Highlights

- Breath analysis for the biological monitoring exposure in non-acute conditions
- At low contamination, ambient air monitoring may underestimate the inhaled dose
- Exposure levels related to chronic health effects are easily reached in laboratories
- Environmental requirements to create safe laboratory environments are needed
- Increased concern about safety precautions should be implemented

AIR AND BREATH ANALYSIS FOR THE ASSESSMENT OF EXPOSURE TO SOLVENT EMISSIONS IN UNIVERSITY CHEMISTRY LABORATORIES

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Tables: 2

Figures: 2

ABSTRACT

In this study, ambient and biological monitoring, through the use of breath analysis, in different university laboratory environments have been performed and compared to assess whether breath analysis is an efficient alternative for exposure monitoring in non-acute conditions. 40 atmospheric samples from four laboratories have been evaluated: three of them used solvents on a daily basis and the manipulation of solvents was forbidden in the other laboratory. 76 breath samples have been analyzed from non-exposed people (n=21) and researchers doing their regular daily routine in each of the tested laboratories (n=55). It was found that ambient levels in the most contaminated laboratory reached values below the recommended occupational exposure limits for acute exposition. However, the levels found for some of the solvents tested were above the proposed inhalation minimum risk levels (MRL) and reference concentrations (RfC) associated to chronic health effects. The results obtained for exhaled breath tend to agree with the air levels detected in the most contaminated environments, but it was found that exhaled breath levels for people working in environments with low contamination levels were not always correlated with air levels. These results indicate that biological monitoring using breath analysis mirror more accurately the dose inhaled in non-acute conditions and may help to assess provable chronic health effects.

Keywords: Exposure; Biological Monitoring; Ambient Monitoring; Breath Analysis; Laboratory

1. Introduction

Conventional occupational exposure studies are based on the analysis of workplace air (ambient monitoring) and the determination of safe limit exposure levels to prevent acute health effects (Ott et al., 2007). Despite exposure assessment with the aim to avert acute effects is relatively simple in occupational evaluation; it can be very complex for more general conditions where low levels of exposure may occur via multiple pathways. Thus, simply monitoring air levels may not be sufficient to assess health effects in non-acute conditions.

Exposure (i.e., the pollutant concentration in the air at the point of contact between the body and the external environment) and dose (i.e., the amount of the pollutant that actually crosses one of the body's boundaries and reaches a target tissue) are distinct. Therefore, mathematical models taking into account the magnitude, duration, and frequency of exposure have to be applied to estimate related health consequences (Sexon and Ryan, 1988; Paustenbach and Galbraith, 2006; Boogaard et al., 2011). Moreover, some confirmation of the exposure estimates through an approved method of biological sampling is often needed to validate the accuracy of the exposure assessment (Paustenbach and Galbraith, 2006).

Biological monitoring of exposure assesses the health risk through the evaluation of the internal dose and determines the amount of a chemical agent or its metabolites in a biological fluid. One of the advantages of biological monitoring is that requires no assumptions regarding exposure parameters (e.g. inhalation rate, and duration and frequency of exposure). The biological parameter of exposure is more directly related to the adverse health effects that one attempts to prevent than any environmental measurement. Biological monitoring is often the most reliable exposure assessment methodology as integrates exposure from all routes (Boogaard et al., 2011). It may

offer a better estimate of the risk than ambient monitoring and can reduce the uncertainty inherent within traditional exposure assessments (Paustenbach and Galbraith, 2006). Urine and blood are the most common fluids evaluated in biological monitoring for assessing the intensity of exposure to a pollutant, and although breath is less frequently used (HSE, 1997; Lauwerys and Hoet, 2001; Paustenbach and Galbraith, 2006), it has been demonstrated that breath analysis is a promising alternative to air analysis in occupational exposure assessment (Coelho et al., 2007; Alonso and Sanchez, 2013; Tang et al., 2015) and non-occupational conditions (Castellanos et al., 2016).

There is a large body of evidence that human exposure to chemicals at low levels (chronic non-cancer effects) can be harmful (Ashford and Miller, 1998a; Colosio et al., 2005; Kortenkamp et al., 2007). Multiple chemical sensitivity is a syndrome that is often initiated by repeated exposures to low levels of certain chemicals, which is followed by the triggering of symptoms by everyday chemical exposures at levels that do not appear to affect most people, and the time between the first and subsequent stages of disease can be long enough to obscure the connection (Ashford and Miller, 1998b). In the US, the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 2017) and the Integrated Risk Information System program (IRIS) of the Environmental Protection Agency (EPA) (EPA, 1994) have developed the inhalation Minimum Risk Levels (MRL) and Reference Concentrations (RfC) associated to chronic health effects. These values are estimates of a continuous inhalation exposure that is likely to be without an appreciable risk of adverse, non-cancer effects over a specified duration of exposure (EPA, 1994), and are well below the conventional occupational exposure limits for 8-hours total weight average (TWA 8-h) proposed by institutions as the National Institute for Occupational Safety and Health

(NIOSH) and the Occupational Safety and Health Administration (OSHA) (CDC, 2007).

In the present study, indoor air levels have been evaluated for different solvents in laboratories of a university building in order to assess the levels of contamination reached in those environments. Breath samples of people working in these laboratories and non-exposed volunteers have also been evaluated to determine whether breath analysis can be used as an effective alternative for monitoring exposure to solvents in non-acute conditions.

2. Materials and Methods

2.1. Chemicals

All reagents were reagent grade with ≥99% purity (Sigma-Aldrich, Steinheim, Germany). Sample stock mixtures were prepared by injecting 1-2 μL of single components into cleaned 10 L Tedlar gas-sampling bags (SKC, Eighty Four, PA, USA) filled with purified nitrogen and equilibrated at room temperature. Working standards were made by taking a fixed amount of the stock gas mixture with a gas-tight syringe and diluting to 10 L with purified nitrogen in a clean Tedlar bag. Stock and standards were freshly prepared for each calibration.

Before using a Tedlar bag, it was cleaned by filling it with purified nitrogen and emptying it with a vacuum pump several times. In order to confirm that a bag was clean, the last portion of nitrogen collected in the cleaning cycle was analyzed in the same conditions as samples to confirm that no detectable levels of target analytes were present.

2.2. Selection of target solvents

In a preliminary inquiry laboratory users were asked to determine the solvents that were used daily with a minimum consumption of 0.1 L/day. As a result of this inquiry, n-pentane, hexane (mixture of isomers), n-heptane, diethyl ether, acetone, ethyl acetate, methylene chloride, and toluene were selected for analysis. Chloroform, which is thought to be a group B2 human carcinogen (NTP, 2016) was also added although it was only used occasionally in two of the laboratories (Lab-1 and -4) at levels that were below the criteria established.

