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1 **Highlights**

2 - A non-invasive sampling of volatile organic compounds in exhaled breath is proposed
3 - A method to determine sevoflurane and isopropyl alcohol in breath is developed
4 - Hospital staff exposure levels to sevoflurane and isopropyl alcohol are assessed
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Determination of sevoflurane and isopropyl alcohol in exhaled breath by thermal desorption gas chromatography-mass spectrometry for exposure assessment of hospital staff.

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6 **Abstract**

7 Volatile anesthetics and disinfection chemicals pose ubiquitous inhalation and dermal exposure
8 risks in hospital and clinic environments. This work demonstrates specific non-invasive breath
9 biomonitoring methodology for assessing staff exposures to sevoflurane (SEV) anesthetic,
10 documenting its metabolite hexafluoroisopropanol (HFIP) and measuring exposures to isopropanol
11 (IPA) dermal disinfection fluid. Methods are based on breath sample collection in Nalophan bags,
12 followed by an aliquot transfer to adsorption tube, and subsequent analysis by thermal desorption
13 gas chromatography-mass spectrometry (TD-GC-MS). Ambient levels of IPA were also monitored.
14 These methods could be generalized to other common volatile chemicals found in medical
15 environments. Calibration curves were linear (r^2 = 0.999) in the investigated ranges: 0.01 - 1000
16 ppbv for SEV, 0.02 - 1700 ppbv for IPA, and 0.001 - 0.1 ppbv for HFIP. The instrumental detection
17 limit was 10 pptv for IPA and 5 pptv for SEV, both estimated by extracted ion-TIC chromatograms,
18 whereas the HFIP minimum detectable concentration was 0.5 pptv as estimated in SIM acquisition
19 mode. The methods were applied to hospital staff working in operating rooms and clinics for blood
20 draws. SEV and HFIP were present in all subjects at concentrations in the range of 0.7-18, and
21 0.002 - 0.024 ppbv for SEV and HFIP respectively. Correlation between IPA ambient air and breath
22 concentration confirmed the inhalation pathway of exposure ($r = 0.95$, $p < 0.001$) and breath-borne
23 IPA was measured as high as 1500 ppbv. The methodology is easy to implement and valuable for
24 screening exposures to common hospital chemicals. Although the overall exposures documented
25 were generally below levels of health concern in this limited study, outliers were observed that
26 indicate potential for acute exposures.

27

28 **Keywords**

29 Occupational exposure, hospital staff, sevoflurane, isopropyl alcohol, breath analysis

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30 **1. Introduction**

31 Hospital staff may be exposed to many potentially harmful substances [1-2]. Volatile anaesthetics
32 and alcoholic disinfectants are two main classes of harmful volatile substances commonly found in
33 hospital environments [2]. Alcohol-containing hand rubs and gels are widely used in the healthcare
34 environment for hand decontamination. Two representative compounds of these classes of volatile
35 pollutants were selected, namely sevoflurane (SEV), a widely used anaesthetic gas, and isopropyl
36 alcohol (IPA), one of the main components of skin antiseptics. Since there is a real risk of
37 contamination for exposed hospital personnel, a monitoring to these substances is very important
38 for the protection of hospital staff' health.

39 SEV (fluoromethyl 2,2,2-trifluoro-1-trifluoromethylethyl ether) is used in anaesthesiology for
40 invasive surgery due to its favourable pharmacokinetic properties, i.e. low blood-gas partition
41 coefficient and tissue solubility, fast metabolism and low cardio-depressant effect [3-4]. The low
42 blood solubility leads to the rapid induction of anaesthesia and a rapid recovery afterwards. SEV is
43 directly eliminated via exhaled breath and indirectly metabolized in the liver by the isoenzyme
44 CYP2E1 [5-7], with the formation of inorganic and organic fluorides such as hexafluoroisopropanol
45 (HFIP) and HFIP-glucuronide. Most HFIP is excreted in 12 hours, and only very low
46 concentrations are found two days after anaesthesia [8]. The unconjugated fraction, which
47 represents less than 15% of total HFIP concentration [5-6], is eliminated via exhaled breath.

48 Occupational exposure by the inhalation of anaesthetics may produce several collateral effects.
49 Hospital staff working in operating and recovery rooms, and dental clinics, are the most exposed
50 workers. Since the early 1980s, various epidemiological studies have suggested that chronic
51 exposure to low doses of anaesthetic gases is an occupational risk factor for spontaneous abortion
52 and congenital defects [9-15]. Furthermore, long-term occupational exposure to trace levels of
53 anaesthetic mixtures, including halogenated compounds, has been shown to affect lymphatic

