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## Wood dust and urinary 15-F2t isoprostane in Italian industry workers

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1 **Wood dust and urinary 15-F<sub>2t</sub> isoprostane in Italian industry workers**

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18

19 **Abstract**

20 Wood dust is one of the most common occupational exposures, with about 3.6 million of workers in  
21 the wood industry in Europe. Wood particles can deposit in the nose and the respiratory tract and  
22 cause adverse health effects. Occupational exposure to wood dust has been associated with  
23 malignant tumors of the nasal cavity and paranasal sinuses. The induction of oxidative stress and  
24 the generation of reactive oxygen species through activation of inflammatory cells could have a  
25 role in the carcinogenicity of respirable wood dust. Therefore, we conducted a cross-sectional  
26 study to evaluate the prevalence of urinary 15-F<sub>2t</sub> isoprostane (15-F<sub>2t</sub>-IsoP), a biomarker of  
27 oxidative stress and peroxidation of lipids, in 123 wood workers compared to 57 unexposed  
28 controls living in Tuscany region, Italy. 15-F<sub>2t</sub>-IsoP generation was measured by ELISA. The main  
29 result of the present study showed that a statistically significant excess of this biomarker occurred  
30 in the workers exposed to 1.48 mg/m<sup>3</sup> of airborne wood dust with respect to the unexposed  
31 controls (0.05 mg/m<sup>3</sup>). The overall mean ratio (MR) between the workers exposed to wood dust  
32 and the controls was 1.36, 95% Confidence Interval (C.I.) 1.18–1.57, after correction for age and  
33 smoking habits. A significant increment of 15-F<sub>2t</sub>-IsoP (43%) was observed in the smokers as  
34 compared to the non-smokers. The urinary excretion of 15-F<sub>2t</sub>-IsoP was significantly associated  
35 with co-exposure to organic solvents and formaldehyde, i.e., MR of 1.41, 95% C.I. 1.17-1.70, after  
36 adjustment for age and smoking habits. A 41% excess was observed in long-term wood workers,  
37 95% C.I. 1.14-1.75. Multivariate regression analysis showed that the level of 15-F<sub>2t</sub>-IsoP was  
38 linearly correlated to the length of exposure, regression coefficient ( $\beta$ ) = 0.244 ± 0.002 (SE). The  
39 overall increment by exposure group persisted after stratification for smoking habits. For instance,  
40 in smokers, a 53% excess was detected in the wood workers as compared to the controls, 95%  
41 C.I. 1.23-1.91. Our data support the hypothesis that oxidative stress and lipid peroxidation can  
42 have a role in the toxicity of wood dust F<sub>2</sub>-IsoP measure can be a tool for the evaluation of the  
43 effectiveness of targeted interventions aimed to reduce exposures to environmental carcinogens.

44 **Key words:** wood dust, organic solvents, formaldehyde, 15-F<sub>2t</sub> isoprostane, primary prevention,  
45 occupational health.

46

## 47 1. Introduction

48 Wood dust is one of the most common occupational exposures, with about 3.6 million of workers in  
49 the wood industry in Europe (Kauppinen et al., 2006). Wood particles can deposit in the nose and  
50 the respiratory tract and cause adverse health effects (Çelik and Kanık, 2006). Epidemiological  
51 studies have indeed associated the exposure to wood dust to sinonasal cancers (SNC) (Acheson  
52 et al., 1968; Ball, 1968). In 1960, the first association with SNC was shown in the wood industry  
53 (Acheson et al., 1968). In 1995, this agent was classified as carcinogenic to humans (Group 1) by  
54 the International Agency for Research on Cancer (IARC) based mostly on a SNC excess (IARC,  
55 1995). In 2012, the IARC confirmed the human carcinogenicity of wood dust and reported the first  
56 link with nasopharynx cancer (IARC, 2012). Considering other types of cancer, a meta-analysis  
57 has suggested a relationship with lung cancer (Hancock et al., 2015), but a significant influence of  
58 the geographic region was apparent.

59 SNC has been under compulsory surveillance since 2008 in Italy, through the “Sinonasal Cancer  
60 National Registry” (Registro Nazionale Tumori Naso-Sinusali: ReNaTuNS), a nationwide cancer  
61 registry coordinated by the National Institute for Insurance Against Accidents at Work (Istituto  
62 Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro: INAIL) (Binazzi et al., 2017).  
63 Currently, the registry covers a proportion of Italy through regional structures devoted to the active  
64 search for cases from hospitals, to the definition of the modalities of exposure and has recorded  
65 1,529 cases between 2000-2016. A study conducted by Demers et al. (Demers et al., 1995) found  
66 a doubled risk statistically significant for sinonasal cancer in men employed in any wood-related job  
67 (OR = 2.0, 95% CI: 1.6 to 2.5) in comparison to men who had never worked in a wood-related job.  
68 The increased risk was found among sawmill workers (OR = 2.5, 95% CI: 1.8 to 3.4), furniture  
69 workers (OR = 4.5, 95% CI: 3.2 to 6.5) and carpenters (OR = 2.9, 95% CI: 2.1 to 3.9), while no  
70 excess risk was observed among forestry, logging, pulp and paper workers. An increasing risk was  
71 detected in relation to the duration of exposure, and lagging exposure by 5, 10 or 20 years  
72 increased the strength of the association between duration of employment and sinonasal  
73 adenocarcinoma. Elevated risk for adenocarcinoma of the nasal cavity and paranasal sinuses  
74 (ADCN), a SNC subtype frequently associated with wood dust exposure (IARC, 2012), OR 58.6,  
75 95% C.I. 23.74-144.8, was even reported among wood workers of the Piedmont region, Italy  
76 (d'Errico et al., 2009). Stronger ADCN risk, OR 179.9, 95% C.I. 55.37-584.4, was found among  
77 those workers exposed to high level of wood dust (d'Errico et al., 2009).

78 Higher levels of oxidative damage, measured by the micronucleus and the comet assays in blood,  
79 buccal and nasal cells, have been detected in wood workers compared to unexposed controls  
80 (Bruschweiler et al., 2016; Palus et al., 1999; Rekhadevi et al., 2009). An enhanced risk for  
81 chromosomal instability was found in wood workers (Bruschweiler et al., 2014; Çelik and Kanık,  
82 2006; Rekhadevi et al., 2009). Discrepant results have been reported (Wultsch et al., 2015). In that  
83 study, no induction of micronuclei was observed in wood workers exposed to 0.39-0.66 mg/m<sup>3</sup>  
84 wood dust levels. Thus, further investigation into wood workers' occupational exposures are  
85 warranted. Furthermore, co-exposures to chrome, organic solvents, tannins, formaldehyde, textile  
86 dust and pesticides have been reported in the wood industry (Binazzi et al., 2017). In 2012, IARC  
87 suggested that the cancer risk of wood workers could be associated with the inflammatory  
88 reactions following wood dust exposure rather than to the direct action of this carcinogen (IARC,  
89 2012). Inflammatory cells can generate a large spectrum of proinflammatory mediators and free  
90 radicals (Pylkkänen et al., 2009). Excessive production of reactive oxygen species (ROS) can  
91 cause damage to lipids, proteins and DNA (Marnett, 2000). Peroxidation of lipids (LPO) can lead to  
92 the production of aldehydes, such as malondialdehyde and 4-hydroxynonenal (Marnett, 2000), as  
93 well as to secondary oxidation products such as a series of prostaglandin-like products termed  
94 isoprostanes (IsoPs) (Roberts and Morrow, 2000).  
95 IsoPs are compounds generated from the non-enzymatic free radical-catalyzed peroxidation of  
96 arachidonic acid and other highly unsaturated polyunsaturated fatty acids (Janicka et al., 2010).  
97 IsoPs can be grouped into 4 subfamilies, denoted as 5-, 12-, 8-, or 15-series regioisomers,  
98 depending on the carbon atom to which the side chain hydroxyl is attached. Among the three major  
99 classes of IsoPs (F<sub>2</sub>-, D<sub>2</sub>- and E<sub>2</sub>-), F<sub>2</sub>-IsoPs are recognized as the most suitable biomarker for

100 their chemical stability (Roberts and Morrow, 2000). The measurement of this biomarker is widely  
101 used for the analysis of endogenous oxidative stress following ROS production and peroxidation of  
102 lipids (Basu, 2008). F<sub>2</sub>-IsoPs are more advantageous over other LPO biomarkers because they  
103 can be detected in a variety of biological samples including plasma, urine, lavage fluid and red  
104 blood cells (Milne et al., 2015). As IsoPs generate from LPO, their amounts provide an integrated  
105 measurement of unbalanced oxidant-antioxidant status (Lowe et al., 2013; Montuschi et al., 2004).

