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Title:
Towards a formalin-free hospital. Levels of 15-F_{2t}-isoprostane and malondialdehyde to monitor exposure to formaldehyde in nurses from operating theatres.

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Keywords:
15-F2t-Isoprostane, formaldehyde, malondialdehyde, oxidative stress, under vacuum sealing

Abstract:

Purpose: Nurses are exposed to Formaldehyde when managing surgical samples to be later transferred to Histopathology. We evaluated conditions favouring the risk of exposure to this toxic reagent and the effect of measures to prevent it.

Methods: we conducted a cross-sectional study where 94 female workers were enrolled as potentially exposed to Formaldehyde. For each nurse were collected: 1) personal air-Formaldehyde by a personal dosimeter (8 hours); 2) a standardized questionnaire; 3) a urine sample to test 15-F_{2t}-Isoprostane, Malondialdehyde, Cotinine.

Results: The results indicate a marked difference related to the adoption of the Under Vacuum Sealing procedure, as an alternative to Formaldehyde for preserving tissues. Nurses using the Under Vacuum Sealing system in the operating rooms are exposed to levels of Formaldehyde 75% lower than those who do not use that system. Oxidative stress biomarkers (15-F_{2t}-Isoprostane, Malondialdehyde) are significantly higher in nurses using Formaldehyde ($p < 0.001$) and in absence of Under Vacuum Sealing system ($p = 0.027$), in particular in those workers that use liquid Formaldehyde in the operating theatre ($p = 0.012$).

Conclusions: Analysis of biological biomarkers confirms a direct responsibility of air Formaldehyde on the onset of oxidative stress while the use of the Under Vacuum Sealing technique is associated with a significant reduction of the exposure to air- Formaldehyde and redox status. Our findings can be useful to characterize the environmental health risk in the operating theatres and to plan preventive measures such as the Under Vacuum Sealing procedure.

INTRODUCTION

Formaldehyde (FA) is an important chemical widely used in many working environments including Hospitals [1]; [2]; [3]; [4]; [5]. Since FA represents an ubiquitous pollutant, breathable at variable levels in every living and working environment, the study of the relationship between exposure to this reagent, its biological effect and related diseases is important, but rather complex.

On the whole, exposure to FA is associated to a wide range of adverse health effects, from mild to severe [6]; [7]. In particular, acute exposure to FA can cause irritation (on eyes, nose, throat, and skin), nasal congestion, sore throats, headaches, coughs, conjunctivitis, fatigue, rashes, shortness of breath, nausea and nosebleeds [8]; [9]. FA is also known as a human carcinogen and as an inducer of chronic toxicity, being endowed with genotoxic and oxidant activity [1]; [10]; [11]; [12]. Among the chronic effects of FA, an increased incidence of nasopharyngeal cancer in definite FA-exposed workers was demonstrated by some authors [13];[14] while others have shown a relationship between FA and leukemia [15]; [16].

Previous studies of our group already showed that FA, breathed in appropriate concentrations, is able to induce an oxidative imbalance [17]. To overcome and counteract this oxidative imbalance induced by FA, detoxifying enzymes are produced through different metabolic pathways [18]; [19]. For example, F2-isoprostanes (F2-IsoPs) are prostaglandin-like bioactive compounds formed in vivo from the free radical-catalyzed peroxidation of essential fatty acids, like arachidonic acid. F2-IsoPs are stable and reliable molecules, detectable in all human tissues and biological fluids, including plasma, urine, fluid of broncho-alveolar lavage and cerebrospinal fluid. Based on their mechanism of synthesis, four F2-IsoP regioisomers (5-, 12-, 8-, or 15- series) may be generated, depending on which side of the chain the carbon atom is connected to. A F2-IsoP, produced abundantly in vivo and extensively tested for biological activity, is the 15-F2t-IsoP (8-iso-PGF2α), where "2t" is due to the trans position of the oriented side chain to the prostane ring [20].

Recent studies stressed the usefulness of 15-F2t-IsoP to assess the oxidant stress in humans [17]; [21]; [22]; [23] but also to highlight pathological conditions [24]; [25]. Since F2-IsoPs can be detected in urine specimen in a non invasive way, these molecules have been proposed as a suitable biomarker for oxidative stress [12]; [26]. Another biomarker of lipid peroxidation is malondialdehyde (MDA), which is generated in vivo via peroxidation of polyunsaturated fatty acids and interacts with proteins, being itself potentially atherogenic [18]. Free radicals are able to activate the lipid peroxidation process in an organism and their increase causes an overproduction of MDA, which represents one of the final products of peroxidation of polyunsaturated fatty acids in the cells. MDA is commonly known as a biomarker of oxidative stress, but is also able to highlight the oxidative status in oncologic patients. Thus, data of epidemiological studies on humans support the significance of MDA as predictor of the imbalance in the oxidative stress status and lipid peroxidation. A recent paper of our group [27]

has shown that the histological process of tissue fixation in FA also implies an oxidative damaged of DNA as revealed by the formation of 3-(2-deoxy-β-d-erythro-pentafuranosyl-pyrimido[1,2-α]purin-10(3H)-one (M1dG). In particular, that paper showed that the percentage of M1dG adducts formed when the formalin-fixation procedures was adopted, was about 4-5 fold greater if compared to frozen tissues, which avoid the use of formaldehyde.