The solvent with the largest consumption in the tested laboratories was commercial-grade hexane, which refers to the hexane petroleum hydrocarbon distillation fraction that, while containing a large proportion of n-hexane (20-80%), is a mixture of different structural hexane isomers, with n-hexane, 2-methylpentane and 3-methylpentane being the main hexane isomers, although significant amounts of methylcyclopentane can also be contained (WHO, 1991; Basilico and Garlanda, 1995; Kuk and Hron, 1998; Milman and Kovrizhnych, 2000; ACGIH, 2001). The manufacturing process of commercial-grade hexane also yields benzene as an impurity, usually <0.1% (Basilico and Garlanda, 1995). Therefore, n-hexane, 2-methylpentane, 3-methylpentane, methylcyclopentane, and benzene have been quantified in the present study to assess the contamination through the use of commercial-grade hexane. Table S1 in Supplementary Materials shows the solvents evaluated with their occupational exposure limits and chromatographic parameters used for detection and quantification.

2.3. Description of sites

The university campus is located at the University of Girona (Girona, north-east Spain). Four research laboratories from the Chemistry Department were evaluated.

These consisted of one synthesis laboratory that manipulates 3-5 L of solvents each day (Lab-1), an analytical instrumentation laboratory where the manipulation of solvents was not permitted (Lab-2), and two synthesis laboratories with a varied but small daily consumption of solvents (<2 L) (Lab-3 and -4). All laboratories were located in the same corridor of the Science Faculty building and all were provided with air ventilation systems, which were only for comfort conditions; therefore laboratory air was re-circulated to control the temperature but no outdoor-indoor air exchange took place. The volume of Lab-1 was 158 m^3, Lab-2 was 82 m^3, and the volumes of Lab-3 and Lab-4 were similar at 102 m^3 and 101 m^3, respectively.

2.4. Air samples

Punctual samples were evaluated instead of TWA samples. Approximately 1 L of air sample was obtained with a 1 L gas tight syringe (SGE JUMBO syringe, SGE Europe, UK) in around 30 s, which was then introduced into a cleaned Tedlar bag and analyzed immediately. A total of 40 air samples (10 for each laboratory: 2 each sampling day during a two-week period) were obtained at the center of each laboratory at a height equivalent to the distance where a person would normally breathe (≈1.6 m) in order to collect samples representative of the breathing zone. The first daily sample was obtained at the beginning of the daily routine in the laboratory (i.e., when low contamination is expected), whereas the second one was taken after 4-5 hours (i.e., when the largest contamination is expected).

2.5. Breath samples

Seventy-six breath samples were evaluated, which were grouped into five groups: 16 samples corresponding to volunteers working in Lab-1, 21 in Lab-2, 11 in Lab-3, 7 in

Lab-4, and 21 corresponding to non-exposed people, whom had no contact with any type of laboratory nor with the Chemistry Department section of the faculty building during at least 48-h before taking the sample. For exposed volunteers, breath samples were obtained after at least 2 hours of starting his/her daily routine.

Forced-expired breath samples were collected for each individual as follows: the first 2-3 s of the expiration were not collected in order to minimize the sampling of dead-space air, and the remaining fraction was collected until about 900 mL of breath had been introduced into a clean 1 L Tedlar bag. Each breath sample was analyzed no more than 30 min after being collected.

2.6. Air and breath analysis

Specific details about the instrumentation used have been described in previous publications (Sanchez and Sacks, 2003; Alonso et al., 2009). Briefly, it consists of a three-bed microtrap sequentially filled with Carboxen 1000, Carbopack X, and Carbopack B (Supelco, Bellefonte, PA, USA). Adsorption of VOCs from samples contained in Tedlar bags was done at 30 $mL \cdot min^{-1}$ over 5 to 25 min (depending on the sample to be analyzed) with a vacuum pump and a mass flow controller. A fast heating pulse at 280-290ºC was applied for the quantitative thermal desorption of all retained compounds, which were automatically directed to a gas chromatography-mass spectrometry (GC-MS) instrument (Thermo Scientific, Waltham, MA, USA). Component separation was achieved by the use of a 30 m long TR-Meta.VOC column with 0.25 mm i.d. and 1.5 µm film thickness (Teknokroma, Barcelona, Spain). The oven temperature program was: 35ºC held for 3 min and then ramped at $5ºC \cdot min^{-1}$ to 210ºC and held for 1 min. Electron impact ionization was applied at 70 eV. Helium carrier gas was used, with a constant inlet pressure of 32 kPa. The

acquisition of chromatographic data was performed by means of Xcalibur software (v. 1.4, Thermo Scientific). See information supplied in Supplementary Materials for the results obtained in the validation of the method and the quality control procedures followed during analyses.

2.7. Statistical data analysis

Statistical analyses were performed using SPSS for Windows, version 15.0. Non-parametric statistics was used as a preliminary assessment of the distribution of the data using the Saphiro-Wilk and Kolmogorov-Smirnov tests showed non-normal distribution for practically all compounds and groups tested. The Kruskal-Wallis (KW) range variance analysis and Mann-Whitney U-tests (MW) were used for data comparison. For calculations of significance, two-sided testing was used and $p<0.05$ was considered as significant.

3. Results

3.1. Laboratory air samples

Table 1 shows the air concentrations obtained for the selected target compounds in each laboratory during the period evaluated. Significant differences (KW, $p<0.05$) were found between the four environments for all target compounds. As expected, taking into account the reported volume of solvents manipulated in each environment, the most contaminated laboratory was Lab-1 (Supplementary Materials: Figure S1a), whereas the analytical instrumentation room, Lab-2 (Sup. Mat: Figure S1b), was the least polluted. When the air levels of the two pair of samples obtained the same day in the same environment were compared, it was found an increase in the air pollution that ranged from 2 to 15 times for each solvent. Only in two specific

cases higher increases were obtained: 41 times for toluene and 37 times for methylene chloride, both cases in Lab-4.

The solvents with a large volume of use during the period evaluated were the mixture of hexane isomers, diethyl ether and ethyl acetate, and more than 80% of the total volume of these solvents were manipulated in Lab-1. The univariate statistical evaluation of the results obtained confirms that Lab-1 was the most contaminated laboratory for these solvents (KW, $p \leq 0.005$, Figure 1a for n-hexane), whereas the levels between the other three environments did not yield significant differences (KW, n-hexane, $p=0.081$; 2-methylpentane, $p=0.400$; 3-methylpentane, $p=0.365$; methylcyclopentane, $p=0.492$; diethyl ether, $p=0.063$; ethyl acetate, $p=0.216$).

Acetone (Figure 1b) also gave higher levels of contamination in Lab-1. This solvent was also widely used in the other synthesis laboratories (Lab-3 and -4) and it was observed that levels between Lab-3 and Lab-4 were equivalent (MW, $p=0.142$), but smaller than in Lab-1. Levels obtained in Lab-2 were significantly smaller than in the other three environments.

There were only three solvents than were not used at large quantities in Lab-1. Similar volumes of two of them, n-heptane (Figure 1c) and chloroform, were daily used in Lab-1 and Lab-4 and the results showed that equivalent levels were obtained between these two environments for these solvents (MW, $p=0.685$ and $p=0.935$, respectively), which were higher than in the other environments. Only one solvent, n-pentane (Figure 1d), was used more extensively in Lab-4 than in Lab-1, which resulted in this solvent showing higher pollution levels in Lab-4 (KW, $p<0.001$). Equivalent levels were obtained between Lab-3 and Lab-2 for n-pentane (MW, $p=0.086$).