106 In the current study, we have investigated the potential effects of occupational exposure to wood  
107 dust in the wood product manufacturing sector in the Tuscany Region of Italy. A cross-sectional  
108 study was conducted to analyze the concentration of a biomarker of oxidative stress and LPO (15-  
109 F<sub>2t</sub>-IsoP) in the workers exposed to wood dust. One of the main advantages of using biomarkers is  
110 that one can study signals of carcinogen exposure without having to wait for health effects as in  
111 classical epidemiological studies (Merlo et al., 1997; Munnia et al., 2017; Munnia et al., 2007;  
112 Peluso et al., 1997; Peluso et al., 2012). Although F<sub>2</sub>-IsoP can be evaluated in different biological  
113 fluids, we employed urine due to its ready availability and the high stability of F<sub>2</sub>-IsoP in this  
114 medium (Morrow et al., 1999). Since obesity has been associated with increased F<sub>2</sub>-IsoP  
115 concentrations (Annor et al., 2017; Il'yasova et al., 2015), we have examined the relationships  
116 between urinary F<sub>2</sub>-IsoPs and weight gain. Further understanding of the link between wood dust  
117 and oxidative stress will improve knowledge of the mechanisms of carcinogenicity of this  
118 occupational agent. Novelty of the current study is based on various items, including larger sample  
119 size, a different geographical area, and a different type of data, i.e., the measurement of F<sub>2</sub>-IsoPs  
120 in urine rather than of micronucleus and DNA strand-breaks in blood, buccal and nasal cells.

## 121 2. Material and methods

### 122 2.1 Subjects and sampling

123 A sample of 44 wood companies of the province of Florence, Tuscany, Italy was randomly selected  
124 among those which are under compulsory health surveillance. Wood companies were contacted in  
125 person by medical doctors with qualifications in occupational medicine. The inclusion criteria were  
126 as follows: (a) only workers exposed to wood dust from wood industry; (b) only workers with a  
127 minimal exposure time of 1 year; (c) only controls without occupational history in industries  
128 entailing exposure to known or suspected carcinogens; and (d) only controls resident in areas with  
129 no proximity to major air pollution sources. All the volunteers involved in the study live and work in  
130 the province of Florence, Tuscany, Italy. A 15-F<sub>2t</sub>-IsoP was determined using spot urine samples  
131 collected in the morning at each workplace. Wood workers and the other subjects were contacted  
132 by the local occupational health services. All the volunteers were informed about the study aim and  
133 gave a written informed consent. A life-style questionnaire was filled by each participant (Peluso et  
134 al., 2015). Detailed information on socio-demographic and anthropometric characteristics,  
135 education level, exposure to active and passive tobacco smoke, occupational exposure to wood  
136 dust, protective gear use, co-exposures to organic solvents, welding and motor exhaust fumes and  
137 occupational history were obtained. Subjects who had never smoked were classified as non-  
138 smokers, smokers who had quit smoking from at least one month prior were classified as ex-  
139 smokers, while individuals who smoked at least one cigarette per day were classified as smokers.  
140 The Body Mass Index (BMI) categories reported from the National Heart, Lung, and  
141 Blood Institute (<https://www.nhlbi.nih.gov/>) were used for grouping the study participants in normal  
142 weight persons (18.5-24.9 kg/m<sup>2</sup>), overweight persons (25-25.99 kg/m<sup>2</sup>) and obese persons (≥30  
143 kg/m<sup>2</sup>). BMI was determined using self-reported weight and height. Study procedures were  
144 performed in accordance with the Declaration of Helsinki for human studies and the guidelines of  
145 the General Hospital Institutional Committee that reviewed and approved the present protocol.

### 146 2.2 Exposure data

147 Data on carcinogen exposure are collected by employers and regularly sent to the Italian Institute  
148 for Occupational Safety and Prevention (ISPESL) (Italian legislative decree no. 626 of 19  
149 September 1994). Such information is named exposure registries and includes quantitative  
150 measurements of wood dust exposure. Companies are responsible for collecting the exposure



151 measurements in accordance with the EN 689:1995 regulation by the European Committee on  
152 Standardization (Scarselli et al., 2008). For the purpose of this research, data on occupational  
153 exposure measurements of wood dust recorded in the Information System for Recording  
154 Occupational Exposures to Carcinogens (SIREP) were used to estimate environmental air  
155 concentrations.

### 156 2.3 Urinary 15-F<sub>2t</sub> isoprostane and creatinine measurement

157 The IsoP under investigation consists of one of the most abundant endogenous F<sub>2</sub>-IsoPs, i.e., the  
158 15-F<sub>2t</sub>-IsoP, a biomarker considered to be representative for human oxidant status (Milne et al.,  
159 2015), also referred to as 8-iso-prostaglandin F<sub>2α</sub> (Roberts and Morrow, 2000). In the current  
160 study, the concentrations of 15-F<sub>2t</sub>-IsoP were analyzed using the competitive enzyme-linked  
161 immunoassay (ELISA) with a specific microplate kit (Oxford, MI, USA), according to the  
162 manufacturer's instructions, as previously reported (Bono et al., 2015; Romanazzi et al., 2013). In  
163 order to normalize urinary dilution rate of 15-F<sub>2t</sub>-IsoP an aliquot of urine was used to quantify the  
164 concentration of creatinine by the kinetic Jaffé procedure (Bartels and Cikes, 1969).

### 165 2.4 Statistical analysis

166 The level of 15-F<sub>2t</sub>-IsoP was expressed as ng/mg creatinine. Given the right-skewed distribution of  
167 this biomarker, the data were log transformed to stabilize the variance and normalize the  
168 distribution. Multivariate statistical analyses were applied using log-normal regression models  
169 including age (continuous), tobacco smoking, i.e., non-smokers, ex-smokers, smokers,  
170 occupational history (years), and BMI, as predictive variables to evaluate the association between  
171 exposure to wood dust and the urinary excretion of 15-F<sub>2t</sub>-IsoP in the study participants. **Results**  
172 **were adjusted for age and smoking.** This was based on a previous study showing potential  
173 associations between these variables and biomarker levels (Ceppi et al., 2011). Wood workers  
174 were classified according to occupational exposures in two additional sub-groups: a) wood workers  
175 exposed to wood dust alone and b) wood workers with co-exposures to organic solvents. The  
176 regression parameters estimated from the models were interpreted as ratios [Means Ratio (MR)]  
177 between the means of 15-F<sub>2t</sub>-IsoPs of each level of the categorical variables with respect to the  
178 reference level, as appropriate. The MR was used as a measure of effect (van Houwelingen et al.,  
179 2002). A p-value of <0.05 (two-tailed) was considered significant. Data were analyzed using  
180 SAS9.3 and SPSS 20.0 (IBM SPSS Statistics, New York, NY).

## 181 3. Results

### 182 3.1 Study population

183 The underlying basic population consisted of workers employed in the wood product manufacturing  
184 sector of the province of Florence, Tuscany Region, Italy. 32 out of 44 consented to participate to  
185 the study. Participation rates were ~95%. The concentration of 15-F<sub>2t</sub>-IsoPs in the wood workers  
186 was evaluated along with control subjects, i.e., 123 wood workers and 57 controls. All participants  
187 were males with a mean age of 45.3 ± 0.85 years and 35% of which were smokers. In the current  
188 study, the wood workers consisted of carpenters and joiners, wood processing-plant operators,  
189 woodworking machine operators, wood products assemblers, manufacturing labourers, industrial  
190 robot operators and other wood related workers. The use of the most common Personal Protective  
191 Equipment (PPE) in woodworking, i.e., disposable respirators, was generally reported from  
192 majority of the wood workers. Controls were living in residential areas with no proximity to major air  
193 pollution sources. The two groups had similar demographic, anthropometric and life-style  
194 characteristics. The mean age of the wood workers and the controls was not statistically different  
195 (Table 1). The average values of BMI were similar among the two groups (Table 1). The frequency  
196 of smokers was similar between the groups, i.e., 36% of the wood workers and 37% of the  
197 controls, respectively. The distribution of subjects with respect to wood dust exposure with – out  
198 co-exposures to other airborne carcinogens and smoking habits was reported in Table 2. Other  
199 variables included length of employment and BMI groups (Tables 1-2).

