Communication

The assessment of DNA damage in lymphocytes of wooden furniture workers[*]

Jadwiga Palus[✉], Elżbieta Dziubałtowska and Konrad Rydzyński

Nofer Institute of Occupational Medicine, Department of Toxicology and Carcinogenesis, Teresy 8, P.O. Box 199, 90-950 Łódź, Poland

Single-strand breaks (SSB) and DNA repair were detected in peripheral lymphocytes derived from workers of a furniture factory in a non-polluted region of Poland. The workers were exposed to wood dust (n = 19), or to the dust and varnishes or lacquers together (n = 5). Four groups were studied simultaneously: (a) exposed workers smokers of cigarettes (n = 14), (b) nonexposed smokers – control (n = 14), (c) exposed workers' nonsmokers (n = 14), (d) exposed nonsmokers (n = 10). In exposed workers DNA SSB and DNA repair were statistically significantly increased. DNA SSB was clearly higher in the smoking workers than in the smoking controls. Cigarette smoking itself has produced no evident increase in the frequency of DNA SSB in the control group. Occupational exposure had a significant effect on DNA repair in non stimulated lymphocytes both in smoking and nonsmoking workers.

Many epidemiological studies performed among woodworkers revealed a marked increase in the incidence of cancers of the nasal cavities and paranasal sinuses. Very high relative risk of adenocarcinoma of these tissues, associated with exposure to wood dust, have beeen observed particularly in Europe (IARC, 1995).

So far the only study that has assessed genotoxic effects in the woodworkers, pointed to an increase in the frequency of chromatid breaks in the peripheral lymphocytes of workers exposed to fumes emitted by heated woods in Finnish factories (Kurttio *et al.*, 1993). Several other studies reported on mutagenicity or genotoxicity of extracts of certain kinds of wood (Mohtashamipur & Norpoth, 1990; Schmezer *et al.*, 1994; Nelson *et al.*, 1993).

The number of workers exposed worldwide to wood dust is estimated to be about 2 million

(IARC, 1995). Data from Poland indicate that about 100000 workers in wood and furniture industries are exposed to wood dust (Macie-jewska *et al.*, 1993) the concentration of which may range from 0.1 to 83.00 mg/m^3, depending on the type and volume of manufacture (unpublished data from the Sanitary and Epidemiological Stations).

So far little is known about the possible genotoxic effect of exposure to wood dust in wood or furniture industry workers.

In this connection, we have undertaken a study to assess DNA damage in lymphocytes of workers exposed to wood dust during furniture making and to further compare it with the DNA damaging action of cigarette smoke. It was based on estimation of the level of DNA SSB using the DNA microfiltration technique and the level of DNA repair measured as incorporation of radioactivity into workers' lymphocytes. It is not the purpose of this publication to give a complete analysis of genetic damage caused by occupational exposure in the wooden furniture industry or to give a detailed description of exposure data, but to concentrate on the combined effect of exposure and cigarette smoking.

MATERIALS AND METHODS

The study and control groups. The study was performed at a wood plant situated at the suburb of a small town in the South-Easter region of Poland. This is a farmers' region with large forests, not industrially polluted. On the premises of the plant there are a sawmill, a wooden furniture manufacture department, a flooring blocks department and a tool-room. The exposed group consisted of 24 volunteers out of the 200 workers at the wooden furniture manufacture department. The mean age of these subjects was 38 ± 9 years (range 21–49); fourteen of them were smokers. Most of them have been working in the wooden furniture productions for more than 10 years. Out of the 24 furniture makers who took part

in this study, 19 persons were exposed to wood dust, and 5 to varnishes or lacquers and wood dust.

The control group consisted of 13 office workers at the wooden furniture plant and 15 laboratory assistants at the Institute of Occupational Medicine in Łódź. The mean age of these volunteers was 41 ± 8 years (range 23–55). For each exposed and control subject a questionnaire was filled prior to the collection of blood samples. The questionnaire was to elicit information on the subject's age, cigarette smoking, work-post duration and medicine intake.

