

Woodworkers and the inflammatory effects of softwood/hardwood dust: evidence from nasal cytology

Andrea Lovato¹ · Claudia Staffieri² · Giancarlo Ottaviano¹ · Rocco Cappellesso³ ·
Luciano Giacomelli³ · Giovanni Battista Bartolucci⁴ · Maria Luisa Scapellato⁴ ·
Gino Marioni¹

Received: 6 April 2015 / Accepted: 15 March 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract Our primary aim was to use nasal cytology to compare a group of woodworkers with a group of unexposed subjects to see whether wood dust exposure correlates with specific patterns of inflammatory or infectious rhinitis. A secondary aim was to seek any differences in nasal symptoms or nasal cytology between workers exposed to softwood vs hardwood dust, thereby comparing the inflammatory harmful potential of the two woods. Among 117 woodworkers at factories in the Veneto region (Italy), 40 exposed to either softwood or hardwood dust were assessed by means of a questionnaire, nasal cytology, and personal wood dust sampling, and compared with 40 unexposed controls. Woodworkers reported significantly more nasal symptoms than controls ($p = 0.0007$). The woodworker group's nasal smears contained significantly more neutrophils ($p < 0.00001$) and lymphocytes ($p = 0.02$) than the control group's. The softwood workers had significantly lower levels of personal exposure to wood dust than the hardwood workers ($p = 0.04$); there were no

significant differences in age, history of cigarette smoking, or period of exposure between these two sub-cohorts of woodworkers. A statistical trend indicated that softwood workers had more eosinophils ($p = 0.05$) and lymphocytes ($p = 0.05$) in their rhinocytograms. Nasal cytology revealed chronic inflammatory rhinitis in a significant proportion of woodworkers' enrolled in this study. It also suggested a different harmful potential for softwood and hardwood dust. Nasal cytology could prove useful in screening woodworkers for chronic inflammatory rhinitis. Further investigations are needed to examine the role of different types of wood dust in nasal inflammation.

Keywords Wood dust · Softwood · Hardwood · Occupational rhinitis · Nasal cytology · Personal exposure

Introduction

Hardwood is the common name given to broad-leaved trees, classified botanically as Angiosperms, while softwood is the common name given to conifers, classified botanically as Gymnosperms. Wood dust is one of the most common causes of occupational exposure to airborne particles, and it is believed to irritate the mucous membranes [1]. Wood dust has been classified as a human carcinogen by the International Agency for Research on Cancer, that states in a footnote that this is based on a marked increase in the rate of sinonasal cancer among workers exposed primarily to hardwood dust [2, 3]. Epidemiological data on the carcinogenic effects of wood dust are weaker for softwoods [4] than for hardwoods, making it very difficult to establish standards for softwood dust exposure.

Previous clinical studies have shown that exposure to both hardwood and softwood dust is also associated with

A. Lovato and C. Staffieri contributed equally to the manuscript's preparation.

✉ Andrea Lovato
andrea.lovato.3@hotmail.it

¹ Department of Neurosciences DNS, Otolaryngology Section, University of Padova, Via Giustiniani 2, 35128 Padua, Italy

² Department of Neurosciences DNS, Otolaryngology Section, Treviso Regional Hospital Branch, University of Padova, Padua, Italy

³ Department of Medicine DIMED, University of Padova, Padua, Italy

⁴ Department of Cardilogic, Thoracic and Vascular Sciences, Preventive Medicine and Risk Assessment Unit, University of Padova, Padua, Italy

several other health effects, such as rhinitis, chronic bronchitis, asthma, and other upper and lower respiratory symptoms [5, 6]; and it has been reported that the incidence of these conditions increases with the intensity of exposure [7]. Nasal obstruction in woodworkers has been confirmed by objective tests using rhinomanometry [8], nasal peak expiratory flow [9], and acoustic rhinometry [10].