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54 systems. Acute headaches, asthenia, neurobehavioral changes and effects on performance have also
55 been reported [16-17].
56 SEV may thus pose a hazard to hospital workers, and the European and United States health
57 authorities recommend exposure limits for volatile anaesthetics. Although an occupational exposure
58 standard for SEV has never been set in Europe, a target level of 20 ppm as an 8-h time-weighted
59 average (TWA) has been recommended [18]. On the other hand, the U.S. National Institute of
60 Occupational Health and Safety (NIOSH) recommends a general exposure limit of 2 ppm for all
61 volatile anaesthetics, which is mostly interpreted as a ceiling value [19].
62 The second widespread contaminant in hospital environments is IPA. It is very often used as a
63 cleaning agent and as a solvent in mild disinfectants, antiseptic solutions and rubbing alcohols [20-
64 21]. This compound is rapidly absorbed and distributed throughout the body after inhalation,
65 ingestion or absorption through the skin, but most intoxication are related to oral ingestion [22]. The
66 volume of distribution of IPA in the human body is 0.5 L Kg^{-1}. A half-life ranging from 2.5 to 6.4
67 hours is estimated, which could be longer in the case of high blood concentrations occurring in
68 intoxication due to the saturation of alcohol dehydrogenase (ADH). Elimination mainly depends on
69 the liver (80-90%) and kidneys [22]. The critical step in the biotransformation of IPA is oxidation to
70 acetone, which is catalysed by the liver enzyme ADH [23-25]. Unlike alcohols such as methanol or
71 ethylene glycol, the toxic effects of IPA are directly related to the molecule rather than its
72 metabolites [21, 23]. IPA has an anaesthetic effect and irritates the respiratory mucosa if inhaled
73 [25-28] as well as the central nervous system (CNS) [20, 22].
74 Different safety and occupational health agencies indicate threshold values for exposure to IPA in
75 ambient air. The European agency for safety and health at work (EU-OSHA) enforces a legal
76 ambient air permissible exposure limit (PEL) of 400 ppmv averaged over an 8-h work shift. The
77 same TWA limit of 400 ppmv and a short-term exposure limit (STEL) of 500 ppmv are reported in
78 the NIOSH guide to chemical hazards and by the American conference of governmental industrial
79 hygienists (ACGIH) [26].

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80 Several analytical methods are suitable for monitoring exposure to potentially dangerous
81 environmental volatile organic compounds (VOCs). The determination of SEV and IPA is mainly
82 implemented in biological matrices such as blood and urine, or in ambient air. Although studies on
83 plasma and urinary biomarkers of low-level occupational exposure to SEV have been carried out in
84 the last few decades [29-32], few studies have been carried out by analysing ambient air and no
85 study to the best of our knowledge has examined the exhaled breath of hospital staff.
86 In two studies, SEV concentrations, ranging from 0.1 to 12 ppm, were determined by photo acoustic
87 infrared spectrometry in the ambient air of operating rooms [33-34]. Room staff exposure was
88 related to the anaesthetic doses as well as the duration of the intervention, the position of the room
89 staff, and the type and functioning of the ventilation system in an operating room. In another study,
90 a portable ambient air analyser called MIRAN 1B, which used a single beam infrared
91 spectrophotometer was applied to measure SEV background concentrations (4 - 17 ppm) in working
92 environments during gaseous induction with the anaesthetic [18]. Other studies involved hospital
93 personnel exposed to volatile anaesthetics, using MIRAN 1B to evaluate halogenated anaesthetic
94 concentrations in the gas samples thus leading to the conclusion that exposures for post-operative
95 nurses may exceed NIOSH ceilings [35]. Nurses who are exposed to volatile anaesthetics exhaled
96 by patients were involved in several studies because they are particularly exposed to anaesthetic
97 gases, as well as the staff working in operating theatres [36].
98 IPA poisoning can be diagnosed by normal acid-base parameters, the evaluation of hyperosmolarity
99 (the most common laboratory abnormality associated with isopropanol poisoning), and positive
100 nitroprusside reactions in urine and/or blood [20-21, 23]. At the same time, a non-invasive
101 biological monitoring of IPA-exposed workers can be carried out by ambient air, saliva, or urine
102 analysis [37-40]. Two important institutions for workplace safety, NIOSH and EU-OSHA, have
103 proposed methods to monitor IPA exposure in ambient air.
104 The analytical method proposed by NIOSH to determine IPA in ambient air was based on sampling
105 air in an adsorption tube (coconut shell charcoal) followed by thermal desorption gas-