Blood sampling and isolation of lymphocytes. The blood samples were collected on Thursdays and Fridays, i.e. at the end of a working week; 10 ml of venous heparinized blood was taken. All blood samples were transported on ice and processed within 3 h. The lymphocytes were separated using Histopaque according to Freeman & Ryan (1988). For each person we have calculated the number of isolated cells in the suspension and adjusted the sample to about 1×10^6 cells/0.5 ml. The cells were stained for viability with trypan blue. The viability of the cells always exceeded 98%.

Determination of DNA single-strand breaks. A simple method for detection DNA SSB in lymphocytes, based on centrifugation of alkaline-lysed cells through microfilters, described by Leanderson *et al.* (1994), with some modifications was used. The lymphocyte suspension from a single person of the study or control group was mixed vigorously with pipette and 500 μl aliquots were transferred onto 0.8 μm cellulose acetate filters (MFS, Dublin, CA, U.S.A.) mounted to MF-1 microfilters devices (Bioanalytical Systems Inc., West Lafayette, IN, U.S.A.) where they were washed, lysed and centrifuged to separate undamaged DNA from damaged DNA. The filtrates containing the single strands of DNA were then transferred to tubes, buffered with 200 μl of SSC solution, pH 7.0 (0.015 M sodium citrate, 0.14 M NaCl) and 200 μl of 0.2 M

KH_2PO_4, pH 7.0, solution. The amount of DNA was measured by the fluorometric method using Hoechst 33258 dye (Sigma) as described by Cesarone et al. (1979). DNA remaining on the filter was rinsed with 10 mM Na_2EDTA solution, pH 12.4 as follows: 200 μl of this solution was added onto the filter, left for 15 min and centrifuged at 2000 r.p.m. for 5 min; this procedure was repeated twice. Then 200 μl of SSC solution (pH 7.0) and 200 μl of 0.2 M KH_2PO_4 (pH 7.0) solution were added to 600 μl of the filtrate obtained from the filter. The amount of DNA was measured using the fluorometric method. To 1 ml aliquots of the buffered fractions containing single strand DNA (filtrate) and DNA washed out from the filter (filter) 1 ml of Hoechst 33258 solution (1.5×10^{-6} M) was added. The samples were then stirred and allowed to stand for 10 min in a dark room. Fluorescence was measured in a Perkin-Elmer spectrophotofluorimeter Model LS-2B at excitation wavelength of 375 nm and emission at 460 nm. As a positive control we used hydrogen peroxide (H_2O_2) (POCh, Poland) which is known to form hydroxyl radicals that could damage the sugar-phosphate backbone and generate DNA SSB (Mello-Filho & Meneghini, 1984; Imlay & Linn, 1988). Two or three lymphocyte suspensions (about 1×10^6 cells/0.5 ml in RPMI 1640) from the study group were exposed to 5 μM H_2O_2 for 5 min in 1.5 ml Eppendorf tubes in darkness at room temperature. After exposure, the cell suspensions were mixed and transferred to 0.8 μm cellulose acetate filters where they were washed, lysed and centrifuged to separate undamaged from the damaged DNA in the same manner as described above.

To assess DNA SSB in lymphocytes of workers from the study and the control groups, the percentages of damaged DNA remaining on the filter were calculated, according to the formula:

$$\% \text{ damaged DNA} = \frac{\text{filtrate fluorescence}}{\text{fluorescence (filtrate + filter)}} \times 100$$

Determination of DNA repair in lymphocytes. Lymphocytes isolated from the workers' and the controls' blood were suspended in RPMI-1640 medium without serum (1×10^6 cells/0.5 ml) and placed in Petri dishes 3.5 mm in diameter, which contained 1 ml medium RPMI-1640 without serum and with 5 μCi/ml of [6-^{3}H]thymidine (40 MBq, Amersham). In order to determine DNA repair, the suspensions were incubated without phytohemagglutinin (mitogen stimulating the lymphocytes division) for 3 h at 37°C in a CO_2 incubator (Celotti et al., 1990; Benigni et al., 1984). Three suspensions on Petri dishes were prepared for one person. Then the cells were washed three times in PBS, resuspended in 1 ml of PBS and transferred to scintillation vials to which 10 ml of Aquasol (New England Nuclear) were added. The incorporation of radioactivity was detected by liquid scintillation counting (LKB, WALLAC 1209 "RACK-BETA", Pharmacia) and recorded as c.p.m./10^6 lymphocytes.