### 200 3.2 Exposure data

201 The exposure measurement of wood dust air concentrations corresponds to a single value  
202 assessed from several consecutive samples by fixed positions (Scarselli et al., 2008). Airborne  
203 levels of industrial contaminants were quantified by daily mean concentration, i.e., 8-h time-  
204 weighted average (TWA-8), of respirable wood dust among exposed workers. The mean level of  
205 TWA-8 concentration of wood dust was 1.48 mg/m<sup>3</sup> in wood workers.

### 206 3.3 Urinary 15-F<sub>2t</sub> isoprostane level, smoking habits and occupational exposure

207 An increased amount of 15-F<sub>2t</sub>-IsoP was found in the urine of wood workers as compared to the  
208 controls (4.2 vs 2.9 ng/mg creatinine, Table 2). The multivariate analysis shows that the 36%  
209 excess of 15-F<sub>2t</sub>-IsoP of the wood workers was significantly higher as compared to the controls,  
210 95% C.I. 1.18–1.57. Smokers had an average concentration of 15-F<sub>2t</sub>-IsoP higher than ex-smokers  
211 and non-smokers. A significant excess was found in the smokers in respect to the non-smokers,  
212 95% Confidence Interval (C.I.) 1.23–1.66, after adjusting for age by statistical analysis.  
213 Subsequently, the effect of co-exposures to other potential occupational carcinogens in the wood  
214 industry on the level of 15-F<sub>2t</sub>-IsoP was investigated. Therefore, workers were stratified into two  
215 additional sub-groups: a) only wood dust exposed workers and b) mixed exposed workers. Table 2  
216 indicates that the highest level of 15-F<sub>2t</sub>-IsoP occurred in the wood workers who were co-exposed  
217 to respirable organic solvents in respect to those who were only exposed to wood dust (4.5 and 4.0  
218 ng/mg creatinine, respectively). After adjusting for age and smoking, the multivariate analysis  
219 shows a 41% increment of 15-F<sub>2t</sub>-IsoP, 95% C.I. 1.17–1.70, in the mixed exposed workers,  
220 whereas a lower increment was observed in the only wood dust exposed workers, 95% C.I. 1.15–  
221 1.56. When we considered occupational history, there was a greater production of 15-F<sub>2t</sub>-IsoP in  
222 the long-term wood workers (4.8 ng/mg creatinine of 15-F<sub>2t</sub>-IsoP) compared to those with shorter  
223 occupational history (3.2 ng/mg creatinine). A 41% excess of 15-F<sub>2t</sub>-IsoP was observed in the  
224 wood workers with longer occupational exposure times, 95% C.I. 1.14–1.75. Then, the excretion of  
225 15-F<sub>2t</sub>-IsoPs was found to be significantly correlated with the length of dust exposure (p-value =  
226 0.007). Table 3 reports the mean concentrations of 15-F<sub>2t</sub>-IsoP and MR and 95% C.I. by exposure  
227 group and smoking stratification. The highest amount of 15-F<sub>2t</sub>-IsoP was found in the wood  
228 workers who were smokers, i.e., 5.0 ng/mg.

### 229 3.4 Urinary 15-F<sub>2t</sub> isoprostane level and BMI groups

230 Since early studies have supported the hypothesis of a relationship between F<sub>2</sub>-IsoP and weight  
231 gain (Annor et al., 2017; Il'yasova et al., 2015), the association of this biomarker of oxidant status  
232 with BMI was investigated. Study participants were divided by three BMI categories: a) normal  
233 weight persons (18.5-24.9 kg/m<sup>2</sup>), b) overweight persons (25-25.99 kg/m<sup>2</sup>) and c) obese persons  
234 (≥30 kg/m<sup>2</sup>) to evaluate the relationship of F<sub>2</sub>-IsoP with increase in body weight that could result in  
235 excessive fat accumulation. Table 2 shows that the mean concentrations of 15-F<sub>2t</sub>-IsoP of obese  
236 and overweight participants were higher than those with normal weight, but, no significant effect  
237 was found.

## 238 4. Discussion

239 Wood processing causes small particles of wood dust to become suspended in the air. Workers  
240 can inhale these particles, which can cause adverse health effects. The main result of this paper  
241 showed that significantly enhanced level of F<sub>2</sub>-IsoP occurred in the workers compared to the  
242 unexposed controls. A 36% excess of 15-F<sub>2t</sub>-IsoP levels was found in the wood workers as  
243 compared with the unexposed controls. Furthermore, the significant excess of 15-F<sub>2t</sub>-IsoP  
244 persisted after smoking habit stratification. Among the wood workers, a 53% excess of 15-F<sub>2t</sub>-IsoP  
245 was found in the smokers, a 48% excess was observed in the ex-smokers and a 27% in the non-  
246 smokers as compared to the appropriate controls. The urinary excretion of this biomarker was  
247 significantly associated with other parameters, including smoking habits, co-exposure to other  
248 airborne carcinogens and length of employment. In particular, multivariate regression analysis  
249 showed that the level of 15-F<sub>2t</sub>-IsoP was linearly correlated to the length of exposure. In

250 agreement with our findings, other studies have previously reported increased oxidative stress  
251 generation in relation to occupational exposure to wood dust (Bruschweiler et al., 2016; Palus et  
252 al., 1999; Rekhadevi et al., 2009). Our findings provide strengthening of the hypothesis that  
253 oxidative stress and LPO can have a main role in the toxicity of wood dust. The analysis of F<sub>2</sub>-IsoP  
254 in urine could offer a unique noninvasive analytic tool to study the role of ROS in chronic  
255 occupational exposures. In the current case, the linkage between urinary 15-F<sub>2t</sub>-IsoPs and wood  
256 dust can be due to an increased production of ROS caused by inflammation after exposure fine  
257 and abundant airborne dust created during wood manipulation, maintenance activities and  
258 cleaning equipment. Increased oxidative stress and LPO can be caused from the oxidative burst of  
259 activated macrophages and neutrophils, cells with a main role in phagocytosis and clearance of  
260 xenobiotic particles, and from increased inflammatory cytokines and activated leukocytes (Gungor  
261 et al., 2010; Vanhees et al., 2013). This is in keeping with the results of previous studies using a  
262 biomarker of oxidative DNA damage and LPO (Bonassi et al., 2017; Bono et al., 2016; Bono et al.,  
263 2010; Peluso et al., 2013; Peluso et al., 2010). In support of our hypothesis, free radicals produced  
264 through chronic inflammatory process and cancer disease have been implicated as the causal  
265 factor in the mutagenesis of the *tumor suppressor gene TP53* (Brancato et al., 2016; Perez-  
266 Escuredo et al., 2012).

267  
268 Next, our study showed an empirical relationship between tobacco smoking and the urinary  
269 excretion of 15-F<sub>2t</sub>-IsoP, possibly related to the inhalation exposure to carcinogens contained in  
270 tobacco smoke. A 43% increment of the level of 15-F<sub>2t</sub>-IsoP was present in overall the smokers as  
271 compared to the non-smokers. This excess is commonly interpreted as an harmful oxidative stress  
272 (Basu, 2008). These findings were somewhat expected as active smokers inhale a broad range of  
273 airborne carcinogens (IARC, 2004). The involvement of altered oxidative stress-related  
274 mechanisms in tobacco smoke carcinogenesis is in line with previous studies using various  
275 biomarkers of oxidative stress and LPO (Munnia et al., 2004; Peluso et al., 2014; Romanazzi et al.,  
276 2013). Various groups have measured the concentrations of F<sub>2</sub>-IsoP in biological fluids of smokers.  
277 The mean level of free and esterified F<sub>2</sub>-IsoP in the urine and plasma of smokers have been found  
278 to be significantly elevated as compared to non-smokers (Lowe et al., 2013). For instance, a  
279 previous cross-sectional study conducted on workers employed in an industry of plastic laminates  
280 in Piedmont, Italy, finds that smoking habits were significantly associated with the urinary  
281 excretion of 15-F<sub>2t</sub>-IsoP (Romanazzi et al., 2013). When the relationship of 15-F<sub>2t</sub>-IsoP with BMI  
282 was investigated, we found that the levels of 15-F<sub>2t</sub>-IsoP tended to increase with fat accumulations.  
283 The 42% of the obese subjects showed indeed higher excretion of 15-F<sub>2t</sub>-IsoP in respect to those  
284 with normal weight. This is partially in keeping with a previous work of Annor et al. (Annor et al.,  
285 2017) on the risk of diabetes and weight gain. In that study, the 35% of the obese individuals  
286 showed greater levels of F<sub>2</sub>-IsoPs as compared to the controls. Additional studies are necessary to  
287 understand if this biomarker can be used as measure of lifestyle habits and intervention targeted to  
288 obesity prevention.