Interest on studies on the exposure of this toxic substance were, if possible, enhanced by the recent process leading to a formal banning of FA in the European countries in 2016, as a consequence of the EC Regulation n.605/2014 of 05.06.2014 that modifies the EC Regulation n.1272/2008. In some working processes, complete banning might be unattainable because of the lack of substitutes and specifically; thus, special exemptions for formalin use are going to be advanced. However, these requests should go in parallel with deeper knowledge of risk of exposure, while technical improvements and plans to reduce it to safe levels should be adopted.

In Healthcare, formalin is commonly used for fixing and preserving biologic specimens for pathologic and histologic examination or as a bactericide in embalming fluid and medical laboratories [15]. This practice is currently effected in two alternative ways, either by pouring liquid FA (3-5 litres) in large containers, or by using prefilled vials (containing 50-100 ml of FA). At variance to the use of FA, and with the specific goal of reducing exposure to this reagent, since a few years our Hospital adopted the practice of the Under-Vacuum Sealing (UVS) which involves the introduction of tissues removed by surgeons into a special plastic bag, afterwards placed under vacuum and chilled at + 4 °C until being transferred in the Pathology Laboratory.

The UVS procedure has potential for introducing some important improvements: a) it avoids the use of FA and the consequent human FA exposition in the operating rooms, b) keeps very well the anatomical and immunehistochemical features of tissues while reducing DNA damage, c) enhances the preservation of both structure and tissue components (proteins, nucleic acids), and, d) lengthens the useful time before the tissue fixation [28;29]. Moreover, tissues processed with UVS are suitable for tissue banking and cell culture [30]. Since the use of the UVS procedure in the Hospitals selected for this study was actually active only in some operating theatres, we intended to check if the adoption of this procedure implied objective differences in exposure to FA and variations in the related biological response. Specifically, we have assessed the intensity of oxidative stress and correlated it to the intensity of exposure to FA vapours.

To achieve this goal, we enrolled as volunteers a group of healthy female nurses, partly smokers, attending different operating theatres adopting or not the UVS system and making or not use of FA. Cohorts with different expositive scenarios have been compared with one another through the quantification of 15- F2t-IsoP and MDA as markers of lipid peroxidation, in order to assess

different FA exposures and the effectiveness of different tissue preservation procedures (UVS *vs.* FA). For each of the workers the exposure to tobacco smoke, a confounder because inducer of oxidative stress, was quantified using cotinine values as a marker.

METHODS

Study-subjects. Ninety-four female workers, recruited in the largest hospital of the Piedmont region in Italy ("Città della Salute e della Scienza" of Torino), were enrolled as subjects potentially exposed to FA in the operating theatre. In agreement with the standards of the institutional Ethical Committee on human experimentation and with the Helsinki declaration, all subjects were informed about the objectives of the study, and gave written, informed consent. Nurses, operating in surgical theatres, are traditionally exposed to FA because of the common and tradition practice of immersing in this preservative liquid (3-5 litres at a time) the surgical samples, of a size ranging between 2 and 30 cm, to be later transferred to Pathology Lab. The preservation technique of which we want verify the effectiveness consists of introducing in special plastic bags the specimen removed by the surgeon and then inducing the complete removal of air from the plastic bag. UVS bags are then preserved at 4°C till transfer to the Pathology Lab. The 94 subjects, according to their professional involvement and exposition, were *a posteriori* grouped in 2 groups, the first composed of nurses working, on the day of sampling, in surgical theatres equipped with the apparatus (Tissue SAFE, Milestone, Bergamo, Italy) for the UVS procedure, the second group of nurses from theatres not engaged in this procedure and where the standard for all surgical specimens was the immersion in large containers where liquid FA (3-5 liters at a time) was poured.

In both type of theatres small biopsies (core or incisional) were immersed in vials (DiaPath, Bergamo, Italy) pre-filled with FA (50-100 ml) and sent to the Pathology Laboratory. Nurses from the first type of surgical theatres (UVS-equipped) were occasionally committed as well to the manage of liquid FA, for filling up containers for specimens / organs >30 cm in size, but most specimens (over 95%) were processed by UVS.