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106 chromatography analysis with flame ionization detector [41]. Two 8-mm o.d. (6-mm i.d.) Anasorb®
107 747 tubes in series were proposed in the OSHA method. Analytes were eluted with a 60/40 N,N-
108 dimethylformamide/carbon disulphide solution which was then analysed by gas-chromatography
109 with flame ionization detector. The detection limit of the overall procedure was 13 ppb [42].
110 In standard methods, SEV and IPA are routinely monitored in working ambient air, however
111 determination in exhaled breath would be a more meaningful measurement of hospital personnel
112 exposure. Based on this background information, we optimized a previously published analytical
113 methodology [8, 43] involving the collection of mixed exhaled breath samples in disposable
114 Nalophan bags and analysis by thermal desorption gas chromatography-mass spectrometry (TD-
115 GC-MS). This methodology was then used to determine SEV and IPA to assess exposure levels in a
116 hospital environment.
117 VOCs found in human breath are linked to various physiological conditions as they represent the
118 products of metabolism in human bodies, and VOCs detected in human breath can be directly
119 correlated to specific diseases or environmental contaminations. In fact, the determination of
120 exogenous substances, or their metabolites, in the exhaled breath may reveal their possible
121 assumption.
122 Being able to detect metabolites uniquely correlated with the intake of exogenous substances is an
123 additional advantage of breath analysis compared to ambient air analysis. The determination of
124 contaminants such as SEV and IPA in exhaled breath should help to better assess exposure levels in
125 the workplace. In addition, compared to traditional specimen testing, breath analysis is a non-
126 invasive approach. It is a simple alternative to traditional specimen testing in both clinical diagnosis
127 and therapeutic monitoring, and when quantifying exposure at work, [44-46]. Breath analysis can
128 also easily be expanded to the analysis of other potentially harmful VOCs that require monitoring in
129 the workplace.
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132 **2. Material and methods**

133 *2.1 Chemical reagents*

134 Fluoromethyl 2,2,2-trifluoro-1-trifluoromethylethyl ether (with a purity > 99.9%) was purchased
135 from Abbott (USA). 1,1,1,3,3,3-hexafluoro-2-propanol (puriss. p.a. standard for GC grade >
136 99.0%) was purchased from Fluka, Sigma-Aldrich (Italy). Isopropyl alcohol was purchased from
137 AccuStandard, Inc. Chemical Reference Standard (USA). Labelled isopropanol-D8 and toluene-D8
138 (both puriss. p.a. standard for GC grade of 99.8%) were purchased from ARMAR Chemicals
139 (Switzerland). Reagents were stored at 4 °C to minimize the risk of evaporation.

140

141 *2.2 Preparation of standards*

142 A gaseous standard of IPA, SEV and HFIP (MIX3) was prepared by evaporating 5 μL of each
143 liquid standard in a pre-evacuated glass flask (2 L) equipped with a septum and held at 37 °C. The
144 calculated concentrations were 830 ppmv for IPA, 480 ppmv for SEV and 610 ppmv for HFIP.
145 MIX3 was diluted injecting known volumes in the flow of pure air (Hydrocarbon free, purity of
146 99.5%, Sol, Italy) at 500 mL min^{-1} during Nalophan bags (5 L) filling. The resulted gaseous
147 standard mixture was further diluted in the same way to obtain mixtures at suitable concentrations
148 for the method performances evaluation.

149 A gaseous mixture of labelled isopropanol-D8 and toluene-D8 (MIX 2D), for use as an internal
150 standard, was prepared by evaporation of 5 μL of both compounds in a 2 L glass flask equipped
151 with a septum, pre-evacuated and held at 37 °C. The corresponding concentrations were 830 ppmv
152 and 600 ppmv, respectively.

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157 *2.3 Air and breath sample collection*

158 The ambient air was collected using a gas-tight cylindrical glass vessel containing a Nalophan bag
159 (approximate volume of 1 L) connected to room air by a short PTFE tube (1/4 inch i.d.) and a
160 bulkhead union mounted on the vessel lid (Fig. 1A). A pump, connected to the vessel by a second
161 bulkhead union on the lid, decreased the pressure in the gap between the bag and the vessel wall,
162 thus inflating the bag. After collection, room air samples were analysed in the same way as the
163 breath samples.
164 Disposable bags (approximate volume of 3 L) were made from a roll of Nalophan tube
165 (polyethylene terephthalate film, thickness 20 μm) supplied by Kalle (Germany). One end of the
166 Nalophan bag was rolled and tightened by nylon cable ties. The other end was wrapped and
167 tightened around a PTFE tube (1/4 inch i.d.) connected to a stopcock, a one-way valve, and a
168 mouthpiece, as shown in Fig. 1B. All parts of the sampling system were made of inert materials and
169 were freshly sterilized before sample collection. Each subject was asked to calmly fill a bag with
170 multiple deep breaths. All subjects who volunteered to join the study gave written informed consent
171 prior to their participation. The breath sampling was carried out in two different applications.
172

173 *2.3.1 Application A: monitoring of SEV exposure levels*

174 Mixed breath samples were collected from five anaesthesiologists (2 males, 3 females), aged
175 between 27 and 33 years (average value 29 ± 2 years), working in different operating rooms at the
176 university hospital in Pisa, Italy. Work shifts were organized on a weekly basis with five
177 consecutive working days followed by two days of rest. Sample collection was designed to assess
178 the effects of both the working day and the working week. Three different samples of exhaled
179 breath were collected from each subject. The first sample was collected when the anaesthesiologist
180 arrived at the hospital at the beginning of the first day of work (t_0), the second sample at the end of

