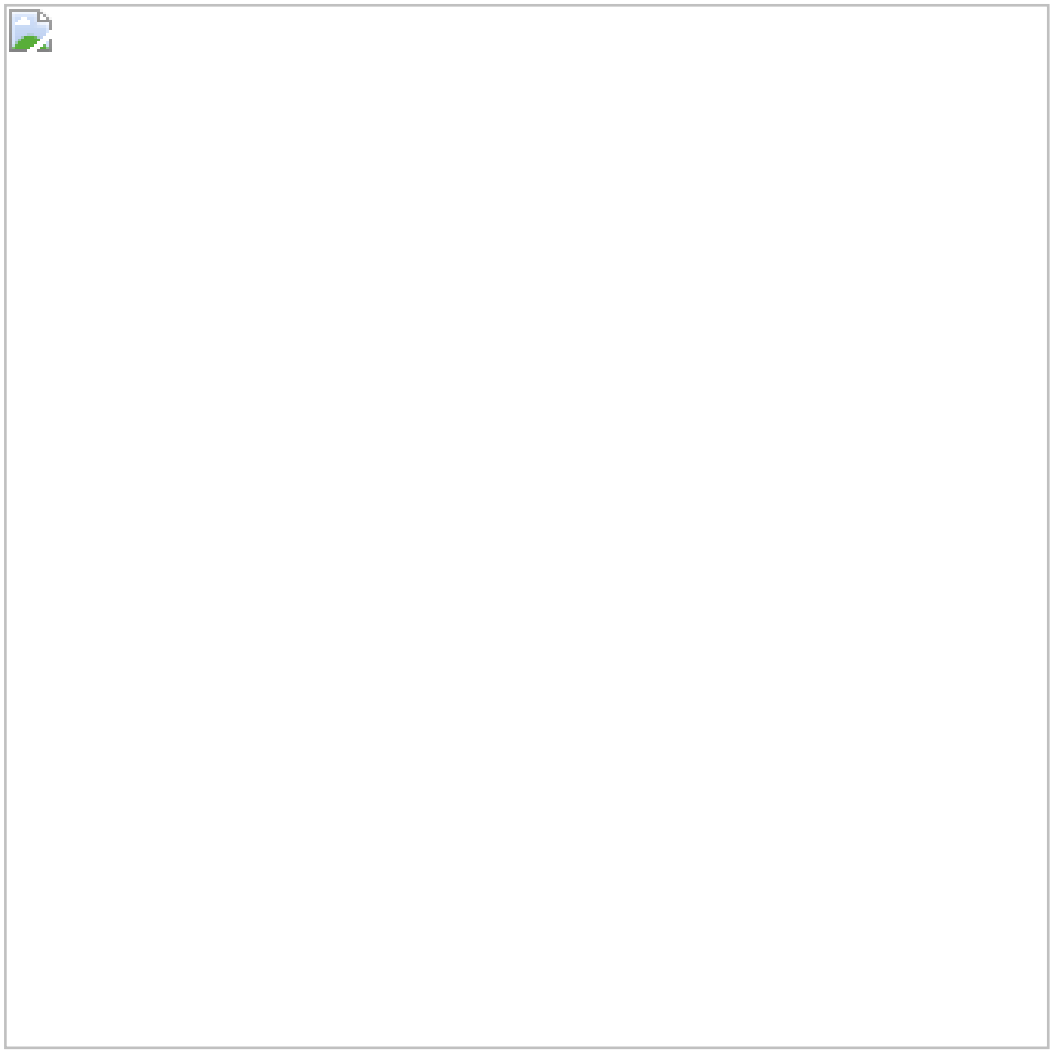

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DNA Damage among Wood Workers Assessed with the Comet Assay

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ABSTRACT: Exposure to wood dust, a human carcinogen, is common in wood-related industries, and millions of workers are occupationally exposed to wood dust worldwide. The comet assay is a rapid, simple, and sensitive method for determining DNA damage. The objective of this study was to investigate the DNA damage associated with occupational exposure to wood dust using the comet assay (peripheral blood samples) among nonsmoking wood workers ($n = 31$, furniture and construction workers) and controls ($n = 19$). DNA damage was greater in the group exposed to composite wood products compared to the group exposed to natural woods and controls ($P < 0.001$). No difference in DNA damage was observed between workers exposed to natural woods and controls ($P = 0.13$). Duration of exposure and current dust concentrations had no effect on DNA damage. In future studies, workers' exposures should include cumulative dust concentrations and exposures originating from the binders used in composite wood products.