Respiratory effects caused by wood dust are assumed to involve much the same pathophysiological mechanisms as in the case of other types of organic dust (such as grain dust and cotton dust), with the release of inflammatory mediators by inflammatory and epithelial cells [11]. In vitro studies analyzing cell lines exposed to softwood and hardwood dust have produced contradictory results [12, 13]. To the best of our knowledge, few clinical investigations have compared woodworkers exposed to either softwood or hardwood dust, and none of the studies performed to date used nasal cytology to do so.

The present investigation concerns a group of 40 woodworkers who only handled either softwood or hardwood species. Nasal cytology was used first to compare the group of woodworkers as a whole with a group of unexposed controls to see whether wood dust exposure correlates with specific patterns of inflammatory or infectious rhinitis. Then, we sought any differences in nasal symptoms or nasal cytology between the workers exposed to softwood vs hardwood dust, to compare the inflammatory harmful potential of the two.

Materials and methods

Subjects

The woodworkers who volunteered for the present study were enrolled during an industrial environment investigation conducted by the Padova University Preventive Medicine and Risk Assessment Service on woodworking factories in the Veneto region (Italy). Informed consent was obtained from all individuals taking part in the study, and all procedures performed for the purposes of the study complied with the ethical standards of our institutional research committee. A total of 117 woodworkers were administered a self-reporting questionnaire to investigate their nasal symptoms (nasal congestion, rhinorrhea, sneezing fits, nasal itching, anosmia/hyposmia, epistaxis, ageusia/hypogeusia, snoring, nocturnal oral breathing, cough, headache, and sore throat) during the previous 6 months, cigarette smoking history, years of exposure to wood dust and the species of wood involved (hardwood or softwood).

The inclusion criteria for the study group were: (1) male gender; (2) negative medical history for rhinitis; (3)

anterior rhinoscopy ruling out nasal polyps or obstructive septal deviations; (4) involvement in woodworking processes that entailed exposure to either hardwood or softwood species; and (5) wood dust exposure persisting for at least 12 months. Forty woodworkers met the above inclusion criteria and were enrolled for the present study.

A group of 40 volunteers meeting the above criteria (1) to (3), but with no history of occupational or other exposure to wood dust or other nasal irritants also answered the questionnaire in as much as concerned nasal symptoms, and their cigarette smoking history.

For the purposes of the statistical analysis, nasal congestion, rhinorrhea, sneezing fits, nasal itching, and anosmia/hyposmia were defined as major nasal symptoms and also considered separately.

Nasal cytology

Nasal cytology was performed by anterior rhinoscopy, using a nasal speculum. The collection technique involved scraping mucosa from the middle portion of the inferior turbinate, using a Rhino-Probe [14]. The sample was placed on a glass slide, fixed by air drying and then stained using the May–Grunwald Giemsa (MGG) method (Carlo Erba, Milan, Italy). This method is the most widely used in diagnostic nasal cytology because all the cellular components of the nasal mucosa—from inflammatory cells (neutrophils, eosinophils, mast cells, lymphocytes) to bacteria, spores, fungal hyphae and mucous secretions—stain easily. The slide was examined under a Nikon E600 light microscope (Nikon, Canada). In each rhinocytogram, 50 microscopic fields were analyzed at 1000× magnification to ascertain the presence of normal and abnormal cellular elements. Cell counts and bacterial and fungal analyses were scored by means of a semiquantitative grading method proposed by Meltzer and Jalowayski [15] as follows: Grade 0 (not visible); Grade 1 (occasional groups); Grade 2 (moderate number); Grade 3 (easily visible); Grade 4 (many of which cover the entire field of view).