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181 the same day (t_1) and the last sample was collected at the end of the anaesthesiologist's working
182 week (t_2).
183 The sampling of ambient air inside the operating room was not taken for safety reasons and in order
184 not to hamper the surgical operations in progress.
185
186 *2.3.2 Application B: monitoring of IPA exposure levels*

187 To assess IPA exposure of hospital staff, mixed breath and ambient air samples were
188 simultaneously collected in a room specifically used for blood drawings, before the beginning of the
189 work shift (t_0), and 90 (t_1) and 180 (t_2) minutes later. For this application, nine nurses (3 males, 6
190 females), aged between 22 and 43 years (average value 29 ± 9 years), were enrolled at the Institute
191 of Clinical Physiology (National Research Council, Pisa, Italy) in a time span of two months.
192
193 *2.4 Sample analysis*

194 Sampling bags containing air or breath samples were stabilised at 37 (±1) °C in a thermostated box
195 for half an hour to prevent water condensation. An aliquot of the sample (250 mL) was then flowed
196 through a drying tube filled with 9 g of anhydrous sodium sulphate (SKC, Italy) for water removal,
197 and transferred into a glass adsorption tube packed with 250 mg of 60⁄80 mesh Tenax GR phase
198 (70% Tenax TA, 2,6-diphenyl-p-phenylene oxide and 30% graphite, Supelco, USA). During the
199 sample transfer, the sampling bag and the drying tube were kept at 37 °C, whereas the adsorption
200 tube was kept at ambient temperature. A low flow pocket pump (210-1002TX, SKC, Italy) was
201 used to transfer breath samples into adsorption tubes, using a constant flow of 50 mL min^{-1} for 5
202 min^{-1}. The adsorption tubes were then thermally desorbed by an automated two-stage thermal
203 desorption unit (STD 1000, DANI Instrument, Italy) equipped with an internal focusing trap packed
204 with 70 mg of Tenax GR. During the first desorption stage, carried out at 250 °C for 5 min under a
205 helium splitless flow of 35 mL min^{-1}, the sample was concentrated in a cold trap at 5 °C. The cold

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206 trap was then flashed at 250 °C to inject the analytes into the capillary column (DB-624, 60 m
207 length, 0.25 mm internal diameter, 1.4 µm film thickness, Agilent Technologies, USA) of the gas
208 chromatograph (Trace GC Ultra, Thermo Electron Corporation, USA) coupled to a quadrupole
209 mass spectrometer (Trace DSQ, Thermo Electron Corporation, USA) operated in the positive
210 electron impact (EI) ionization mode (70 eV). Chromatograms were collected in both total ion
211 current (TIC) and selected ion monitoring (SIM) acquisition modes. The ions at *m/z* 45, 131 and 99
212 were used for IPA, SEV and HFIP identification and quantification, respectively. The ions at *m/z* 49
213 were used for isopropanol-D8 and *m/z* 98 for toluene-D8. The oven temperature program was 35 °C
214 for 10 min, 4 °C min^{-1} to 130 °C, 2 min hold, 20 °C min^{-1} to 250 °C, 10 min hold, 25 °C min^{-1} to
215 260 °C, 15 min hold. The total GC-MS run time was 56 minutes. The temperature of the injector
216 was set at 200 °C. Helium (constant pressure 210 kPa, split flow of 10 mL min^{-1}) was used as
217 carrier gas. Dedicated software controlled the thermal desorption unit (TD Manager, DANI
218 Instrument, Italy) and the GC-MS (Xcalibur, Thermo Electron Corporation, USA). The GC-MS
219 response factor stability unit was checked daily by injecting 50 µL of labelled MIX2D. The analysis
220 of all the collected samples was carried out in triplicate.
221 The chemical stability of IPA, SEV and HFIP was evaluated by filling Nalophan bags with standard
222 mixtures and then analysed soon after filling (t_0) and after 0.5, 2.5, 5 and 24 hours. For this purpose,
223 2.5 mL of MIX3 were injected in the aspiration flow during the filling of Nalophan bags (5 L) with
224 pure air at 500 mL min^{-1}. The calculated concentration of SEV, HFIP and IPA in the bag was 240,
225 305 and 415 ppbv, respectively. 250 mL of gaseous mixture were loaded into the adsorption tube at
226 50 mL min^{-1} and 50 µL of MIX2D were injected in the aspiration flow during the sample transfer.
227 Adsorption tube was finally analysed in the same way as the breath samples.
228 Relative response factors to labelled internal standards were calculated according to the following:
229
230 $K = (A_i \times m_{D8})/(A_{D8} \times m_i)$ (1)
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232 where A_i and m_i are the chromatographic peak areas (a.u.) and the theoretical amounts (ng loaded in
233 the adsorption tube) of the ith compound, respectively. A_{D8} and m_{D8} are the chromatographic peak
234 areas (a.u.) and the theoretical amounts (ng loaded in the adsorption tube) of the internal labelled
235 standards, respectively. To determine K, 50 μL of each standard (MIX3 and MIX2D) were injected
236 in the aspiration flow during the transfer of 250 mL of pure air into the adsorption tube at 50 mL
237 min^{-1}. Five adsorption tubes were analysed in the same way as the breath samples.
238 Isopropanol-D8 was used as internal standard for the quantification of IPA and SEV, whereas
239 toluene-D8 was used for the quantification of HFIP..
240