KEYWORDS: comet assay, wood dust, DNA damage, occupational exposure

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Introduction

Wood dust has been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer[1] based mostly on an excess of sinonasal cancers. To reduce the risk of cancer, it is, therefore, necessary to find better ways of prevention, early diagnosis, and treatment of wood dust-related diseases.[2]

The genotoxic effects of wood dust among exposed workers have been assessed using biomarkers of effects, such as the micronucleus (MN) assay in blood,[3] buccal,[4,5] and nasal cells.[5] Although the genotoxic effects of wood dust have been known for some time, only two studies[3,6] have measured DNA damage using the comet assay among wood workers.

Dust generation depends on the type of wood material, size, and surface area of the aerosolized particles. Workers' wood dust exposures depend on the wood processing task performed (sanding, sawing, or cutting). The more enclosed a workspace space is, the more the dust will build up. A wood worker's dust exposure also increase with frequency and time spent on a dusty task. Although controls to reduce concentrations of airborne dust exist, such as ventilation (general, local, and on-tool extraction) and general housekeeping (dust removal/cleaning), the dust concentrations reported for the furniture making and construction industry are high (>5 mg m^3) in Ref. 7.

Complicating the exposure scenario further is the unknown intrinsic genotoxic properties of different types of wood dust. Wood dust is a complex substance composed of a varying number of substances with different toxic properties. The furniture making and construction industries mainly use natural woods (untreated) and composite wood products. Composite wood products refer to plywood, particle board, fiberboard, medium density fiberboard (MDF), and other boards. MDF is produced from wood broken down into fibers and combined with wax and a resin (glue). One common resin is made from melamine and formaldehyde. Natural woods are often divided into softwood (coniferous species) or hardwood (deciduous species), reflecting their density and not toxicity. Tree species used in the woodworking industry vary considerable between geographical areas. The demand for sustainable growth in this industry may change the use of wood material in the future, as has been observed with the increasing use of the fast growing bamboo (grass family).

In *in vitro* studies, several techniques have been used to assess DNA damage from wood dust exposure. Beech wood dust was shown to be mutagenic using the salmonella/mammalian microsome assay.[8] Human epithelial cell line (A549) exposed to dust from beech, teak, pine, and MDF, separately, has all shown detectable DNA damage.[9]

Comet assay. The comet assay is a visual and quantitative method for DNA damage measured in blood cells.[10–13] The comet assay has been suggested as an important tool for rapidly detecting DNA damage in exposed populations.[14] It is sensitive for DNA damage detection at the cellular level[15]; it requires only a small number of cells per sample (<10,000) and can be used on any eukaryote single-cell population. The comet assay, under alkaline conditions (pH > 13) can detect DNA strand breaks and alkali labile sites; and the extent of DNA migration correlates with the amount of DNA damage.[12] The alkaline version (pH > 13) of the comet assay has been identified as a sensitive version for the detection of induced DNA damage.[13] There is currently a lack of studies using the comet assay to measure DNA damage in an occupational context. Only two studies have investigated DNA damage using the comet assay among occupationally exposed wood workers.[3,6] Both found comet scores to be greater among wood dust-exposed workers compared to controls. Smoking contributed to the comet score in both studies.

Although the comet assay is not specific with respect to exposure agent – of relevance to the wood working industry with coexposures to other carcinogens, such as formaldehyde, wood preservatives, and also polycyclic aromatic hydrocarbons (PAH)[16] – it does indicate an overall genotoxic risk. In addition, smoking has been shown to be a confounding factor in a meta-analysis of occupational studies (including 38 studies) using the comet assay to assess the DNA damage.[17]

Biological monitoring of occupational cancer risk among the millions of workers worldwide exposed to wood dust[1] is of importance but is often not used because it is poorly understood by occupational hygienists and occupational physicians. In this article, we seek to advance the scientific understanding of the comet assay test as a tool to assess cancer risk associated with wood dust exposure, especially exposures to natural wood and composite wood.

The aims of the present study were to (1) determine the genotoxic effects among nonsmoking wood workers exposed to wood dust using the comet assay; (2) investigate the relationship between DNA damage with present day exposure to wood dust, total PAHs, and benzo[a]pyrene (BaP); and (3) compare the increase in MN score that we observed in this population with the results of the comet assay.