Personal exposure

Personal exposure to inhalable wood dust was assessed using an Institute of Occupational Medicine (IOM) sampler equipped with PVC membranes (diameter 25 mm, pore diameter 1.2 µm) and connected to an air sampling pump (SKC Inc., Pennsylvania, USA, PCXR4-model, Fig. 1) operating at a flow rate of 2 l/min. Sampling was done during a full 8-h working day. The PVC membranes were conditioned in the Aquaria Climatic hood (Aquaria, Milan, Italy) at 20 ± 1 °C and 50 ± 5 % relative humidity for 48 h before and after the sampling period. The dust



Fig. 1 Woodworker wearing the IOM sampler connected to the air sampling pump

collected was measured gravimetrically on an MC-5 microscale with a detection threshold of 0.001 mg (Sartorius Mechatronics, Muggio, Milan, Italy).

Statistical analysis

Given the purposes of the investigation, we conducted two inter-group analyses, one to compare exposed and unexposed subjects, the other between the two sub-cohorts of woodworkers exposed to softwood and hardwood dust. Most of the variables were dichotomized: age [<38 vs ≥ 38 years (38 being the median age of the group of woodworkers)], smoking (no vs yes), nasal symptoms (absent vs present), major nasal symptoms (absent vs present), neutrophil grade (0 vs 1–4), eosinophil grade (0 vs 1–4), mast cell grade (0 vs 1–4), lymphocyte grade (0 vs 1–4), bacterial grade (0 vs 1–4), and personal exposure level (≤ 1 mg/m³ vs >1 mg/m³). Fisher's exact test was used to compare the dichotomized variables. When a significant p value was disclosed, the odds ratio and the corresponding 95 % confidence interval (CI) were calculated.

The Mann–Whitney test and the Kendall rank correlation coefficient were also used. A p value <0.05 was considered significant, while values in the range of $0.07 > p \geq 0.05$ were assumed to indicate a statistical trend. The STATATM 8.1 (Stata Corp, College Station, TX, USA) statistical package was used for all analyses.

Results

Woodworkers vs unexposed controls

The mean age of our sample was 40.8 years for the woodworkers [standard deviation (SD) 11.6 years] and

39.6 years for the controls (SD 11.7 years); age did not differ significantly between the two groups (Fisher's exact test; $p = 0.82$). Eleven woodworkers and 11 controls reported a history of cigarette smoking (Fisher's exact test; $p = 1.00$). Nasal symptoms and major nasal symptoms were reported by 27 (67 %) and 19 (47 %) woodworkers, and by 11 (27 %) and 9 (20 %) controls, respectively. Fisher's exact test disclosed a significant difference between the two groups in the distribution of nasal symptoms [$p = 0.0007$; odds ratio (OR) = 5.47, 95 % CI = 1.90–16.02], and of major nasal symptoms ($p = 0.02$; OR = 3.6, 95 % CI = 1.21–11.25).

The cell counts on nasal cytology (Fig. 2), and the presence of bacteria and fungi in the woodworkers and unexposed controls are shown in Table 1. Neutrophils were seen significantly more frequently in the woodworkers' nasal smears (Fisher's exact test; $p < 0.00001$; OR = 9.33, 95 % CI = 3.01–30.05) than in the control group's. Lymphocytes were also more common in the woodworkers' rhinocytograms (Fisher's exact test; $p = 0.02$). No other significant differences emerged between the two groups in terms of the other cytotype counts (Fisher's exact test; eosinophils, $p = 1.00$; mast cells, $p = 1.00$), or the presence of bacteria (Fisher's exact test; $p = 0.75$).

Workers exposed to softwood vs hardwood dust

Of the 40 woodworkers enrolled in the present study, 13 were exposed exclusively to softwoods (mainly pine and spruce) and 27 to hardwoods (mainly beech, oak, and teak). The mean age of the former group was 39.1 years (SD 14.7 years), while for the latter it was 41.5 years (SD 10.0 years) (Fisher's exact test; $p = 0.18$). One of the softwood workers and 10 of the hardwood workers reported a history of cigarette smoking (Fisher's exact test; $p = 0.07$).