241 **3. Results and discussion**

242 *3.1 Analytical performance*

243 The analytical method had already been proposed and validated in previous studies [8, 43]. In this
244 study, the application to assess workers' exposure to potentially harmful VOCs in a hospital
245 environment was tested. Stability test showed that within 5 h there were no significant variations of
246 IPA (415 ppbv), SEV (240 ppbv) and HFIP (305 ppbv) concentrations in the Nalophan bags and a
247 decrease of about 10% within 24 h was observed. Standard gaseous mixtures prepared according to
248 the method described in 2.2 were analysed as described in 2.4. Seven points calibration curves
249 showed a good linearity (r^2 = 0.999) in the ranges 0.01-1000 ppbv for SEV, 0.001 - 0.1 ppbv for
250 HFIP, and 0.02-1700 ppbv for IPA.
251 The instrumental detection limits (IDLs), calculated considering the concentrations producing a
252 signal-to-noise ratio equal to 3, were 10 pptv for IPA, and 5 pptv for SEV. The HFIP minimum
253 detectable concentration was 0.5 pptv as estimated in SIM acquisition mode.
254 In the experimental conditions, the retention time of IPA, SEV, HFIP, isopropanol-D8 and toluene-
255 D8 was 9.14, 7.35, 23.43, 8.86 and 25.37 minutes, respectively.

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256 The mean relative response factors (n=5), with a RSD of about 6%, were 0.87 for IPA, 1.34 for
257 SEV and 0.54 for HFIP.

258 *3.2 Determination of SEV in breath samples*

259 All subjects involved in the study were working eight hours per day, and participated in surgeries of
260 different types and length carried out in different operating rooms for a maximum of six hours each
261 shift. They were of a similar age but were of different sexes and body weights. Potential exposures
262 were also different, depending on the kinds of surgery and doses of anaesthetic used.

263 Fig. 2 shows SEV (A) and HFIP (B) concentrations measured in the collected breath samples. . A
264 mean SEV/HFIP breath concentration ratio of 170 ± 150 was observed, in good agreement with the
265 pharmacokinetic model previously described [8]. SEV was measured in most samples, but in four
266 out of five t_0 samples, the concentration was below the IDL. This reasonably means that usually
267 concentrations lower than 5 pptv remained in the subject's breath after the weekend rest.
268 Concentration profiles during the week did not seem to follow the same pattern for all the
269 anaesthesiologists. In three cases, SEV concentrations were higher at the end of the first working
270 day (t_1) than at the end of the working week (t_2). If time between two consecutive working days is
271 insufficient to let SEV concentrations drop below the IDL, then a cumulative increase in
272 concentration during the working week would be observed, resulting in higher SEV levels in t_2
273 samples. Instead, our results appeared more compatible with a highly variable exposure, in which
274 daily variability plays a major role.

275 Note that SEV concentration in breath was always very low, far below the limit of 2 ppm suggested
276 by NIOSH in ambient air [19].

277 Our statistics are insufficient to draw ultimate conclusions and we lack data concerning SEV
278 concentrations in ambient air, since we were not allowed to access surgery rooms during surgeries
279 as this was considered to pose risks for patients. However, it seems that the exposure of workers
280 involved in the study was of acceptable levels. This is very important, because the risk of harmful

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281 effects from chronic occupational exposure to SEV is so serious that NIOSH declared that a safe
282 level of exposure for waste-anaesthetic gases could not be identified, and recommended that risks
283 should be minimized by "reducing exposures to the greatest extent possible" [19].
284
285 *3.3 Determination of IPA in air and breath samples*