On Wednesday and Thursday, for each of 94 subjects, were collected the following items: 1) a personal air-FA sampling for one entire working shift (8 hours); 2) a standardized questionnaire; 3) an urine sample for the quantification of 15-F2t-IsoP, MDA, urinary cotinine and creatinine (CREA). A specimen of urine at the end of the working shift was collected from each volunteer and stored at -80 °C until analysis. On the whole, the only exclusion criteria was thus not to recruit males whereas all the females who have joined voluntarily in the study were included and recruited.

Personal Air-FA. FA air samples were collected for a working shift (8 hours) using passive, personal air samplers working with radial symmetry (Radiello®). The sampler was clipped near the

breathing zone of the subject to quantify as accurately as possible the air exposure during a work shift. Each sampler was equipped with a specific sorbent tube containing silica gel coated with 2,4-dinitrophenylhydrazine – DNPH – (NIOSH 2016). This last, reacting with FA, changes by derivatization to the 2,4-dinitrophenylhydrazone specific of FA derivative. Subsequently, the tube were quantified with a HPLC Perkin-Elmer equipped with an UV detector regulated at 360 nm [31]; [32].

Questionnaire. On the same day of the personal air sampling, a questionnaire (a synthesis of the most extensive "GEIRD" questionnaire, www.geird.org) was administered to all subjects by one interviewer obtaining information on individual, clinical features and smoking habits. Thus, the following individual and clinical information were acquired: age, sex, residence, hobbies, therapies, smoking habits, profession (qualifications, seniority, and job-specific work), use of FA in the operating theatre during the sampling day, and the presence and use of UVS system and environmental and personal devices to prevent FA exposure and health risks.

Urinary cotinine. Urinary cotinine was measured aiming to consider the possible role played by tobacco smoke in the onset of an oxidative stress status. An aliquot of fresh urine was collected in the morning and approximately at the same time from each volunteers, and stored at −80◦C prior to analysis, performed within 20 working days. The enzyme immunoassay for cotinine is based on the competition between the cotinine in the urine sample and the cotinine-alkaline phosphatase conjugate: the sample containing the cotinine and the cotinine-alkaline phosphatase conjugate compete for binding to a limiting number of antibody sites. The bound enzymatic activity was measured by the addition of a chromogenic substrate. Therefore, the intensity of the color developed is inversely proportional to the concentration of cotinine in the sample. The concentration is calculated on the basis of a standard curve. The declared limit of detection is 1 ng/ml.

Urinary isoprostane. 15-F2t-IsoP in urine was quantified by means of ELISA technique per-formed with a specific microplate kit, according to manufacturer's instructions, (Oxford, MI, USA). 15-F2t IsoP in urine was measured by ELISA technique performed with a specific microplate kit (Oxford, MI, USA), according to manufacturer's instructions. 15-F2t-IsoP in the samples or standards competed with 15-F2t-IsoP conjugated to horseradish peroxidase (HRP) for binding to a polyclonal antibody specific for 15-F2t-IsoP coated on the microplate. The HRP activity resulted in color development when the substrate was added, with the intensity inversely proportional to the amount of unconjugated 15-F2t-IsoP in the samples or standards. The declared limit of detection is 0.2 ng/ml. Dilution 1:4 was adopted to achieve better accuracy in the competitive ELISA method. Because of the high percentage of 15-F2t IsoP excreted in human urine conjugated to glucuronic

acid (over 50%), a preliminary incubation with β-glucuronidase for 2 h at 37 °C was performed, in order to detect the entire quantity of 15-F2t IsoP present in each urine sample.

Urinary malondialdehyde. A TBARS Assay kit (Abnova), according to manufacturer's instructions, measured MDA in urine. An aliquot of fresh urine was collected and stored at −80°C prior to analysis performed within 20 days. Urine does not require any special treatments before analysis. The principle of the method is based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA at 25°C. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid with a Knoevenagel-type condensation to yield a chromophore with absorbance maximum at 532 nm.

Creatinine quantification. In order to normalize the excretion rate of cotinine and 15-F2t-IsoP an aliquot of fresh urine was used to quantify the concentration of creatinine (CREA) by the kinetic Jaffé procedure.

Statistical analysis. Data were analyzed using STATA® vs 13.0 statistical package (StataCorp, Tex, USA). Appropriate linear transformations were applied on data whenever suggested by distributional diagnostic plots (symmetry plot, quantile plot) and descriptive statistic inspection (looking at variance stability among categories). A log-transformation was performed to find the power transformation that stabilize the variance and normalize the distribution. To compare the values among the resulted groups, a median test (non-parametric tests on the equality of medians) was applied, checking the null hypothesis that the K samples were drawn from populations with the same median.