RESULTS

DNA single-strand breaks in peripheral blood lymphocytes of workers from the study and control groups

For the assessment of the level of DNA single strand breaks the isolated lymphocytes were alkaline lysed on microfilters and DNA single-strand fragments were separated from undamaged DNA using the microfiltration method. The percentage of damaged DNA was calculated from the fluorometric measurements of the filtrate and filter DNA and regarded as an DNA SSB index in the lymphocytes of workers and control subjects.

The results are summarized in Table 1. The mean percentage of damaged DNA in the lymphocytes of 24 exposed workers was 24.1 ± 11.5 compared to 13.3 ± 5.9 calculated from the 28 control subjects. In the study group, the increase in the percentage of damaged DNA

Table 1. The level of DNA single-strand breaks in the lymphocytes of wooden furniture workers and control persons

	Number of individuals	Number of lymphocytes studied (mean ± S.D.)	Fluorescence (mean ± S.D.) Filtrate	Fluorescence (mean ± S.D.) Filter	Percentage of damaged DNA (mean ± S.D.)
Control persons	24	914.000 ± 67.000	29.1 ± 11.2	191.1 ± 37.0	13.3 ± 5.9
Workers	28	900.000 ± 62.000	58.5 ± 33.5	164.1 ± 49.1	24.1 ± 11.5[a]
Control smokers	14	928.000 ± 56.000	31.7 ± 9.0	193.6 ± 22.4	14.1 ± 5.1
Exposed smokers	14	905.000 ± 66.000	67.0 ± 32.2	153.2 ± 33.6	29.5 ± 10.0[bc]
Control nonsmokers	14	901.000 ± 72.000	27.5± 9.0	201.2 ± 44.7	12.2 ± 6.6
Exposed nonsmokers	10	889.000 ± 59.000	46.0 ± 32.9	200.1 ± 16.2	16.6 ± 9.0

Statistically significant difference between: [a]workers and control persons ($P < 0.05$); [b]exposed and control smokers ($P < 0.05$); [c]smoking and nonsmoking exposed workers ($P < 0.05$).

was statistically significant ($P < 0.05$, t-test) (Table 1). In smoking workers the level of DNA SSB was significantly higher compared to smoking controls and nonsmoking workers ($P < 0.05$, t-test) (Table 1). Table 2 shows the percentage of damaged DNA in the smoking and nonsmoking exposed workers after exposure of their lymphocytes to 5 μM H_2O_2 in vitro. The exposure to H_2O_2 has brought an about 40% increase in the level of DNA SSB in the lymphocytes both of the nonsmoking and smoking workers.

DNA repair in the lymphocytes of the exposed workers and the controls

The amount of incorporated radioactivity, regarded as the DNA repair, was determined and averaged for the lymphocytes and expressed as counts per minute (c.p.m.) per 10^6 cells.

The incorporation of radioactivity to the lymphocytes of the individual exposed workers and controls is presented in Fig. 1. In the exposed workers, the label of DNA was significantly higher compared to that for the control group. The radioactivity in the smoking and nonsmoking exposed workers was 5 and 6 times, respectively, higher than in their controls. Cigarette smoking in the exposed and

the control groups caused a relatively small increase in DNA label (1.6 and 1.8 times, respectively) compared to the group of their nonsmoking colleagues.

