IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

15-F2t Isoprostane as biomarker of oxidative stress induced by tobacco smoke and occupational exposition to formaldehyde in workers of plastic laminates

This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/119093 since 2015-07-24T09:10:35Z

Published version:

DOI:10.1016/j.scitotenv.2012.10.057

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

09 November 2026

UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:
Questa è la versione dell'autore dell'opera:
[The Science of the total environment, 442, 2013 , 10.1016/j.scitotenv.2012.10.057]

The definitive version is available at:
La versione definitiva è disponibile alla URL:
[http://www.sciencedirect.com/science/article/pii/S0048969712013393]

15-F_{2t} Isoprostane as biomarker of oxidative stress induced by tobacco smoke and occupational exposition to formaldehyde in workers of plastic laminates

Valeria Romanazzi[a*], Valentina Pirro[b], Valeria Bellisario[a], Giulio Mengozzi[c], Marco Peluso[d], Marco Pazzi[b], Massimiliano Bugiani[a], Giuseppe Verlato[e], and Roberto Bono[a].

[a] Department of Public Health and Pediatric Sciences, University of Torino; Via Santena 5 bis, 10126 Torino; ITALY
[b] Department of Chemistry, University of Torino; Via Pietro Giuria 5, 10126 Torino; ITALY
[c] Clinical Chemistry Laboratory, San Giovanni Battista Hospital; C.so Bramante 88, 10126 Torino; ITALY
[d] Cancer Risk Factor Branch, ISPO-Cancer Prevention and Research Institute; Via Cosimo il Vecchio 2, 50139 Firenze; ITALY
[e] Unit of Epidemiology and Medical Statistics, University of Verona; Strada Le Grazie 8, 37134 Verona; ITALY

***CORRESPONDENCE TO:**

Dr. Valeria Romanazzi, Ph.D.
University of Torino
Department of Environmental Health and Microbiology
Via Santena 5 bis,
10126 Torino - ITALY
Phone: (+39) 011-670-5818
Fax: (+39) 011-236-5818
e-mail: valeria.romanazzi@unito.it

ABSTRACT

Background: Formaldehyde (FA) is a suspected human carcinogen capable of inducing oxidative stress through different metabolic ways. FA may origin from tobacco smoke, several environmental sources, as well as occupational sources, like furnishing industries specialized in the production of pressed-wood and laminate products.

Object: Our aim was to investigate the role of tobacco smoke and occupational exposure to air-FA in the induction of oxidative stress status by comparing FA-exposed with non-exposed subjects who smoked or did not.

Methods: Enrollment of 105 subjects was made in an industry of plastic laminates, including both workers directly exposed to FA and non-exposed office personnel, as control group. 15-F_{2t} Isoprostane (15-F_{2t} IsoP), detected by ELISA technique and urinary cotinine, detected by GC-MS, were used for evaluating oxidative stress and tobacco smoke exposure, respectively. Air-FA levels were detected by GC-MS.

Results: FA concentrations were significantly higher in subjects occupationally exposed than those in controls. Smoking habits and air-FA exposures independently induce the formation of 15-F_{2t} IsoP and increase the oxidative stress level.

Conclusions: Our findings show, for the first time, that 15-F_{2t} IsoP presents a dependency from both the smoking habit and air-FA exposures, and consequently, that these breathable pollutants could be considered as two important independent risk factors in increasing the oxidative stress in human beings.

KEYWORDS

15-F_{2t} Isoprostane, oxidative stress, formaldehyde, tobacco smoke

ABBREVIATION

FA (formaldehyde), air-FA (professional exposure to formaldehyde), ELISA (enzyme-linked immuno-sorbent assay), GC-MS (gas chromatography–mass spectrometry), IsoP (15-F_{2t} Isoprostane), crea (creatinine).

ACKOWLEDGEMENTS

This study was made possible by a grant from Regione Piemonte, Italy (grants 2008). Thanks are given to doctors: Giovanni Monchiero, Davide Bogetti, Piero Corino, (ASL CN2); Massimo Favilla, Elisa Suppo (University of Torino) for their help. The authors kindly thank Mr. Mario Cravero and all the workers (exposed and non-exposed) voluntarily collaborated on this study.

