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Wood dust and urinary 15-F_{2t} isoprostane in Italian industry workers 1

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19 Abstract

Wood dust is one of the most common occupational exposures, with about 3.6 million of workers in 20 the wood industry in Europe. Wood particles can deposit in the nose and the respiratory tract and 21 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 the generation of reactive oxygen species through activation of inflammatory cells could have a 24 25 role in the carcinogenicity of respirable wood dust. Therefore, we conducted a cross-sectional study to evaluate the prevalence of urinary 15-F_{2t} isoprostane (15-F_{2t}-IsoP), a biomarker of 26 oxidative stress and peroxidation of lipids, in 123 wood workers compared to 57 unexposed 27 controls living in Tuscany region, Italy. 15-F_{2t}-IsoP generation was measured by ELISA. The main 28 result of the present study showed that a statistically significant excess of this biomarker occurred 29 in the workers exposed to 1.48 mg/m³ of airborne wood dust with respect to the unexposed 30 controls (0.05 mg/m³). The overall mean ratio (MR) between the workers exposed to wood dust 31 32 and the controls was 1.36, 95% Confidence Interval (C.I.) 1.18-1.57, after correction for age and smoking habits. A significant increment of 15-F2t-IsoP (43%) was observed in the smokers as 33 compared to the non-smokers. The urinary excretion of 15-F_{2t}-IsoP was significantly associated 34 35 with co-exposure to organic solvents and formaldehyde, i.e., MR of 1.41, 95% C.I. 1.17-1.70, after adjustment for age and smoking habits. A 41% excess was observed in long-term wood workers, 36 37 95% C.I. 1.14-1.75. Multivariate regression analysis showed that the level of 15-F_{2t}-IsoP was linearly correlated to the length of exposure, regression coefficient (β) = 0.244 ± 0.002 (SE). The 38 overall increment by exposure group persisted after stratification for smoking habits. For instance, 39 in smokers, a 53% excess was detected in the wood workers as compared to the controls, 95% 40 41 C.I. 1.23-1.91. Our data support the hypothesis that oxidative stress and lipid peroxidation can have a role in the toxicity of wood dust F2-IsoP measure can be a tool for the evaluation of the 42 effectiveness of targeted interventions aimed to reduce exposures to environmental carcinogens. 43

44 **Key words:** wood dust, organic solvents, formaldehyde, 15-F_{2t} isoprostane, primary prevention, 45 occupational health.

47 **1. Introduction**

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

59 SNC has been under compulsory surveillance since 2008 in Italy, through the "Sinonasal Cancer National Registry" (Registro Nazionale Tumori Naso-Sinusali: ReNaTuNS), a nationwide cancer 60 registry coordinated by the National Institute for Insurance Against Accidents at Work (Istituto 61 Nazionale per l'Assicurazione contro gli Infortuni sul Lavoro: INAIL) (Binazzi et al., 2017). 62 63 Currently, the registry covers a proportion of Italy through regional structures devoted to the active search for cases from hospitals, to the definition of the modalities of exposure and has recorded 64 1,529 cases between 2000-2016. A study conducted by Demers et al. (Demers et al., 1995) found 65 66 a doubled risk statistically significant for sinonasal cancer in men employed in any wood-related job (OR = 2.0, 95% CI: 1.6 to 2.5) in comparison to men who had never worked in a wood-related job. 67 The increased risk was found among sawmill workers (OR = 2.5, 95% CI: 1.8 to 3.4), furniture 68 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 69 excess risk was observed among forestry, logging, pulp and paper workers. An increasing risk was 70 71 detected in relation to the duration of exposure, and lagging exposure by 5, 10 or 20 years increased the strength of the association between duration of employment and sinonasal 72 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, 95% C.I. 23.74-144.8, was even reported among wood workers of the Piedmont region, Italy 75 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. buccal and nasal cells, have been detected in wood workers compared to unexposed controls 79 80 (Bruschweiler et al., 2016; Palus et al., 1999; Rekhadevi et al., 2009). An enhanced risk for chromosomal instability was found in wood workers (Bruschweiler et al., 2014; Celik and Kanık, 81 2006; Rekhadevi et al., 2009). Discrepant results have been reported (Wultsch et al., 2015). In that 82 83 study, no induction of micronuclei was observed in wood workers exposed to 0.39-0.66 mg/m³ wood dust levels. Thus, further investigation into wood workers' occupational exposures are 84 85 warranted. Furthermore, co-exposures to chrome, organic solvents, tannins, formaldehyde, textile dust and pesticides have been reported in the wood industry (Binazzi et al., 2017). In 2012, IARC 86 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 radicals (Pylkkänen et al., 2009). Excessive production of reactive oxygen species (ROS) can 90 91 cause damage to lipids, proteins and DNA (Marnett, 2000). Peroxidation of lipids (LPO) can lead to the production of aldehydes, such as malondialdehyde and 4-hydroxynonenal (Marnett, 2000), as 92 93 well as to secondary oxidation products such as a series of prostaglandin-like products termed isoprostanes (IsoPs) (Roberts and Morrow, 2000). 94 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,

depending on the carbon atom to which the side chain hydroxyl is attached. Among the three major classes of IsoPs (F_{2^-} , D_{2^-} and E_{2^-}), F_2 -IsoPs are recognized as the most suitable biomarker for their chemical stability (Roberts and Morrow, 2000). The measurement of this biomarker is widely
 used for the analysis of endogenous oxidative stress following ROS production and peroxidation of
 lipids (Basu, 2008). F₂-IsoPs are more advantageous over other LPO biomarkers because they
 can be detected in a variety of biological samples including plasma, urine, lavage fluid and red
 blood cells (Milne et al., 2015). As IsoPs generate from LPO, their amounts provide an integrated
 measurement of unbalanced oxidant-antioxidant status (Lowe et al., 2013; Montuschi et al., 2004).

