pyridinyl)-ethanone *n*-Ethyl-benzenamine Benzeneethanamine $OP(N(CH_3)_2)(CH_3)_2$ *N*,*N*-dimethyl-benzenamine 4-(*i*-C_3H_7)-C5H4N 2-(C_3H_7)-pyridine 2-(*i*-C_3H_7)-pyridine	Significant; concentration $<$ 1 ppb	Not significant; concentration $<$ 1 ppb
123	**(Methoxymethyl)-benzene** **Benzoic acid** **Carbonodithioic acid O, S-dimethyl ester** **2-Methoxy-1,3,2-dioxaphospholane** **Niacinamide** ***N*,*N*-dimethyl-2-pyridinamine** ***N*,*N*-dimethyl-3-pyridinamine** ***N*,*N*-dimethyl-4-pyridinamine**	**Significant (but relatively low concentrations $<$ 1 ppb)**	**Significant (but relatively low concentrations $<$ 1 ppb)**
124	Nitro-benzene $CF_2HCON(CH_3)_2$ 4-Methoxy-benzenamine 2-Methoxy-benzenamine 3-Methoxy-benzenamine 2-(CH_3OCH_2)-pyridine 3-Methylene 1-azabicyclo [2.2.2]octane	Already difference in inhaled air, isotope of m/z 123	Already difference in inhaled air, isotope of m/z 123

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	3-Methylene 1-azabicyclo [2.2.2]octane		
125	$O(CH_2CH_2CN)_2$ 3-FC_6H_4CHO 4-Fluoro-benzaldehyde 5,5-Dimethyl-2-cyclohexenone (Methylthio)-benzene 4-Nitropyridine 2,3,4,5-Tetramethylfuran 3(5)-*t*-butylpyrazole Phosphorous acid trimethyl ester *N*-butylpyrazole 2,6-Dimethyl-4H-Pyran-4-one 1-*t*-Butylimidazole 1-Diazabicyclo[4.3.0]non-5-ene	Not significant; concentration smoker < concentration non-smoker	Not significant; concentration smoker < concentration non-smoker
126	2-Bromo-ethanol 1-Azabicyclo[2.2.2]octan-3-one 2-(Methylthio)-pyridine 3-(Methylthio)-pyridine 4-(Methylthio)-pyridine $(CH_3)_2N$-CH=N-CH_2CH_2CN 4-Methyl-1-azabicyclo[2.2.2]octane 1,4,4-$(CH_3)_3$_1,2,3,4-tetrahydropyridine 3-Methyl1-azabicyclo[2.2.2]-octane 2-Methyl1-azabicyclo[2.2.2]-octane	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb
127	1-Chloro-4-methyl-benzene 1-Chloro-2-methyl-benzene 1-Chloro-3-methyl-benzene *c*-$C_6H_{11}COCH_3$ Cyclooctanone $(c\text{-}C_3H_5)_2CS$ 3-Amino-1-azabicyclo[2.2.2]octane 2-Methyl-1,2-diazabicyclo[2.2.2]octane 2,3-Dimethyl-2,3-diazabicyclo[2.2.1]heptane $(CH_3)_2N$-C(CH_3)=N(c-C_3H_5)	Not significant; concentration smoker < concentration non-smoker	Not significant; concentration smoker < concentration non-smoker
128	1-Methyl-3-nitropyrazole 1-Methyl-5-nitropyrazole *m*-Chloroaniline *p*-Chloroaniline 1-Methyl-5-nitroimidizole Dimethyl(2,2-difluoroethyl)amine 4,4,4-Trifluorobutylamine 2-Cl-4-(CH_3)-pyridine 2-Cl-6-(CH_3)-pyridine *N*,3,5-trimethylpiperidine *N*,3,5-trimethylpiperidine 1,4,4-Trimethylpiperidine *N*,*N*-dimethyl-cyclohexanamine	Not significant; concentration < 1 ppb; concentration smoker < concentration non-smoker	Not significant; concentration < 1 ppb
129	$CF_3C(O)OCH_3$ 2,2,2-Trifluoroethyl formate Ethyl 2,2,2-trifluoroethyl ether 1,4-Benzenedicarbonitrile 1,3-Benzenedicarbonitrile Naphthalene Cyclohexanecarboxylic acid $C_6H_{11}CH_2OCH_3$ 2,2,4-Trimethyl-3-pentanone Azulene Hexahydro-1,2-dimethyl 1H-1,2-diazepine $(CH_3)_2N$-CH=N-(*n*-butyl) $(CH_3)_2N$-CH=N-(2-methylpropyl) $(CH_3)_2N$-CH=N-(1-methylpropyl)	Not significant; concentration < 1 ppb; concentration smoker < concentration non-smoker	Not significant; concentration < 1 ppb