286 An antiseptic water-alcohol solution containing 70% of IPA was identified as the likely source of
287 isopropyl alcohol in the air of the blood sampling room. Cotton balls were typically soaked with the
288 solution and used to disinfect the skin before blood sampling, then thrown into a basket.
289 Fig. 3 shows IPA levels in breath versus concentrations measured in the air. The good correlation (r
290 $= 0.95$, $p < 0.001$) between these concentrations suggests that breath levels can be used at the same
291 time to monitor exposure and to have an idea of the level of ambient contamination. In a hospital,
292 where largely variable conditions are found in different rooms depending on use, breath levels will
293 be a weighted average of the concentrations based on the time spent by the subjects in the different
294 areas.
295 During our study, an accidental exposure to higher than normal IPA concentration levels occurred
296 to a subject who probably remained very close to the basket containing the waste cotton balls. In
297 this case, a concentration of 1500 ± 70 ppbv was measured, compared to a mean value of 20 ± 20
298 ppbv determined in the other nurses' exhaled breath. This level is in any case far below the TWA
299 limit of 400 ppmv and a STEL of 500 ppmv recommended by NIOSH for ambient air [26], and
300 concentration in breath decreased more than seven times in about half an hour, suggesting that the
301 risks for health remained quite low.
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303
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305 **4. Conclusions**

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307 In hospitals, anaesthetic gases, antiseptics and disinfectants are a primary source of air
308 contamination. The consequent presence of potentially harmful VOCs in the air poses a risk for
309 hospital staff in terms of acute and chronic exposure.
310 Our method was successfully used to measure the two representative contaminants - SEV and IPA -
311 in breath, and may also be exploited to determine volatile metabolites of xenobiotics, such as HFIP
312 in the breath samples. HFIP is a biologically damped metabolite of SEV, and so longer-term
313 chronic exposures would eventually appear as the metabolite despite the fact that the on-board
314 original SEV may have already been lost to exhaled volatilization.
315 Managing cotton balls soaked with IPA undoubtedly requires careful storage pending disposal. In
316 addition there should be a ventilation system in any environment where potentially harmful volatile
317 substances are used, such as anaesthetic gases.
318 However, appropriate precautions are taken in the workplace environment of the subjects involved
319 in our study, as demonstrated by the fact that the concentrations of the analytes of interest were all
320 below the recommended legal limits.
321 The main advantages of our method are non-invasiveness and the simple sampling procedure
322 compared to conventional biological fluids (e.g. blood and urine). In addition, the method enables
323 the determination of both SEV and IPA at concentration levels far below the occupational exposure
324 limits in both exhaled breath and ambient air. This indicates that in conjunction with other
325 monitoring programs our method could be used for sensitive, short-term monitoring of hospital
326 personnel exposed to potentially harmful VOCs as well as for monitoring staff exposure to other
327 potentially harmful VOCs.
328

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334 **Captions**

335

336 **Fig. 1.** (A) Air sampling system composed by (1) pump, (2) PTFE tube, (3) three-ways stop valve,
337 (4) Nalophan bag, (5) vessel; (B) Breath sampling system composed by (1) disposable mouthpiece,
338 (2) non-return valve, (3) stopcock, (4) Nalophan bag.

339

340 **Fig. 2.** SEV (A) and HFIP (B) levels (ppbv) in trainees' exhaled breath. Each trainee filled the
341 sampling bags at the beginning of the working week (t_0), at the end of the first day of work (t_1), and
342 at the end of the last working day (t_2).

343

344 **Fig. 3**. IPA concentrations in workers' breath samples versus IPA concentrations in ambient air.