3.2. Breath samples

In order to mimic real conditions of exposure to solvents, volunteers were asked to follow their daily routines. Table 2 shows the breath concentrations obtained for the target compounds in each group of volunteers. All solvents gave significant differences between the five groups evaluated (KW, $p<0.05$) and the lowest breath levels were always detected in the non-exposed group (Sup. Mat: Figure S2b). The group of volunteers working in Lab-1 gave, in general, higher levels of contaminants in their exhaled breath (Sup. Mat: Figure S2a), whereas those volunteers performing their work in Lab-2 gave the lower levels, which were sometimes equivalent to those found in the non-exposed group.

All compounds related to the manipulation of commercial-grade hexane showed the same behavior (Figure 2a for n-hexane). The group of volunteers from Lab-1 gave higher levels in their breath (KW, $p<0.001$), whereas people from Lab-3 and Lab-4 had equivalent levels (MW, $p=0.441$ for 2-methylpentane, $p=0.342$ for 3-methylpentane, $p=0.497$ for n-hexane, $p=0.298$ for methylcyclopentane, and $p=0.128$ for benzene). For these compounds, breath samples from Lab-2 volunteers always gave lower levels than Lab-3 and Lab-4, which were sometimes higher than non-exposed (KW, $p=0.025$ for 3-methylpentane, $p=0.031$ for n-hexane, and $p=0.043$ for methylcyclopentane) and others were equivalent (KW, $p=0.051$ for 2-methylpentane and $p= 0.615$ for benzene).

Similar results were obtained for toluene (Figure 2b), ethyl acetate, and acetone. Breath samples showed significant higher levels for Lab-1 volunteers (KW, $p<0.001$). In the case of toluene and ethyl acetate, levels detected between Lab-3 and Lab-4 were equivalent (MW, $p=0.856$ for toluene and $p=0.077$ for ethyl acetate) but higher than for Lab-2 volunteers, which were equivalent to non-exposed (MW, $p=0.705$ for

toluene and p=0.308 for ethyl acetate). In the case of acetone, similar levels were found between Lab-2, Lab-3 and Lab-4 (KW, p=0.142), and lower levels were obtained for non-exposed people when compared to Lab-2 (MW, p=0.014).

Three solvents (chloroform, methylene chloride -Figure 2c-, and n-heptane) showed significant differences (KW, p<0.001) between all groups, but breath levels between all synthesis laboratories (Lab-1, -3, and -4) did not show significant differences (KW, p=0.241 for chloroform, p=0.389 for methylene chloride, and p=0.087 for n-heptane). Lab-2 volunteers gave lower breath levels than volunteers from other laboratories. In the case of chloroform and n-heptane, Lab-2 results were significantly higher than controls (MW, p=0.001 and p=0.007, respectively), whereas equivalent levels between Lab-2 and non-exposed were found in the case of methylene chloride (MW, p=0.213).

Diethyl ether and n-pentane (Figure 2d) gave higher levels for Lab-4 volunteers (p<0.001). Smaller and equivalent levels were detected for Lab-1 and Lab-3 (MW, p=0.844 and p=0.980, respectively). These two compounds gave the lower levels for Lab-2, which were equivalent to non-exposed (MW, p=0.116 and p=0.267, respectively).

4. Discussion

4.1. Ambient monitoring

Contamination levels obtained in the different laboratories (Table 1) agreed with the amount of solvents manipulated in each environment. Lab-1 was the room with the largest volume of solvents used during the evaluated period. Moreover, it was observed that in this environment, despite having fume hoods, many reactions were performed outside the hoods due to the number of reactions being manipulated at

the same time in this environment. This fact helps to explain the high contamination levels reached for practically all solvents in Lab-1 (from 2 to 125 times higher than in Lab-2, the least contaminated environment, when median values were compared). Solvents with a large consumption in this environment were commercial-grade hexane, acetone, ethyl acetate, diethyl ether, and methylene chloride, which were the compounds that reached air contamination in the $mg \cdot m^{-3}$ level for practically all samples, whereas all other solvents and laboratories evaluated gave air concentrations in the $\mu g \cdot m^{-3}$ level (Table 1). These results do not seem to be a particular situation of the laboratories evaluated. Other studies performing ambient monitoring of university chemistry laboratories where solvents were manipulated also found that those compounds used routinely can reach values in the $mg \cdot m^{-3}$ level (Valavanidis and Vatista, 2006; Ugranli et al., 2015; Rastkari et al., 2016).

Commercial-grade hexane was the solvent with the largest consumption in the laboratories evaluated, with a maximum of 5 L/day in Lab-1. Highly significant correlations were found between n-hexane and 2-methylpentane (Spearman rho (ρ)=0.970, p<0.001), 3-methylpentane (ρ=0.989, p<0.001), and methylcyclopentane (ρ=0.981, p<0.001), confirming that the source of contamination for these four compounds was the use of the commercial-grade hexane. Benzene levels were also significantly correlated with n-hexane (ρ=0.905, p<0.001) and the other hexane isomers, which also confirms that the benzene contamination detected in these laboratories was mainly due to the presence of benzene as an impurity of the commercial-grade hexane used.

Despite n-pentane and n-heptane can also be present as impurities in commercial-grade hexane (Basilico and Garlanda, 1995; Kuk and Hron, 1998; Milman and Kovrizhnych, 2000), a significant but poor correlation was found between n-hexane

and n-heptane (ρ=0.397, p=0.045), which indicates that, although a part of the n-heptane contamination was due to its presence as an impurity in the commercial-grade hexane, there were another sources of contamination. In this case, it was observed that n-heptane was used for some specific applications in Lab-1 and Lab-4. In the case of n-pentane, no significant correlation was obtained with n-hexane, which was attributed to the fact that n-pentane was routinely used in both environments.

The only solvent that did not give the largest contamination in Lab-1 was n-pentane (Figure 1d), but the preliminary information obtained from the staff of each laboratory indicated that large volumes of this solvent were used in Lab-4 during the period evaluated.

From an occupational point of view, it was found that laboratory air levels detected for all compounds (Table 1) were clearly below the recommended occupational exposure limits (Sup. Mat: Table S1) (CDC, 2007). However, it was observed that, despite the small volume of solvents manipulated in the laboratories when compared to industrial applications, 100% samples analyzed in Lab-1 gave n-hexane and methylene chloride air levels above the proposed RfC and MRL limits for chronic effects (0.7 and 2.1 $mg \cdot m^{-3}$, respectively, for n-hexane; and 0.6 and 1.0 $mg \cdot m^{-3}$ for methylene chloride (ATSDR, 2017; EPA, 1994)). In the case of the second most contaminated laboratory, Lab-4, 60% of the samples also gave methylene chloride values above the RfC and MRL limits. As previously indicated, chloroform was only used occasionally in Lab-1 and Lab-4 but this solvent is classified as B2 human carcinogen (NTP, 2016). This compound has not a RfC limit but it has a MRL of 0.098 $mg \cdot m^{-3}$. It was found that chloroform air levels detected in Lab-1 were always above this limit, whereas 80% of the samples evaluated in Lab-4 overtook this limit.