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	$(CH_3)_2N$-CH=N(t-C_4H_9) $(CH_3)_2N$-C(CH_3)=N(n-C_3H_7) $(CH_3)_2N$-C(CH_3)=N(i-C_3H_7)		
130	3-Chloro-pyridine-1-oxide 1-Octanamine Isoquinoline Quinoline 2-Methyl-*N*-(2-methylpropyl)-1-propanamine *n*-Butyl-1-butanamine *N*-(1-methylpropyl)-2-butanamine $(t\text{-}C_4H_9)_2NH$ $(i\text{-}C_3H_7)_2(C_2H_5)N$	Not significant; concentration $<$ 1 ppb; concentration smoker $<$ concentration non-smoker	Not significant; concentration $<$ 1 ppb
131	$c\text{-}C_6H_{11}CH_2SH$ *n*-Butyl ether 5-$(CH_3)_2$–C_6H_3–CCH_3 Heptamethylenesulfide $c\text{-}C_6H_{11}SCH_3$ di-*sec*-butyl ether di-*tert*-butyl ether Quinoxaline $CH_3C(OCH_3)$=CHCOOCH_3 CH_2=(CH_3)OSi$(CH_3)_3$ Cinnoline *tert*-Butyl trimethylhydrazine Butyltrimethylhydrazine 1,7-Diaminoheptane $(CH_3)_2N$–CH=N-(2-methoxyethyl) *N*,*N*,*N*′,*N*′-tetramethyl-1,3-propanediamine	Not significant; concentration $<$ 1 ppb	Not significant; concentration $<$ 1 ppb
132	4-Formyl-benzonitrile $CH_3CONHCH_2COOCH_3$ *N*,*N*-di-2-propynyl-2-propyn-1-amine Dimethyl(trimethylsilylmethyl)amine $NH_2(CH_2)_3NH(CH_2)_3NH_2$	Not significant; concentration $\ll$ 1 ppb; concentration smoker $<$ concentration non-smoker	Not significant; concentration $\ll$ 1 ppb; concentration smoker $<$ concentration non-smoker
133	1,2,3-Trifluorobenzene 1,2,4-Trifluorobenzene 1,3,5-Trifluorobenzene $(C_2H_5)_3SiOH$ 1-Cyclopropyl-3-methyl-benzene 1-Cyclopropyl-2-methyl-benzene 1-Cyclopropyl-4-methyl-benzene 1-Methyl-2-(1-methylethenyl)-benzene 2-Methylbenzofuran 1-Methyl-3-(1-methylethenyl)-benzene 3-$CH_3C_6H_4C(CH_3)$=CH_2 4-$CH_3C_6H_4C(CH_3)CH_2$ 4-CH_3O–C_6H_4–CCH 1-Methylindazole $CH_3O(CH_2)_5OCH_3$ 2-Methyl-2H-indazole Tetramethyl-thiourea 1-Methylbenzimidazole $(n\text{-}C_3H_7)_2(CH_3)P$ 5-Methylimidazo(1,2-a)pyridine 2-Methylimidazo(1,2-a)pyridine 7-Methylimidazo(1,2-a)pyridine	Not significant; concentration $<$ 1 ppb; concentration smoker $<$ concentration non-smoker	Not significant; concentration $<$ 1 ppb; concentration smoker $<$ concentration non-smoker
134	2-Methyl-2H-benzotriazole Aspartic acid 4-$H_2NC_6H_4C(CH_3)$=CH_2 1-Methylbenzotriazole *N*-phenylazetidine 5,6,7,8-Tetrahydro-quinoline	Not significant; concentration $\ll$ 1 ppb; concentration smoker $<$ concentration non-smoker	Not significant; concentration $\ll$ 1 ppb; concentration smoker $<$ concentration non-smoker