1. INTRODUCTION

Oxidants, anti-oxidants and free radicals often play a useful role in cellular signalling, control of vascular tones, cell generation, and defence against microorganisms. Their formation is the result of an evolutive process. The oxidative balance can be disturbed by several adverse environmental and/or occupational conditions, causing an uncompensated increase of pro-oxidants (Basu, 2010). As a consequence, oxidative modification of cellular macromolecules, induction of cell death by apoptosis or necrosis, damage of structural tissue may occur (Lykkesfeldt, 2007). Cells living under aerobic conditions are continuously exposed to a large number of oxidizing compounds from several endogenous and exogenous sources. Urban air is a typical exogenous mixture of chemical compounds that can induce carcinogenic activity, oxidative stress and toxicity. For example, several pollutants (PM, PAHs, benzene, etc.), emitted from cars (Rusconi et al., 2011) may: (i) inhibit cell-mediated immunity toward infectious agents, (ii) exacerbate respiratory allergy, (iii) cause DNA damage and (iv) induce lung cancer, after a long-term exposure (Rossner et al., 2008a, b). Many epidemiologists related the presence of an oxidative stress status in human beings with traffic emissions and air pollution (Rossner et al., 2011).

Formaldehyde (FA) is a breathable pollutant present in both living and working environments and is considered the prevalent carbonyl specie in urban atmosphere. FA is emitted by several primary sources (Bono et al., 2010a), it is formed in the troposphere by photochemical hydrocarbon-oxidation processes (Correa et al., 2003; Flyvholm and Andersen, 1993; Salthammer et al., 2010), and is also a component of tobacco smoke (Godish, 1989; Uchiyama et al., 2010). Production and use of FA in the manufacture of resins, paints, disinfectants, preservatives, and a variety of other chemicals or industrial products make this chemical compound potentially breathable in several (indoor) working and living environments (Kelly et al., 1999; Zhang et al., 2010b). Thus, FA is

nowadays a relevant topic to be studied in the environmental and occupational health studies (Nielsen and Wolkoff, 2010; Zhang et al., 2010b).

FA can induce local irritations, acute and chronic toxicity, and genotoxic and carcinogenic activity (IARC, 2006; Schmid and Speit, 2007; Speit et al., 2007), as confirmed by an increased incidence of nasopharyngeal cancer in some types of FA-exposed workers (Duhayon et al., 2008; Hauptmann et al., 2004), the relationship reported between FA and leukemia (Zhang et al., 2010a; Zhang et al., 2009), and a significant positive association between FA exposure and childhood asthma (McGwin et al., 2010).

Rager et al. have recently suggested that FA alters the expression of 89 microRNA (miRNA) profiles targeting mRNAs linked to numerous biological pathways, including those involved in the inflammatory response (Rager et al., 2011). In some molecular epidemiology studies, oxidative properties of FA have been reported in rats and humans (Bono et al., 2010b). Different metabolic pathways, i.e., the production of FA detoxifying enzymes after FA exposure, appeared to be involved. (Im et al., 2006; Kum et al., 2007). F_2-isoprostanes (F_2-IsoPs) are prostaglandin-like bioactive compounds formed *in vivo* from the free radical-catalyzed peroxidation of essential fatty acids. F2-IsoPs are stable, robust molecules and are detectable in all human tissues and biological fluids analyzed, including plasma, urine, broncho-alveolar lavage fluid, cerebrospinal fluid, and bile. Based on their mechanism of formation, four F_2-IsoP regioisomers are generated. Compounds are denoted as 5-, 12-, 8-, or 15-series regioisomers depending on the carbon atom to which the side chain hydroxyl is attached; thus the compound under investigation belongs to the last regioisomer. Moreover, "*2t*" is for the – *trans* position of the oriented side chain to the prostane ring in the 15-F_{2t}-IsoP (Roberts and Milne, 2009).

The metabolic fate of 15-F2t-IsoP in humans has been assessed in previous studies (Mitsumoto et al., 2008; Morrow et al., 1999; Roberts and Morrow, 2000). Findings of these studies show the usefulness of 15-F_{2t}-IsoP to assess oxidant stress in vivo both in animal models and in humans.

In fact, they appear to be related to quite a number of human diseases, although a clear correlation between those pathological conditions and an oxidative stress status is far from being proven (Giustarini et al., 2009). Moreover, F_2-IsoPs seem to play a role in acute and sub-clinical chronic inflammations (Basu et al., 2009). Since F_2-IsoPs can be detected in urine samples, which sampling is possible with a non-invasive procedure, they have been proposed as a suitable biomarker for airways inflammation (Basu, 2008) and asthma diagnosis (Wedes et al., 2009), with the scientific community agreement.

Considering that FA and tobacco smoke have toxic and cancerogenic activities in different biological districts and may play a role in the onset of oxidative stress (Bono et al., 2010b; Campos et al., 2011), the potentiality of urinary 15-F_{2t} IsoP as indicator of oxidative stress induced by FA and tobacco smoke was investigated in this study, for the first time.