DISCUSSION

In the present study we have determined both single strand breaks and DNA repair in the same lymphocyte samples either from the study or the control groups. The increase both in DNA SSB (Table 1) and DNA repair (Fig. 1) in peripheral lymphocytes of the exposed workers was statistically significant as compared to the controls. We have found that the level of DNA SSB was clearly higher in the smoking workers than in smoking controls. In nonsmokers a slight tendency towards a higher level of DNA SSB could be noted, but this has never reached statistical significance. Moreover, cigarette smoking itself has not produced an evident increase in the frequency of DNA SSB in the control group (Table 1). A significant effect of occupational exposure to wood dust and the substances used during the varnishing and lacquering, on DNA repair in the unstimulated lymphocytes has been found both in the smoking and nonsmoking exposed workers. Our findings indicate that the effect
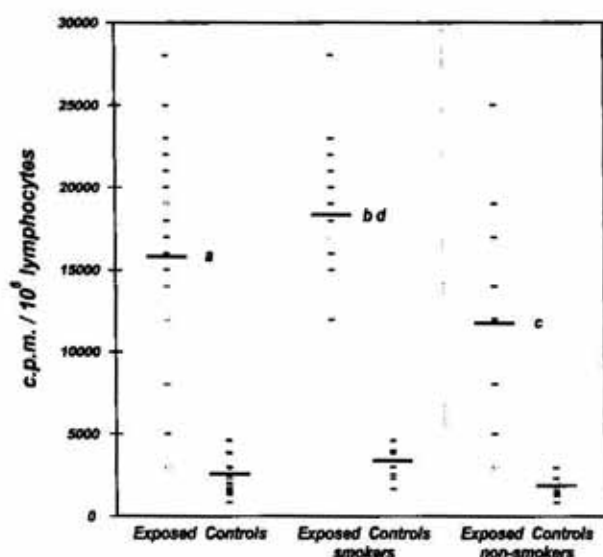
Figure 1. Interindividual variation in the incorporation of radioactive [3H]thymidine into the lymphocytes DNA of wooden furniture workers and control subjects.

Statistically significant differences between: [a]exposed workers and control ($P < 0.05$), [b]exposed and control smokers ($P < 0.05$), [c]exposed and control nonsmokers ($P < 0.05$), [d]smoking and nonsmoking exposed workers ($P < 0.05$).

of cigarette smoking (control persons) on DNA repair may be less significant than it is commonly considered (Fig. 1).

Oesch *et al.* (1994) also reported a small but statistically significant increase in the frequency of DNA SSB in smoking control individuals in comparison to nonsmoking, only if a large number of individuals was tested. They suggested that smoking may induce some kind of a protective factor which reduces the formation in mononuclear blood cells of DNA SSB that may be associated with a variety of other genotoxic factors than smoking. Contrary to this view, Reitz *et al.* (1994) pointed to cigarette smoking as the main cause of the increase in DNA SSB in the lymphocytes. Therefore, it seems that an increased cancer incidence in smokers cannot be directly related to DNA SSB, because many additional factors (e.g., promoters) may also be involved.

Our results indicate that the combination of occupational exposure to wood dust, technological chemicals and cigarette smoking might have intensified other lesions, and not only the generation of DNA SSB. The interaction between smoking and occupational exposure to genotoxic agents is very complex. Cigarette smoking itself is a well-known risk factor for several types of cancer, notably for lung cancer (World Health Organization, 1989). Moreover, an increased incidence of cancer and of DNA damaging effects after co-exposure to cigarette smoke and certain toxic substances has been demonstrated (Balarajan & McDowall, 1988; Hogstedt, 1988; Selikoff *et al.*, 1968). So far there are no reports on the biomonitoring of the genotoxic effects in wood industry workers (IARC, 1995). One study has revealed an increase in the fre-

Table 2. Effect of H_2O_2 on the level of DNA single-strand breaks in the lymphocytes of workers in the wooden furniture plant (positive control)

Number of individuals	Age (yr) (mean ± S.D.)	Habit of smoking	Number of lympho- cytes studied (mean ± S.D.)	Fluorescence (mean ± S.D.) Filtrate	Filter	Percentage of damaged DNA (mean ± S.D.)
			UNEXPOSED TO H_2O_2			
3	34 ± 13	−	853500 ± 24000	62.6 ± 26.4	243.0 ± 48.6	19.9 ± 9.4
3	47 ± 3	+	948000 ± 35000	77.0 ± 8.6	200.7 ± 47.0	28.3 ± 5.3
			EXPOSED TO 5 μM H_2O_2			
3	34 ± 13	−	853500 ± 24000	206.5 ± 46.1	116.0 ± 30.2	63.6 ± 10.7
3	47 ± 3	+	948000 ± 35000	194.0 ± 25.8	92.5 ± 17.8	67.5 ± 2.2

quency of chromatid breaks in peripheral lymphocytes in nonsmoking workers exposed to fumes emitted from heated wood (Kurttio *et al.*, 1993).