These results indicate that although acute health effects are not expected due to occupational exposure in these environments, some chronic effects may take place for people developing their routine work in these environments.

4.2. Biological monitoring (breath samples)

People undertaking their routine work in Lab-1 gave the highest levels of contaminants in their exhaled breath for practically all target compounds, which agrees with the results obtained for ambient monitoring. When compared to non-exposed people, the increase in median values ranged from 11 times for benzene to >200 times for all hexane isomers. In the case of n-pentane, Lab-4 volunteers gave the largest levels in their exhaled breath, which also agrees with ambient monitoring results.

Diethyl ether, however, showed a different trend between air and exhaled breath levels and people developing their work in Lab-4 gave higher levels for this compound in exhaled breath than those working in Lab-1; which shows that although the concentration of this solvent was always higher in Lab-1, the dose inhaled by people working in Lab-4 was larger. This can be associated to a higher duration and frequency of direct exposure to this solvent in Lab-4, which suggests that biological monitoring using breath analysis seems to reflect more accurately the dose inhaled and probable chronic health effects in situations of low occupational contamination. Another factor that confirm this fact is that all volunteers that gave their breath sample <15 min after being manipulation a solvent yielded breath levels higher than ambient levels.

Correlations were evaluated for assessing presence of different sources of exposure for the compounds evaluated. A highly significant correlation between n-hexane and its isomers (including methylcyclopentane) was observed in exhaled breath samples of people working in the different laboratories ($\rho \geq 0.98$, $p < 0.001$), which confirms that the only source of contamination for all these compounds is the use of commercial-grade hexane.

In the case of benzene, a significant but slight correlation with all hexane isomers was observed ($\rho \geq 0.58$, $p < 0.001$). The distribution of the data suggests the existence of different sources for the benzene contamination in exhaled breath that cannot be observed using only air levels. It is well known that breath benzene levels increase significantly after smoking a cigarette (Alonso et al., 2010). Therefore, smoking status is another factor affecting the exhaled breath levels of this compound. However, despite the confounding smoking factor and the reduced exposure to benzene, given that this compound was only present as a minor impurity in the mixture of hexane isomers, a mean increase of one order of magnitude was obtained for exhaled benzene in Lab-1 volunteers. It was also found that benzene breath levels in non-smokers working in Lab-1 reached equivalent levels to smokers non-exposed to solvents.

Exhaled breath levels for Lab-2 users were higher than for non-exposed volunteers for practically all target compounds, although users of Lab-2 were not manipulating solvents and no direct occupational exposure was expected. Breath results confirmed a cross-contamination between the different sections of the Chemistry Department. A risk problem observed during the period evaluated was that doors in the most contaminated laboratory were maintained open at all times, which facilitates

the diffusion of the gases generated in this environment through nearby environments.

5. Conclusions

In the present study, it has been confirmed that the manipulation of very small volumes of a solvent (<100 mL) are sufficient to produce a significant increase in the contamination of a laboratory. Other studies have also demonstrated that these laboratories are special micro-environments where specific pollutant concentrations in air can reach relatively high levels depending on the nature of the experiments conducted (Valavanidis and Vatista, 2006; Ugranli et al., 2015; Rastkari et al., 2016; Park et al., 2014). Occupational exposure limits for acute effects were never reached in the environments evaluated, which allows management to considerer these laboratories as safe occupational environments. However, inhalation RfC and MRL limits established for chronic effects were clearly exceeded for some solvents in some laboratories, which may lead to adverse chronic health effects. Moreover, it has been observed that breath analysis mirrors more accurately the dose inhaled in conditions of low occupational exposure than ambient monitoring. In these conditions the dose inhaled for specific manipulations can be significantly larger than the exposure determined from air levels, which could lead to wrong conclusions about the safety of these environments.

In the environments evaluated, the solvent with the highest level of exposure was n-hexane and its isomers. Hexane is a solvent commonly manipulated in different types of industries, such as vehicle repair, shoe-markers, plastic factories, and furniture finishers, where median exposure levels have been detected in the range of 30 to 70 $mg \cdot m^{-3}$ (Mayan et al., 2002; Prieto et al., 2003; Wilson et al., 2007; Kutlu et

al., 2009). Despite the relatively small volume of commercial-grade hexane manipulated in the environments evaluated when compared with industrial uses, air levels in Lab-1 reached maximum levels of 7.1, 3.0, 3.5, and 3.0 $mg \cdot m^{-3}$ for n-hexane, 2-methylpentane, 3-methylpentane, and methylcyclopentane, respectively, only one order of magnitude below values reported for industrial applications. From a health point of view, n-hexane is absorbed rapidly through the lungs following inhalation, and distributed through the body. Once inside the organism, it is mediated to a neurotoxic species, 2,5-hexanedione. The most widely reported health effect of exposure to n-hexane is peripheral neuropathy (Mayan et al., 2002; Prieto et al., 2003; Wilson et al., 2007; Kutlu et al., 2009; Takeuchi et al., 1980; ATSDR, 1999; Dick, 2006). Therefore, good occupational hygiene is recommended during the manipulation of n-hexane as, although treatment for its neuropathy mainly involves cessation of exposure, symptoms may progress for several months after exposure has ceased, and only people who develop a mild neuropathy usually make a complete and satisfactory recovery (Kutlu et al., 2009).

The levels reached for many solvents in this study in laboratories manipulating solvents suggest that some preventive actions should be taken. This requirement has also been pointed out by the US-EPA, which recommends that responsible staff in small laboratories should take steps to minimize emissions because even small, unregulated amounts of pollutants can be harmful to the environment (EPA, 2000a). Moreover, EPA issued an Enforcement Alert indicating that “colleges and universities are required to comply with all applicable environmental requirements like their counterparts in the regulated industry to create a safe haven for human health and the environment” (EPA, 2000b), and has proposed that all laboratories should prepare and maintain a list of actual and potential air emissions in the laboratories,

including the source and location of emissions, and an estimate of the type and quantity of emissions (EPA, 2000a). The results obtained in the present study clearly indicate that these requirements ideally should be implemented in all countries. Unfortunately, many university lecturers and researchers still tend to consider that the reduced volume of solvents used in university laboratories, when compared with industry use, results in no significant emissions of hazardous compounds in these environments, which also leads to a reduced concern about safety precautions.