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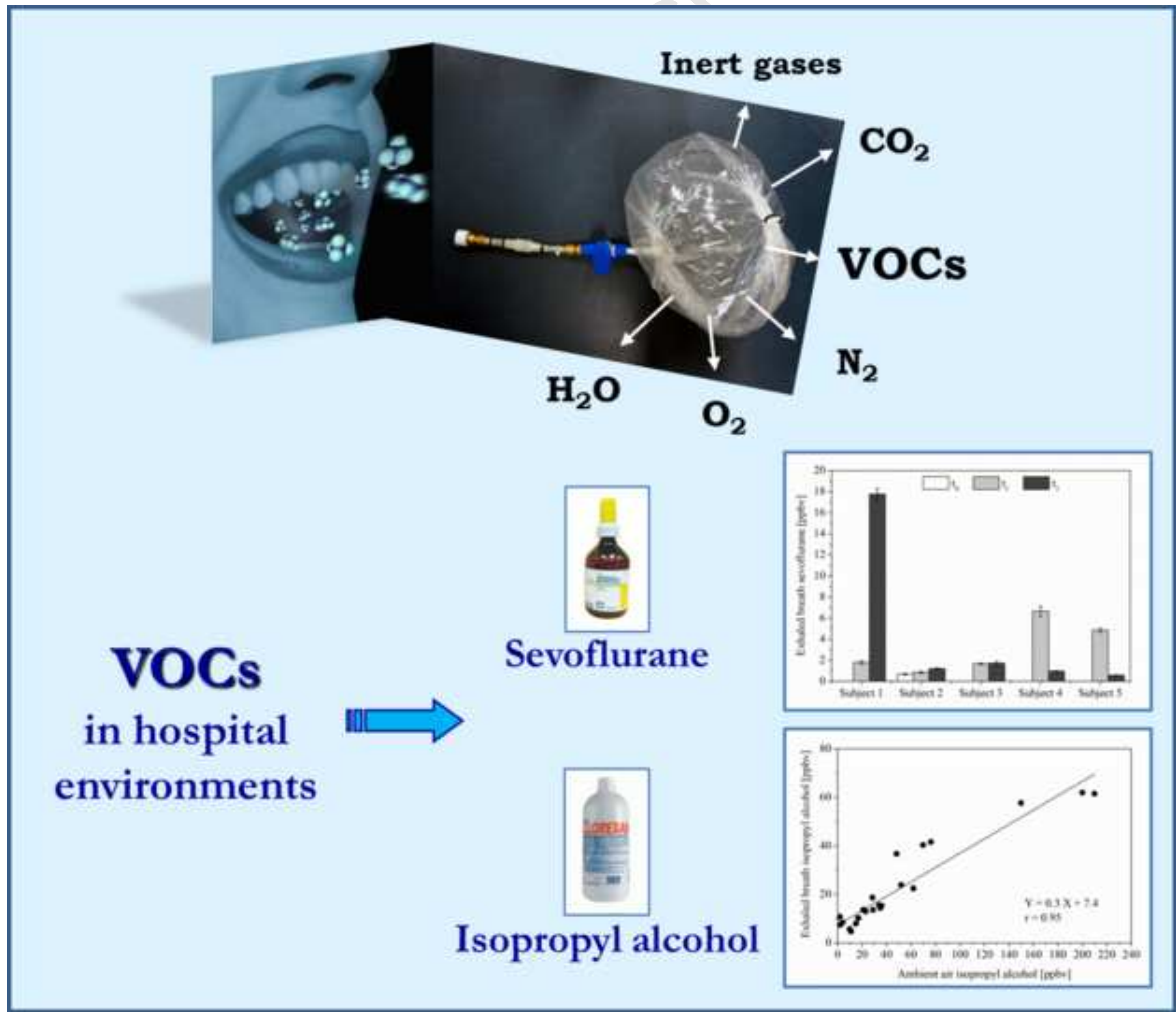
*Graphical Abstract



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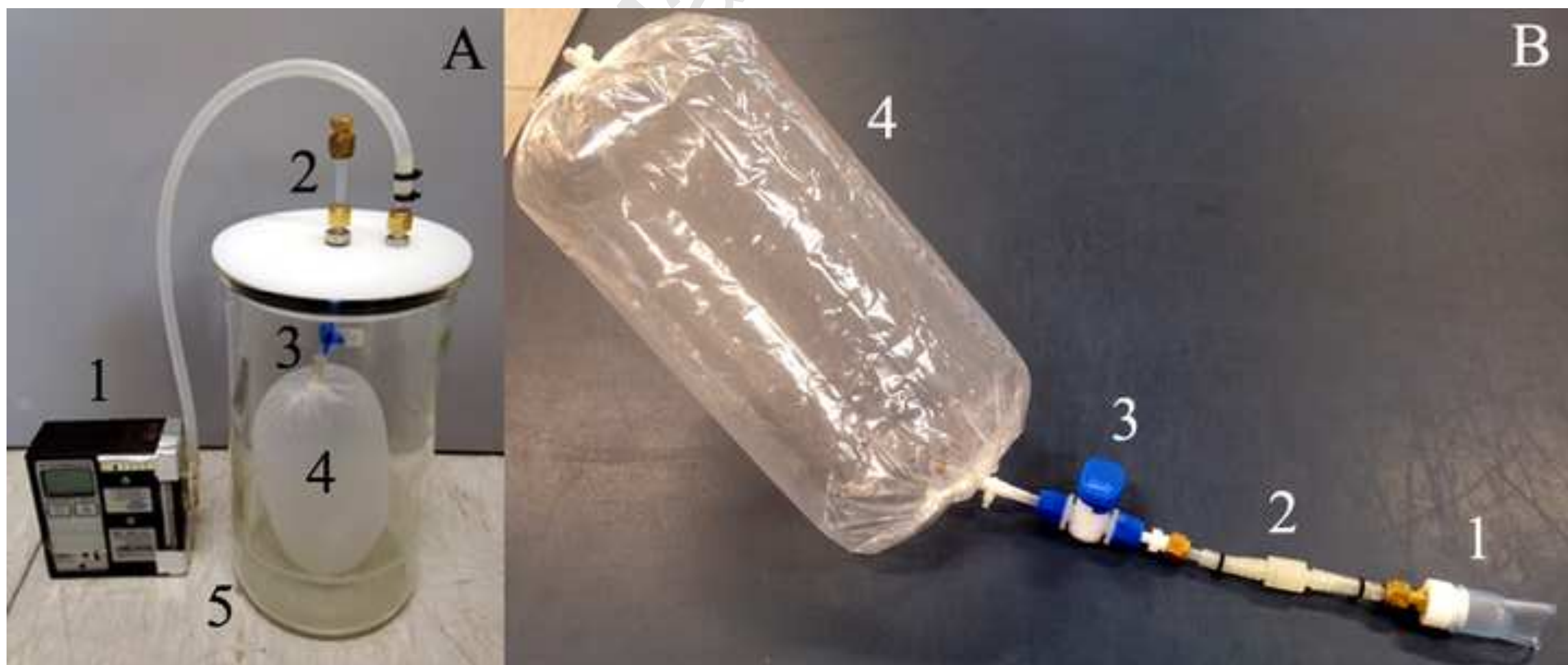