Healthy subjects, working in one of the world's leading manufacturers of decorative laminate located in the north-western part of Italy, were enrolled as volunteers. Workers exposed to FA and non-exposed office personnel, as control group, were statistically compared. To evaluate the FA exposure, air-FA was quantified, while tobacco exposure was measured by urinary cotinine, a metabolite of nicotine.

2. MATERIAL AND METHODS

2.1 Epidemiological sample.

51 healthy male workers of an industry of decorative laminates were recruited as subjects potentially exposed to FA. The decorative laminate sheeting is made of melamine and phenolic resins reacting with aldehydes during the thermosetting process. The resins are laminated onto layers of kraft paper topped with a decorative sheet. Other 54 male subjects were enrolled as controls from some offices and laboratories of the National Health System, where FA was not used.

160 All the subjects involved in this study live and work in Bra (town located in Piedmont region, Italy,
161 counting 28.000 inhabitants, 250 meters a.s.l.) or in its immediate surroundings. Only males were
162 selected for this epidemiological investigation, since gender is actually debated as confounding
163 factor in the 15-F_{2t} IsoP formation, moreover only male workers are usually employed in plastic
164 laminate industries. All subjects were informed about the objective of the study and gave a written
165 informed consent, voluntarily.
166 All samplings were executed during summer. For each subject, air-FA samples were passively
167 collected for an entire working shift (i.e., from 6 a.m. to 2 p.m.; about 8 h) in the middle of the
168 working week (Wednesday). A spot of the first urine in the morning was also collected for urinary
169 cotinine and 15-F_{2t} IsoP determinations.
170
171 *2.2 Questionnaire.*
172 At the end of the working shift, a questionnaire was administered to each subject to acquire
173 information about individual and clinical features (age, place of residence, hobbies, and therapies),
174 smoking habits, profession (qualifications, seniority, and job-specific work), the presence and use of
175 environmental and personal devices to prevent air exposure and health risks. More in detail, the
176 description of smoking habits for all subjects was established *a-priori*. Both subjects who never
177 smoked and smokers who had ceased smoking from at least 1 month were classified as "non-
178 smokers", while subjects who smoked at least one cigarette per day were classified as "smokers".
179
180 *2.3 Air-FA sampling and analysis.*
181 FA air sample was collected from each subject for the whole working shift (8 h) using a passive,
182 personal air sampler working with radial symmetry (Radiello®), clipped near the breathing zone of
183 the subject. Samplers were equipped with a specific sorbent tube containing 35–50 florisil mesh
184 coated with 2,4-dinitrophenylhydrazine (DNPH). DNPH reacts with FA yielding 2,4-

dinitrophenylhydrazone which was subsequently quantified by a GC-MS method, within the following 10 days. Cartridges were stored at -80°C before GC-MS analysis. Each sample was eluted with 3 ml of toluene and shaken at room temperature for 15 min. An aliquot was transferred into a vial and then injected in a capillary Agilent Technologies 6890 gas chromatograph, interfaced to a 5973 MSD Inert Agilent single quadrupole mass spectrometer. A Gerstel CIS4 PTV injection system utilized an initial temperature of 65 °C followed by heating at 5 °C/s; with a final temperature of 320 °C, held for 10 min. The injection volume was 2 µl in splitless mode. The capillary column used was a HP-5MS 30m×0.25mm×0.25µm film thickness. Initial column temperature was 70°C, and increased at 20°C/min up to 220°C and at 30°C/min up to 300°C. The carrier gas was ultrapure He (1.0 ml/min). The transfer-line temperature was set at 280°C. The mass spectrometer operated in electron impact and Selected Ion Monitoring (SIM) mode. The monitored *m/z* values for FA were 63, 79, 180 and 210, while the ones for the internal standard (isovaleraldehyde-DNPH) were 177, 206, 223 and 166. The FA calibration curve was built by fortifying 3 ml of toluene so as to obtain a concentration range from 0.10 µg/ml to 10 µg/ml. The fortified toluene was analyzed as for the samples.

The limit of detection (LOD) was calculated as the concentration of the analyte that gives a signal equal to the average background of the blank (Sblank) plus three times its standard deviation (LOD = Sblank + 3*SD Sblank), while the limit of quantification (LOQ) was estimated as twice of the LOD value.

LOD and LOQ were respectively 0.05 µg/ml and 0.10 µg/ml. Coefficients of variation (CV%) calculated to test repeatability were below 5%.