There was no significant difference (Mann–Whitney test; $p = 0.60$) in the period of exposure to wood dust (in years) between the softwood (median 15 years; SD 17.6 years) and the hardwood (median 10 years; SD 10.4 years) groups. Personal exposure levels were ≤ 1 mg/m³ in 31 woodworkers and >1.0 mg/m³ in 9 (all in the hardwood group). None of the woodworkers in either group had levels of exposure to wood dust in excess of 5.0 mg/m³. The levels of exposure were significantly higher in the group working with hardwoods (Kendall rank correlation coefficient; $p = 0.04$).

When we compared the groups exposed to softwoods vs hardwood, we found a trend towards a significant difference in mean eosinophil (Mann–Whitney test; $p = 0.05$) and lymphocyte (Mann–Whitney test; $p = 0.05$) scores, but no significant differences in terms of the other cytotypes (Fisher's exact test; neutrophils, $p = 0.71$; mast cells,

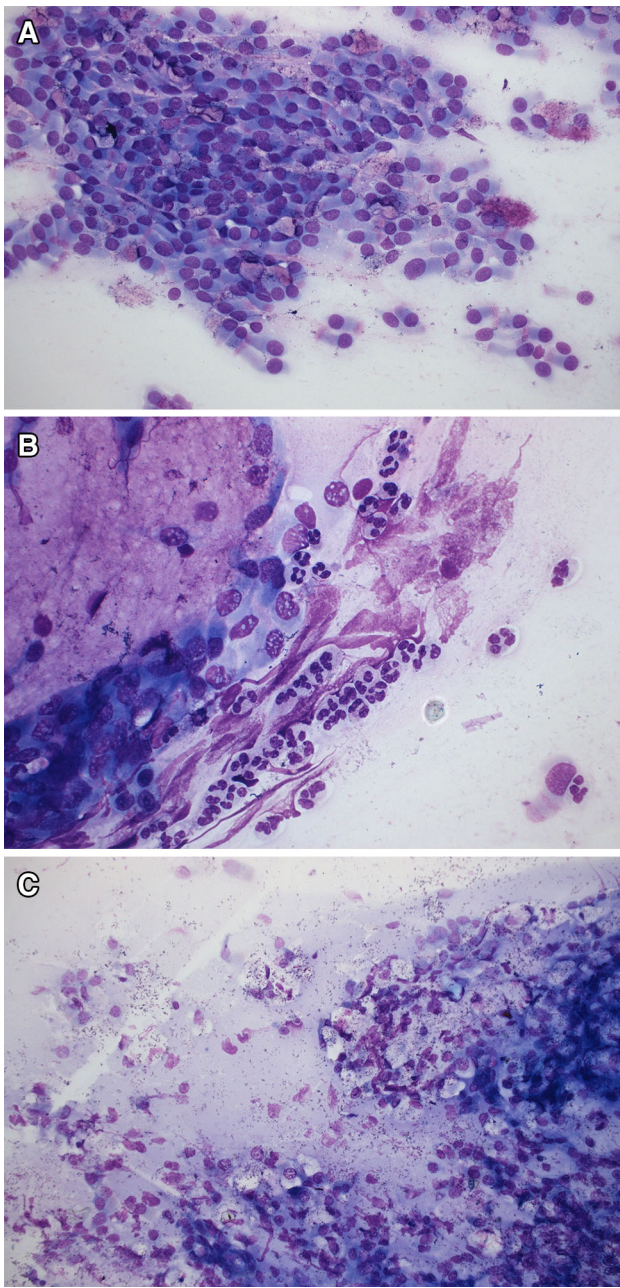


Fig. 2 May-Grunwald Giemsa (MGG) staining shows: **a** normal rhinocytogram (original magnification $\times 50$); **b** neutrophils (original magnification $\times 50$); **c** bacteria (original magnification $\times 20$)

$p = 0.58$), the presence of bacteria (Fisher's exact test; $p = 0.47$) (see Table 1), or nasal symptoms (Fisher's exact test; $p = 0.48$).