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Conflict of interest

The author declares that there is no conflict of interest

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Table 1. Statistics obtained in the analysis of laboratory air levels. Concentrations are in $\mu g \cdot m^{-3}$. sd: standard deviation, IQR: interquartile range

Solvent	Lab-1		Lab-2		Lab-3		Lab-4	
	Mean (sd)	**Median [IQR]**	**Mean (sd)**	**Median [IQR]**	**Mean (sd)**	**Median [IQR]**	**Mean (sd)**	**Median [IQR]**
n-pentane	6.1 (4.1)	4.6 [4.0-5.2]	0.9 (1.5)	0.5 [0.4-1.4]	1.5 (0.9)	1.4 [1.0-1.5]	94.0 (30.9)	77.9 [77.4-91.5]
Diethyl ether	1219.7 (812.4)	974.2 [693.6-1555.6]	87.5 (175.3)	10.0 [8.4-84.5]	270.2 (123.8)	308.9 [179.8-360.6]	223.6 (252.2)	83.6 [37.2-366.7]
Acetone	7703.0 (5094.6)	8299.9 [2636.7-11842.6]	230.1 (314.5)	120.0 [57.3-200.3]	1469.0 (573.8)	1527.4 [992.5-1945.5]	936.4 (577.8)	732.8 [469.9-1447.0]
2-methylpentane	1740.0 (773.6)	1570.0 [1366.4-2068.4]	67.5 (108.5)	12.6 [5.7-66.3]	23.1 (25.1)	11.6 [9.3-25.7]	39.7 (39.2)	25.4 [18.0-31.0]
Methylene chloride	3104.0 (1232.8)	3083.0 [2651.5-3289.9]	358.8 (314.5)	315.1 [108.7-468.7]	1333.7 (1969.4)	197.8 [194.5-1429.1]	1052.5 (584.6)	1317.0 [469.9-1443.0]
3-methylpentane	2381.4 (776.6)	2129.6 [1951.7-2850.5]	95.4 (133.0)	28.4 [15.7-111.1]	36.5 (14.6)	20.9 [18.9-40.8]	73.9 (54.3)	57.0 [40.6-64.4]
n-hexane	4892.9 (1737.3)	4405.7 [3626.1-6900.5]	343.9 (364.0)	255.8 [134.6-323.7]	147.7 (144.9)	85.1 [74.4-156.2]	526.9 (404.4)	382.4 [270.6-503.7]
Methylcyclopentane	1975.3 (715.3)	1838.8 [1461.2-2434.3]	125.5 (194.3)	24.6 [17.5-113.2]	47.4 (55.8)	23.8 [16.5-47.7]	62.6 (56.4)	44.6 [27.0-55.7]
Ethyl acetate	2664.2 (746.1)	2629.2 [2154.3-2933.2]	91.1 (100.7)	25.3 [14.8-171.2]	141.2 (137.3)	80.0 [68.7-166.4]	280.5 (304.3)	158.7 [121.9-178.5]
Chloroform	143.0 (28.0)	142.0 [121.7-160.0]	69.2 (59.7)	48.3 [20.8-112.4]	56.3 (10.9)	56.6 [47.0-65.6]	178.6 (99.6)	142.1 [105.1-238.9]
n-heptane	168.5 (67.4)	136.8 [131.1-209.2]	5.0 (6.4)	1.4 [0.9-10.8]	52.8 (57.3)	24.3 [17.2-63.9]	232.3 (265.2)	114.3 [44.8-299.6]
Benzene	24.5 (12.2)	20.7 [15.1-34.7]	4.4 (1.4)	4.4 [3.8-5.6]	2.2 (0.6)	2.0 [1.7-2.4]	1.9 (1.1)	1.7 [1.1-2.2]
Toluene	139.1 (70.7)	116.7 [95.2-168.4]	20.0 (16.6)	13.3 [7.8-26.3]	20.1 (16.1)	16.4 [9.6-23.7]	94.3 (131.0)	29.8 [11.9-104.0]

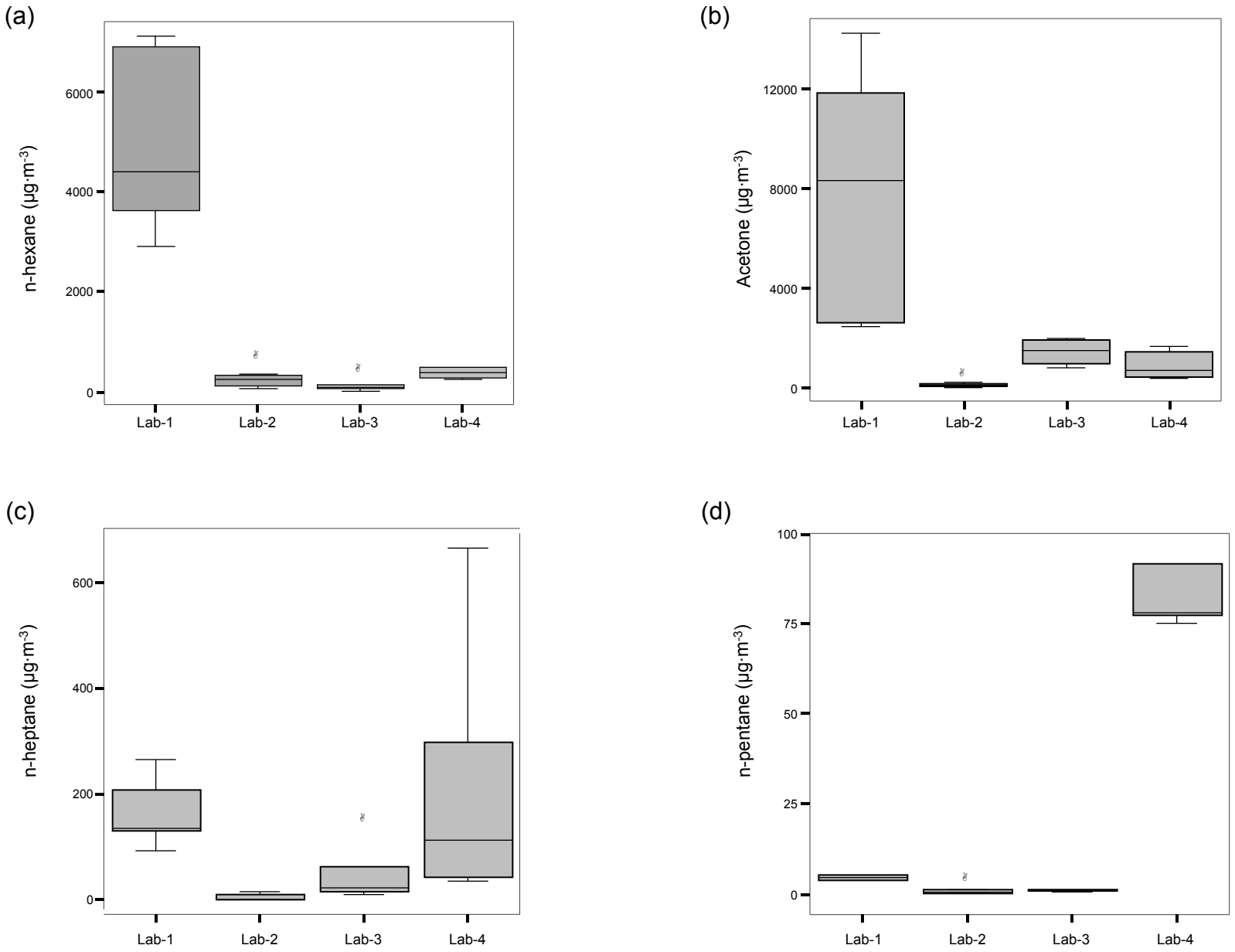
Table 2. Statistics obtained in the analysis of breath samples. Concentrations are in ppbv. IQR: interquartile range