In the current study, we have investigated the potential effects of occupational exposure to wood 106 107 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-F_{2t}-IsoP) in the workers exposed to wood dust. One of the main advantages of using biomarkers is 109 110 that one can study signals of carcinogen exposure without having to wait for health effects as in classical epidemiological studies (Merlo et al., 1997; Munnia et al., 2017; Munnia et al., 2007; 111 Peluso et al., 1997; Peluso et al., 2012). Although F₂-IsoP can be evaluated in different biological 112 fluids, we employed urine due to its ready availability and the high stability of F₂-IsoP in this 113 medium (Morrow et al., 1999). Since obesity has been associated with increased F2-IsoP 114 concentrations (Annor et al., 2017; Il'yasova et al., 2015), we have examined the relationships 115 between urinary F₂-IsoPs and weight gain. Further understanding of the link between wood dust 116 and oxidative stress will improve knowledge of the mechanisms of carcinogenicity of this 117 118 occupational agent. Novelty of the current study is based on various items, including larger sample size, a different geographical area, and a different type of data, i.e., the measurement of F_{2} -lsoPs 119 in urine rather that of micronucleus and DNA strand-breaks in blood, buccal and nasal cells. 120

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 among those which are under compulsory health surveillance. Wood companies were contacted in 124 person by medical doctors with qualifications in occupational medicine. The inclusion criteria were 125 126 as follows: (a) only workers exposed to wood dust from wood industry: (b) only workers with a minimal exposure time of 1 year; (c) only controls without occupational history in industries 127 entailing exposure to known or suspected carcinogens; and (d) only controls resident in areas with 128 no proximity to major air pollution sources. All the volunteers involved in the study live and work in 129 130 the province of Florence, Tuscany, Italy. A 15-F₂t-IsoP was determined using spot urine samples collected in the morning at each workplace. Wood workers and the other subjects were contacted 131 by the local occupational health services. All the volunteers were informed about the study aim and 132 gave a written informed consent. A life-style questionnaire was filled by each participant (Peluso et 133 134 al., 2015). Detailed information on socio-demographic and anthropometric characteristics, education level, exposure to active and passive tobacco smoke, occupational exposure to wood 135 dust, protective gear use, co-exposures to organic solvents, welding and motor exhaust fumes and 136 137 occupational history were obtained. Subjects who had never smoked were classified as non-138 smokers, smokers who had guitted smoking from at least one month prior were classified as exsmokers, while individuals who smoked at least one cigarette per day were classified as smokers. 139 The Body Mass Index (BMI) categories reported from the National Heart National Heart, Lung, and 140 Blood Institute (https://www.nhlbi.nih.gov/) were used for grouping the study participants in normal 141 142 weight persons (18.5-24.9 kg/m²), overweight persons (25-25.99 kg/m²) and obese persons (\geq 30 143 kg/m²). BMI was determined using self-reported weight and height. Study procedures were performed in accordance with the Declaration of Helsinki for human studies and the guidelines of 144 145 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

- 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
- 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_{2t} isoprostane and creatinine measurement

The IsoP under investigation consists of one of the most abundant endogenous F_2 -IsoPs, i.e., the 158 15- F_{2t} -IsoP, a biomarker considered to be representative for human oxidant status (Milne et al., 2015), also referred to as 8-iso-prostaglandin $F_{2\alpha}$ (Roberts and Morrow, 2000). In the current study, the concentrations of 15- F_{2t} -IsoP were analyzed using the competitive enzyme-linked immunoassay (ELISA) with a specific microplate kit (Oxford, MI, USA), according to the manufacturer's instructions, as previously reported (Bono et al., 2015; Romanazzi et al., 2013). In order to normalize urinary dilution rate of 15- F_{2t} -IsoP an aliquot of urine was used to quantify the concentration of creatinine by the kinetic Jaffé procedure (Bartels and Cikes, 1969).

165 2.4 Statistical analysis

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

181 **3. Results**

182 3.1 Study population

The underlying basic population consisted of workers employed in the wood product manufacturing 183 sector of the province of Florence, Tuscany Region, Italy. 32 out of 44 consented to participate to 184 the study. Participation rates were ~95%. The concentration of $15-F_{21}$ -IsoPs in the wood workers 185 was evaluated along with control subjects, i.e., 123 wood workers and 57 controls. All participants 186 were males with a mean age of 45.3 ± 0.85 years and 35% of which were smokers. In the current 187 study, the wood workers consisted of carpenters and joiners, wood processing-plant operators, 188 woodworking machine operators, wood products assemblers, manufacturing labourers, industrial 189 robot operators and other wood related workers. The use of the most common Personal Protective 190 Equipment (PPE) in woodworking, i.e., disposable respirators, was generally reported from 191 majority of the wood workers. Controls were living in residential areas with no proximity to major air 192 pollution sources. The two groups had similar demographic, anthropometric and life-style 193 characteristics. The mean age of the wood workers and the controls was not statistically different 194 (Table 1). The average values of BMI were similar among the two groups (Table 1). The frequency 195 196 of smokers was similar between the groups, i.e., 36% of the wood workers and 37% of the controls, respectively. The distribution of subjects with respect to wood dust exposure with - out 197 co-exposures to other airborne carcinogens and smoking habits was reported in Table 2. Other 198 variables included length of employment and BMI groups (Tables 1-2). 199

200 3.2 Exposure data

The exposure measurement of wood dust air concentrations corresponds to a single value assessed from several consecutive samples by fixed positions (Scarselli et al., 2008). Airborne levels of industrial contaminants were quantified by daily mean concentration, i.e., 8-h timeweighted average (TWA-8), of respirable wood dust among exposed workers. The mean level of TWA-8 concentration of wood dust was 1.48 mg/m³ in wood workers.

3.3 Urinary 15- F_{2t} isoprostane level, smoking habits and occupational exposure

An increased amount of 15-F_{2t}-IsoP was found in the urine of wood workers as compared to the 207 controls (4.2 vs 2.9 ng/mg creatinine, Table 2). The multivariate analysis shows that the 36% 208 excess of 15-F_{2t}-lsoP of the wood workers was significantly higher as compared to the controls, 209 95% C.I. 1.18–1.57. Smokers had an average concentration of 15-F_{2t}-IsoP higher than ex-smokers 210 and non-smokers. A significant excess was found in the smokers in respect to the non-smokers, 211 95% Confidence Interval (C.I.) 1.23-1.66, after adjusting for age by statistical analysis. 212 Subsequently, the effect of co-exposures to other potential occupational carcinogens in the wood 213 industry on the level of 15-F_{2t}-IsoP was investigated. Therefore, workers were stratified into two 214 additional sub-groups: a) only wood dust exposed workers and b) mixed exposed workers. Table 2 215 indicates that the highest level of 15-F_{2t}-IsoP occurred in the wood workers who were co-exposed 216 217 to respirable organic solvents in respect to those who were only exposed to wood dust (4.5 and 4.0 ng/mg creatinine, respectively). After adjusting for age and smoking, the multivariate analysis 218 shows a 41% increment of 15-F_{2t}-IsoP, 95% C.I. 1.17-1.70, in the mixed exposed workers, 219 220 whereas a lower increment was observed in the only wood dust exposed workers, 95% C.I. 1.15-1.56. When we considered occupational history, there was a greater production of $15-F_{2t}$ -IsoP in 221 the long-term wood workers (4.8 ng/mg creatinine of 15-F_{2t}-IsoP) compared to those with shorter 222 occupational history (3.2 ng/mg creatinine). A 41% excess of 15-F_{2t}-IsoP was observed in the 223 wood workers with longer occupational exposure times, 95% C.I. 1.14-1.75. Then, the excretion of 224 225 15- F_{2t} -lsoPs was found to be significantly correlated with the length of dust exposure (p-value = 0.007). Table 3 reports the mean concentrations of 15-F_{2t}-IsoP and MR and 95% C.I. by exposure 226 group and smoking stratification. The highest amount of 15-F2t-IsoP was found in the wood 227 workers who were smokers, i.e., 5.0 ng/mg. 228