Multiple Linear Regression (MLR) analysis with robust standard error estimate was used to analyze the relationship between log transformed personal air-FA ($\mu g/m^3$) as dependent variable and use of UVS and the type of container of FA (prefilled or large container) as predictive variables and the relationship of log transformed 15-F2t- and MDA with the personal air-FA. The models were adjusted for cotinine, and age. For all tests, a p value of ≤ 0.05 (two-tailed) was considered significant. All the variables proving a significativity $\geq 5\%$ were excluded

RESULTS

The 94 subjects, on the basis of the results of the questionnaire, were divided into 2 groups. The first group declared that he had worked the day of sampling in operating theatres equipped with the UVS device, the second group stated that he had worked in operating theatres without such device. In both type of theatres small biopsies (core or incisional) were immersed by nurses in vials pre-filled with FA (50-100 ml) and sent to the Pathology Laboratory. By studying the results of the questionnaires we observed that nurses from the first type of surgical theatres (UVS-equipped) were

occasionally committed as well to the manage of liquid FA, for filling up containers for specimens / organs >30 cm in size, but most specimens (over 95%) were processed by UVS.

Table 1 describes the numerousness of groups of subjects who have used the FA during the sampling day according to the smoking habit. In the lower part of table 1, the subjects were also sub-grouped according to the availability of UVS in the operating theatres and, in both cases, to the epidemiological characteristics and smoking habits.

Table 2 describes the personal air-FA concentrations ($\mu g/m^3$), which came out to be higher in the 64 subjects who used FA in the sampling day ($p = 0.032$) and related to the use of the UVS technique ($p = 0.040$) and to the use of FA (liquid or prefilled). The results indicate a significant difference related to the adoption of the UVS system when the FA was not used ($p = 0.002$) but obviously even more so when the FA was used in liquid form ($p = 0.001$) and not using prefilled vials. Furthermore, workers who use the liquid FA without UVS technique show overall an exposition to FA more than three times higher when compared to those who do not use this procedure.

For the further analysis, the FA concentrations were log-transformed to normalize the distribution and improve the homoscedasticity. Thus, the robust regression shows on one hand a concentration of air-FA, when adjusted by UVS use, directly proportional to the amount of FA used (liquid > prefilled) and, on the other hand a lower level of air-FA when UVS technique is adopted, with a significant interaction in the intensity of exposure to air-FA between use of FA and the adoption of UVS technique (**table 3**). Furthermore, given the significant interaction before mentioned, the effect of UVS is higher in subjects exposed to liquid FA than in those exposed to prefilled FA (coef. log -0.51 vs -0.15). Overall, results of the linear regression of FA shown in **figure 1,** underlines that the nurses who use the UVS system in the operating rooms are exposed to levels of FA 75% lower than those who do not use that system. Furthermore, as can be seen on the left side of the figure the adoption of the UVS allows halving the level of air-FA in the surgical theatre also in days when FA was not in use (no FA).

In the higher part of **table 4**, nurses who use FA show concentrations of 15-F2t-IsoP significantly higher if compared to not users of FA ($p < 0.001$). The concentrations of 15-F2t-IsoP underline differences when the subjects are sub-grouped according to the adoption of the UVS procedure. Overall, 15-F2t-IsoP is higher in absence of UVS system ($p = 0.027$) and, in particular, in those workers that use FA in the operating theatre without UVS ($p = 0.029$). Besides, 15-F2t-IsoP levels are two times higher in subjects that used liquid FA in absence of UVS system ($p = 0.012$). In the middle part of table 3 the concentrations of MDA are reported. The concentrations are significantly higher in subjects who use liquid FA and are not provided with UVS ($p = 0.012$). Additionally, in

the lower part of table 3 the ability of cotinine to quantify effectively the intensity of exposure to tobacco smoke is confirmed in the present subjects, since a significantly higher level was observed in smokers ($p = 0.035$) but this factor did not mask the use of different amounts of FA.

To deepen the positive relationship between FA exposure and synthesis of oxidative stress biomarkers, a multiple robust regression was calculated considering 15-F2t-IsoP, as the dependent variable, MDA, as a covariate, log-FA, UVS, urinary cotinine, age of subjects, and BMI as independent variables and confounding factors (**table 5**). After adjustment for UVS, cotinine, and age, findings show a positive and significant relationship between air-FA and oxidative stress biomarkers.

DISCUSSIONS

Since its introduction as a histological fixative back in the 19th century[33], the 4% formaldehyde solution in water called Formalin has been adopted as the fixative of choice in histopathology. Besides its superior properties in guaranteeing structural preservation, FA fixation allows an immuno-histochemical and genetic definition of pathological lesions and this multi-faceted characterization carries paramount importance in planning therapies [34]. This implies that Health Authorities would object that dismissal of the use of FA would generate a major harm to the quality of diagnosis for patients.