Materials and Methods

Study population. This study used the same population of workers and controls that were used in a previous study using a MN test to assess the DNA damage in nasal turbinate and buccal cell samples.[5] Blood samples used for the comet assay were collected at the same time. However, due to the lack of resources, it is not until now that we were able to present the comet assay results. Briefly, exposed workers (31 male parquet layers, installers and carpenters) were grouped in seven exposure groups, ie, the five traditional factories (using common wood working tools) and the two workshops in a modern factory (using an automated wood router). They were regrouped according to the predominant wood type used: *natural woods*, such as untreated fir, spruce, beech, and oak (12 workers), and *composite wood products*, such as MDF (19 workers). The nonexposed group (n = 20) was all male, working as pantry chefs preparing cold dishes, such as salads, canapés, pâtés, and terrines, in a cool and well-ventilated area away from the cooking part of the commercial kitchen and computer engineers.

Study participants were nonsmokers or exsmokers (minimum one year) and occasional alcohol consumers (1–2 glasses/week). They completed a questionnaire to determine demographic and lifestyle data (smoking, alcohol intake, and dietary intake of PAHs), medical status (taking medication yes/no), and work history information. Types of wood materials used during the visit were recorded. All subjects signed a consent form before entering the study. The study was approved by the Ethics Committee of the Faculty of Biology and Medicine at the University of Lausanne. Our research complied with the principles of the Declaration of Helsinki.

Wood dust, PAHs, and BaP exposure assessment. Personal inhalable dust concentrations were determined for two consecutive work shifts for all exposed workers as described in the study by Bruschweiler et al.[5] The wood dust was collected on a 37 mm closed-face cassette sampler equipped with glass fiber filters (GF/B, Ø37 mm, Whatman) that operated with a flow rate of 2 L/minute (Esscort ELF pump; MSA). Wood dust concentrations were determined by gravimetric analysis. PAH concentrations in the dust samples were determined as previously described by Vu-Duc et al.[18,19] Wood dust samples were extracted, followed by microcolumn purification, and analyzed using a capillary gas chromatography (Varian Saturn 2000 MS) ion trap mass spectrometry. All samples were analyzed for 21 PAHs: naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, benzo[a]fluoranthene, benzo[e]pyrene, BaP, perylene, indeno[1,2,3-cd]pyrene, benzo[b]chrysene, benzo[ghi]perylene, dibenzo[a,j]anthracene, dibenzo[a,h]anthracene, and dibenzo[a,c]anthracene. The sum of these 21 PAHs is later referred to as total PAHs.

Chemical reagents. Sodium hydroxide (NaOH, no. 1.06498.1000), sodium chloride (NaCl, no. 1.06404.1000), tris (hydroxynetyl)-aminomethan (no. 1.08382.0500), triton (no. 11869), and acetic acid (no. 1.00063) were bought from Merck, dimethyl silfoxide (no. D5879) and ethylenediaminetetraacetic acid (EDTA) tetra sodium salt (no. E6758) from Sigma-Aldrich, phosphate-buffered saline (no. 4870-500)-free magnesium from Trevigen, regular agarose (no. 161-3103), low melt agarose (no. 161–3111), and silver stain kit from Bio-Rad (no. 161-0461-5).

Comet assay. To reduce variability due to time for DNA repair, we standardized the collection time and processing of

the blood samples. The blood samples were collected at the end of work shift, and comet assay analyses were performed within three hours in order to respect optimal sample time for detecting DNA damage. Whole blood (40 µL) was obtained by finger puncture with a safety lancet normal, 21 G (Sarstedt) at the end of the work shift. The blood samples were immediately mixed with phosphate-buffered saline (500 µL) in a standard micro test tube 3810X (Eppendorf, DE). This cell suspension was stored (4 °C) in standard micro test tube 3810X until processed (within three hours).