3.4 Urinary 15-F_{2t} isoprostane level and BMI groups

Since early studies have supported the hypothesis of a relationship between F₂-IsoP and weight 230 gain (Annor et al., 2017; Il'yasova et al., 2015), the association of this biomarker of oxidant status 231 with BMI was investigated. Study participants were divided by three BMI categories: a) normal 232 weight persons (18.5-24.9 kg/m²), b) overweight persons (25-25.99 kg/m²) and c) obese persons 233 $(\geq 30 \text{ kg/m}^2)$ to evaluate the relationship of F₂-IsoP with increase in body weight that could result in 234 235 excessive fat accumulation. Table 2 shows that the mean concentrations of 15-F_{2t}-lsoP of obese and overweight participants were higher than those with normal weight, but, no significant effect 236 237 was found.

238 4. Discussion

Wood processing causes small particles of wood dust to become suspended in the air. Workers 239 can inhale these particles, which can cause adverse health effects. The main result of this paper 240 showed that significantly enhanced level of F2-IsoP occurred in the workers compared to the 241 242 unexposed controls. A 36% excess of 15-F2t-IsoP levels was found in the wood workers as compared with the unexposed controls. Furthermore, the significant excess of 15-F2t-IsoP 243 persisted after smoking habit stratification. Among the wood workers, a 53% excess of 15-F2t-IsoP 244 245 was found in the smokers, a 48% excess was observed in the ex-smokers and a 27% in the nonsmokers as compared to the appropriate controls. The urinary excretion of this biomarker was 246 significantly associated with other parameters, including smoking habits, co-exposure to other 247 248 airborne carcinogens and length of employment. In particular, multivariate regression analysis showed that the level of 15-F2t-IsoP was linearly correlated to the length of exposure. In 249

agreement with our findings, other studies have previously reported increased oxidative stress 250 251 generation in relation to occupational exposure to wood dust (Bruschweiler et al., 2016; Palus et al., 1999; Rekhadevi et al., 2009). Our findings provide strengthening of the hypothesis that 252 oxidative stress and LPO can have a main role in the toxicity of wood dust. The analysis of F2-IsoP 253 254 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-F2t-IsoPs and wood 256 dust can be due to an increased production of ROS caused by inflammation after exposure fine and abundant airborne dust created during wood manipulation, maintenance activities and 257 258 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 xenobiotic particles, and from increased inflammatory cytokines and activated leukocytes (Gungor 260 et al., 2010; Vanhees et al., 2013). This is in keeping with the results of previous studies using a 261 biomarker of oxidative DNA damage and LPO (Bonassi et al., 2017; Bono et al., 2016; Bono et al., 262 2010; Peluso et al., 2013; Peluso et al., 2010). In support of our hypothesis, free radicals produced 263 264 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-Escuredo et al., 2012). 266

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Next, our study showed an empirical relationship between tobacco smoking and the urinary 268 excretion of $15-F_{2t}$ -IsoP, possibly related to the inhalation exposure to carcinogens contained in 269 tobacco smoke. A 43% increment of the level of 15-F_{2t}-IsoP was present in overall the smokers as 270 compared to the non-smokers. This excess is commonly interpreted as an harmful oxidative stress 271 (Basu, 2008). These findings were somewhat expected as active smokers inhale a broad range of 272 273 airborne carcinogens (IARC, 2004). The involvement of altered oxidative stress-related mechanisms in tobacco smoke carcinogenesis is in line with previous studies using various 274 275 biomarkers of oxidative stress and LPO (Munnia et al., 2004; Peluso et al., 2014; Romanazzi et al., 2013). Various groups have measured the concentrations of F_2 -IsoP in biological fluids of smokers. 276 The mean level of free and esterified F₂-lsoP in the urine and plasma of smokers have been found 277 278 to be significantly elevated as compared to non-smokers (Lowe et al., 2013). For instance, a previous cross-sectional study conducted on workers employed in an industry of plastic laminates 279 in Piedmont, Italy, founds that smoking habits were significantly associated with the urinary 280 excretion of 15-F_{2t}-IsoP (Romanazzi et al., 2013). When the relationship of 15-F_{2t}-IsoP with BMI 281 was investigated, we found that the levels of 15-F_{2t}-IsoP tended to increase with fat accumulations. 282 283 The 42% of the obese subjects showed indeed higher excretion of 15-F_{2t}-IsoP in respect to those with normal weight. This is partially in keeping with a previous work of Annor et al. (Annor et al., 284 2017) on the risk of diabetes and weight gain. In that study, the 35% of the obese individuals 285 286 showed greater levels of F₂-IsoPs as compared to the controls. Additional studies are necessary to understand if this biomarker can be used as measure of lifestyle habits and intervention targeted to 287 288 obesity prevention.

The threshold exposure limit recommended by the Italian law is 5 mg/m³ (Legislative Decree No 289 66/2000). This value will remain until the 2020th, after the entry into force of the new threshold 290 exposure limit of 3 mg/m³ for five years and thereafter of 2 mg/m³ (European Directive Decree No 291 2017/2398). In this context, the SIREP database aims to facilitate analysis of occupational 292 exposure figures for carcinogenic agents. In or study, the average amount of wood dust 293 concentrations experienced from the wood workers was lower than threshold exposure limit of 3 294 295 mq/m^3 (i.e., 1.48 mq/m^3). This result is consistent with that reported from a previous study of Scarselli et al. (Scarselli et al., 2008), where the mean concentrations of wood dust was of 1.44 296 mg/m³ for 1.181 companies in Italy. Although our static measurements of the concentrations of 297 298 industrial contaminants by fixed positions provide evidence of wood workers' exposure via air, they are not well representative of individual exposures to wood dust due to spatial and temporal 299 300 variations. Therefore, we could not assess the potential relationships of airborne measurements 301 with biomarker urinary excretion in exposed workers.