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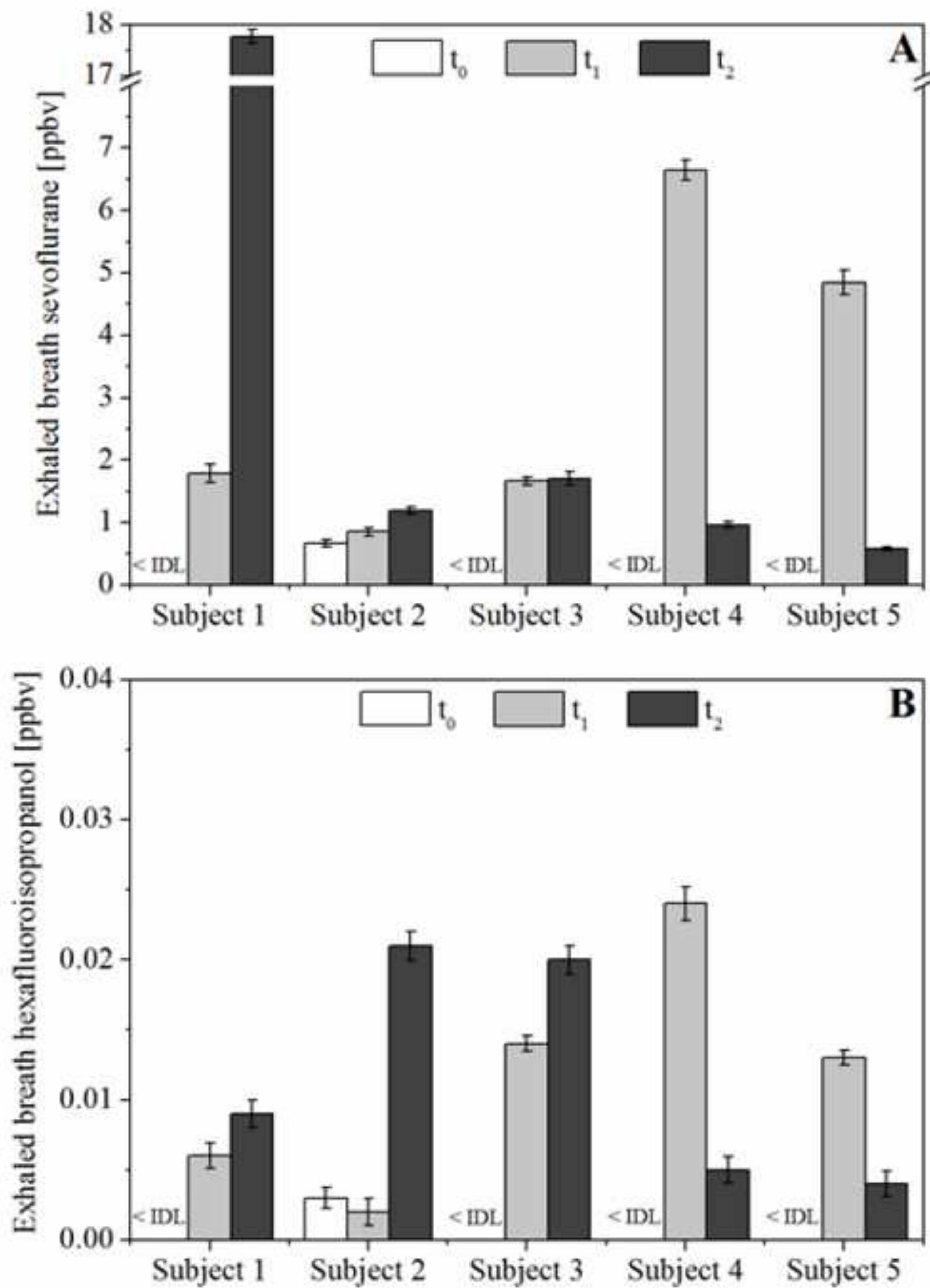

Figure(s)