The comet assay was performed as described by Singh et al.[11] and Speit and Schmid.[20] Briefly, three agarose layers per gel bond were used. The bottom layer was prepared with 0.5% regular agarose, which was transferred into a gel bond film (Gel Bond® Film; Lonza) (100 mm × 150 mm). This regular agarose layer was covered with microscopic cover (20 mm × 20 mm) and left for 10 minutes at 4 °C. The microscopic cover was removed after agarose polymerization. For the cell containing layer, we mixed the cell suspension (50 µL) with 0.7% low melt agarose (325 µL). An aliquot (70 µL) of this mixture (cell suspension and low melt agarose) was transferred onto the regular agarose as the second layer and then covered with the microscopic cover. This layer solidified at 4 °C after 20 minutes, and the microscopic cover removed. The cell-containing layer was then covered with 0.7% low melt agarose (70 µL) (third layer) and immediately covered with a microscopic cover. This third layer was left for 15 minutes at 4 °C. The microscopic cover was removed. The GelBond film containing the samples were placed in lysis solution (2 M NaCl, 0.15 M NaOH, 100 mM Na2 EDTA, 10 mM Tris, 10% dimethyl silfoxide, and 1% triton X-100) for one hour at 4 °C. The film was then immersed in cold electrophoresis buffer (0.3 M NaOH, 0.9 mM EDTA, pH > 13) for 20 minutes to allow the unwinding of DNA. Electrophoresis was conducted for 24 minutes at 23 V, 270 mA with fresh electrophoresis buffer. The film was washed with neutralizing buffer (400 mM Tris solution, pH 7.5) for 15 minutes and left to dry at room temperature overnight. Samples were stained with silver stain following the manufacturer's instructions. Briefly, samples were fixed (10% acetic acid, 10% fixative entrancer concentrate, 50% methanol, and 30% H_2O) during 20 minutes on a horizontal shaker and then washed twice with distilled water for 10 minutes. The GelBond was placed in coloration and developing solution for 15 minutes. The GelBond was washed with 5% acetic acid for 15 minutes, followed by distilled water for 5 minutes, and left to dry at room temperature. The GelBond film for each sample was placed on a slide and comets scored under microscope.

The DNA damage was manually quantified by visual scoring of cells into four categories (0–3) (Fig. 1) corresponding to tail size with 0 representing *no damage*, 1 representing *low* damage level (a beginning of a tail), 2 representing *medium* damage level (an obvious tail was visible), 3 representing *high* damage level (the comet tail started to fray; low density). For each subject, 100 cells were scored. The extent of the DNA damage was expressed as the sum of the cell-specific comet classification (0–3) so that the maximal possible comet score was 300. Samples are examined at 500 × magnifications using a bright field microscope (Diaplan; Wild Leitz GmbH). No software was used in the scoring of the comets. The comet assay scorers were blinded to the identity of the samples.

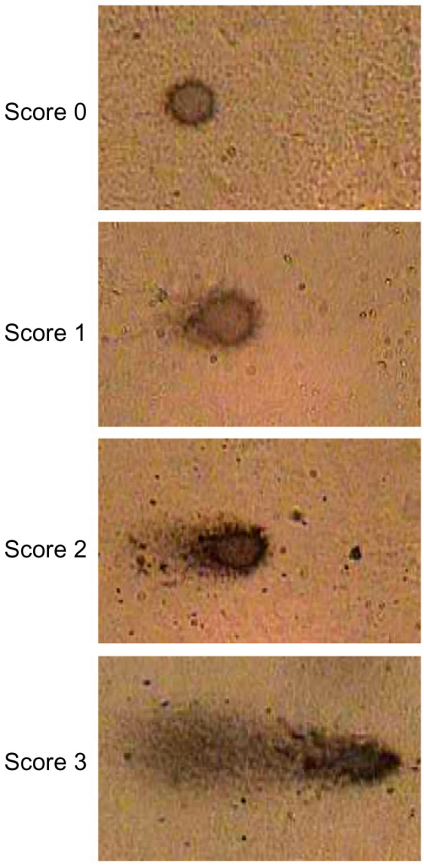

Figure 1. The DNA damage was quantified by visual classification of cells into four categories (0–3); corresponding to tail size with 0 representing no damage, 1 representing low level damage, 2 representing medium level damage, and 3 representing high level damage.

Statistical analysis. The statistical distribution of the comet scores was graphically presented as box plots that show for each group, the three quartiles as the *box* and the adjacent values defined by Tukey (1977)[21] as the *whiskers*. Scores outside these adjacent values are represented as dots.

Linear regression models – or equivalently analysis of variance (ANOVA) when only discreet (as opposed to continuous) variables were included as independent factors – were performed on the comet score, which was log transformed in order to yield normally distributed residuals.