Discussion

Occupational exposure to wood dust is known to be associated with a higher risk of many malignant and other respiratory diseases [16]. Nasal symptoms have frequently

been reported in occupational groups exposed to wood dust. The inhaled particles appear to modify nasal function in terms of mucociliary activity and airflow resistance on rhinomanometry [17]. Evidence from analyses on woodworkers' nasal lavage fluids has suggested that wood dust may act as a mucous membrane irritant, triggering a chronic inflammatory response [18]. The primary goal of the present investigation was to use nasal cytology to compare a group of woodworkers with a group of controls not exposed to wood dust to see whether exposure correlated with specific patterns of inflammatory or infectious rhinitis. In this setting, the main advantages of nasal cytology on mucosa obtained by scraping lie in that is inexpensive and easy to perform in an outpatient setting using a light microscope.

In our sample, 67 % of the woodworkers reported nasal symptoms—considerably more than in the control group (27 %). Our figure is slightly higher than in other reports, according to which approximately 50 % of workers in the wooden furniture industry complain of nasal discomfort [19, 20]. This difference might reasonably be explained by the limited number of subjects included in our sample. On nasal cytology, our woodworkers also had significantly more neutrophils and lymphocytes in their rhinocytograms than controls, consistently with a picture of chronic inflammatory rhinitis. Holness et al. [21] likewise found more inflammatory cells in cabinet-makers' nasal smears than in those of control workers. Other studies [20, 22] on the nasal cellularity of woodworkers analyzed nasal lavage fluids, but the authors found no significant differences between woodworkers and controls. Such contradictory results may be due to the different sampling methods used (we used nasal mucosa scraped from the inferior turbinate, not nasal lavage). We preferred nasal cytology to nasal lavage fluid analysis mainly because we had to complete our sampling procedure at the workplace, and smears are easier to handle than lavage fluids.

Personal sampling of the inhalable fractions of wood dust disclosed no cases among our woodworkers in which the values exceeded the 5 mg/m^3 limit imposed by European and Italian legislation. On the other hand, 9 of our hardwood workers had personal exposure levels beyond the limit of 1 mg/m^3 permitted by the American Conference of Governmental Industrial Hygienists [23].

The European Directive 2004/37 [24] that establishes the exposure limit of 5 mg/m^3 only considers dust from hardwood and mixed wood species. Workers are usually exposed simultaneously to dust from several wood species, making it difficult to compare the harmful potential of dust from specific wood species, or from groups such as hardwoods vs softwoods [1]. In addition, the epidemiological data concerning the harmful potential of softwood dust is weaker than for other wood species, so it is not easy to set

Table 1 Nasal cytology scores in softwood (SW) and hardwood (HW) workers and controls

Grades ^a	Neutrophils		Lymphocytes		Eosinophils		Mast cells		Bacteria		Fungi	
	SW/ HW no. of cases	Controls no. of cases	SW/ HW no. of cases	Controls no. of cases	SW/ HW no. of cases	Controls no. of cases	SW/ HW no. of cases	Controls no. of cases	SW/ HW no. of cases	Controls no. of cases	SW/ HW no. of cases	Controls no. of cases
0	3/9	32	9/25	40	10/26	37	11/25	35	11/22	35	13/27	39
1–4	10/18	8	4/2	0	3/1	3	2/2	5	2/5	5	0/0	1

^a Grades were assigned using a semi-quantitative method proposed by Meltzer and Jalowayski [15] as follows: Grade 0 (not visible); Grade 1 (occasional groups); Grade 2 (moderate number); Grade 3 (easily visible); Grade 4 (many of which cover the entire field of view)

standards for softwood dust. Hence, our secondary aim to seek any differences in nasal symptoms or nasal cytology between workers only exposed to dust from either softwoods or hardwoods. Our sample consisted of 13 workers only exposed to softwood dust, and 27 only exposed to hardwood dust. While the limited number of individuals in each subgroup constitutes the main weakness of our study, its main strength lies in the homogeneity of the two subgroups in all other respects, i.e., they were all males with no significant differences in their age, history of cigarette smoking, or period of exposure to wood dust, and no medical history of rhinitis, and they all underwent personal sampling of the inhalable wood dust in their working environment.