FA is know to be toxic and is classified as a category 1B/2 carcinogen and a significant association was demonstrated between formalin-fixation procedures and the generation of oxidatively damaged DNA testified also by the formation of the molecular adduct M1dG [27]. This would justify its banning, as recently proposed by EU authorities. FA can induce increased levels of oxidative stress and enhanced formation of ROS by different ways, including the activation of oxidases and the inhibition of scavenger systems. For instance, FA is a substrate for the action of cytochrome P-450 monooxygenase system II E1 isozyme and can be oxidized by peroxidase, aldehyde oxidase, and xanthine oxidase with subsequent ROS formation. However, given that to date a reagent able to guarantee the same performance in histopathology is not available, a reasonable policy is to reduce the risk, by creating working conditions in which the exposure of the personnel involved is limited to an acceptable minimum.

The present study shows that the adoption of the UVS procedure for the transfer of surgical specimens to the Pathology labs results in a sharp decline of exposure of nurses to FA. In fact, we give evidence of reduction of oxidative stress in nurses adopting the UVS technique as an alternative to the use of FA in operating theatres. This aspect has to be added to those already acquired, consisting of the approval of this procedure by pathologists who see improved their

chances of diagnosis and research, and in the greater facility with which the nursing staff can operate [29].

The present study indicates that the major source of exposure to FA is not its use in pre-filled vials for fixing small biopsies, since in fact this procedure was carried out in both types of surgical theatres, while the bulk of exposure is related to the habit of pouring liquid FA (3-5 litres at a time) in large containers. In fact, the volunteers enrolled for this study who used the FA in liquid form showed a significantly higher exposure to FA than those who have not used it. Moreover, in preventive terms, the use of FA prefilled and, even more the use of the technique UVS, shows a significant reduction of the exposure to air-FA. Furthermore, the use of UVS technique fosters a lower level of air-FA as compared to that of theatres not equipped with the UVS apparatus, thus demonstrating a long-term "environmental efficiency" of UVS.

The more macroscopic effect of UVS technique is anyway observable when the FA is currently used in liquid form, since its breathable concentration is, in surgical theatres not equipped with UVS, 3 times higher. The robust regression (table 3) confirms a significant and independent interaction of air-FA exposition and UVS technique, underlining that the human intake of FA increases both as a result of use of a higher amount of FA and by the unavailability of the UVS technique. The linear regression of air-FA sub-grouped according by the UVS use and adjusted by cotinine and BMI highlighted in figure 1, allowed us to observe a greater effectiveness (-75%) of UVS technique on air-FA levels. In particular, these findings were evident among those who use the FA liquid than the FA in prefilled vials (table 3).

Analysis of biological oxidative stress biomarkers confirms a direct responsibility of air FA on the onset of oxidative stress. 15-F2t-IsoP is synthesized in significantly higher quantity when FA is used, in theatres where the UVS technique is not available, and when, without UVS, FA is used in liquid form. MDA seems to respond in a less sensitive way, proving to be significantly higher only when nurses are exposed to liquid FA that is to say to FA at higher concentrations. This aspect may depend on the biochemical characteristics of MDA, sensitive to FA in direct way, but most easily degradable, especially in the case of healthy subjects exposed in spot form. Thus, in future studies we believe that the measure of 15-F2t-IsoP is largely sufficient to quantify the extent of oxidative stress in the populations occupationally or environmentally exposed to formaldehyde.

In this study, cotinine was confirmed as a very sensitive and specific internal dose marker of smoking habits, able to exclude a role of this confounding factor among the subpopulations studied. In fact, cotinine is a metabolite of nicotine and nicotine is a chemical present only in the tobacco leaves. Finally, a definitive evidence of the direct relationship between exposure to air-FA and increase of oxidative stress is provided by the robust multiple regression that describes this

relationship for 15-F2t-IsoP and MDA (table 5), after adjustment for use of the UVS technique, exposure to smoking and age.

The principal finding of this paper is to underline the preventive role of adoption of the UVS system, bound to eliminate exposure to formalin in the operating rooms. Thus, the adoption of the UVS procedure appears to offer both, environmental and technical advantages. In fact, on the one hand pathologists declare themselves largely satisfied for the histological characteristics of the tissues preserved under vacuum at +4 °C and, on the other hand, our results highlight drastic reduction of workers' exposure to airborne FA, both in environmental and biological terms. This indicates that adoption of the UVS procedure lead to the elimination of the FA in operating rooms and a significant reduction of FA in pathology departments receiving the tissues.

Our findings can be useful to characterize the risk in terms of imbalance of redox status, experienced from the subjects working in the operating theatre engaged or not in the UVS procedure. However, predictive role of the biomarkers of early biological effects are quite limited to assess individual risk. This is because the complex processes that lead from the exposure to the formalin to diseases are affected by many factors, many of which are still unknown or whose real impact is not estimable (e.g., individual genetic profile, age, life and working style, health status, etc.).