Independent factors considered were:

- Length of lifelong occupational wood exposure, which was the duration of wood-related exposures, obtained from the work histories provided by the participants.

- Measured concentrations of inhalable wood dust, BaP, and total PAHs.
- The seven initial exposure groups and the two wood-type groups (natural woods vs composite wood products).

Potential confounders, such as age and body mass index (BMI) defined as the weight in kilogram divided by the square of the height in meters, were also investigated.

The relationship between comet and MN scores was based on a grouped logistic regression model of the MN data including the number of cells scored for MN as an offset. The comet score is introduced as an independent variable in this logistic regression.

Results

Demographic characteristics of study participants ($n = 50$) are presented in Table 1 as well as exposure data for the exposed wood workers ($n = 31$) according to their regrouped exposure (natural wood vs composite wood products). The individual data are displayed in Appendix.

The workers in the composite wood products group were older and had correspondingly a longer duration of wood dust exposures than workers in the natural wood group. Current exposure, measured as inhalable dust or total PAH concentrations, was lower in the composite wood products group compared to workers in the natural wood group. None of these exposure measurement differences were, however, statistically significant at the 5% level (data not shown). The BaP concentrations were always below the limit of detection (LOD = 0.001 ppm) in the composite wood products group but were quantifiable in the natural wood group.

When modeling the log-transformed comet score, none of the independent factors, such as age, BMI, duration of exposure, and the present day inhalable wood dust concentrations, had statistically significant effects on the measured DNA damages. When comparing the initial groups using post hoc comparisons after a Bonferroni multiplicity adjustment (data not shown), all groups of workers in the composite wood products group had greater comet scores than both the natural wood group ($P < 0.001$) and the nonexposed group ($P < 0.001$). The natural wood groups, on the other hand, did not differ in comet score, when adjusted for multiplicity, from the nonexposed group ($P > 0.05$). Overall, after grouping the exposed workers in the two categories defined by the wood type, the comet score was different according to the exposure groups ($P < 0.001$; Table 2). Thus, the main feature in the data was a higher log-transformed comet scores in the composite wood product workers compared to both natural wood workers and nonexposed workers. This feature is illustrated in the box plot of the comet score presented in Figure 2.

When exploring the relationship between the numbers of MN and the comet score, the latter was found to be related to both the number of buccal and nasal MNs (Table 2 and Fig. 3A and B). However, these associations became nonsignificant after adjustment for the duration of exposure.

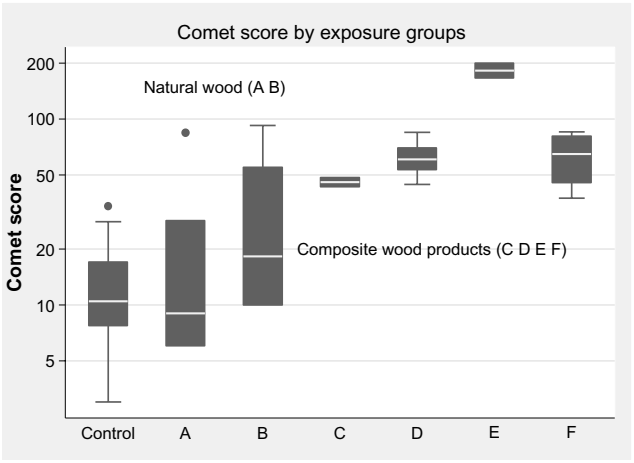

Figure 2. Box plot of comet assay scores (*y*-axis) by exposure group (*x*-axis): nonexposed group, natural wood exposed group (fir, spruce, beech, and oak), and composite wood products group.

Table 1. Demographic characteristics and exposure data for study participants by wood dust exposure status.

VARIABLES	NON EXPOSED (n = 20)		EXPOSED (n = 21)			
			NATURAL WOOD GROUP[a] (n = 12)		COMPOSITE WOOD PRODUCTS GROUP[b] (n = 19)	
	MEDIAN	25th–75th	MEDIAN	25th–75th	MEDIAN	25th–75th
Age [years]	38	31–45	39	29–45	39	31–54
BMI[c]	24.1	22.1–26.4	24.7	23.0–26.5	24.3	21.8–25.6
Duration [years][d]	–	–	21	24.5–28	20	10–30
Wood dust [mg/m^3][e]	–	–	3.6	1.9–12.4	1.7	1.2–3.3
Total PAHs [ng/m^3]	–	–	78.9	64.5–95.9	65.0	48.5–81.5
BaP [ng/m^3]	–	–	4.5	2.8–5.4	< lod	< lod

Notes: [a]Natural wood (fir, spruce, beech, and oak). [b]Composite wood boards (MDF and other composite products). [c]BMI, body mass index. [d]Duration of exposures to wood dust. [e]Gravimetric wood dust concentrations of the inhalable fraction.