289 The threshold exposure limit recommended by the Italian law is 5 mg/m<sup>3</sup> (Legislative Decree No  
290 66/2000). This value will remain until the 2020<sup>th</sup>, after the entry into force of the new threshold  
291 exposure limit of 3 mg/m<sup>3</sup> for five years and thereafter of 2 mg/m<sup>3</sup> (European Directive Decree No  
292 2017/2398). In this context, the SIREP database aims to facilitate analysis of occupational  
293 exposure figures for carcinogenic agents. In our study, the average amount of wood dust  
294 concentrations experienced from the wood workers was lower than threshold exposure limit of 3  
295 mg/m<sup>3</sup> (i.e., 1.48 mg/m<sup>3</sup>). This result is consistent with that reported from a previous study of  
296 Scarselli et al. (Scarselli et al., 2008), where the mean concentrations of wood dust was of 1.44  
297 mg/m<sup>3</sup> for 1.181 companies in Italy. Although our static measurements of the concentrations of  
298 industrial contaminants by fixed positions provide evidence of wood workers' exposure via air, they  
299 are not well representative of individual exposures to wood dust due to spatial and temporal  
300 variations. Therefore, we could not assess the potential relationships of airborne measurements  
301 with biomarker urinary excretion in exposed workers.

302 The airborne wood-dust concentrations from exposure registries are commonly used for the  
303 purposes of hazard control, exposure surveillance and assessment of health risks (Kauppinen et



304 al., 2006). Nevertheless, a limitation of our study is that no data on the variability of wood dust  
305 concentrations within a facility were available. The bias due to the variability of airborne carcinogen  
306 levels in occupational settings is difficult to predict, but a large variation can be present in one spot  
307 of a factory versus another. There could be an underestimation of the exposure to wood dust  
308 associated to some woodworking operations. For instance, local exhaust ventilation is used widely  
309 with fixed woodworking machinery, but it is generally lacking for hand tools (Pisaniello et al., 1991).  
310 The effects of poor work practices, such as the use of compressed air for cleaning, the lack of local  
311 exhaust ventilation for hand tools, that are commonly associated to high exposure levels to wood  
312 dust (Alwis et al., 1999), could be missed. Variations in the use of PPE (Alwis et al., 1999) and in  
313 the effective application of WorkSafe procedures at work places could have influenced the  
314 personal levels of exposure to wood dust of our workers.

315 Our subsequent finding shows that the urinary excretion of 15-F<sub>2t</sub>-IsoP in the workers exposed to  
316 wood dust can aggravate with co-exposure to other respiratory carcinogens. An excess of 41%  
317 was detected in the wood workers that were co-exposed to organic solvents compared to the  
318 controls. Conversely, a lower excess was determined in the only wood dust exposed workers. High  
319 biosynthesis of F<sub>2</sub>-IsoP can be due to frequent free radical-catalyzed reactions induced by  
320 alterations of oxidative stress, antioxidant defence and inflammation especially caused by  
321 occupational exposures to complex mixtures of airborne carcinogens. This is consistent with a  
322 cross-sectional study of workers exposed to dust containing silica (Peluso et al., 2015). In this  
323 study, the amount of oxidative stress and LPO biomarker of the workers exposed to airborne silica  
324 dust was greater in the case of occupational co-exposures to organic solvents, welding and motor  
325 exhaust fumes. Constituents of organic solvents, such as benzene and formaldehyde can be  
326 involved in the generation of oxidative stress and ROS (Bono et al., 2016; Bono et al., 2010;  
327 Sorensen et al., 2003) and cause the production of 15-F<sub>2t</sub>-IsoP determined in the workers exposed  
328 to wood dust. Our results suggest that the urinary level of F<sub>2</sub>-IsoP resulting from exposures to  
329 airborne wood dust can be affected from concomitant carcinogen exposures. **Levels of oxidative  
330 stress can increase with exposures to organic solvents (Salimi et al., 2017; Singh et al., 2010),  
331 leading to a greater imbalance between excessive ROS generation and their degradation by  
332 antioxidants. The induction of reactive species can increase damage to membrane lipids, cellular  
333 proteins and DNA.**

334 A significant difference in the amount of 15-F<sub>2t</sub>-IsoP was then observed among sub-groups of wood  
335 workers with different occupational history. The urinary excretion of this biomarker of oxidant status  
336 was significantly elevated in those subjects with longer exposure time. An 41% excess of 15-F<sub>2t</sub>-  
337 IsoP was found in the long-term wood workers as compared to those with shorter exposures, used  
338 as the reference level. Multivariate regression analysis showed that the level of 15-F<sub>2t</sub>-IsoP was  
339 significantly linearly correlated to the length of employment, in agreement with a previous study on  
340 asbestos workers (Yoshida et al., 2001). In that study, the generation of an urinary biomarker of  
341 oxidative stress correlated positively with the length of exposure. Rekhadevi et al. (Rekhadevi et  
342 al., 2009) have similarly found an association between length of occupational exposure and  
343 increase frequency of micronuclei. Taken together, the occurrence of elevated oxidative stress in  
344 long-term wood workers can be possibly due to chronic inflammatory conditions. **Our study  
345 suggests that the measure of urinary F<sub>2</sub>-IsoPs can serve as a biomarker for assessing  
346 occupational carcinogen exposure and improving workplace safety. Particular effort should  
347 be devoted to studying long term health effects of exposure to wood dust, such as SNC.**

348 Particular effort should be devoted to study delayed reactions such as diseases that take a long  
349 time to develop, like SNC, that can be caused by long-term exposure to this carcinogenic agent.

## 350 5. Conclusions

351 Our study provides a valuable contribution to the issue of oxidative stress in woodworking. **An  
352 excessive ROS generation was demonstrated in exposed workers. Furthermore, we showed that  
353 exposure to organic solvents can increase the levels of urinary biomarkers of oxidative stress in  
354 wood workers.** Results provide a basis for worker surveillance in occupational settings. F<sub>2</sub>-IsoP  
355 measure could be used for the evaluation of the effectiveness of targeted interventions aimed to

356 reduce exposures to various environmental carcinogens. A more effective control of occupational  
357 health risks could decrease the incidence of illness at work and improve the health of the  
358 workforce.

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365 **Declarations of interest**

366 None

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1 **Wood dust and urinary 15-F<sub>2t</sub> isoprostane in Italian industry workers**

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19 **Abstract**

1  
20 Wood dust is one of the most common occupational exposures, with about 3.6 million of workers in  
21 the wood industry in Europe. Wood particles can deposit in the nose and the respiratory tract and  
22 cause adverse health effects. Occupational exposure to wood dust has been associated with  
23 malignant tumors of the nasal cavity and paranasal sinuses. The induction of oxidative stress and  
24 the generation of reactive oxygen species through activation of inflammatory cells could have a  
25 role in the carcinogenicity of respirable wood dust. Therefore, we conducted a cross-sectional  
26 study to evaluate the prevalence of urinary 15-F<sub>2t</sub> isoprostane (15-F<sub>2t</sub>-IsoP), a biomarker of  
27 oxidative stress and peroxidation of lipids, in 123 wood workers compared to 57 unexposed  
28 controls living in Tuscany region, Italy. 15-F<sub>2t</sub>-IsoP generation was measured by ELISA. The main  
29 result of the present study showed that a statistically significant excess of this biomarker occurred  
30 in the workers exposed to 1.48 mg/m<sup>3</sup> of airborne wood dust with respect to the unexposed  
31 controls (0.05 mg/m<sup>3</sup>). The overall mean ratio (MR) between the workers exposed to wood dust  
32 and the controls was 1.36, 95% Confidence Interval (C.I.) 1.18–1.57, after correction for age and  
33 smoking habits. A significant increment of 15-F<sub>2t</sub>-IsoP (43%) was observed in the smokers as  
34 compared to the non-smokers. The urinary excretion of 15-F<sub>2t</sub>-IsoP was significantly associated  
35 with co-exposure to organic solvents and formaldehyde, i.e., MR of 1.41, 95% C.I. 1.17-1.70, after  
36 adjustment for age and smoking habits. A 41% excess was observed in long-term wood workers,  
37 95% C.I. 1.14-1.75. Multivariate regression analysis showed that the level of 15-F<sub>2t</sub>-IsoP was  
38 linearly correlated to the length of exposure, regression coefficient ( $\beta$ ) = 0.244  $\pm$  0.002 (SE). The  
39 overall increment by exposure group persisted after stratification for smoking habits. For instance,  
40 in smokers, a 53% excess was detected in the wood workers as compared to the controls, 95%  
41 C.I. 1.23-1.91. Our data support the hypothesis that oxidative stress and lipid peroxidation can  
42 have a role in the toxicity of wood dust F<sub>2</sub>-IsoP measure can be a tool for the evaluation of the  
43 effectiveness of targeted interventions aimed to reduce exposures to environmental carcinogens.  
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29 **Key words:** wood dust, organic solvents, formaldehyde, 15-F<sub>2t</sub> isoprostane, primary prevention,  
30 occupational health.  
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## 47 1. Introduction