The softwood workers revealed significantly lower levels of personal exposure to wood dust than the hardwood workers. The former reported nasal symptoms more often (77 %) than the latter (63 %), however, though the difference did not reach statistical significance. We found a trend towards statistical significance indicating that softwood workers had more eosinophils and lymphocytes in their rhinocytograms than hardwood workers, findings consistent with a picture on nasal cytology of chronic eosinophilic rhinitis in softwood workers. Dahlqvist et al. [25] analyzed the acute effects of exposure to softwood dust (Scots pine) in the nasal lavage fluids of a group of 19 volunteers, finding a significantly increased concentration of interleukin-6 (IL-6), a cytokine also secreted by the eosinophils. Määttä et al. [12] used an in vitro model (the murine macrophage cell line RAW 264) to characterize and compare the effects of dust from hardwood (beech and teak) and softwood (pine and spruce) on the expression of cytokines IL-1 β , IL-6, and TNF- α : all the tested hardwood and softwood dust induced TNF- α expression and inhibited IL-1 β expression, while IL-6 was induced by all, but beech wood dust. The authors concluded that hardwoods and softwoods had quite similar effects on cytokine expression in the RAW 264.7 cell line. Bornholdt et al. [13] tested the human epithelial cell line A549, finding an increased expression of cellular IL-6 and IL-8 mRNA induced by hardwood and softwood dust. Like us, the authors

identified a difference between the effects of the dust from the different wood species: the dust from spruce and pine (softwoods) seemed to stimulate a more severe inflammatory response than the dust from beech and oak, the typical hardwood species associated with cancer.

In conclusion, a significant proportion of the woodworkers considered in the present investigation revealed a chronic inflammatory rhinitis on nasal cytology, with higher neutrophil and lymphocyte counts than in controls. Nasal cytology seems to disclose a greater inflammatory harmful potential for softwood dust, which is associated with higher eosinophil and lymphocyte scores at lower levels of personal exposure. Nasal cytology may be a useful method for screening woodworkers for chronic inflammatory rhinitis. Further investigations on larger cohorts are needed to assess the role of dust from different wood species in nasal inflammation.

Acknowledgments The authors thank Frances Coburn for correcting the English version of this paper.

Compliance with ethical standards

Conflict of interest This was not an industry supported study. The authors have no conflicts of interest to disclose. The study was supported partly by Grant No. 60A07-8485/13 (G. Marioni) from the University of Padova, and partly by Grant No. 15/2009 (G. B. Bartolucci) from the Italian Workers' Compensation Authority (INAIL).

Statement of human rights All procedures performed in the study were in accordance with the ethical standards of our institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent Informed consent was obtained from all individuals participating in the study.

References

1. Demers PA, Teschke K, Kennedy SM (1997) What to do about softwood? A review of respiratory effects and recommendations regarding exposure limits. *Am J Ind Med* 31:385–398
2. International Agency for Research on Cancer (IARC) (1995) Wood dust and formaldehyde. IARC Monographs, vol 2. IARC, Lyon