Table 2. Median and quartiles of the comet score according to exposure groups and categories of number of nasal and buccal cells with micronuclei.

COMET SCORE	n	MEDIAN	25th–75th	MAXIMUM	*P*
Exposure group					
Non exposed	19	11.0	8.0–18.0	34.0	–
Natural wood	12	11.3	8.8–26.3	92.5	–
Wooden board	19	61.5	49.5–85	200.5	<0.0001[a]
Groups of nasal MN					
0 MN cells/1000	11	9.0	8.0–53.5	200.5	–
1–3 MN cells/1000	23	18.0	10.0–49.5	85.5	–
4+ MN cells/1000	16	51	18.3–73.3	182.5	0.001[b]
Groups of buccal MN					
1–3 MN cells/2000	18	12.0	9.0–37.5	53.5	–
4–5 MN cells/2000	18	33.5	9.0–70.0	200.5	–
6+ MN cells/2000	12	58.3	36.5–84.5	182.5	–

Notes: [a]Based on one-way ANOVA of the log-transformed comet score. In a post hoc multiplicity corrected test, the comet score was significantly larger in the wooden board group than in the two other groups that did not differ significantly. [b]Based on a test for linear trend with the log-transformed comet score in a logistic regression model of the number of MN cells. When comparing the log-transformed comet score between the three groups in a one-way ANOVA, these groups did not differ significantly.

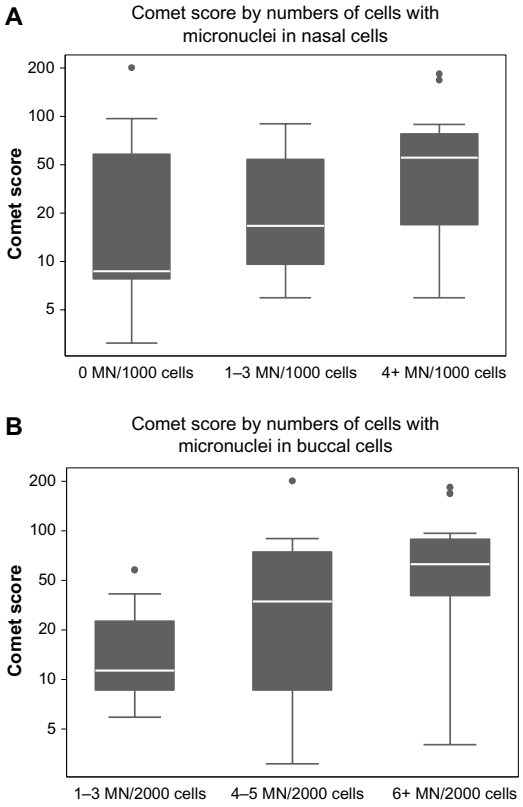

Figure 3. Comet score by number of cells with micronuclei in nasal (**A**) and buccal (**B**) cells.

Discussion

Elevated levels of DNA damage were observed among workers exposed to dust from composite wood products but not for workers exposed to natural wood. No correlations were found between comet score and wood dust concentration or with duration of exposure (years). The comet assay test can be recommended as a biomarker of effect for use in cancer prevention programs among workers exposed to composite wood products.

The comet assay can detect initial or acute DNA damage following a short-term exposure (one to four hours), but this damage can be repaired or undergo programmed cell death (apoptosis) and/or mutations, leading to a reduced detectable DNA damage.[22] It is, therefore, not surprising that the duration of exposure (years) to wood dust was not associated with DNA damage detected by the comet assay in our study. Similar results have been observed among workers exposed to formaldehyde with a relatively long exposure duration (13.6 ± 8.7 years).[23] The comet assay has been used in an *in vitro* study for assessing DNA damage related to fiber exposures, such as asbestos, and demonstrated DNA damage after a short-term exposure (3 and 24 hours).[24] This finding supports the hypothesis that DNA damage detected by the comet assay is related to fairly recent exposure.