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

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

Our subsequent finding shows that the urinary excretion of 15-F_{2t}-IsoP in the workers exposed to 315 wood dust can aggravate with co-exposure to other respiratory carcinogens. An excess of 41% 316 was detected in the wood workers that were co-exposed to organic solvents compared to the 317 controls. Conversely, a lower excess was determined in the only wood dust exposed workers. High 318 319 biosynthesis of F₂-IsoP can be due to frequent free radical-catalyzed reactions induced by alterations of oxidative stress, antioxidant defence and inflammation especially caused by 320 occupational exposures to complex mixtures of airborne carcinogens. This is consistent with a 321 322 cross-sectional study of workers exposed to dust containing silica (Peluso et al., 2015). In this study, the amount of oxidative stress and LPO biomarker of the workers exposed to airborne silica 323 dust was greater in the case of occupational co-exposures to organic solvents, welding and motor 324 exhaust fumes. Constituents of organic solvents, such as benzene and formaldehyde can be 325 326 involved in the generation of oxidative stress and ROS (Bono et al., 2016; Bono et al., 2010; Sorensen et al., 2003) and cause the production of 15-F_{2t}-IsoP determined in the workers exposed 327 to wood dust. Our results suggest that the urinary level of F₂-IsoP resulting from exposures to 328 airborne wood dust can be affected from concomitant carcinogen exposures. Levels of oxidative 329 330 stress can increase with exposures to organic solvents (Salimi et al., 2017; Singh et al., 2010), leading to a greater imbalance between excessive ROS generation and their degradation by 331 antioxidants. The induction of reactive species can increase damage to membrane lipids, cellular 332 proteins and DNA. 333

A significant difference in the amount of 15-F_{2t}-IsoP was then observed among sub-groups of wood 334 workers with different occupational history. The urinary excretion of this biomarker of oxidant status 335 336 was significantly elevated in those subjects with longer exposure time. An 41% excess of $15-F_{2t}$ -IsoP was found in the long-term wood workers as compared to those with shorter exposures, used 337 as the reference level. Multivariate regression analysis showed that the level of 15-F_{2t}-IsoP was 338 339 significantly linearly correlated to the length of employment, in agreement with a previous study on asbestos workers (Yoshida et al., 2001). In that study, the generation of an urinary biomarker of 340 oxidative stress correlated positively with the length of exposure. Rekhadevi et al. (Rekhadevi et 341 al., 2009) have similarly found an association between length of occupational exposure and 342 increase frequency of micronuclei. Taken together, the occurrence of elevated oxidative stress in 343 344 long-term wood workers can be possibly due to chronic inflammatory conditions. Our study suggests that the measure of urinary F2-IsoPs can serve as a biomarker for assessing 345 occupational carcinogen exposure and improving workplace safety. Particular effort should 346 bedevoted to studyinglong termhealth effects of exposure to wood dust, such as SNC. 347 Particular effort should be devoted to study delayed reactions such as diseases that take a long 348

time to develop, like SNC, that can be caused by long-term exposure to this carcinogenic agent.

350 **5. Conclusions**

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. F₂-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

368 **References**

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1	Wood dust and urinary 15-F _{2t} isoprostane in Italian industry workers
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19 Abstract

Wood dust is one of the most common occupational exposures, with about 3.6 million of workers in the wood industry in Europe. Wood particles can deposit in the nose and the respiratory tract and cause adverse health effects. Occupational exposure to wood dust has been associated with malignant tumors of the nasal cavity and paranasal sinuses. The induction of oxidative stress and 725 826 1027 the generation of reactive oxygen species through activation of inflammatory cells could have a role in the carcinogenicity of respirable wood dust. Therefore, we conducted a cross-sectional study to evaluate the prevalence of urinary 15-F_{2t} isoprostane (15-F_{2t}-IsoP), a biomarker of oxidative stress and peroxidation of lipids, in 123 wood workers compared to 57 unexposed controls living in Tuscany region, Italy. 15-F_{2t}-IsoP generation was measured by ELISA. The main result of the present study showed that a statistically significant excess of this biomarker occurred in the workers exposed to 1.48 mg/m³ of airborne wood dust with respect to the unexposed **431** controls (0.05 mg/m³). 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 1734 1835 2036 smoking habits. A significant increment of 15-F2t-IsoP (43%) was observed in the smokers as compared to the non-smokers. The urinary excretion of 15-F_{2t}-IsoP was significantly associated with co-exposure to organic solvents and formaldehyde, i.e., MR of 1.41, 95% C.I. 1.17-1.70, after 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_{2t}-IsoP was linearly correlated to the length of exposure, regression coefficient (β) = 0.244 ± 0.002 (SE). The **38 339** overall increment by exposure group persisted after stratification for smoking habits. For instance, in smokers, a 53% excess was detected in the wood workers as compared to the controls, 95% ²541 C.I. 1.23-1.91. Our data support the hypothesis that oxidative stress and lipid peroxidation can have a role in the toxicity of wood dust F2-IsoP measure can be a tool for the evaluation of the effectiveness of targeted interventions aimed to reduce exposures to environmental carcinogens.

Key words: wood dust, organic solvents, formaldehyde, 15-F_{2t} isoprostane, primary prevention, occupational health.

1. Introduction 47

1 ₂48 Wood dust is one of the most common occupational exposures, with about 3.6 million of workers in the wood industry in Europe (Kauppinen et al., 2006). Wood particles can deposit in the nose and 349 450 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 £2 et al., 1968; Ball, 1968). In 1960, the first association with SNC was shown in the wood industry ⁷⁵³ ⁷⁵³ ⁹⁵⁴ 10⁵⁵ (Acheson et al., 1968). In 1995, this agent was classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) based mostly on a SNC excess (IARC, 1995). In 2012, the IARC confirmed the human carcinogenicity of wood dust and reported the first link with nasopharynx cancer (IARC, 2012). Considering other types of cancer, a meta-analysis 1156 1**57** has suggested a relationship with lung cancer (Hancock et al., 2015), but a significant influence of 1358 the geographic region was apparent.