Studies *in vitro* have shown that the reverse mutation in *Salmonella typhimurium* can be induced by the extracts of beech (Mohtashamipur & Norpoth, 1990), but not by those of birch and spruce wood (Kurttio *et al.*, 1990). Moreover the extracts of beech, oak and pasteboard produced an increased number of DNA SSB in rat hepatocytes *in vitro* (Schmezer *et al.*, 1994). An experiment *in vivo* revealed increase frequency of micronuclei in the crypts of the small intestine of mice and in the nasal epithelium of rats after treatment by gavage or topical application of beech wood extracts (Nelson *et al.*, 1993).

At present, in the light of our findings we can only suggest that occupational exposure to wood dust may be the factor that has produced an increase of DNA damage such as single-strand breaks in the workers' lymphocytes. Furthermore, cigarette smoking may have intensified this kind of DNA damage. The initial results of the mutagenicity testing of workers' urinary extracts, carried on volunteers, provide evidence that the occupational exposure to wood dust leads to an increased mutagenic activity of urine but not to such a high extent as does cigarette smoking (unpublished). We suggest that biomonitoring of the genotoxic effects in wood industry should be undertaken in future.

REFERENCES

Balarajan, R. & McDowall, M.E. (1988) *Br. J. Ind. Med.* **45**, 438–486.

Benigni, R., Calcagnile, M., Fabri, G., Giuliani, A., Leopardi, P., & Paoletti, A. (1984) *Mutation Res.* **138**, 105–111.

Celotti, L., Biasin, R., Ferraro, P. & Fiorentino, M.(1990) *Mutat. Res.* **245**, 217–222.

Cesarone, C.F., Bolognesi, C. & Santi, L. (1979) *Anal. Biochem.* **100**, 188–197.

Freeman, S.E. & Ryan, L. (1988) *Mutation Res.* **194**, 143–150.

IARC (1995) *Monographs on the evaluation of carcinogenic risks to humans.* Vol. 62., Lyon.

Imlay, J.A. & Linn, S. (1988) *Science* **240**, 1302–1309.

Hogstedt, L.C. (1988) *IARC Sci. Publ.* **89**, 265–270.

Kurttio, P., Kalliokoski, P., Lampelo S. & Jantunen, M.J. (1990) *Mutation Res.* **242**, 9–15.

Kurttio, P., Norppa, H., Jarventaus, H., Sorsa, M. & Kalliokoski, P. (1993) *Scand. J. Work Environ. Health* **19**, 132–134.

Leanderson, P., Wennerberg, K. & Tagesson, Ch. (1994) *Carcinogenesis* **15**, 137–139.

Maciejewska, A., Wojtczak, J., Bielichowska-Cybula, G., Domanska, A., Dutkiewicz, J. & Molocznik, A. (1993) *Occup. Med.* **44**, 277–288 (in Polish).

Mello-Filho, A.C. & Meneghini, R. (1984) *Biochim. Biophys. Acta* **781**, 56–63.

Mohtashamipur, E. & Norpoth, K. (1990) *Toxicol. Lett.* **51**, 277–285.

Nelson, E., Zhou, Z., Carmichael, P.L., Norpoth, K. & Fu, J. (1993) *Arch. Toxicol.* **67**, 586–589.

Oesch, F., Hengstler, J.G. & Fucks, J. (1994) *Mutation Res.* **321**, 175–185.

Reitz, M., Coen, R. & Lanz, E. (1994) *Environ. Res.* **65**, 12–21.

Schmezer, P., Kuchenmeister, F., Klein, R.G., Pool-Zobel, R.G., Fengel, B.L., Stehlin, D. & Wolf, J. (1994) *Arbeitsmed. Sozialmed. Umweltmed.* **21**, 13–17.

Selikoff, I.J., Hammond, E.C. & Churg, J. (1968) *J. Am. Med. Assoc.* **204**, 104–110.

World Health Organization (1989) Biennial Report 1988-1989; pp. 16–17, International Agency for Research on Cancer, Lyon.