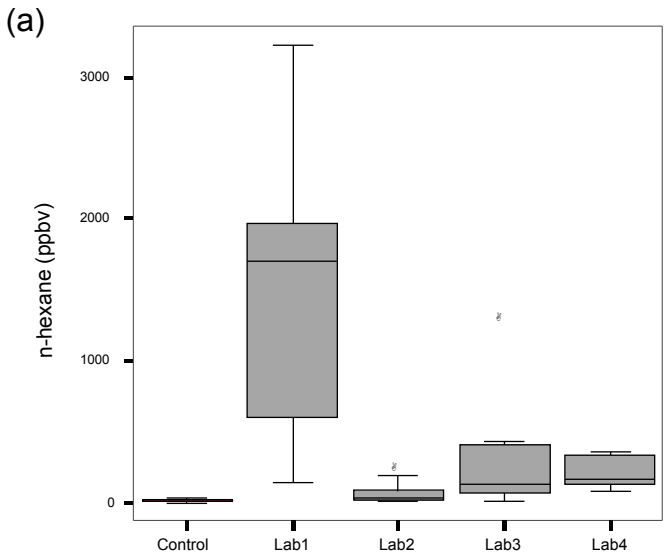
	Lab-1	**Lab-2**	**Lab-3**	**Lab-4**	**Non-exposed**
	Median [IQR]	**Median [IQR]**	**Median [IQR]**	**Median [IQR]**	**Median [IQR]**
n-pentane	3.2 [1.0-4.8]	0.3 [0.2-0.6]	2.7 [1.4-5-0]	6.7 [1.7-10.7]	0.2 [0.1-0.5]
Diethyl ether	75.0 [19.3-88.1]	12.2 [5.3-24.7]	57.5 [28.1-91.4]	155.3 [78.1-303.6]	5.6 [0.7-30.8]
Acetone	1014.8 [399.6-1920.3]	414.8 [92.8-566.0]	593.1 [389.9-703.4]	545.0 [249.4-755.9]	71.8 [42.9-185.9]
2-methylpentane	691.6 [180.5-985.0]	4.5 [2.1-17.6]	31.7 [14.6-91.0]	50.1 [27.3-106.5]	2.9 [1.0-4.2]
Methylene chloride	374.1 [345.1-730.8]	10.2 [2.4-46.9]	305.6 [160.6-464.1]	207.7 [164.8-677.6]	6.9 [1.8-26.4]
3-methylpentane	809.4 [256.0-927.9]	10.5 [3.0-27.5]	60.2 [27.2-139.6]	74.1 [58.87-157.7]	3.5 [1.3-6.7]
n-hexane	1697.7 [599.6-1971.5]	28.3 [6.9-91.0]	131.9 [62.3-411.0]	164.0 [132.6-336.9]	8.1 [3.1-17.0]
Methylcyclopentane	523.5 [210.3-639.8]	6.5 [3.2-24.2]	57.3 [30.6-113.2]	61.7 [43.5-202.4]	3.0 [1.3-7.8]
Ethyl acetate	218.3 [170.4-506.0]	10.2 [4.0-33.6]	13.6 [7.8-42.4]	50.7 [30.6-109.5]	8.5 [5.0-15.7]
Chloroform	42.9 [19.2-106.2]	6.2 [2.0-29.8]	55.6 [28.5-171.0]	31.7 [14.4-47.2]	1.1 [0.4-5-9]
n-heptane	24.0 [9.7-30.4]	5.4 [0.7-8.7]	3.7 [1.9-18.4]	17.6 [2.4-62.8]	1.5 [0.1-1.9]
Benzene	6.8 [3.1-24.8]	0.7 [0.4-1.3]	1.0 [0.6-1.9]	1.3 [0.9-1.4]	0.6 [0.2-1.6]
Toluene	74.7 [17.7-143.6]	4.1 [1.8-9.9]	12.6 [4.0-18.9]	10.0 [6.1-16.1]	3.3 [1.8-10.1]

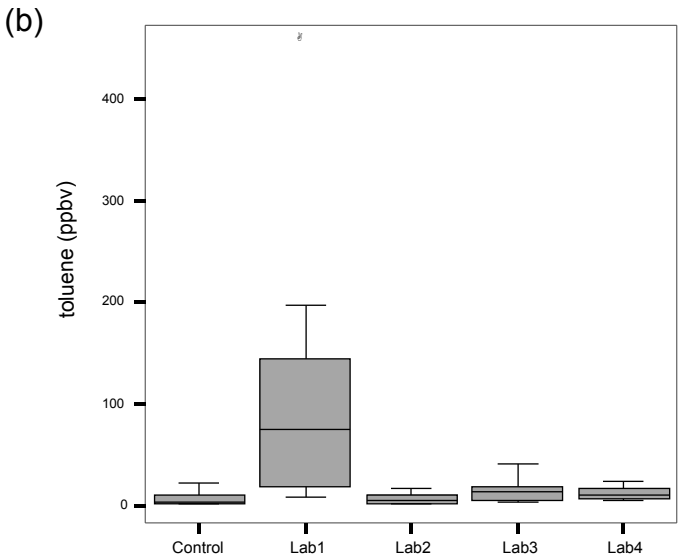
Figure Captions

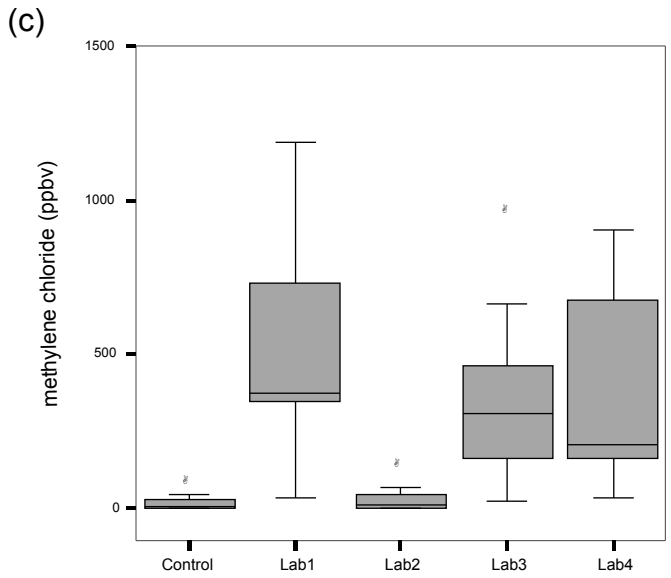
Figure 1. Atmospheric concentration distribution (in $\mu g \cdot m^{-3}$) of individual solvents between the four laboratories tested (a: n-hexane, b: acetone, c: n-heptane, d: n-pentane).