14 SNC has been under compulsory surveillance since 2008 in Italy, through the "Sinonasal Cancer 1559 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 20 21 22 64 22 65 Currently, the registry covers a proportion of Italy through regional structures devoted to the active search for cases from hospitals, to the definition of the modalities of exposure and has recorded 1,529 cases between 2000-2016. A study conducted by Demers et al. (Demers et al., 1995) found a doubled risk statistically significant for sinonasal cancer in men employed in any wood-related job 2366 (OR = 2.0, 95% CI: 1.6 to 2.5) in comparison to men who had never worked in a wood-related job. 24**67** The increased risk was found among sawmill workers (OR = 2.5, 95% CI: 1.8 to 3.4), furniture 2**568** 2669 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 2770 excess risk was observed among forestry, logging, pulp and paper workers. An increasing risk was ²⁸71 ²⁹72 ³⁰73 ³¹73 ³²74 detected in relation to the duration of exposure, and lagging exposure by 5, 10 or 20 years increased the strength of the association between duration of employment and sinonasal adenocarcinoma. Elevated risk for adenocarcinoma of the nasal cavity and paranasal sinuses (ADCN), a SNC subtype frequently associated with wood dust exposure (IARC, 2012), OR 58.6, 95% C.I. 23.74-144.8, was even reported among wood workers of the Piedmont region, Italy 3 3**75** (d'Errico et al., 2009). Stronger ADCN risk, OR 179.9, 95% C.I. 55.37-584.4, was found among 3476 3577 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 4081418242834383chromosomal instability was found in wood workers (Bruschweiler et al., 2014; Celik and Kanık, 2006; Rekhadevi et al., 2009). Discrepant results have been reported (Wultsch et al., 2015). In that study, no induction of micronuclei was observed in wood workers exposed to 0.39-0.66 mg/m³ 4484 wood dust levels. Thus, further investigation into wood workers' occupational exposures are warranted. Furthermore, co-exposures to chrome, organic solvents, tannins, formaldehyde, textile 4585 4686 dust and pesticides have been reported in the wood industry (Binazzi et al., 2017). In 2012, IARC 4787 suggested that the cancer risk of wood workers could be associated with the inflammatory 4888 reactions following wood dust exposure rather than to the direct action of this carcinogen (IARC. 4989 5090 5191 5291 5392 2012). Inflammatory cells can generate a large spectrum of proinflammatory mediators and free radicals (Pylkkänen et al., 2009). Excessive production of reactive oxygen species (ROS) can cause damage to lipids, proteins and DNA (Marnett, 2000). Peroxidation of lipids (LPO) can lead to the production of aldehydes, such as malondialdehyde and 4-hydroxynonenal (Marnett, 2000), as well as to secondary oxidation products such as a series of prostaglandin-like products termed 5**₽**3 5594 isoprostanes (IsoPs) (Roberts and Morrow, 2000).

5**695** IsoPs are compounds generated from the non-enzymatic free radical-catalyzed peroxidation of 5796 arachidonic acid and other highly unsaturated polyunsaturated fatty acids (Janicka et al., 2010). 5897 IsoPs can be grouped into 4 subfamilies, denoted as 5-, 12-, 8-, or 15-series regioisomers, ⁵⁹98 depending on the carbon atom to which the side chain hydroxyl is attached. Among the three major 60 61 99 classes of IsoPs (F₂-, D₂- and E₂-), F₂-IsoPs are recognized as the most suitable biomarker for 62

their chemical stability (Roberts and Morrow, 2000). The measurement of this biomarker is widely 100 101 used for the analysis of endogenous oxidative stress following ROS production and peroxidation of lipids (Basu, 2008). F₂-IsoPs are more advantageous over other LPO biomarkers because they 1202 103 can be detected in a variety of biological samples including plasma, urine, lavage fluid and red **1**04 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).

1706 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-F_{2t}-IsoP) in the workers exposed to wood dust. One of the main advantages of using biomarkers is 1109 1**1**210 that one can study signals of carcinogen exposure without having to wait for health effects as in classical epidemiological studies (Merlo et al., 1997; Munnia et al., 2017; Munnia et al., 2007; 11311 11412 Peluso et al., 1997; Peluso et al., 2012). Although F₂-IsoP can be evaluated in different biological 1¢13 fluids, we employed urine due to its ready availability and the high stability of F₂-IsoP in this 1614171518161916medium (Morrow et al., 1999). Since obesity has been associated with increased F2-IsoP concentrations (Annor et al., 2017; Il'yasova et al., 2015), we have examined the relationships between urinary F₂-IsoPs and weight gain. Further understanding of the link between wood dust and oxidative stress will improve knowledge of the mechanisms of carcinogenicity of this $\frac{1}{2}$ 2<u>1</u>18 occupational agent. Novelty of the current study is based on various items, including larger sample size, a different geographical area, and a different type of data, i.e., the measurement of F_{2} -lsoPs <u>21/</u>219 in urine rather that of micronucleus and DNA strand-breaks in blood, buccal and nasal cells. 21320 24

2. Material and methods 21521

26 21722 2.1 Subjects and sampling

28 21923 A sample of 44 wood companies of the province of Florence, Tuscany, Italy was randomly selected 31/24 among those which are under compulsory health surveillance. Wood companies were contacted in 3125 person by medical doctors with qualifications in occupational medicine. The inclusion criteria were ³126 as follows: (a) only workers exposed to wood dust from wood industry: (b) only workers with a ³127 34 35 35 minimal exposure time of 1 year; (c) only controls without occupational history in industries entailing exposure to known or suspected carcinogens; and (d) only controls resident in areas with 3**1**29 no proximity to major air pollution sources. All the volunteers involved in the study live and work in 3**1/30** the province of Florence, Tuscany, Italy. A 15-F₂t-IsoP was determined using spot urine samples collected in the morning at each workplace. Wood workers and the other subjects were contacted 31831 31932 by the local occupational health services. All the volunteers were informed about the study aim and 4_1233 gave a written informed consent. A life-style questionnaire was filled by each participant (Peluso et ⁴134 al., 2015). Detailed information on socio-demographic and anthropometric characteristics, 42354336413641374537education level, exposure to active and passive tobacco smoke, occupational exposure to wood dust, protective gear use, co-exposures to organic solvents, welding and motor exhaust fumes and occupational history were obtained. Subjects who had never smoked were classified as non-41,38 smokers, smokers who had guitted smoking from at least one month prior were classified as exsmokers, while individuals who smoked at least one cigarette per day were classified as smokers. 41/39 The Body Mass Index (BMI) categories reported from the National Heart National Heart, Lung, and 41840 41941 Blood Institute (https://www.nhlbi.nih.gov/) were used for grouping the study participants in normal 51/42 weight persons (18.5-24.9 kg/m²), overweight persons (25-25.99 kg/m²) and obese persons (\geq 30 ⁵143 ⁵143 ⁵144 ⁵345 ⁵145 ⁵145 ⁵146 kg/m²). BMI was determined using self-reported weight and height. Study procedures were performed in accordance with the Declaration of Helsinki for human studies and the guidelines of the General Hospital Institutional Committee that reviewed and approved the present protocol.