In conclusion, given that complete elimination of FA from the Health Care System could hardy be adopted since it would impact on the quality of diagnosis for patients, reduction of exposure seems a reasonable compromise. The present study demonstrates that preventive measures can be effective and the behaviour of the oxidative stress biomarkers highlights the feasibility of this approach. The crucial preventive role of the adoption of the UVS technique in the operating theatres [27]; [35] is here demonstrated.

COMPLIANCE WITH ETHICAL STANDARDS

The study was submitted to the competent Ethics Committee of the "Azienda Ospedaliera Città della Salute e della Scienza" of Torino that approved the study (prot. 0071900, 25.6.2013 and prot. 0094007, 09/05/2013).

The manuscript does not contain report on clinical studies. The enrolled subjects are healthy adults who have voluntary participated in the study. Informed consent was obtained before the study from all individual participants included in the study. The study was conducted in accordance with the 1964 Helsinky declaration and its later amendments, all the data were treated anonymously and all the biological samples were destroyed after measurements.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest

REFERENCES

1 Committee to Review the Formaldehyde Assessment in the National Toxicology Program 12th Report on Carcinogens, Board on Environmental Studies and Toxicology, Division on Earth and Life Sciences and National Research Council, *Review of the Formaldehyde Assessment in the National Toxicology Program 12th Report on Carcinogens*, National Academies Press (US), Washington (DC), 2014.
2 H. M. Bolt, G. H. Degen and J. G. Hengstler, *Arch. Toxicol.*, 2010, **84**, 421–422.
3 IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, *IARC Monogr. Eval. Carcinog. Risks Hum. World Health Organ. Int. Agency Res. Cancer*, 2012, **100**, 9–562.
4 R. Bono, V. Bellisario, V. Romanazzi, V. Pirro, P. Piccioni, M. Pazzi, M. Bugiani and M. Vincenti, *Int. J. Hyg. Environ. Health*, 2014, **217**, 287–293.
5 R. Bono and V. Romanazzi, in *General Methods in Biomarker Research and their Applications*, eds. V. R. Preedy and V. B. Patel, Springer Netherlands, 2015, pp. 383–404.
6 M. Hulin, D. Caillaud and I. Annesi-Maesano, *Indoor Air*, 2010, **20**, 502–514.
7 M. Hulin, M. Simoni, G. Viegi and I. Annesi-Maesano, *Eur. Respir. J.*, 2012, **40**, 1033–1045.
8 R. Dales and M. Raizenne, *J. Asthma Off. J. Assoc. Care Asthma*, 2004, **41**, 259–270.
9 J. H. E. Arts, M. A. J. Rennen and C. de Heer, *Regul. Toxicol. Pharmacol. RTP*, 2006, **44**, 144–160.
10 IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, *IARC Monogr. Eval. Carcinog. Risks Hum. World Health Organ. Int. Agency Res. Cancer*, 2006, **88**, 1–478.
11 O. Schmid and G. Speit, *Mutagenesis*, 2007, **22**, 69–74.
12 V. Romanazzi, V. Pirro, V. Bellisario, G. Mengozzi, M. Peluso, M. Pazzi, M. Bugiani, G. Verlato and R. Bono, *Sci. Total Environ.*, 2013, **442**, 20–25.
13 S. Duhayon, P. Hoet, G. Van Maele-Fabry and D. Lison, *Int. Arch. Occup. Environ. Health*, 2008, **81**, 695–710.
14 M. Hauptmann, J. H. Lubin, P. A. Stewart, R. B. Hayes and A. Blair, *Am. J. Epidemiol.*, 2004, **159**, 1117–1130.
15 L. Zhang, C. Steinmaus, D. A. Eastmond, X. K. Xin and M. T. Smith, *Mutat. Res.*, 2009, **681**, 150–168.
16 L. Zhang, L. E. B. Freeman, J. Nakamura, S. S. Hecht, J. J. Vandenberg, M. T. Smith and B. R. Sonawane, *Environ. Mol. Mutagen.*, 2010, **51**, 181–191.
17 R. Bono, V. Romanazzi, A. Munnia, S. Piro, A. Allione, F. Ricceri, S. Guarrera, C. Pignata, G. Matullo, P. Wang, R. W. Giese and M. Peluso, *Chem. Res. Toxicol.*, 2010, **23**, 1342–1348.
18 K. Uchida, *Free Radic. Biol. Med.*, 2000, **28**, 1685–1696.
19 C. Kum, F. Kiral, S. Sekkin, K. Seyrek and M. Boyacioglu, *Exp. Anim. Jpn. Assoc. Lab. Anim. Sci.*, 2007, **56**, 35–42.
20 L. J. Roberts and G. L. Milne, *J. Lipid Res.*, 2009, **50 Suppl**, S219-223.
21 H. Mitsumoto, R. M. Santella, X. Liu, M. Bogdanov, J. Zipprich, H.-C. Wu, J. Mahata, M. Kilty, K. Bednarz, D. Bell, P. H. Gordon, M. Hornig, M. Mehrazin, A. Naini, M. Flint Beal and P. Factor-Litvak, *Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis.*, 2008, **9**, 177–183.
22 J. D. Morrow, A. R. Tapper, W. E. Zackert, J. Yang, S. C. Sanchez, T. J. Montine and L. J. Roberts, *Adv. Exp. Med. Biol.*, 1999, **469**, 343–347.
23 L. J. Roberts and J. D. Morrow, *Free Radic. Biol. Med.*, 2000, **28**, 505–513.
24 D. Giustarini, I. Dalle-Donne, D. Tsikas and R. Rossi, *Crit. Rev. Clin. Lab. Sci.*, 2009, **46**, 241–281.
25 G. Ferretti and T. Bacchetti, *J. Neurol. Sci.*, 2011, **311**, 92–97.
26 S. Basu, *Antioxid. Redox Signal.*, 2008, **10**, 1405–1434.
27 M. E. M. Peluso, A. Munnia, M. Tarocchi, R. W. Giese, L. Annaratone, G. Bussolati and R. Bono, *Toxicol. Res.*, 2014, **3**, 341–349.
28 G. Bussolati, L. Chiusa, A. Cimino and G. D'Armento, *Virchows Arch. Int. J. Pathol.*, 2008, **452**, 229–231.
29 C. Di Novi, D. Minniti, S. Barbaro, M. G. Zampirolo, A. Cimino and G. Bussolati, *Sci. Total Environ.*, 2010, **408**, 3092–3095.
30 L. Annaratone, C. Marchiò, R. Russo, L. Ciardo, S. M. Rondon-Lagos, M. Goia, M. S. Scalzo, S. Bolla, I. Castellano, L. Verdun di Cantogno, G. Bussolati and A. Sapino, *PLoS ONE*, 2013, **8**.
31 R. Bono, V. Romanazzi, A. Munnia, S. Piro, A. Allione, F. Ricceri, S. Guarrera, C. Pignata, G. Matullo, P. Wang, R. W. Giese and M. Peluso, *Chem. Res. Toxicol.*, 2010, **23**, 1342–1348.
32 R. Bono, V. Romanazzi, V. Pirro, R. Degan, C. Pignata, E. Suppo, M. Pazzi and M. Vincenti, *Sci. Total Environ.*, 2012, **414**, 701–707.
33 F. Blum, 1893, 314–315.
34 G. Bussolati, L. Annaratone, E. Medico, G. D'Armento and A. Sapino, *PloS One*, 2011, **6**, e21043.
35 F. Veglia, S. Loft, G. Matullo, M. Peluso, A. Munnia, F. Perera, D. H. Phillips, D. Tang, H. Autrup, O. Raaschou-Nielsen, A. Tjønneland, P. Vineis and Genair-EPIC Investigators, *Carcinogenesis*, 2008, **29**, 932–936.