2.4 Urine: collection and analyses.

208 Two aliquots of fresh urine were collected in the early morning and approximately at the same time
209 from each volunteer, and stored at −80 °C prior to analysis, performed within 20 working days. One
210 aliquot of urine was used for cotinine quantification, the other one for 15-F_{2t} IsoP determination.
211 First, urinary creatinine (crea) was determined by the kinetic Jaffé procedure (Bartels and Cikes,
212 1969) so as to normalize the excretion rate of urinary cotinine and 15-F2t IsoP.
213 Urinary cotinine was measured in order to consider the possible role played by tobacco smoke for
214 the subjects under study. 10 ml of urine was transferred into a glass tube and 4 g of NaCl, 500 µl of
215 NaOH (5M) and 10 µl of cotinine-d3 (internal standard) were added. Subsequently, for two times, 2
216 ml of trichloromethane ($CHCl_3$) were added to the sample to perform liquid–liquid extraction which
217 was carried out in a shaking wheel for 15 min. Sample was then centrifuged for 10 min at 1000 g
218 and the resulting organic phase was collected in a new glass tube and evaporated to dryness in a
219 rotary evaporator at room temperature. The dry residue was reconstitute in 200 µl of $CHCl_3$ and
220 transferred into a conical vial for GC-MS determination (Bono et al., 2005). GC-MS analysis was
221 performed using an Agilent Technologies 6890 gas chromatograph, interfaced to a 5973 MSD Inert
222 Agilent mass spectrometer. A Gerstel CIS4 PTV injection system utilized an initial temperature of
223 50 °C followed by heating at 10 °C/s; with a final temperature of 300 °C, held for 10 min. The
224 injection volume was 1 µl in the split mode. The capillary column used was a HP-5MS
225 30m×0.25mm×0.25 µm film thickness. The initial column temperature was 50 °C, increased at 15
226 °C/min up to 300 °C. The carrier gas was ultrapure Helium (1.0 ml/min). The transfer-line
227 temperature was set at 280 °C. The mass spectrometer operated in electron impact and SIM mode.
228 The monitored *m/z* values for cotinine were: 98, 118, 176; while the ones for the internal standard
229 were 101, 121, 179.
230 The cotinine calibration curve was built by fortifying a blank urine pool of non-smoking subjects, to
231 obtain a concentration range from 0.02 µg/ml to 2 µg/ml. The fortified urine was extracted as for
232 the samples. LOD and LOQ (calculated as previously described) were respectively 0.01 µg/ml and

0.02 µg/ml. Coefficients of variation calculated to test repeatability were below 5% for both cotinine and the internal standard.

15-F_{2t} IsoP in urine was measured by ELISA technique performed with a specific microplate kit (Oxford, MI, USA), according to manufacturer's instructions. The declared limit of detection is 0.2 ng/ml and cross-reactivity can likely occur for some other isoprostanes: prostaglandin E_2, prostaglandin D_2, and arachidonic acid (< 0.01%), 9α,11β prostaglandin $F_{2\alpha}$ (4.1%), 13,14 dihydro-15-keto-$PGF_{2\alpha}$ (3.0%) and. Dilution 1:4 was adopted to achieve better accuracy in the competitive ELISA method.

Because of the high percentage of 15-F_{2t} 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-F_{2t} IsoP present in each urine sample.

2.5 Statistical analysis.

The analysis was performed by means of Stata 12 Statistical Package (StataCorp LP, Lakeway Drive, TX, USA). Table 1 shows some general epidemiological aspects of the resulting groups of volunteers (according to their FA exposure and smoking habits).

Where suggested by distributional diagnostic plots (symmetry plot, quantile plot) and descriptive statistic inspection (looking at variance stability among categories), appropriate linear transformation was applied on data. Untransformed 15-F_{2t} IsoP , urinary cotinine and air-FA values were compared among smoking and exposure categories by means of Nonparametric equality-of-medians test.

Multiple regression analysis with robust standard error estimation was performed to assess the relationship between urinary 15-F_{2t} IsoP values with urinary cotinine and air-FA concentrations. For each category, means and 95% confidence intervals of transformed 15-F_{2t} IsoP values were

calculated as margins of responses from predictions of the complete fitted model integrating over the covariates.

3. RESULTS

As reported in Table 1, subjects were classified according to their professional exposure to FA (exposed and non-exposed), and their smoking habits (smokers and non-smokers). As reported above, gender was not considered for describing the epidemiological sample population. Among the non-exposed subjects, 14 out of 54 are smokers (26%), while the percentage of smokers into the FA-exposed population grows up to 47%. The difference between the number of smokers into the FA-exposed and non-exposed categories appears to be statistically significant.