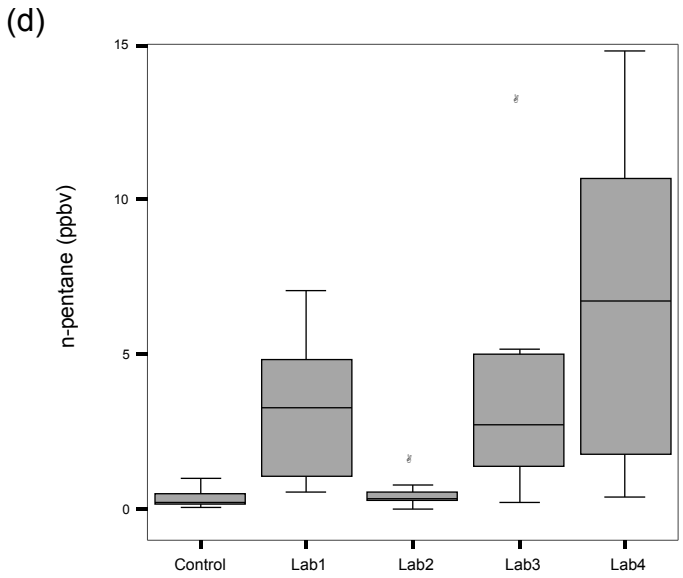
Figure 2. Breath concentration distribution (in ppbv) of individual solvents from researchers developing their work in the tested laboratories and controls without contact with solvents (a: n-hexane, b: toluene, c: methylene chloride, d: n-pentane).


(a)
n-hexane (µg·m-3)
6000
4000
2000
0
Lab-1
Lab-2
Lab-3
Lab-4
(b)
Acetone (µg·m-3)
12000
8000
4000
0
Lab-1
Lab-2
Lab-3
Lab-4
(c)
n-heptane (µg·m-3)
600
400
200
0
Lab-1
Lab-2
Lab-3
Lab-4
(d)
n-pentane (µg·m-3)
100
75
50
25
0
Lab-1
Lab-2
Lab-3
Lab-4


(a)
n-hexane (ppbv)
3000
2000
1000
0
Control
Lab1
Lab2
Lab3
Lab4


(b)
toluene (ppbv)
400
300
200
100
0
Control
Lab1
Lab2
Lab3
Lab4


(c)
methylene chloride (ppbv)
1500
1000
500
0
Control
Lab1
Lab2
Lab3
Lab4


(d)
n-pentane (ppbv)
15
10
5
0
Control
Lab1
Lab2
Lab3
Lab4

SUPPLEMENTARY MATERIALS

Air and Breath Analysis for the Assessment of Exposure to Solvent Emissions in University Chemistry Laboratories

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Table S1. List of solvents evaluated in the present study with their exposure limits ($mg \cdot m^{-3}$) set by different organizations.

Solvent	CAS number	retention time (min)	m/z[a]	TWA 8-h ($mg \cdot m^{-3}$)		
				NIOSH REL[1]	OSHA PEL[1]	Spain OEL[2]
n-pentane	109-66-0	4.0	**57**,72	350, C=1800	2950	3000
Diethyl ether	60-29-7	4.6	45, **59**, 74	NE	1200	308, ST=616
Acetone	67-64-1	5.1	**58**, 43	590	2400	1210
2-methylpentane (hexane isomer 1)	107-83-5	5.8	**71**, 57, 86	350[b], C=1800[b]	---	1790[b], ST=3580[b]
Methylene chloride	75-09-2	6.2	49, **84**, 86	Ca	87, ST=434	105, ST=210
3-methylpentane (hexane isomer 2)	96-14-0	6.3	**57**, 71, 86	350[b], C=1800[b]	---	1790[b], ST=3580[b]
n-hexane	110-54-3	6.9	**57**, 71, 86	180	1800	72
Methylcyclopentane (hexane impurity)	96-37-7	8.6	**56**, 69, 84	Not available	Not available	Not available
Ethyl acetate	141-78-6	9.2	**43**, 61, 70	1400	1400	1460
Chloroform	67-66-3	9.5	47, **83**, 85	Ca, ST =9.78	240	10
n-heptane	142-82-5	11.1	**57**, 71, 100	350, C=1800	2000	2085
Benzene	71-43-2	11.5	**78**, 77, 51	Ca, 0.325 ST= 3.25	3.25 ST=16.25	3.25
Toluene	108-88-3	16.3	**91**, 92	375, ST=560	754, C=1131	192, ST=384

[a] quantification mass in bold

[b] exposure limit value for hexane isomers, excluding n-hexane

NE: No established NIOSH REL

C: ceiling value

ST: STEL value (short term exposure level), usually expressed as TWA 15-min

Ca: Classified by NIOSH as potential occupational carcinogen

Validation of the method

The method was validated by analysing breath and synthetic air samples with the developed microtrap and a conventional thermal desorption method. Statistical comparison of the results obtained confirmed that no significant differences were found between the proposed and the standard method (p>0.05, n=5 for each group).

Trueness of the method, measured as the recovery obtained from three breath samples from non-exposed volunteers fortified with 10-20 ppbv for each of the target compounds and three non-contaminated air samples fortified with 50-100 $\mu g \cdot m^{-3}$, yielded values in the 85-119% range. Inter-day precision (repeatability) was measured in terms of relative standard deviation and values between 1-18% were obtained, which are considered satisfactory.

The method detection limit (MDL) was determined by analysing a standard using purified nitrogen as a matrix and spiked at 15-30 pptv for each solvent (n=7). The standard deviation obtained was taken as the standard deviation of the blank (SD_{bl}) [2,3]. The $3 \cdot SD_{bl}$ criterion was applied to calculate the MDLs. The results obtained gave MDLs in the range 5-10 pptv for all target compounds.

Quality controls

Traps were daily conditioned by passing nitrogen (99.9990% purity), purified for hydrocarbons, oxygen and water, at about 280ºC during 15-20 minutes at the beginning of each session. Once conditioned, traps were analysed without sample collection to verify the absence of memory effects.

A method blank (prepared using nitrogen as the blank matrix) was analyzed after conditioning the trap and after the analysis of every five samples or standards.

The stability of some compounds in the Tedlar bags is limited because losses may occur after some hours of storage in the bags [4,5]. We checked the stability for some of the compounds evaluated. In the case of benzene, toluene and hexane, losses <5% were found after 24-h of storage, whereas higher losses were found for methylene chloride and chloroform after 3-h. To avoid the bias by effusion, only benzene, toluene and n-hexane were quantified in the control samples. Duplicate standards and duplicate samples, all prepared and obtained the same day, were used as a controls. A maximum error of ±10% was considered acceptable for controls.