1  
248 Wood dust is one of the most common occupational exposures, with about 3.6 million of workers in  
349 the wood industry in Europe (Kauppinen et al., 2006). Wood particles can deposit in the nose and  
450 the respiratory tract and cause adverse health effects (Çelik and Kanık, 2006). Epidemiological  
551 studies have indeed associated the exposure to wood dust to sinonasal cancers (SNC) (Acheson  
652 et al., 1968; Ball, 1968). In 1960, the first association with SNC was shown in the wood industry  
753 (Acheson et al., 1968). In 1995, this agent was classified as carcinogenic to humans (Group 1) by  
854 the International Agency for Research on Cancer (IARC) based mostly on a SNC excess (IARC,  
955 1995). In 2012, the IARC confirmed the human carcinogenicity of wood dust and reported the first  
1056 link with nasopharynx cancer (IARC, 2012). Considering other types of cancer, a meta-analysis  
1157 has suggested a relationship with lung cancer (Hancock et al., 2015), but a significant influence of  
1258 the geographic region was apparent.

14  
1559 SNC has been under compulsory surveillance since 2008 in Italy, through the “Sinonasal Cancer  
1660 National Registry” (Registro Nazionale Tumori Naso-Sinusali: ReNaTuNS), a nationwide cancer  
1761 registry coordinated by the National Institute for Insurance Against Accidents at Work (Istituto  
1862 Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro: INAIL) (Binazzi et al., 2017).  
1963 Currently, the registry covers a proportion of Italy through regional structures devoted to the active  
2064 search for cases from hospitals, to the definition of the modalities of exposure and has recorded  
2165 1,529 cases between 2000-2016. A study conducted by Demers et al. (Demers et al., 1995) found  
2266 a doubled risk statistically significant for sinonasal cancer in men employed in any wood-related job  
2367 (OR = 2.0, 95% CI: 1.6 to 2.5) in comparison to men who had never worked in a wood-related job.  
2468 The increased risk was found among sawmill workers (OR = 2.5, 95% CI: 1.8 to 3.4), furniture  
2569 workers (OR = 4.5, 95% CI: 3.2 to 6.5) and carpenters (OR = 2.9, 95% CI: 2.1 to 3.9), while no  
2670 excess risk was observed among forestry, logging, pulp and paper workers. An increasing risk was  
2771 detected in relation to the duration of exposure, and lagging exposure by 5, 10 or 20 years  
2872 increased the strength of the association between duration of employment and sinonasal  
2973 adenocarcinoma. Elevated risk for adenocarcinoma of the nasal cavity and paranasal sinuses  
3074 (ADCN), a SNC subtype frequently associated with wood dust exposure (IARC, 2012), OR 58.6,  
3175 95% C.I. 23.74-144.8, was even reported among wood workers of the Piedmont region, Italy  
3276 (d'Errico et al., 2009). Stronger ADCN risk, OR 179.9, 95% C.I. 55.37-584.4, was found among  
3377 those workers exposed to high level of wood dust (d'Errico et al., 2009).

36  
3778 Higher levels of oxidative damage, measured by the micronucleus and the comet assays in blood,  
3879 buccal and nasal cells, have been detected in wood workers compared to unexposed controls  
3980 (Bruschweiler et al., 2016; Palus et al., 1999; Rekhadevi et al., 2009). An enhanced risk for  
4081 chromosomal instability was found in wood workers (Bruschweiler et al., 2014; Çelik and Kanık,  
4182 2006; Rekhadevi et al., 2009). Discrepant results have been reported (Wultsch et al., 2015). In that  
4283 study, no induction of micronuclei was observed in wood workers exposed to 0.39-0.66 mg/m<sup>3</sup>  
4384 wood dust levels. Thus, further investigation into wood workers' occupational exposures are  
4485 warranted. Furthermore, co-exposures to chrome, organic solvents, tannins, formaldehyde, textile  
4586 dust and pesticides have been reported in the wood industry (Binazzi et al., 2017). In 2012, IARC  
4687 suggested that the cancer risk of wood workers could be associated with the inflammatory  
4788 reactions following wood dust exposure rather than to the direct action of this carcinogen (IARC,  
4889 2012). Inflammatory cells can generate a large spectrum of proinflammatory mediators and free  
4990 radicals (Pylkkänen et al., 2009). Excessive production of reactive oxygen species (ROS) can  
5091 cause damage to lipids, proteins and DNA (Marnett, 2000). Peroxidation of lipids (LPO) can lead to  
5192 the production of aldehydes, such as malondialdehyde and 4-hydroxynonenal (Marnett, 2000), as  
5293 well as to secondary oxidation products such as a series of prostaglandin-like products termed  
5394 isoprostanes (IsoPs) (Roberts and Morrow, 2000).

5495 IsoPs are compounds generated from the non-enzymatic free radical-catalyzed peroxidation of  
5596 arachidonic acid and other highly unsaturated polyunsaturated fatty acids (Janicka et al., 2010).  
5697 IsoPs can be grouped into 4 subfamilies, denoted as 5-, 12-, 8-, or 15-series regioisomers,  
5798 depending on the carbon atom to which the side chain hydroxyl is attached. Among the three major  
5899 classes of IsoPs (F<sub>2</sub>-, D<sub>2</sub>- and E<sub>2</sub>-), F<sub>2</sub>-IsoPs are recognized as the most suitable biomarker for  
6000

100 their chemical stability (Roberts and Morrow, 2000). The measurement of this biomarker is widely  
101 used for the analysis of endogenous oxidative stress following ROS production and peroxidation of  
102 lipids (Basu, 2008). F<sub>2</sub>-IsoPs are more advantageous over other LPO biomarkers because they  
103 can be detected in a variety of biological samples including plasma, urine, lavage fluid and red  
104 blood cells (Milne et al., 2015). As IsoPs generate from LPO, their amounts provide an integrated  
105 measurement of unbalanced oxidant-antioxidant status (Lowe et al., 2013; Montuschi et al., 2004).

106 In the current study, we have investigated the potential effects of occupational exposure to wood  
107 dust in the wood product manufacturing sector in the Tuscany Region of Italy. A cross-sectional  
108 study was conducted to analyze the concentration of a biomarker of oxidative stress and LPO (15-  
109 F<sub>2t</sub>-IsoP) in the workers exposed to wood dust. One of the main advantages of using biomarkers is  
110 that one can study signals of carcinogen exposure without having to wait for health effects as in  
111 classical epidemiological studies (Merlo et al., 1997; Munnia et al., 2017; Munnia et al., 2007;  
112 Peluso et al., 1997; Peluso et al., 2012). Although F<sub>2</sub>-IsoP can be evaluated in different biological  
113 fluids, we employed urine due to its ready availability and the high stability of F<sub>2</sub>-IsoP in this  
114 medium (Morrow et al., 1999). Since obesity has been associated with increased F<sub>2</sub>-IsoP  
115 concentrations (Annor et al., 2017; Il'yasova et al., 2015), we have examined the relationships  
116 between urinary F<sub>2</sub>-IsoPs and weight gain. Further understanding of the link between wood dust  
117 and oxidative stress will improve knowledge of the mechanisms of carcinogenicity of this  
118 occupational agent. Novelty of the current study is based on various items, including larger sample  
119 size, a different geographical area, and a different type of data, i.e., the measurement of F<sub>2</sub>-IsoPs  
120 in urine rather than of micronucleus and DNA strand-breaks in blood, buccal and nasal cells.