Means and standard deviations for age, urinary cotinine, urinary 15-F_{2t} IsoP and air-FA levels are reported in Table 2. Both the overall and single category values are reported.

From Table 2 outcomes, it is worth noting that the mean age of studied groups were comparable, and any statistical differences were recorded excluding "age of the subjects" as possible confounding factor. Considering the air-FA concentrations (ranged from 49 to 444 µg/m^3 for FA-exposed subjects, and from 16 to 110 µg/m^3 for the control group), three FA-exposed workers presented air-FA concentrations greater than 370 µg/m^3, namely the absolute exposure limit that should not be exceeded at any time (TLV-ceiling), suggested by the American Conference Governmental Industrial Hygienists (ACGIH). Moreover, the air-FA concentrations for the FA-exposed workers appear to be significantly higher at 5% probability than those for the non-exposed subjects (Fisher exact p <0,0001). Conversely, urinary cotinine concentrations appear to be significantly higher for smokers than those for non-smokers (Fisher exact p <0,0001). Finally, considered the four kind of exposures, 15-F_{2t} IsoP levels appear to be higher for FA-exposed workers and smokers than those for non-exposed subjects and non-smokers, respectively; instead,

282 15-F_{2t} IsoP in the two groups having only one exposure shows intermediate mean levels (Fisher
283 exact p <0,0001).
284 Due to non-normal distribution and heteroskedasticity (the variance increases linearly with the
285 mean), for further analysis appropriate log-transformation was applied to 15-F_{2t} IsoP levels, air-FA
286 and cotinine concentrations, in order to stabilize the variance and normalize the distributions.
287 Table 3 shows the outcomes for the linear regression analyses performed considering the
288 transformed 15-F_{2t} IsoP levels *versus* transformed air-FA and urinary cotinine concentrations, first
289 singly (Mod.1 and Mod.2, respectively), and then in association between them (Mod.3).
290 Despite the higher air-FA values for smokers than those for non-smokers, there are no significant
291 linear relationship between urinary cotinine and air-FA concentrations (p=0.24; R=0.04; B= 0.018;
292 C.I. 95% = -0.054, 0.09). The standardized regression coefficient (Beta) show the smoking
293 contribution to IsoP biosynthesis as equal in subjects exposed and not exposed to FA; in particular,
294 each increase of one S.D. unit both for Log-FA and Log-cotinine induces an Log-IsoP increase of
295 approximately 35%.
296 15-F_{2t} IsoP levels increase significantly with both air-FA and urinary cotinine concentrations. The
297 complete model confirms the multivariate relationship without substantial confounding effects. No
298 age main effect significant at 5% level and no significant at 5% level interactions were detected.
299 Table 4 reports the means and 95% confidence intervals of 15-F_{2t} IsoP levels for all the studied
300 categories, as estimated by the multiple regression analysis. The smoking effect is not significantly
301 different from the FA exposure effect. In addition, the two effects seem to be less than additive in
302 logarithmic scale (2.3 *versus* 3.3 expected, p <0.05).
303 Figure 1 shows the added-variable plots - or partial regression plot – in which the effect of adding
304 one variable (FA or tobacco smoke exposure) to the model containing the other one can be
305 evaluated. Although both regression lines are significantly different from 0, the residuals are very
306 scattered around them, suggesting (as confirmed by the low R^2 value) that the model is able to

307 predict only part (20%) of the large 15-F_{2t} IsoP variability. Evident outliers (influential position or
308 evident lack of residual normality distribution) from the regression lines are not evident.
309
310 ## 4. DISCUSSION
311
312 In this study we investigated the relationship occurring between human exposure to FA and
313 oxidative stress - measured by urinary 15-F_{2t} IsoP - in a population of male subjects. These subjects
314 were recruited in a laminate industry in Piedmont region (Italy) and information about FA and
315 tobacco smoke exposures was collected. They were classified as FA-exposed and non-exposed
316 (control group) subjects and as smokers or non-smokers too.
317 If compared with controls, FA-exposed workers reported significantly higher concentrations of air-
318 FA, although the mean concentration was lower than the ACGIH limit (370 µg/m^3) (Table 2). Good
319 environmental work conditions and health status suggest that these high values of air-FA could
320 essentially depend on improper work behaviour by those workers, rather than improper working
321 conditions. On the other hand, non-exposed subjects presented an average air-FA concentration
322 equal to 40 µg/m^3 (Table 2) and 10% of these subjects even exceed 70 µg/m^3 concentration,
323 suggesting that other non-controlled domestic or environmental FA exposures may influence the
324 results.
325 As previously mentioned, urinary 15-F_{2t} IsoP were investigated as indicators of an oxidative stress
326 status due to FA and tobacco smoke exposure. The presence of aldehydes by lipid peroxydation
327 (LPO) processes is one of the factors related to the "physiological" oxidative stress status (Qujeq et
328 al., 2004; Sohal et al., 2002). FA exposure can increase the oxidative stress status with further
329 release of non-specific cellular membrane inflammation mediators, especially at the upper
330 respiratory tract (Bartsch and Nair, 2006). In the last decade, many experimental evidences
331 suggested that cellular damage, mediated by reactive species, may play a crucial role in the