The decrease in the extent of DNA damage may occur with increasing sampling time after exposure termination due to DNA repair processes, the loss of heavily damage cells via apoptosis, necrosis, and cells turnover. The blood sample collection times were, therefore, standardized in our study.

Using the comet assay, several studies have demonstrated elevated DNA damage in workers occupationally exposed to wood dust compared to control subjects.[3,6] Our study supports these previous findings. However, the elevated levels of DNA damage among Polish wooden furniture workers (n = 35) were associated with natural wood dust exposures (pine, oak, and beech), which is contrary to our findings.[6] The authors indicated that their workers may have had additional exposures, such as *solvents and hardener vapors during the varnishing and lacquering*. The genotoxicity of these chemicals was not assessed and could potentially have contributed to the genotoxicity observed. Thus, these Polish workers might be more similar to our workers in the composite wood product group than the natural wood group. Smoking could also have been a confounder in this Polish study as both smokers and nonsmokers were included. To eliminate smoking as a confounder, we included only nonsmokers in this study.

A second study reported a positive relationship between natural wood exposure and DNA damage among wood workers. Wood dust exposure was assessed among Indian furniture workers (n = 60) exposed to a mixture of wood species,[3] especially to hardwoods, such as teak wood, ash wood, mango, neem, tamarind, sandal wood, rose wood, and satin wood, and softwoods, such as guava and deodar. A significant effect of wood dust exposure ($P < 0.05$) in the exposed group when compared to the control group was observed. The airborne dust concentration found in this study cannot be compared with our study as sampling methods differed; surface wipe

sampling was used to assess air concentration of dust in the Indian factory, while we sampled inhalable dust with personal sampling trains equipped with filters. Moreover, years of exposure to wood dust did not have a significant effect on DNA damage. However, the wood species accounted for in this study differ from our study, and genotoxicity of wood dust may be related to wood species or be confounded with smoking.

Seven types of wood material dust have been compared for genotoxic effects using human alveolar epithelial cells *in vitro* and the comet assay.[9] A 1.2–1.4-fold increase in DNA strand breaks was observed after three hours of separate incubation with beech, teak, pine, and MDF dust compared to the levels in the untreated cells. This research team found that both hardwood and softwood induced similar levels of DNA damage. They concluded that although dust from hardwood is considered more harmful than softwood, there is no evidence for the distinction of hardwood and softwood dust toxicity using the comet assay. Interestingly, when cells were incubated for six hours with natural woods, such as oak, beech, birch, teak, pine, and spruce, there were no effects of dust exposure from those wood types. MDF represented the only wood material that was capable of inducing DNA damage after six hours of incubation. This result supports the findings of our study, with low DNA damage among workers exposed to natural wood during an eight-hour work shift and elevated DNA damage in workers exposed to composite wood products dust. In other words, workers exposed to dust from composite wood products had higher DNA damage than both workers exposed to natural wood and the nonexposed group ($P < 0.001$). Exposures to formaldehyde have been suggested as an explanation for elevated comet scores.[23,25] The exact constituents of an MDF board will vary from one product to another, and thus, their potential to emit formaldehyde differs as well.

One possible risk factor for DNA damage is exposure to PAHs. When wood workers manipulate wood melamine using power tools, PAHs are generated as shown in our previous study.[16] Total PAH concentrations decreased in the following order of wood dust collected after use of different power tools under controlled conditions: wood melamine > oak > fir > sipo > beech > MDF.[16] Thus, PAHs are unlikely to play a major role in the DNA damage measured with the comet assay for wood workers as the lowest exposure group showed the highest comet score.

An alternative possible risk factor for the DNA damage observed is exposure to formaldehyde, which is present in MDF, as have been suggested by several authors.[23,25] The carcinogenic mechanism of formaldehyde involves DNA strand breaks, while wood dusts vary in their potential to cause DNA damage. Exposure to the type of wood species is important but is rarely specified in the studies because they vary depending on the production. We did not determine formaldehyde concentrations, which is a limitation of our study, and thus cannot relate this exposure to the comet score. We believe that in our study, formaldehyde caused the DNA strand breaks measured by the comet assay among the composite wood product workers and that the wood species used by our natural wood workers did not cause measureable levels of DNA strand breaks as measured by the comet assay.