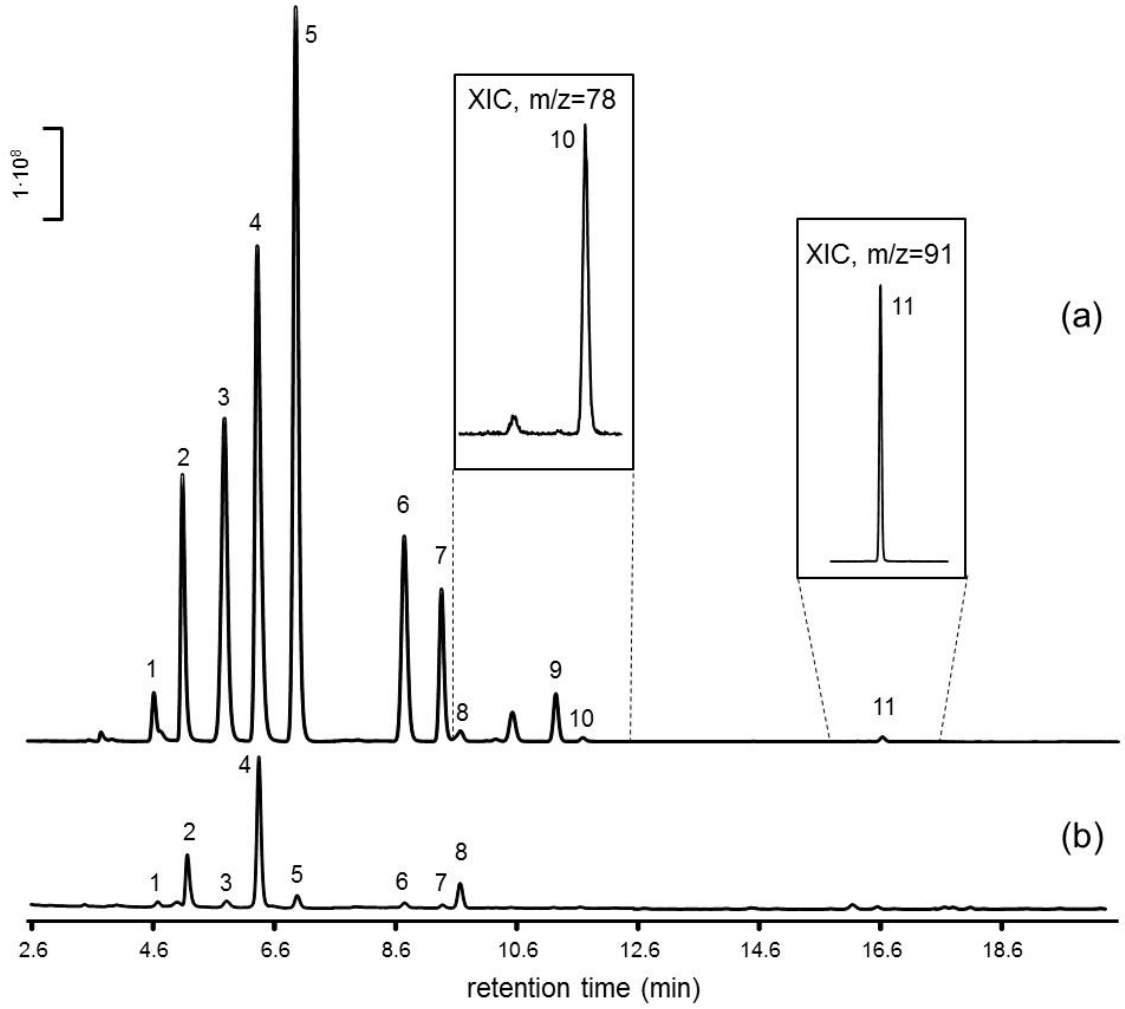

Figure S1. Chromatograms (TIC: total ion chromatogram) obtained in the analysis of atmospheric air in Lab-1 (a) and Lab-2 (b). Insets shows the extracted ion chromatograms (XIC) for a better view of benzene (m/z=78) and toluene (m/z=91).

1: diethylether; 2: acetone; 3: 2-methylpentane; 4: methylene chloride + 3-methylpentane; 5: n-hexane; 6: methylcyclopentane; 7: ethylacetate; 8: chloroform; 9: n-heptane; 10: benzene; 11: toluene

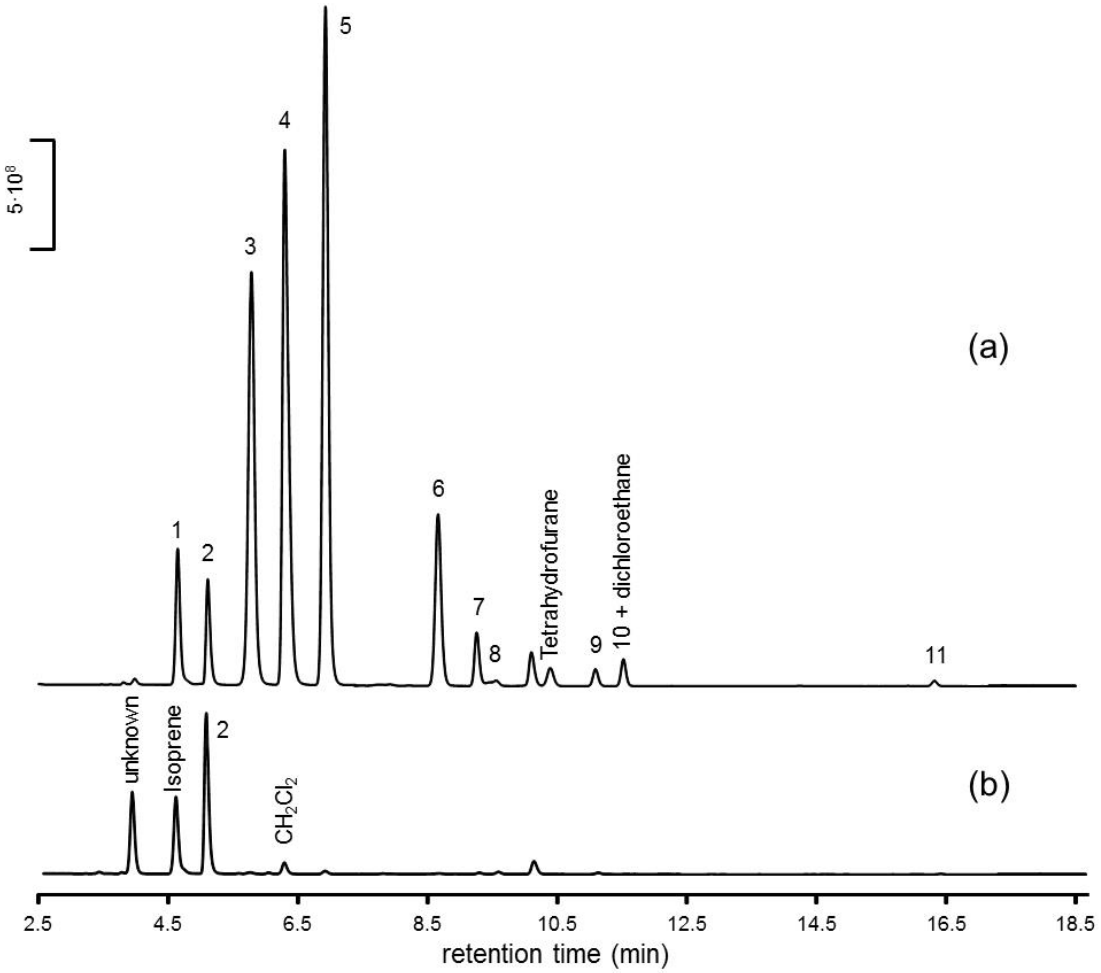

Figure S2. Chromatograms (TIC: total ion chromatogram) obtained in the analysis of exhaled breath of a volunteer from Lab-1 (a) and a non-exposed person (b). Peak number assignment as in Figure S1.

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