pathogenesis of respiratory disorders (Wedes et al., 2009; Yang and Omaye, 2009) - that cover a wide range of conditions: from simple acute effects due to tobacco smoke exposure in healthy subjects to chronic damages characterized by interstitial lung destruction (emphysema) or irreversible thickening.

The outcomes of this study globally show the effectiveness of urinary 15-F_{2t} IsoP in describing the *in vivo* exposure to air-FA (Table 3 and Figure 1a). Age usually does not linearly correlate with 15-F_{2t} IsoP levels (Basu et al., 2009) and, within the studied population, this tendency appeared to be confirmed. The influence of tobacco smoke habits on 15-F_{2t} IsoP formation was also reported - as previously described by Campos (Campos et al., 2011) – although it appeared to be independent from the FA exposure effect (Table 3 and Figure 1b). Indeed, no statistically significant interaction was detected between FA and tobacco smoke exposures, at least in the logarithmic scale. In addition to FA, cigarette smoke contains quite a number of different carcinogenic compounds and reactive oxygen species (ROS) - due to tobacco pyrolisis - that potentially can lead to an oxidative stress status (Bhalla et al., 2009; Faux et al., 2009). Furthermore, FA and tobacco smoke exposures seem not to have an additive effect in logarithmic scale (multiplicative in natural scale) on the 15-F_{2t} IsoP formation and not to play a reciprocal confounding effect.

It is important to remark that urinary 15-F_{2t} IsoP is a non-specific oxidative stress indicator, and therefore, other exogenous *stimuli*, other than FA and tobacco smoke, may influence their urinary levels, as the wide dispersion of the residuals around the regression line (Figure 1 a and b) and by the relatively low values of determination coefficient (<0,20 in the complete model) seem to suggest.

The means of 15-F_{2t} IsoP, as predicted by the multiple regression model (as shown in Table 4), are similar for FA-exposed/non-smokers and non-exposed/smokers, but the means of 15-F_{2t} IsoP for subjects exposed both to FA and tobacco smoke (FA-exposed/smokers) are less than expected for the additive model.

5. CONCLUSION

In this study, urinary 15-F_{2t} IsoP levels were measured as indicator of oxidative stress status induced by FA and tobacco smoke exposures. Several endogenous/biological and exogenous/environmental or occupational factors, such as FA and tobacco smoke exposures - here investigated - affect the onset of oxidative stress and probably act synergistically in different ways. In this study a relationship between urinary 15-F_{2t} IsoP levels with FA and urinary cotinine concentrations has been reported, suggesting that primary prevention towards professional (and environmental) exposure to FA and tobacco smoke plays an important role for health care, as well as primary prevention towards several pathogenic conditions.

Finally, an enzyme-linked immunoassay procedure was adopted and proved to be appropriate for the purpose of this study. Comparison of the ELISA procedure with a GC-NCI-MS protocol for 15-F_{2t} IsoP determination can be considered as a perspective goal.

6. REFERENCES

Bartels H, Cikes M. Chromogens in the creatinine determination of Jaffè. Clin Chim Acta 1969 26(1): 1-10.

Bartsch H, Nair J. Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair. Langenbecks Archives of Surgery 2006 391(5): 499-510.

Basu S. F-2-isoprostanes in human health and diseases: From molecular mechanisms to clinical implications. Antioxidants & Redox Signaling 2008 10(8): 1405-1434.

Basu S. Fatty acid oxidation and isoprostanes: oxidative strain and oxidative stress. Prostaglandins Leukot Essent Fatty Acids 2010 82(4-6): 219-225.

Basu S, Helmersson J, Jarosinska D, Sallsten G, Mazzolai B, Barregard L. Regulatory factors of basal F2-isoprostane formation: Population, age, gender and smoking habits in humans. Free Rad Res 2009 43(1): 85-91.

Bhalla DK, Hirata F, Rishi AK, Gairola CG. Cigarette smoke, inflammation, and lung injury: a mechanistic perspective. J Toxicol Environ Health B Crit Rev 2009 12(1): 45-64.