FA total (number)	Subjects NOT using FA during the sampling day	**64**		
	Subjects USING FA during the sampling day	**30**	FA prefilled	**12**
			FA liquid	**18**
Smoking habits (number)	No smokers	**51**		
	Passive smokers	**20**		
	Active smokers	**23**		

	Yes UVS		**No UVS**		***p***
Total number	**38**		**56**		-
	Means ± sd				
Height (number)	**163.1 ± 5.1**		**162.8 ± 6.1**		NS
Weight (number)	**61.9 ± 9.9**		**66.6 ± 16.4**		NS
BMI	**23.3± 3.7**		**25.1± 6.0**		NS
Age (years)	**45 ± 8.6**		**46.2 ± 7.6**		NS
	Absolute (%) frequencies				
Smoking habits	No smokers	**25 (46%)**	No smokers	**26 (66%)**	NS
	Passive smokers	**6 (25%)**	Passive smokers	**14 (16%)**	
	Active smokers	**7 (29%)**	Active smokers	**16 (18%)**	
FA (number)	Subjects using FA **15**		Subjects using FA **15**		-
	Prefilled	5	Prefilled	7	-
	liquid	10	liquid	8	
	Subjects <u>not</u> using FA* **23**		Subjects <u>not</u> using FA* **41**		-

Table 1. Number of subjects according to the use of FA and subjects characteristics (means and standard deviations for variables in interval scales and frequencies absolute and % for variables categoricals) subgrouped by vacuum presence. *on the day of sampling.
Statistical significativity estimated with non parametric statistical tests for two indipent samples
NS = not significant