2.2 Exposure data

57 58 58 Data on carcinogen exposure are collected by employers and regularly sent to the Italian Institute ₅1,48 for Occupational Safety and Prevention (ISPESL) (Italian legislative decree no. 626 of 19 September 1994). Such information is named exposure registries and includes guantitative 61049 61150 measurements of wood dust exposure. Companies are responsible for collecting the exposure

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measurements in accordance with the EN 689:1995 regulation by the European Committee on 151 Standardization (Scarselli et al., 2008). For the purpose of this research, data on occupational 1152 1253 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** 5 concentrations.

156 7 157 2.3 Urinary 15- F_{2t} isoprostane and creatinine measurement

The IsoP under investigation consists of one of the most abundant endogenous F₂-IsoPs, i.e., the 15715810115911591160126015-F_{2t}-IsoP, a biomarker considered to be representative for human oxidant status (Milne et al., 2015), also referred to as 8-iso-prostaglandin $F_{2\alpha}$ (Roberts and Morrow, 2000). In the current study, the concentrations of 15-F2t-IsoP were analyzed using the competitive enzyme-linked immunoassay (ELISA) with a specific microplate kit (Oxford, MI, USA), according to the 1<u>1</u>61 manufacturer's instructions, as previously reported (Bono et al., 2015; Romanazzi et al., 2013). In 1**1**62 order to normalize urinary dilution rate of 15-F_{2t}-IsoP an aliquot of urine was used to quantify the 1163 11664 concentration of creatinine by the kinetic Jaffé procedure (Bartels and Cikes, 1969). 17

11665 2.4 Statistical analysis 19

The level of 15-F_{2t}-IsoP was expressed as ng/mg creatinine. Given the right-skewed distribution of 21066 ²167 this biomarker, the data were log transformed to stabilize the variance and normalize the ²168 distribution. Multivariate statistical analyses were applied using log-normal regression models 2169240247025702571including age (continuous), tobacco smoking, i.e., non-smokers, ex-smokers, smokers, occupational history (years), and BMI, as predictive variables to evaluate the association between exposure to wood dust and the urinary excretion of 15-F2t-IsoP in the study participants. Results were adjusted for age and smoking. This was based on a previous study showing potential 2**1**72 associations between these variables and biomarker levels (Ceppi et al., 2011). Wood workers 21873 21974 were classified according to occupational exposures in two additional sub-groups: a) wood workers 31075 exposed to wood dust alone and b) wood workers with co-exposures to organic solvents. The 3176 regression parameters estimated from the models were interpreted as ratios [Means Ratio (MR)] ³177 ³178 ³479 ³480 ³7 ³480 ³7 ³480 ³7 ³481 ³9 4482 between the means of 15-F2t-IsoPs of each level of the categorical variables with respect to the reference level, as appropriate. The MR was used as a measure of effect (van Houwelingen et al., 2002). A p-value of <0.05 (two-tailed) was considered significant. Data were analyzed using SAS9.3 and SPSS 20.0 (IBM SPSS Statistics, New York, NY).

3. Results

3.1 Study population

41 41283 The underlying basic population consisted of workers employed in the wood product manufacturing sector of the province of Florence, Tuscany Region, Italy. 32 out of 44 consented to participate to 4**1**₃84 the study. Participation rates were ~95%. The concentration of 15-F₂₁-IsoPs in the wood workers 41485 41786 was evaluated along with control subjects, i.e., 123 wood workers and 57 controls. All participants 41787 were males with a mean age of 45.3 ± 0.85 years and 35% of which were smokers. In the current 41788 study, the wood workers consisted of carpenters and joiners, wood processing-plant operators, 489 49 190 50 5191 woodworking machine operators, wood products assemblers, manufacturing labourers, industrial robot operators and other wood related workers. The use of the most common Personal Protective Equipment (PPE) in woodworking, i.e., disposable respirators, was generally reported from majority of the wood workers. Controls were living in residential areas with no proximity to major air 5192 pollution sources. The two groups had similar demographic, anthropometric and life-style 51;93 51494 characteristics. The mean age of the wood workers and the controls was not statistically different 51595 (Table 1). The average values of BMI were similar among the two groups (Table 1). The frequency 5196 of smokers was similar between the groups, i.e., 36% of the wood workers and 37% of the ⁵1797 controls, respectively. The distribution of subjects with respect to wood dust exposure with - out ⁵198 co-exposures to other airborne carcinogens and smoking habits was reported in Table 2. Other 6199 variables included length of employment and BMI groups (Tables 1-2).

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200 3.2 Exposure data

201 The exposure measurement of wood dust air concentrations corresponds to a single value assessed from several consecutive samples by fixed positions (Scarselli et al., 2008). Airborne 202 levels of industrial contaminants were quantified by daily mean concentration, i.e., 8-h time-2103 204 weighted average (TWA-8), of respirable wood dust among exposed workers. The mean level of **205** 7 TWA-8 concentration of wood dust was 1.48 mg/m³ in wood workers.

3.3 Urinary 15-F_{2t} isoprostane level, smoking habits and occupational exposure

206 9 1207 1208 1208 1209 1309 1210 An increased amount of 15-F_{2t}-IsoP was found in the urine of wood workers as compared to the controls (4.2 vs 2.9 ng/mg creatinine, Table 2). The multivariate analysis shows that the 36% excess of 15-F_{2t}-lsoP of the wood workers was significantly higher as compared to the controls, 95% C.I. 1.18–1.57. Smokers had an average concentration of 15-F_{2t}-IsoP higher than ex-smokers and non-smokers. A significant excess was found in the smokers in respect to the non-smokers, 1**2**11 95% Confidence Interval (C.I.) 1.23-1.66, after adjusting for age by statistical analysis. 12/12 12/13 Subsequently, the effect of co-exposures to other potential occupational carcinogens in the wood 12814 industry on the level of 15-F2t-IsoP was investigated. Therefore, workers were stratified into two ¹2²15 additional sub-groups: a) only wood dust exposed workers and b) mixed exposed workers. Table 2 2215 2216 2217 2217 2217 2218 2419 2420 indicates that the highest level of 15-F_{2t}-IsoP occurred in the wood workers who were co-exposed to respirable organic solvents in respect to those who were only exposed to wood dust (4.5 and 4.0 ng/mg creatinine, respectively). After adjusting for age and smoking, the multivariate analysis shows a 41% increment of 15-F_{2t}-IsoP, 95% C.I. 1.17-1.70, in the mixed exposed workers, whereas a lower increment was observed in the only wood dust exposed workers, 95% C.I. 1.15-2221 1.56. When we considered occupational history, there was a greater production of $15-F_{2t}$ -IsoP in 22722 the long-term wood workers (4.8 ng/mg creatinine of 15-F_{2t}-IsoP) compared to those with shorter occupational history (3.2 ng/mg creatinine). A 41% excess of 15-F_{2t}-IsoP was observed in the 22823 ²224 ³225 ³226 ³227 ³27 ³ wood workers with longer occupational exposure times, 95% C.I. 1.14-1.75. Then, the excretion of 15- F_{2t} -lsoPs was found to be significantly correlated with the length of dust exposure (p-value = 0.007). Table 3 reports the mean concentrations of 15-F_{2t}-IsoP and MR and 95% C.I. by exposure group and smoking stratification. The highest amount of 15-F2t-IsoP was found in the wood workers who were smokers, i.e., 5.0 ng/mg.