Bono R, Degan R, Pazzi M, Romanazzi V, Rovere R. Benzene and formaldehyde in air of two Winter Olympic venues of "Torino 2006". Environ Int 2010a 36(3): 269-275.

Bono R, Romanazzi V, Munnia A, Piro S, Allione A, Ricceri F, Guarrera S, Pignata C, Matullo G, Wang P, Giese RW, Peluso M. Malondialdehyde-deoxyguanosine adduct formation in workers of pathology wards: the role of air formaldehyde exposure. Chem Res Toxicol 2010b 23(8): 1342-1348.

Bono R, Vincenti M, Schiliro T, Traversi D, Pignata C, Scursatone E, Dotti G, Gilli G. Cotinine and N-(2-hydroxyethyl)valine as markers of passive exposure to tobacco smoke in children. J Expo Anal Environ Epidemiol 2005 15(1): 66-73.

Campos C, Guzman R, Lopez-Fernandez E, Casado A. Urinary biomarkers of oxidative/nitrosative stress in healthy smokers. Inhal Toxicol 2011 23(3): 148-156.

Correa SM, Martins EM, Arbilla G. Formaldehyde and acetaldehyde in a high traffic street of Rio de Janeiro, Brazil. Atmospheric Environment 2003 37(1): 23-29.

Duhayon S, Hoet P, Van Maele-Fabry G, Lison D. Carcinogenic potential of formaldehyde in occupational settings: a critical assessment and possible impact on occupational exposure levels. Int Arch Occup Environ Health 2008 81(6): 695-710.

Faux SP, Tai T, Thorne D, Xu Y, Breheny D, Gaca M. The role of oxidative stress in the biological responses of lung epithelial cells to cigarette smoke. Biomarkers 2009 14 Suppl 1: 90-96.

Flyvholm MA, Andersen P. Identification of formaldehyde releasers and occurrence of formaldehyde and formaldehyde releasers in registered chemical products. Am J Ind Med 1993 24(5): 533-552.

Giustarini D, Dalle-Donne I, Tsikas D, Rossi R. Oxidative stress and human diseases: Origin, link, measurement, mechanisms, and biomarkers. Crit Rev Clin Lab Sci 2009 46(5-6): 241-281.

Godish T. Formaldehyde exposures from tobacco smoke: a review. Am J Public Health 1989 79(8): 1044-1045.

Hauptmann M, Lubin JH, Stewart PA, Hayes RB, Blair A. Mortality from solid cancers among workers in formaldehyde industries. Am J Epidemiol 2004 159(12): 1117-1130.

IARC, ed. 2006. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. 2007/03/21 ed. Lyon, France: International Agency for Research on Cancer.

Im H, Oh E, Mun J, Khim JY, Lee E, Kang HS, Kim E, Kim H, Won NH, Kim YH, Jung WW, Sul D. Evaluation of toxicological monitoring markers using proteomic analysis in rats exposed to formaldehyde. Journal of Proteome Research 2006 5(6): 1354-1366.

Kelly TJ, Smith DL, Satola J. Emission rates of formaldehyde from materials and consumer products found in California homes. Environmental Science & Technology 1999 33(1): 81-88.

Kum C, Kiral F, Sekkin S, Seyrek K, Boyacioglu M. Effects of xylene and formaldehyde inhalations on oxidative stress in adult and developing rats livers. Experimental Animals 2007 56(1): 35-42.

Lykkesfeldt J. Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin Chim Acta 2007 380(1-2): 50-58.

McGwin G, Lienert J, Kennedy JI. Formaldehyde exposure and asthma in children: a systematic review. Environ Health Perspect 2010 118(3): 313-317.

Mitsumoto H, Santella RM, Liu XH, Bogdanov M, Zipprich J, Wu HC, Mahata J, Kilty M, Bednarz K, Bell D, Gordon PH, Hornig M, Mehrazin M, Naini A, Beal MF, Factor-Litvak P. Oxidative stress biomarkers in sporadic ALS. Amyotrophic Lateral Sclerosis 2008 9(3): 177-183.

Morrow JD, Zackert WE, Yang JP, Kurhts EH, Callewaert D, Dworski R, Kanai K, Taber DF, Moore K, Oates JA, Roberts LJ. Quantification of the major urinary metabolite of 15-F-2t-isoprostane (8-iso-PGF(2 alpha)) by a stable isotope dilution mass spectrometric assay. Analytical Biochemistry 1999 269(2): 326-331.

Nielsen GD, Wolkoff P. Cancer effects of formaldehyde: a proposal for an indoor air guideline value. Arch Toxicol 2010 84(6): 423-446.