By manually scoring the comets and categorizing them only from 0 to 3, we might have missed some nuances compared to using a scoring scheme from 0 to 4 and a computational software. Even with our simple approach, the comet assay showed to be a robust method in detecting a difference between exposure groups.

An interpretation of the significant relationship between the nasal MNs and the comet score is a validation that both biomarkers signal that an agent has reached the target tissue and has caused changes in the genetic material and changes that may possibly precede the development of cancer. The disappearance of the biomarker relationship when the duration of exposure was considered could be because the comet assay reflects recent exposure to clastogens,[26] and MNs are a result of a clastogenic effect arising from a whole lagging chromosome or an acentric chromosome fragment detaching from a chromosome after breakage,[27] thus after chronic exposure to the genotoxic agent, not just repairable DNA breakage or alkali-labile sites.

In contrast to our previous study among the same study participants where we assessed MN in buccal and nasal cells, the present study explores DNA damage in systemically circulating cells that requires diffusion of the damaging agent and possibly metabolism. Here, we show that workers exposed to natural wood materials have a less acute DNA damage as assessed by the comet assay test compared to workers exposed to composite wood products. This indicates that composite wood products contain or produce, during power tool manipulations, substances leading to acute DNA damage. This has not previously been shown. This suggests that the genotoxicity of wood dust is related to wood material type. MDF is known to contain binders that can potentially emit carcinogenic substances [eg, formaldehyde (urea formaldehyde and melamine urea formaldehyde), isocyanate (polymeric diphenylmethane diisocyanate), and epichlorohydrine (soy-based binders)]. To better understand the effect that wood materials play in the mechanism of genotoxicity, future studies among wood workers should collect information not only on the concentration of wood dust but also on the exposure originating from the binders.

Author Contributions

Design of the work: BD, CKH, EDB, DSB. Analysis: EDB, DSB, CKH. Interpretation of data for the work: NBH, PW, BD, CKH, DSB, EDB. Drafted the work: NBH, EDB, PW. BD and CKH revised it critically for important intellectual content. All authors approve of the version to be published and agree to be accountable for all aspects of the work.

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Appendix

	EXPOSURE GROUP	AGE	BMI	DURATION OF EXPOSURE	COMET SCORE
Non exposed		23	21	0	4
Non exposed		27	22	0	6
Non exposed		31	24	0	18
Non exposed		31	21	0	12
Non exposed		31	25	0	9
Non exposed		32	17	0	10
Non exposed		35	26	0	8
Non exposed		36	30	0	34
Non exposed		38	20	0	16
Non exposed		38	23	0	12
Non exposed		39	26	0	9
Non exposed		43	24	0	25
Non exposed		45	29	0	3
Non exposed		45	24	0	8
Non exposed		47	24	0	8
Non exposed		47	29	0	11
Non exposed		50	24	0	6
Non exposed		50	22	0	14
Non exposed		55	–	0	19
Non exposed		62	27	0	28
Natural wood	A	16	22	0.5	85
Natural wood	A	18	23	5	9
Natural wood	A	18	23	5	9
Natural wood	A	25	24	9	29
Natural wood	A	36	25	20	18
Natural wood	A	44	26	28	6
Natural wood	A	47	25	31	6
Natural wood	B	33	20	9	93
Natural wood	B	36	30	17	10
Natural wood	B	42	27	22	10
Natural wood	B	43	20	25	13
Natural wood	B	46	26	23	24
Natural wood	B	52	27	24	55
Composite wood boards	C	37	24	34	43
Composite wood boards	C	39	24	30	49
Composite wood boards	D	26	25	10	85
Composite wood boards	D	30	26	15	60
Composite wood boards	D	31	22	15	50
Composite wood boards	D	35	31	19	60
Composite wood boards	D	39	22	24	85
Composite wood boards	D	46	22	23	62
Composite wood boards	D	47	24	20	54
Composite wood boards	D	54	24	25	63
Composite wood boards	D	58	22	30	70
Composite wood boards	D	59	23	43	45
Composite wood boards	E	27	21	6	201
Composite wood boards	E	55	26	30	167
Composite wood boards	E	60	20	40	183
Composite wood boards	F	20	28	5	54
Composite wood boards	F	33	22	5	38
Composite wood boards	F	36	24	7	86
Composite wood boards	F	45	37	20	77

(*Continued*)