3.4 Urinary 15-F_{2t} isoprostane level and BMI groups

Since early studies have supported the hypothesis of a relationship between F₂-IsoP and weight 3231 gain (Annor et al., 2017; Il'yasova et al., 2015), the association of this biomarker of oxidant status 42332 with BMI was investigated. Study participants were divided by three BMI categories: a) normal 42133 weight persons (18.5-24.9 kg/m²), b) overweight persons (25-25.99 kg/m²) and c) obese persons $(\geq 30 \text{ kg/m}^2)$ to evaluate the relationship of F₂-IsoP with increase in body weight that could result in excessive fat accumulation. Table 2 shows that the mean concentrations of 15-F_{2t}-IsoP of obese and overweight participants were higher than those with normal weight, but, no significant effect was found.

4. Discussion

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Wood processing causes small particles of wood dust to become suspended in the air. Workers can inhale these particles, which can cause adverse health effects. The main result of this paper showed that significantly enhanced level of F2-IsoP occurred in the workers compared to the 52;42 unexposed controls. A 36% excess of 15-F2t-IsoP levels was found in the wood workers as 52443 compared with the unexposed controls. Furthermore, the significant excess of 15-F2t-IsoP 52544 persisted after smoking habit stratification. Among the wood workers, a 53% excess of 15-F2t-IsoP 52645 was found in the smokers, a 48% excess was observed in the ex-smokers and a 27% in the non-⁵2⁷46 ⁵2⁸47 ⁵248 ⁶248 smokers as compared to the appropriate controls. The urinary excretion of this biomarker was significantly associated with other parameters, including smoking habits, co-exposure to other airborne carcinogens and length of employment. In particular, multivariate regression analysis showed that the level of 15-F2t-IsoP was linearly correlated to the length of exposure. In 6249

250 agreement with our findings, other studies have previously reported increased oxidative stress generation in relation to occupational exposure to wood dust (Bruschweiler et al., 2016; Palus et 251 2252 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 F2-IsoP **2**54 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-F2t-IsoPs and wood 256 dust can be due to an increased production of ROS caused by inflammation after exposure fine 2⁄57 and abundant airborne dust created during wood manipulation, maintenance activities and 2,58 cleaning equipment. Increased oxidative stress and LPO can be caused from the oxidative burst of 1**2**59 activated macrophages and neutrophils, cells with a main role in phagocytosis and clearance of xenobiotic particles, and from increased inflammatory cytokines and activated leukocytes (Gungor 12160 12261 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 15 16 16 16 16 1265 1265 1265 1266through chronic inflammatory process and cancer disease have been implicated as the causal factor in the mutagenesis of the tumor suppressor gene TP53 (Brancato et al., 2016; Perez-Escuredo et al., 2012).

1**2**67 Next, our study showed an empirical relationship between tobacco smoking and the urinary 2268 22169 excretion of 15-F_{2t}-IsoP, possibly related to the inhalation exposure to carcinogens contained in 22270 tobacco smoke. A 43% increment of the level of 15-F_{2t}-IsoP was present in overall the smokers as ²²²71 ²²72 ²²73 ²73 ²74 ²75 ²75 compared to the non-smokers. This excess is commonly interpreted as an harmful oxidative stress (Basu, 2008). These findings were somewhat expected as active smokers inhale a broad range of airborne carcinogens (IARC, 2004). The involvement of altered oxidative stress-related mechanisms in tobacco smoke carcinogenesis is in line with previous studies using various biomarkers of oxidative stress and LPO (Munnia et al., 2004; Peluso et al., 2014; Romanazzi et al., 2**2**976 2013). Various groups have measured the concentrations of F_2 -IsoP in biological fluids of smokers. 32077 The mean level of free and esterified F₂-IsoP in the urine and plasma of smokers have been found 32178 to be significantly elevated as compared to non-smokers (Lowe et al., 2013). For instance, a 32279 previous cross-sectional study conducted on workers employed in an industry of plastic laminates ³2⁸0 ³2⁸1 ³2⁸1 ³2⁸2 ³2⁸2 ³2⁸3 in Piedmont, Italy, founds that smoking habits were significantly associated with the urinary excretion of 15-F_{2t}-IsoP (Romanazzi et al., 2013). When the relationship of 15-F_{2t}-IsoP with BMI was investigated, we found that the levels of 15-F_{2t}-IsoP tended to increase with fat accumulations. The 42% of the obese subjects showed indeed higher excretion of 15-F_{2t}-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., 2017) on the risk of diabetes and weight gain. In that study, the 35% of the obese individuals 3285 42786 showed greater levels of F₂-IsoPs as compared to the controls. Additional studies are necessary to 42187 understand if this biomarker can be used as measure of lifestyle habits and intervention targeted to 4288 obesity prevention. 43

4**24**89 The threshold exposure limit recommended by the Italian law is 5 mg/m³ (Legislative Decree No 4290 4291 4292 4292 4292 4293 66/2000). This value will remain until the 2020th, after the entry into force of the new threshold exposure limit of 3 mg/m³ for five years and thereafter of 2 mg/m³ (European Directive Decree No 2017/2398). In this context, the SIREP database aims to facilitate analysis of occupational exposure figures for carcinogenic agents. In or study, the average amount of wood dust 5294 concentrations experienced from the wood workers was lower than threshold exposure limit of 3 52195 mg/m^3 (i.e., 1.48 mg/m^3). This result is consistent with that reported from a previous study of 52296 Scarselli et al. (Scarselli et al., 2008), where the mean concentrations of wood dust was of 1.44 5297 mg/m³ for 1.181 companies in Italy. Although our static measurements of the concentrations of ⁵2498 industrial contaminants by fixed positions provide evidence of wood workers' exposure via air, they 52599 5300 5301 are not well representative of individual exposures to wood dust due to spatial and temporal variations. Therefore, we could not assess the potential relationships of airborne measurements with biomarker urinary excretion in exposed workers.