	FA (µg/m^3)				Non parametric test
			Means ± sd		
A	Personal air-FA in the population n=94)		**20.7** ± 23.3		
	Subjects USING FA (n=64)		**33.7** ± 37.9		*p = 0.032*
	Subjects NOT using FA (n=30)		**14.6** ± 4.6		
B	**UVS**		**NO UVS**		**Non parametric test UVS vs NO UVS**
	All the subjects	**15.5** ± 7.4	All the subjects	**24.2** ± 29.1	*p = 0.040*
	Subjects not using FA	**12.1** ± 2.6	Subjects not using FA	**16.0** ± 4.9	*p = 0.002*
C	Subjects using FA	**20.7** ± 9.3	Subjects using FA	**46.7** ± 50.3	*p = 0.001*
	FA prefilled	**18.4** ± 5.4	FA prefilled	**25.6** ± 5.5	*N.S.*
	FA liquid	**20.9** ± 9.7	FA liquid	**65.0** ± 64.0	*p = 0.001*

Table 2. Personal air-FA (µg/m^3) in the whole population (A), quantified according to the availability of UVS in the surgical theatres (B), to the use of FA on the day of sampling (C), and the robust regression between air-FA and use of UVS subgrouped by the type of container of FA (prefilled or large container). C.I. = confidence interval

log FA	Regression coefficient B	exponential exp(B)	Std. err.	*p*
Prefilled (adj. by UVS)	0.49 [0.32 - 0.67]	1.65	0.08	**0.000**
Liquid (adj. by UVS)	1.06 [0.83-1.29]	2.90	0.11	**0.000**
Personal air-FA with UVS	-0.26 [0.38 - -0.12]	0.77	0.06	**0.000**
Prefilled	-0.15 [-0.60 - 0.30]	0.86	0.22	N.S.
liquid	-0.51 [-0.84 - -0.18]	0.60	0.16	**0.003**

Table 3 The robust regression between air-FA and use of UVS end interaction ((*) between UVS use and the type of container of FA (prefilled or large container). C.I. = confidence interval within square brackets. NB exponentiation of coefficients express the proportion of variation by group.

15-F_{2t}-IsoP (ng/mg CREA)				
Subjects USING FA		**12.9** ± 6.9		$p = 0.001$
Subjects NOT using FA		**3.8** ± 3.1		
UVS		**NO UVS**		
6.3 ± 4.5		**7.0** ± 7.3		$p = 0.027$
Subjects using FA	**10.1** ± 4.3	Subjects using FA	**15.8** ± 8.0	$p = 0.029$
Subjects NOT using FA	**3.7** ± 3.3	Subjects NOT using FA	**3.9** ± 2.7	N.S.
FA prefilled	**6.4** ± 2.2	FA prefilled	**10.9** ± 6.2	N.S.
FA liquid	**11.9** ± 3.9	FA liquid	**20.1** ± 7.1	0.012
MDA (μM)				
Subjects using FA		**1.9** ± 0.7		N.S.
Subjects not using FA		**1.2** ± 0.6		
UVS		**NO UVS**		
1.3 ± 0.8		**1.5** ± 0.6		N.S.
Subjects using FA	**1.7** ± 0.5	Subjects using FA	**2.1** ± 0.9	*N.S.*
Subjects NOT using FA	**1.1** ± 0.5	Subjects NOT using FA	**1.3** ± 0.6	N.S.
FA prefilled	**1.4** ± 0.3	FA prefilled	**1.5** ± 0.2	N.S.
FA liquid	**1.8** ± 0.4	FA liquid	**2.6** ± 0.9	$p = 0.012$
Cotinine (ng/mg CREA)				
The whole population		**32.8** ± 59.5		
No smokers		**3.1** ± 2.0		$p = 0.035$
Passive smokers		**6.2** ± 10.7		
Active smokers		**109.0** ± 68.2		
Subjects using FA		**28.9** ± 61.8		N.S.
Subjects not using FA		**34.1** ± 58.8		

Table 4. 15-F_{2t}-Isop, MDA and cotinine subgrouped according the UVS availability, use of FA and smoking exposure (CREA = creatinine). The models were adjusted by age, gender, BMI and cotinine.

independent	Regresion coefficient B	Esponential exp (B)	p
log [MDA]	0.77 [0.38 - 1.17]	2.18	**0.002**
Costant	-2.04 [-3.04 - -1.05]	.13	
log [15-F_{2t}-IsoP]	1.02 [0.66 - 1.38]	2.78	**0.001**
Costant	-1.45 [-2.64 - -2.47]	.23	

Table 5. Robust regression between log-15-F_{2t}-IsoP and MDA as dependent variables and log-FA as independent variable. UVS, cotinine, and age effect were not significant at 5% level. C.I. = confidence interval

NB exponentiation of coefficients express the proportion of variation for unit of variation of log(FA)