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	5,6,7,8-Tetrahydro-isoquinoline		
135	Butyl-benzene Methyltrioxaphosphabicycloheptane Benzyl methyl ketone 1,2,3,5-tetramethyl-benzene $((CH_3)_2SiH)_2O$ 1-Phenyl-1-propanone 1-(3-Methylphenyl)-ethanone 2,6,7-Trioxa-1-phosphabicyclo[2.2.2]octane 1-(4-Methylphenyl)-ethanone 1,1′-Oxybis[2-methoxy-ethane $(C_2H_5)_3PO$ 6-Methyl-1H-purine	Not significant; concentration smoker < concentration non-smoker	Not significant; concentration < 1 ppb; concentration smoker < concentration non-smoker
136	4-Methyl-benzamide *m*-Toluamide 4′-Amino-acetophenone *N*-ethyl-*N*-methylaniline *N*,*N*,3-trimethyl-benzenamine Adenine *N*,*N*,4-trimethyl-benzenamine *N*,*N*,2-trimethyl-benzenamine 4-(1,1-Dimethylethyl)-pyridine 2-(t-C_4H_9)-pyridine *N*,*N*,dimethyl-benzenemethanamine 2,6-$(C_2H_5)_2$-pyridine	Not significant; concentration < 1 ppb	Not significant; concentration < 1 ppb; concentration smoker < concentration non-smoker
137	Isoprene (1.6% of m/z 69) 3-ClC_6H_4CCH 3-Methyl-benzoic acid 1-Chloro-4-ethynyl-benzene 4-Methyl-benzoic acid 2-Methyl-benzoic acid 3-$FC_6H_4C(CH_3)=CH_2$ 3-Methoxy-benzaldehyde Benzoic acid methyl ester 4-$FC_6H_4C(CH_3)=CH_2$ 1-(3-hydroxyphenyl)-ethanone $CH_2=C(CH_3)$-$SeCH_3$ 4-Methoxy-benzaldehyde 4′-Hydroxy-acetophenone 3-NH_2–$C_6H_4CONH_2$ 1,5,5-Trimethyl-3-methylenecyclohexene Hypoxanthine 2-Methoxy-1,3,2-dioxaphosphorinane 4-Amino-benzamide 2-Cyano1-azabicyclo[2.2.2]-octane 1-Azabicyclo[2.2.2]octane-4-carbonitrile 3-Cyano1-azabicyclo[2.2.2]-octane *n*,*n*-Dimethyl-14-benzenediamine	Not significant	Not significant
138	1-Methyl-4-nitro-benzene *p*-Aminobenzoic acid 3-Amino-benzoic acid Anthranilic acid 1-(3-Pyridinyl-1-oxide)ethanone Pyridine-4-carboxylic acid methyl ester Methyl nicotinate *N*,*N*-di-2-propenyl-2-propen-1-amine	Not significant; concentration <1 ppb	Not significant; concentration <1 ppb
139	3-$ClC_6H_4CH=CH_2$ 1-(3-Fluorophenyl)-ethanone 1-(4-Fluorophenyl)-ethanone	Not significant; concentration <1 ppb	Not significant; concentration <1 ppb

Table 4. (Continued.)

m/z	Possible substances	After filtering	Before filtering
	p-Nitroaniline 3,5,5-Trimethyl-2-cyclohexen-1-one 1-Methyl-5-*t*-butylpyrazole 1-Methyl-3-*t*-butylpyrazole 3(5)-Methyl-5(3)-*t*-butylpyrazole 3,5-Diethyl-4-methylpyrazole Dimethylphenylphosphine 1,5-Diazabicyclo[4.4.0]dec-6-ene (DBD)		
140	*p*-Fluorobenzamide 3-Fluoro-benzamide 3-$CH_3SC_6H_4NH_2$ *N*,*N*-Dimethyl-4-fluoroaniline 5,5-Dimethyl-3-amino 2-Cyclohexenone 1-(2-Methyl-1-propenyl)-piperidine 1-Cyclopentylpyrrolidine Lanthanum *N*‴, *N*⁗-dimethylhistamine 1,5,7-Triazabicyclo[4.4.0]dec-5-ene	Not significant; concentration $\ll$1 ppb	Not significant; concentration $\ll$ 1 ppb
141–230		Not significant; concentration $\ll$ 1 ppb;	Not significant; concentration $\ll$ 1 ppb;

compounds the protonated form (e.g., $HCN{\cdot}H^+$) partly loses its proton to water again [67] in moist samples:

$$HCN \cdot H^+ + H_2O \rightarrow HCN + H_3O^+.$$

Recently, it has been shown by SIFT-MS experiments[9] that HCN is present in the breath of healthy persons at a median level of about 10 ppb, which is much greater than the median value indicated by the present PTR-MS measurements. Incidentally, HCN along with acetonitrile and benzene is known to be present in inhaled cigarette smoke [68], which is the most likely reason for its higher levels in exhaled breath of smokers as compared to non-smokers.

This pilot study has identified seven volatile organic compounds, VOCs, which are at significantly higher concentrations in the exhaled breath of smokers than non-smokers. Of these compounds, acetonitrile is confirmed as the clearest indicator, as previously shown by other studies [15, 23]. Our results for this compound in exhaled breath of non-smokers are higher than in other studies [15, 23, 52]. This may be an indicator of passive smoking, a subject of great topical interest.

Although our seven selected VOCs in breath occur following cigarette smoking and decrease with the time after the last smoke, their presence still must be interpreted with caution, since some may also have their origins in adverse clinical conditions such as lung cancer or COPD.

Thus, our findings should be regarded as tentative, and validation studies with the analysis of alveolar air samples, taking into consideration the amount of pack-years, respiratory and heart rates and level of blood pressure, including control groups of healthy probands and COPD patients, need to be carried out, ideally employing additional analytical techniques such as SIFT-MS and GC/MS, which allow precise (not tentative) identification of the detected compounds.

[9] Spanel P: personal communication.

Acknowledgments

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