3. International Agency for Research on Cancer (IARC) (2012) A review of human carcinogens: arsenic, metals, fibres, and dusts. IARC Monographs, vol 100C. IARC, Lyon
4. Vaughan TL, Davis S (1991) Wood dust exposure and squamous cell cancers of the upper respiratory tract. *Am J Epidemiol* 133:560–564
5. Jacobsen G, Schaumburg I, Sigsgaard T, Schlunssen V (2010) Non-malignant respiratory diseases and occupational exposure to wood dust. Part I. Fresh wood and mixed wood industry. *Ann Agric Environ Med* 17:15–28
6. Jacobsen G, Schaumburg I, Sigsgaard T, Schlunssen V (2010) Non-malignant respiratory diseases and occupational exposure to wood dust. Part II. Dry wood industry. *Ann Agric Environ Med* 17:29–44
7. Hessel PA, Herbert FA, Melenka LS, Yoshida K, Michaelchuk D, Nakaza M (1995) Lung health in sawmill workers exposed to pine and spruce. *Chest* 108:642–646
8. Wilhelmsson B, Drettner B (1984) Nasal problems in wood furniture workers. A study of symptoms and physiological variables. *Acta Otolaryngol* 98:548–555
9. Ahman M, Söderman E (1996) Serial nasal peak expiratory flow measurements in woodwork teachers. *Int Arch Occup Environ Health* 68:177–182
10. Schlunssen V, Schaumburg I, Andersen NT, Sigsgaard T, Pedersen OF (2002) Nasal patency is related to dust exposure in woodworkers. *Occup Environ Med* 59:23–29
11. Määttä J, Majuri ML, Luukkonen R et al (2005) Characterization of oak and birch dust-induced expression of cytokines and chemokines in mouse macrophage RAW 264.7 cells. *Toxicology* 215:25–36
12. Määttä J, Luukkonen R, Husgafvel-Pursiainen K, Alenius H, Savolainen K (2006) Comparison of hardwood and softwood dust-induced expression of cytokines and chemokines in mouse macrophage RAW 264.7 cells. *Toxicology* 218:13–21
13. Bornholdt J, Saber AT, Sharma AK, Savolainen K, Vogel U, Wallin H (2007) Inflammatory response and genotoxicity of seven wood dusts in the human epithelial cell line A549. *Mutat Res* 632:78–88
14. Gelardi M, Passalacqua G, Fiorella ML, Mosca A, Quaranta N (2011) Nasal cytology: the “infectious spot”, an expression of a morphological-chromatic biofilm. *Eur J Clin Microbiol Infect Dis* 30:1105–1109
15. Meltzer EO, Jalowayski AA (1988) Nasal cytology in clinical practice. *Am J Rhinol* 2:47–54
16. Enarson DA, Chan-Yeung M (1990) Characterization of health effects of wood dust exposures. *Am J Ind Med* 17:33–38
17. Holmström M, Rosén G, Wilhelmsson B (1991) Symptoms, airway physiology and histology of workers exposed to medium-density fiber board. *Scand J Work Environ Health* 17:409–413
18. Granstrand P, Nylander-French L, Holmström M (1998) Biomarkers of nasal inflammation in wood-surface coating industry workers. *Am J Ind Med* 33:392–399
19. Holmström M, Rosén G, Wähländer L (1997) Effect of nasal lavage on nasal symptoms and physiology in wood industry workers. *Rhinology* 35:108–112
20. Ahman M, Holmström M, Ingelman-Sundberg H (1995) Inflammatory markers in nasal lavage fluid from Industrial Arts teachers. *Am J Ind Med* 28:541–550
21. Holness DL, Sass-Kortsak AM, Pilger CW, Nethercott JR (1985) Respiratory function and exposure-effect relationships in wood dust-exposed and control workers. *J Occup Med* 27:501–506
22. Priha E, Pennanen S, Rantio T, Uitti J, Liesivuori J (2004) Exposure to and acute effects of medium-density fiber board dust. *J Occup Environ Hyg* 1:738–744
23. ACGIH (2012) Threshold limit values and biological exposure indices for 2012. Cincinnati: American Conference of Governmental Industrial Hygienists
24. Council Directive 2004/37/EC of 29 April 2004 on the protection of workers from the risk related to exposure to carcinogens or mutagens at work. Available at: <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:229:0023:0034:EN:PDF>. Accessed 15 Mar 2015
25. Dahlqvist M, Palmberg L, Malmberg P, Sundblad BM, Ulfvarson U, Zhiping W (1996) Acute effects of exposure to air contaminants in a sawmill on healthy volunteers. *Occup Environ Med* 53:586–590