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

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al., 2006). Nevertheless, a limitation of our study is that no data on the variability of wood dust 304 305 concentrations within a facility were available. The bias due to the variability of airborne carcinogen levels in occupational settings is difficult to predict, but a large variation can be present in one spot 3206 307 of a factory versus another. There could be an underestimation of the exposure to wood dust 3408 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 3,11 exhaust ventilation for hand tools, that are commonly associated to high exposure levels to wood dust (Alwis et al., 1999), could be missed. Variations in the use of PPE (Alwis et al., 1999) and in 3,12 1**3**13 the effective application of WorkSafe procedures at work places could have influenced the personal levels of exposure to wood dust of our workers. 13114 12

Our subsequent finding shows that the urinary excretion of 15-F_{2t}-IsoP in the workers exposed to 13315 13416 wood dust can aggravate with co-exposure to other respiratory carcinogens. An excess of 41% ¹3717 was detected in the wood workers that were co-exposed to organic solvents compared to the ¹318 ¹319 ¹⁸20 ¹⁹21 ²⁰21 controls. Conversely, a lower excess was determined in the only wood dust exposed workers. High biosynthesis of F₂-IsoP can be due to frequent free radical-catalyzed reactions induced by alterations of oxidative stress, antioxidant defence and inflammation especially caused by occupational exposures to complex mixtures of airborne carcinogens. This is consistent with a 2<u>3</u>22 cross-sectional study of workers exposed to dust containing silica (Peluso et al., 2015). In this study, the amount of oxidative stress and LPO biomarker of the workers exposed to airborne silica 23223 23324 dust was greater in the case of occupational co-exposures to organic solvents, welding and motor 2**3£2**5 exhaust fumes. Constituents of organic solvents, such as benzene and formaldehyde can be ²526 involved in the generation of oxidative stress and ROS (Bono et al., 2016; Bono et al., 2010; ²327 ²327 ²328 ²328 ²329 ²329 Sorensen et al., 2003) and cause the production of 15-F_{2t}-IsoP determined in the workers exposed to wood dust. Our results suggest that the urinary level of F₂-IsoP resulting from exposures to airborne wood dust can be affected from concomitant carcinogen exposures. Levels of oxidative _33530 stress can increase with exposures to organic solvents (Salimi et al., 2017; Singh et al., 2010), leading to a greater imbalance between excessive ROS generation and their degradation by 3<u>3</u>31 antioxidants. The induction of reactive species can increase damage to membrane lipids, cellular 33232 33333 proteins and DNA. 34

A significant difference in the amount of 15-F_{2t}-IsoP was then observed among sub-groups of wood 33534 3435 workers with different occupational history. The urinary excretion of this biomarker of oxidant status ³3⁷36 was significantly elevated in those subjects with longer exposure time. An 41% excess of 15-F_{2t}-³337 ³337 ³338 ⁴020 IsoP was found in the long-term wood workers as compared to those with shorter exposures, used as the reference level. Multivariate regression analysis showed that the level of 15-F_{2t}-IsoP was ₄3<u></u>39 significantly linearly correlated to the length of employment, in agreement with a previous study on asbestos workers (Yoshida et al., 2001). In that study, the generation of an urinary biomarker of ₄3,40 43;41 oxidative stress correlated positively with the length of exposure. Rekhadevi et al. (Rekhadevi et 4344.2 al., 2009) have similarly found an association between length of occupational exposure and 43543 increase frequency of micronuclei. Taken together, the occurrence of elevated oxidative stress in 43644 long-term wood workers can be possibly due to chronic inflammatory conditions. Our study 43745 43745 43946 3947 5948 suggests that the measure of urinary F2-IsoPs can serve as a biomarker for assessing occupational carcinogen exposure and improving workplace safety. Particular effort should bedevoted to studyinglong termhealth effects of exposure to wood dust, such as SNC. 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. ₅3249

53 53₽0 **5. Conclusions**

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⁵⁵ ⁵³⁵¹Our study provides a valuable contribution to the issue of oxidative stress in woodworking. An ⁵³⁵²excessive ROS generation was demonstrated in exposed workers. Furthermore, we showed that ⁵³⁵³exposure to organic solvents can increase the levels of urinary biomarkers of oxidative stress in ⁵³⁵⁴wood workers. Results provide a basis for worker surveillance in occupational settings. F₂-IsoP ⁶³⁵⁵measure 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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Declarations of interest

None

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Study Po	pulation	
	Controls	Wood workers
I	z	z
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_{2t} isoprostane (15-F_{2t}-IsoP) and Mean Ratio (MR) and 95% Confidence Interval (C.I.) by exposure group and other variables.

Urinary 15-F _{2t} Isopro	stan	e and Wood Du	ist Exposure	
8		15-F _{2t} IsoP ± SE	MR, 95% C.I.	P-value ^a
	z	ng/mg creatinine		
Smoking habits				
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
Exposure group				
Controls	57	2.9 ± 0.19	Reference level	
Wood workers	123	4.2 ± 0.21	1.36, 1.18-1.57	<0.0001
Co-carcinogen occupational exposures				
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	38	4.5 ± 0.43	1.41, 1.17-1.70	0.0002
formaldehyde exposed workers				
Occupational history				
s8 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
Body mass index categories				
Normal weight (18.5-24.9 kg/m ²)	94	3.6 ± 0.16	Reference level	
Overweight (25-24.99 kg/m ²)	74	4.0 ± 0.32	1.05, 0.90-1.21	0.5393
Obese (≥30 kg/m²)	12	4.4 ± 0.84	1.10, 0.84-1.44	0.5018
^a P-values (Test of Wald) were adjusted	for ag	e and smoking, as a	ppropriate.	

Table 3. Average level of 15- F_{2t} isoprostane (15- F_{2t} -lsoP) and Mean Ratio (MR) and 95% Confidence Interval (C.I.) by exposure group after smoking stratification.

Excess Risk o	of Ur	inary Biomarker	in Wood Dust	Workers
		15-F _{2t} -IsoP ± SE	MR, 95% C.I.	P-value ^a
	z	ng/mg creatinine		
Non-smokers				
Controls	26	2.8 ± 0.37	Reference level	
Wood workers	56	3.5 ± 0.23	1.27, 1.01-1.59	0.0353
Ex-smokers				
Controls	12	2.7 ± 0.30	Reference level	
Wood workers	23	4.3 ± 0.53	1.48, 1.09-2.01	0.01
Smokers				
Controls	19	3.3 ± 0.17	Reference level	
Wood workers	44	50 + 042	153 1 23-1 91	0 0001

^a P-values (Test of Wald) were adjusted for age.