Qujeq D, Aliakbarpour HR, Kalavi K. Relationship between malondialdehyde level and glutathione peroxidase activity in diabetic rats. Clin Chim Acta 2004 340(1-2): 79-83.

Rager JE, Smeester L, Jaspers I, Sexton KG, Fry RC. Epigenetic Changes Induced by Air Toxics: Formaldehyde Exposure Alters miRNA Expression Profiles in Human Lung Cells. Environ Health Perspect 2011 119(4): 494-500.

Roberts LJ, 2nd, Milne GL. Isoprostanes. J Lipid Res 2009 50 Suppl: S219-223.

Roberts LJ, Morrow JD. Measurement of F-2-isoprostanes as an index of oxidative stress in vivo. Free Radical Biology and Medicine 2000 28(4): 505-513.

Rossner P, Jr., Rossnerova A, Sram RJ. Oxidative stress and chromosomal aberrations in an environmentally exposed population. Mutat Res 2011 707(1-2): 34-41.

Rossner P, Jr., Svecova V, Milcova A, Lnenickova Z, Solansky I, Sram RJ. Seasonal variability of oxidative stress markers in city bus drivers. Part I. Oxidative damage to DNA. Mutat Res 2008a 642(1-2): 14-20.

Rossner P, Jr., Svecova V, Milcova A, Lnenickova Z, Solansky I, Sram RJ. Seasonal variability of oxidative stress markers in city bus drivers. Part II. Oxidative damage to lipids and proteins. Mutat Res 2008b 642(1-2): 21-27.

Rusconi F, Catelan D, Accetta G, Peluso M, Pistelli R, Barbone F, Di Felice E, Munnia A, Murgia P, Paladini L, Serci A, Biggeri A. Asthma Symptoms, Lung Function, and Markers of Oxidative Stress and Inflammation in Children Exposed to Oil Refinery Pollution. Journal of Asthma 2011 48(1): 84-90.

Salthammer T, Mentese S, Marutzky R. Formaldehyde in the indoor environment. Chem Rev 2010 110(4): 2536-2572.

Schmid O, Speit G. Genotoxic effects induced by formaldehyde in human blood and implications for the interpretation of biomonitoring studies. Mutagenesis 2007 22(1): 69-74.

Sohal RS, Mockett RJ, Orr WC. Mechanisms of aging: an appraisal of the oxidative stress hypothesis. Free Radic Biol Med 2002 33(5): 575-586.

Speit G, Schutz P, Hogel J, Schmid O. Characterization of the genotoxic potential of formaldehyde in V79 cells. Mutagenesis 2007 22(6): 387-394.

Uchiyama S, Inaba Y, Kunugita N. Determination of acrolein and other carbonyls in cigarette smoke using coupled silica cartridges impregnated with hydroquinone and 2,4-dinitrophenylhydrazine. J Chromatogr A 2010 1217(26): 4383-4388.

Wedes SH, Khatri SB, Zhang R, Wu W, Comhair SA, Wenzel S, Teague WG, Israel E, Erzurum SC, Hazen SL. Noninvasive markers of airway inflammation in asthma. Clin Transl Sci 2009 2(2): 112-117.

Yang W, Omaye ST. Air pollutants, oxidative stress and human health. Mutat Res 2009 674(1-2): 45-54.

Zhang L, Freeman LE, Nakamura J, Hecht SS, Vandenberg JJ, Smith MT, Sonawane BR. Formaldehyde and leukemia: epidemiology, potential mechanisms, and implications for risk assessment. Environ Mol Mutagen 2010a 51(3): 181-191.

Zhang L, Steinmaus C, Eastmond DA, Xin XK, Smith MT. Formaldehyde exposure and leukemia: a new meta-analysis and potential mechanisms. Mutat Res 2009 681(2-3): 150-168.

Zhang L, Tang X, Rothman N, Vermeulen R, Ji Z, Shen M, Qiu C, Guo W, Liu S, Reiss B, Freeman LB, Ge Y, Hubbard AE, Hua M, Blair A, Galvan N, Ruan X, Alter BP, Xin KX, Li S, Moore LE, Kim S, Xie Y, Hayes RB, Azuma M, Hauptmann M, Xiong J, Stewart P, Li L, Rappaport SM, Huang H, Fraumeni JF, Jr., Smith MT, Lan Q. Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. Cancer Epidemiol Biomarkers Prev 2010b 19(1): 80-88.

Caption Figure 1: Partial regression plot: regression of Log (15-F2t IsoP) as dependent *vs.* (a) Log (Air-Fa) and (b) Log (Cotinine) as predictive variables.