## 2. Material and methods

### 2.1 Subjects and sampling

123 A sample of 44 wood companies of the province of Florence, Tuscany, Italy was randomly selected  
124 among those which are under compulsory health surveillance. Wood companies were contacted in  
125 person by medical doctors with qualifications in occupational medicine. The inclusion criteria were  
126 as follows: (a) only workers exposed to wood dust from wood industry; (b) only workers with a  
127 minimal exposure time of 1 year; (c) only controls without occupational history in industries  
128 entailing exposure to known or suspected carcinogens; and (d) only controls resident in areas with  
129 no proximity to major air pollution sources. All the volunteers involved in the study live and work in  
130 the province of Florence, Tuscany, Italy. A 15-F<sub>2t</sub>-IsoP was determined using spot urine samples  
131 collected in the morning at each workplace. Wood workers and the other subjects were contacted  
132 by the local occupational health services. All the volunteers were informed about the study aim and  
133 gave a written informed consent. A life-style questionnaire was filled by each participant (Peluso et  
134 al., 2015). Detailed information on socio-demographic and anthropometric characteristics,  
135 education level, exposure to active and passive tobacco smoke, occupational exposure to wood  
136 dust, protective gear use, co-exposures to organic solvents, welding and motor exhaust fumes and  
137 occupational history were obtained. Subjects who had never smoked were classified as non-  
138 smokers, smokers who had quit smoking from at least one month prior were classified as ex-  
139 smokers, while individuals who smoked at least one cigarette per day were classified as smokers.  
140 The Body Mass Index (BMI) categories reported from the National Heart Lung, and  
141 Blood Institute (<https://www.nhlbi.nih.gov/>) were used for grouping the study participants in normal  
142 weight persons (18.5-24.9 kg/m<sup>2</sup>), overweight persons (25-25.99 kg/m<sup>2</sup>) and obese persons (≥30  
143 kg/m<sup>2</sup>). BMI was determined using self-reported weight and height. Study procedures were  
144 performed in accordance with the Declaration of Helsinki for human studies and the guidelines of  
145 the General Hospital Institutional Committee that reviewed and approved the present protocol.

### 2.2 Exposure data

146 Data on carcinogen exposure are collected by employers and regularly sent to the Italian Institute  
147 for Occupational Safety and Prevention (ISPESL) (Italian legislative decree no. 626 of 19  
148 September 1994). Such information is named exposure registries and includes quantitative  
149 measurements of wood dust exposure. Companies are responsible for collecting the exposure  
150



151 measurements in accordance with the EN 689:1995 regulation by the European Committee on  
152 Standardization (Scarselli et al., 2008). For the purpose of this research, data on occupational  
153 exposure measurements of wood dust recorded in the Information System for Recording  
154 Occupational Exposures to Carcinogens (SIREP) were used to estimate environmental air  
155 concentrations.

### 156 2.3 Urinary 15-F<sub>2t</sub> isoprostane and creatinine measurement

157 The IsoP under investigation consists of one of the most abundant endogenous F<sub>2</sub>-IsoPs, i.e., the  
158 15-F<sub>2t</sub>-IsoP, a biomarker considered to be representative for human oxidant status (Milne et al.,  
159 2015), also referred to as 8-iso-prostaglandin F<sub>2α</sub> (Roberts and Morrow, 2000). In the current  
160 study, the concentrations of 15-F<sub>2t</sub>-IsoP were analyzed using the competitive enzyme-linked  
161 immunoassay (ELISA) with a specific microplate kit (Oxford, MI, USA), according to the  
162 manufacturer's instructions, as previously reported (Bono et al., 2015; Romanazzi et al., 2013). In  
163 order to normalize urinary dilution rate of 15-F<sub>2t</sub>-IsoP an aliquot of urine was used to quantify the  
164 concentration of creatinine by the kinetic Jaffé procedure (Bartels and Cikes, 1969).

### 165 2.4 Statistical analysis

166 The level of 15-F<sub>2t</sub>-IsoP was expressed as ng/mg creatinine. Given the right-skewed distribution of  
167 this biomarker, the data were log transformed to stabilize the variance and normalize the  
168 distribution. Multivariate statistical analyses were applied using log-normal regression models  
169 including age (continuous), tobacco smoking, i.e., non-smokers, ex-smokers, smokers,  
170 occupational history (years), and BMI, as predictive variables to evaluate the association between  
171 exposure to wood dust and the urinary excretion of 15-F<sub>2t</sub>-IsoP in the study participants. Results  
172 were adjusted for age and smoking. This was based on a previous study showing potential  
173 associations between these variables and biomarker levels (Ceppi et al., 2011). Wood workers  
174 were classified according to occupational exposures in two additional sub-groups: a) wood workers  
175 exposed to wood dust alone and b) wood workers with co-exposures to organic solvents. The  
176 regression parameters estimated from the models were interpreted as ratios [Means Ratio (MR)]  
177 between the means of 15-F<sub>2t</sub>-IsoPs of each level of the categorical variables with respect to the  
178 reference level, as appropriate. The MR was used as a measure of effect (van Houwelingen et al.,  
179 2002). A p-value of <0.05 (two-tailed) was considered significant. Data were analyzed using  
180 SAS9.3 and SPSS 20.0 (IBM SPSS Statistics, New York, NY).

## 181 3. Results

### 182 3.1 Study population

183 The underlying basic population consisted of workers employed in the wood product manufacturing  
184 sector of the province of Florence, Tuscany Region, Italy. 32 out of 44 consented to participate to  
185 the study. Participation rates were ~95%. The concentration of 15-F<sub>2t</sub>-IsoPs in the wood workers  
186 was evaluated along with control subjects, i.e., 123 wood workers and 57 controls. All participants  
187 were males with a mean age of 45.3 ± 0.85 years and 35% of which were smokers. In the current  
188 study, the wood workers consisted of carpenters and joiners, wood processing-plant operators,  
189 woodworking machine operators, wood products assemblers, manufacturing labourers, industrial  
190 robot operators and other wood related workers. The use of the most common Personal Protective  
191 Equipment (PPE) in woodworking, i.e., disposable respirators, was generally reported from  
192 majority of the wood workers. Controls were living in residential areas with no proximity to major air  
193 pollution sources. The two groups had similar demographic, anthropometric and life-style  
194 characteristics. The mean age of the wood workers and the controls was not statistically different  
195 (Table 1). The average values of BMI were similar among the two groups (Table 1). The frequency  
196 of smokers was similar between the groups, i.e., 36% of the wood workers and 37% of the  
197 controls, respectively. The distribution of subjects with respect to wood dust exposure with – out  
198 co-exposures to other airborne carcinogens and smoking habits was reported in Table 2. Other  
199 variables included length of employment and BMI groups (Tables 1-2).



### 200 3.2 Exposure data

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201 The exposure measurement of wood dust air concentrations corresponds to a single value  
202 assessed from several consecutive samples by fixed positions (Scarselli et al., 2008). Airborne  
203 levels of industrial contaminants were quantified by daily mean concentration, i.e., 8-h time-  
204 weighted average (TWA-8), of respirable wood dust among exposed workers. The mean level of  
205 TWA-8 concentration of wood dust was 1.48 mg/m<sup>3</sup> in wood workers.

### 206 3.3 Urinary 15-F<sub>2t</sub> isoprostane level, smoking habits and occupational exposure

207 An increased amount of 15-F<sub>2t</sub>-IsoP was found in the urine of wood workers as compared to the  
208 controls (4.2 vs 2.9 ng/mg creatinine, Table 2). The multivariate analysis shows that the 36%  
209 excess of 15-F<sub>2t</sub>-IsoP of the wood workers was significantly higher as compared to the controls,  
210 95% C.I. 1.18–1.57. Smokers had an average concentration of 15-F<sub>2t</sub>-IsoP higher than ex-smokers  
211 and non-smokers. A significant excess was found in the smokers in respect to the non-smokers,  
212 95% Confidence Interval (C.I.) 1.23–1.66, after adjusting for age by statistical analysis.  
213 Subsequently, the effect of co-exposures to other potential occupational carcinogens in the wood  
214 industry on the level of 15-F<sub>2t</sub>-IsoP was investigated. Therefore, workers were stratified into two  
215 additional sub-groups: a) only wood dust exposed workers and b) mixed exposed workers. Table 2  
216 indicates that the highest level of 15-F<sub>2t</sub>-IsoP occurred in the wood workers who were co-exposed  
217 to respirable organic solvents in respect to those who were only exposed to wood dust (4.5 and 4.0  
218 ng/mg creatinine, respectively). After adjusting for age and smoking, the multivariate analysis  
219 shows a 41% increment of 15-F<sub>2t</sub>-IsoP, 95% C.I. 1.17–1.70, in the mixed exposed workers,  
220 whereas a lower increment was observed in the only wood dust exposed workers, 95% C.I. 1.15–  
221 1.56. When we considered occupational history, there was a greater production of 15-F<sub>2t</sub>-IsoP in  
222 the long-term wood workers (4.8 ng/mg creatinine of 15-F<sub>2t</sub>-IsoP) compared to those with shorter  
223 occupational history (3.2 ng/mg creatinine). A 41% excess of 15-F<sub>2t</sub>-IsoP was observed in the  
224 wood workers with longer occupational exposure times, 95% C.I. 1.14–1.75. Then, the excretion of  
225 15-F<sub>2t</sub>-IsoPs was found to be significantly correlated with the length of dust exposure (p-value =  
226 0.007). Table 3 reports the mean concentrations of 15-F<sub>2t</sub>-IsoP and MR and 95% C.I. by exposure  
227 group and smoking stratification. The highest amount of 15-F<sub>2t</sub>-IsoP was found in the wood  
228 workers who were smokers, i.e., 5.0 ng/mg.

### 229 3.4 Urinary 15-F<sub>2t</sub> isoprostane level and BMI groups

230 Since early studies have supported the hypothesis of a relationship between F<sub>2</sub>-IsoP and weight  
231 gain (Annor et al., 2017; Il'yasova et al., 2015), the association of this biomarker of oxidant status  
232 with BMI was investigated. Study participants were divided by three BMI categories: a) normal  
233 weight persons (18.5-24.9 kg/m<sup>2</sup>), b) overweight persons (25-25.99 kg/m<sup>2</sup>) and c) obese persons  
234 (≥30 kg/m<sup>2</sup>) to evaluate the relationship of F<sub>2</sub>-IsoP with increase in body weight that could result in  
235 excessive fat accumulation. Table 2 shows that the mean concentrations of 15-F<sub>2t</sub>-IsoP of obese  
236 and overweight participants were higher than those with normal weight, but, no significant effect  
237 was found.

## 238 4. Discussion

239 Wood processing causes small particles of wood dust to become suspended in the air. Workers  
240 can inhale these particles, which can cause adverse health effects. The main result of this paper  
241 showed that significantly enhanced level of F<sub>2</sub>-IsoP occurred in the workers compared to the  
242 unexposed controls. A 36% excess of 15-F<sub>2t</sub>-IsoP levels was found in the wood workers as  
243 compared with the unexposed controls. Furthermore, the significant excess of 15-F<sub>2t</sub>-IsoP  
244 persisted after smoking habit stratification. Among the wood workers, a 53% excess of 15-F<sub>2t</sub>-IsoP  
245 was found in the smokers, a 48% excess was observed in the ex-smokers and a 27% in the non-  
246 smokers as compared to the appropriate controls. The urinary excretion of this biomarker was  
247 significantly associated with other parameters, including smoking habits, co-exposure to other  
248 airborne carcinogens and length of employment. In particular, multivariate regression analysis  
249 showed that the level of 15-F<sub>2t</sub>-IsoP was linearly correlated to the length of exposure. In

250 agreement with our findings, other studies have previously reported increased oxidative stress  
251 generation in relation to occupational exposure to wood dust (Bruschweiler et al., 2016; Palus et  
252 al., 1999; Rekhadevi et al., 2009). Our findings provide strengthening of the hypothesis that  
253 oxidative stress and LPO can have a main role in the toxicity of wood dust. The analysis of F<sub>2</sub>-IsoP  
254 in urine could offer a unique noninvasive analytic tool to study the role of ROS in chronic  
255 occupational exposures. In the current case, the linkage between urinary 15-F<sub>2t</sub>-IsoPs and wood  
256 dust can be due to an increased production of ROS caused by inflammation after exposure fine  
257 and abundant airborne dust created during wood manipulation, maintenance activities and  
258 cleaning equipment. Increased oxidative stress and LPO can be caused from the oxidative burst of  
1259 activated macrophages and neutrophils, cells with a main role in phagocytosis and clearance of  
1260 xenobiotic particles, and from increased inflammatory cytokines and activated leukocytes (Gungor  
1261 et al., 2010; Vanhees et al., 2013). This is in keeping with the results of previous studies using a  
1262 biomarker of oxidative DNA damage and LPO (Bonassi et al., 2017; Bono et al., 2016; Bono et al.,  
1263 2010; Peluso et al., 2013; Peluso et al., 2010). In support of our hypothesis, free radicals produced  
1264 through chronic inflammatory process and cancer disease have been implicated as the causal  
1265 factor in the mutagenesis of the *tumor suppressor gene TP53* (Brancato et al., 2016; Perez-  
1266 Escuredo et al., 2012).

1267  
2268 Next, our study showed an empirical relationship between tobacco smoking and the urinary  
2269 excretion of 15-F<sub>2t</sub>-IsoP, possibly related to the inhalation exposure to carcinogens contained in  
2270 tobacco smoke. A 43% increment of the level of 15-F<sub>2t</sub>-IsoP was present in overall the smokers as  
2271 compared to the non-smokers. This excess is commonly interpreted as an harmful oxidative stress  
2272 (Basu, 2008). These findings were somewhat expected as active smokers inhale a broad range of  
2273 airborne carcinogens (IARC, 2004). The involvement of altered oxidative stress-related  
2274 mechanisms in tobacco smoke carcinogenesis is in line with previous studies using various  
2275 biomarkers of oxidative stress and LPO (Munnia et al., 2004; Peluso et al., 2014; Romanazzi et al.,  
2276 2013). Various groups have measured the concentrations of F<sub>2</sub>-IsoP in biological fluids of smokers.  
3277 The mean level of free and esterified F<sub>2</sub>-IsoP in the urine and plasma of smokers have been found  
3278 to be significantly elevated as compared to non-smokers (Lowe et al., 2013). For instance, a  
3279 previous cross-sectional study conducted on workers employed in an industry of plastic laminates  
3280 in Piedmont, Italy, finds that smoking habits were significantly associated with the urinary  
3281 excretion of 15-F<sub>2t</sub>-IsoP (Romanazzi et al., 2013). When the relationship of 15-F<sub>2t</sub>-IsoP with BMI  
3282 was investigated, we found that the levels of 15-F<sub>2t</sub>-IsoP tended to increase with fat accumulations.  
3283 The 42% of the obese subjects showed indeed higher excretion of 15-F<sub>2t</sub>-IsoP in respect to those  
3284 with normal weight. This is partially in keeping with a previous work of Annor et al. (Annor et al.,  
3285 2017) on the risk of diabetes and weight gain. In that study, the 35% of the obese individuals  
4286 showed greater levels of F<sub>2</sub>-IsoPs as compared to the controls. Additional studies are necessary to  
4287 understand if this biomarker can be used as measure of lifestyle habits and intervention targeted to  
4288 obesity prevention.

4289  
4290 The threshold exposure limit recommended by the Italian law is 5 mg/m<sup>3</sup> (Legislative Decree No  
4291 66/2000). This value will remain until the 2020<sup>th</sup>, after the entry into force of the new threshold  
4292 exposure limit of 3 mg/m<sup>3</sup> for five years and thereafter of 2 mg/m<sup>3</sup> (European Directive Decree No  
4293 2017/2398). In this context, the SIREP database aims to facilitate analysis of occupational  
4294 exposure figures for carcinogenic agents. In our study, the average amount of wood dust  
4295 concentrations experienced from the wood workers was lower than threshold exposure limit of 3  
4296 mg/m<sup>3</sup> (i.e., 1.48 mg/m<sup>3</sup>). This result is consistent with that reported from a previous study of  
4297 Scarselli et al. (Scarselli et al., 2008), where the mean concentrations of wood dust was of 1.44  
4298 mg/m<sup>3</sup> for 1.181 companies in Italy. Although our static measurements of the concentrations of  
4299 industrial contaminants by fixed positions provide evidence of wood workers' exposure via air, they  
4300 are not well representative of individual exposures to wood dust due to spatial and temporal  
4301 variations. Therefore, we could not assess the potential relationships of airborne measurements  
4302 with biomarker urinary excretion in exposed workers.

4303  
59 The airborne wood-dust concentrations from exposure registries are commonly used for the  
60 purposes of hazard control, exposure surveillance and assessment of health risks (Kauppinen et  
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304 al., 2006). Nevertheless, a limitation of our study is that no data on the variability of wood dust  
305 concentrations within a facility were available. The bias due to the variability of airborne carcinogen  
306 levels in occupational settings is difficult to predict, but a large variation can be present in one spot  
307 of a factory versus another. There could be an underestimation of the exposure to wood dust  
308 associated to some woodworking operations. For instance, local exhaust ventilation is used widely  
309 with fixed woodworking machinery, but it is generally lacking for hand tools (Pisaniello et al., 1991).  
310 The effects of poor work practices, such as the use of compressed air for cleaning, the lack of local  
311 exhaust ventilation for hand tools, that are commonly associated to high exposure levels to wood  
312 dust (Alwis et al., 1999), could be missed. Variations in the use of PPE (Alwis et al., 1999) and in  
313 the effective application of WorkSafe procedures at work places could have influenced the  
314 personal levels of exposure to wood dust of our workers.

12  
1315 Our subsequent finding shows that the urinary excretion of 15-F<sub>2t</sub>-IsoP in the workers exposed to  
1316 wood dust can aggravate with co-exposure to other respiratory carcinogens. An excess of 41%  
1517 was detected in the wood workers that were co-exposed to organic solvents compared to the  
1618 controls. Conversely, a lower excess was determined in the only wood dust exposed workers. High  
1719 biosynthesis of F<sub>2</sub>-IsoP can be due to frequent free radical-catalyzed reactions induced by  
1820 alterations of oxidative stress, antioxidant defence and inflammation especially caused by  
1921 occupational exposures to complex mixtures of airborne carcinogens. This is consistent with a  
2022 cross-sectional study of workers exposed to dust containing silica (Peluso et al., 2015). In this  
2123 study, the amount of oxidative stress and LPO biomarker of the workers exposed to airborne silica  
2224 dust was greater in the case of occupational co-exposures to organic solvents, welding and motor  
2325 exhaust fumes. Constituents of organic solvents, such as benzene and formaldehyde can be  
2426 involved in the generation of oxidative stress and ROS (Bono et al., 2016; Bono et al., 2010;  
2527 Sorensen et al., 2003) and cause the production of 15-F<sub>2t</sub>-IsoP determined in the workers exposed  
2628 to wood dust. Our results suggest that the urinary level of F<sub>2</sub>-IsoP resulting from exposures to  
2729 airborne wood dust can be affected from concomitant carcinogen exposures. Levels of oxidative  
2830 stress can increase with exposures to organic solvents (Salimi et al., 2017; Singh et al., 2010),  
2931 leading to a greater imbalance between excessive ROS generation and their degradation by  
3032 antioxidants. The induction of reactive species can increase damage to membrane lipids, cellular  
3133 proteins and DNA.

34  
3534 A significant difference in the amount of 15-F<sub>2t</sub>-IsoP was then observed among sub-groups of wood  
3635 workers with different occupational history. The urinary excretion of this biomarker of oxidant status  
3736 was significantly elevated in those subjects with longer exposure time. An 41% excess of 15-F<sub>2t</sub>-  
3837 IsoP was found in the long-term wood workers as compared to those with shorter exposures, used  
3938 as the reference level. Multivariate regression analysis showed that the level of 15-F<sub>2t</sub>-IsoP was  
4039 significantly linearly correlated to the length of employment, in agreement with a previous study on  
4140 asbestos workers (Yoshida et al., 2001). In that study, the generation of an urinary biomarker of  
4241 oxidative stress correlated positively with the length of exposure. Rekhadevi et al. (Rekhadevi et  
4342 al., 2009) have similarly found an association between length of occupational exposure and  
4443 increase frequency of micronuclei. Taken together, the occurrence of elevated oxidative stress in  
4544 long-term wood workers can be possibly due to chronic inflammatory conditions. Our study  
4645 suggests that the measure of urinary F<sub>2</sub>-IsoPs can serve as a biomarker for assessing  
4746 occupational carcinogen exposure and improving workplace safety. Particular effort should  
4847 be devoted to studying long term health effects of exposure to wood dust, such as SNC.

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Particular effort should be devoted to study delayed reactions such as diseases that take a long  
time to develop, like SNC, that can be caused by long-term exposure to this carcinogenic agent.

## 5. Conclusions

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Our study provides a valuable contribution to the issue of oxidative stress in woodworking. An  
excessive ROS generation was demonstrated in exposed workers. Furthermore, we showed that  
exposure to organic solvents can increase the levels of urinary biomarkers of oxidative stress in  
wood workers. Results provide a basis for worker surveillance in occupational settings. F<sub>2</sub>-IsoP  
measure could be used for the evaluation of the effectiveness of targeted interventions aimed to

356 reduce exposures to various environmental carcinogens. A more effective control of occupational  
357 health risks could decrease the incidence of illness at work and improve the health of the  
358 workforce.

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365 **Declarations of interest**

366 None

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**Table 1.** Demographics and other variables by exposure group.

	<b>Study Population</b>	
	<b>Controls</b>	<b>Wood workers</b>
	<b>N</b>	<b>N</b>
Study population	57	123
Age (years)	47.2 ± 11	44.4 ± 11
Smoking habits		
Non-smokers	26	56
Ex-smokers	12	23
Smokers	19	44
Body mass index (BMI)	25 ± 0.40	25 ± 0.27
BMI categories		
Normal weight (18.5-24.9)	27	67
Overweight (25-24.99)	28	46
Obese (≥30)	2	10

**Table 2.** Mean level of 15-F<sub>2t</sub> isoprostane (15-F<sub>2t</sub>-IsoP) and Mean Ratio (MR) and 95% Confidence Interval (C.I.) by exposure group and other variables.

<b>Urinary 15-F<sub>2t</sub> Isoprostane and Wood Dust Exposure</b>			
	<b>N</b>	<b>15-F<sub>2t</sub> IsoP ± SE</b>	<b>MR, 95% C.I. P-value<sup>a</sup></b>
<b>Smoking habits</b>			
Non-smokers	82	3.3 ± 0.19	Reference level
Ex-smokers	35	3.7 ± 0.38	1.15, 0.96-1.01 0.092
Smokers	63	4.5 ± 0.31	1.43, 1.23-1.66 <0.0001
<b>Exposure group</b>			
Controls	57	2.9 ± 0.19	Reference level
Wood workers	123	4.2 ± 0.21	1.36, 1.18-1.57 <0.0001
<b>Co-carcinogen occupational exposures</b>			
Controls	57	2.9 ± 0.19	Reference level
Only wood dust exposed workers	85	4.1 ± 0.25	1.34, 1.15-1.56 0.0001
Wood dust with organic solvents and formaldehyde exposed workers	38	4.5 ± 0.43	1.41, 1.17-1.70 0.0002
<b>Occupational history</b>			
≤8 years	38	3.2 ± 0.18	Reference level
9-25 years	43	4.4 ± 0.38	1.27, 1.04-1.55 0.017
≥26 years	42	4.8 ± 0.44	1.41, 1.14-1.75 0.0014
<b>Body mass index categories</b>			
Normal weight (18.5-24.9 kg/m <sup>2</sup> )	94	3.6 ± 0.16	Reference level
Overweight (25-24.99 kg/m <sup>2</sup> )	74	4.0 ± 0.32	1.05, 0.90-1.21 0.5393
Obese (≥30 kg/m <sup>2</sup> )	12	4.4 ± 0.84	1.10, 0.84-1.44 0.5018

<sup>a</sup> P-values (Test of Wald) were adjusted for age and smoking, as appropriate.

**Table 3.** Average level of 15-F<sub>2t</sub> isoprostane (15-F<sub>2t</sub>-IsoP) and Mean Ratio (MR) and 95% Confidence Interval (C.I.) by exposure group after smoking stratification.

<b>Excess Risk of Urinary Biomarker in Wood Dust Workers</b>			
	<b>N</b>	<b>15-F<sub>2t</sub>-IsoP ± SE</b> ng/mg creatinine	<b>MR, 95% C.I.</b> <b>P-value<sup>a</sup></b>
<b>Non-smokers</b>			
Controls	26	2.8 ± 0.37	Reference level
Wood workers	56	3.5 ± 0.23	1.27, 1.01-1.59    0.0353
<b>Ex-smokers</b>			
Controls	12	2.7 ± 0.30	Reference level
Wood workers	23	4.3 ± 0.53	1.48, 1.09-2.01    0.01
<b>Smokers</b>			
Controls	19	3.3 ± 0.17	Reference level
Wood workers	44	5.0 ± 0.42	1.53, 1.23-1.91    0.0001

<sup>a</sup>P-values (Test of Wald) were adjusted for age.