Arch Toxicol (2013) 87:145–153
DOI 10.1007/s00204-012-0961-9

IMMUNOTOXICOLOGY

Assessment of immunotoxicity and genotoxicity in workers exposed to low concentrations of formaldehyde

Sevtap Aydın · Hande Canpınar · Ülkü Ündeğer · Dicle Güç · Mustafa Çolakoğlu · Ayşe Kars · Nurşen Başaran

Received: 23 May 2012 / Accepted: 10 October 2012 / Published online: 26 October 2012

Abstract Formaldehyde (FA), which is an important chemical with a wide commercial use, has been classified as carcinogenic to humans by International Research on Cancer (IARC). The genotoxic and carcinogenic potential of FA has been documented in mammalian cells and in rodents. A recent evaluation by the E.U. Scientific Committee for Occupational Exposure Limits (SCOEL) anticipated that an 8-h time-weighted average exposure to 0.2 ppm FA would not be irritating and not genotoxic in humans. In order to verify this prediction, a field study was performed that aimed at evaluating immune alterations and genetic damage in peripheral lymphocytes of workers in medium density fiberboard plants exposed to a level of FA equivalent to the OEL recommended by SCOEL (0.2 ppm). Subsets of peripheral lymphocytes, immunoglobulins (IgG, IgA, IgM), complement proteins, and tumor necrosis factor-alpha (TNF-α) levels were evaluated. DNA damage of the workers was assessed by the Comet assay. The absolute numbers and the percentages of T lymphocytes and of natural killer cells, and the levels of TNF-α were higher than the controls, whereas IgG and IgM levels were found to be lower in workers. Other examined immunological parameters were not different from those of the controls. There was no increased DNA damage in the workers compared to controls.

Keywords Formaldehyde · Lymphocytes · Serum immunoglobulins · Complements proteins · TNF alpha · DNA damage · Occupational exposure

Introduction

Formaldehyde (FA) is an important chemical with a wide commercial use. FA is used in molding compounds, glass wool and rock wool insulation, decorative laminates, textiles treatments, and in the production of resins, plastics, plywood, and chemical intermediates. FA has applications in medicine as a tissue preservative and bactericide. Occupational exposure involves individuals in the manufactures of FA and FA containing products, and users (IPCS 1989; WHO 2000).

Human studies have shown that chronic exposure to FA by inhalation is associated with upper airway irritation (DFG 2000; Kim et al. 2011; Salonen et al. 2009). Epidemiological studies of industrial workers, embalmers, and pathologists have provided some evidence of elevated risks for cancers at various sites, including nasal cavities, lung, and hematopoietic system (McGregor et al. 2006; Thompson and Grafström 2011). The International Agency for Research on Cancer (IARC) has classified FA as being carcinogenic to humans (Group I) (IARC 2006).

FA is a DNA-reactive chemical that does not require biotransformation for this reactivity. Inhaled FA undergoes chemical reactions with organic compounds such as DNA,

Electronic supplementary material The online version of this article (doi:10.1007/s00204-012-0961-9) contains supplementary material, which is available to authorized users.

S. Aydın · Ü. Ündeğer · N. Başaran (✉)
Department of Pharmaceutical Toxicology, Faculty of Pharmacy, University of Hacettepe, 06100 Ankara, Turkey
e-mail: nbasaran@hacettepe.edu.tr

H. Canpınar · D. Güç · A. Kars
Department of Fundamental Oncology, Faculty of Medicine, University of Hacettepe, 06100 Ankara, Turkey

M. Çolakoğlu
Department of Public Health, Republic of Turkey Ministry of Health, 06100 Ankara, Turkey

nucleotides, nucleosides, and proteins, by addition and condensation. FA is mutagenic in Drosophila larvae, in bacteria, and in yeast (Benyajati et al. 1983; Chanet and von Borstel 1979; Takahashi et al. 1985).

A genotoxic and carcinogenic potential of FA has been documented in mammalian cells and in rodents (Sul et al. 2007; Ren et al. 2012). Increased chromosomal aberration frequencies and sister chromatid exchanges (SCEs) have been observed in Chinese hamster ovary (CHO) cells and in cultured human lymphocytes (Kreiger and Garry 1983; Natarajan et al.1983). Previous studies have shown that DNA–protein cross-links (DPX) occur in the nasal mucosa and in the upper respiratory tracts of humans and animals exposed to FA, but increased amounts of DPX were not found in tissues other than the respiratory tract (Heck and Casanova 2004; Lu et al. 2010; Speit et al. 2009; Ye et al. 2005; Zeller et al. 2012).

Recently, it has been argued that the genotoxicity and carcinogenicity of FA has a practical threshold, and health-based exposure limits were derived for occupational settings (SCOEL 2008) and for indoor exposures (Nielsen and Wolkoff 2010). It was anticipated that an 8-h time-weighted average exposure to 0.2 ppm FA with a short-term exposure limit of 0.4 ppm would be not irritating and not genotoxic in humans. These conditions were therefore proposed as Occupational Exposure Limit (OEL; TWA and STEL, respectively) by SCOEL (2008). The purpose of the present field study was to verify this in persons exposed occupationally under exactly these conditions. The study also aimed at evaluating immune alterations and genetic damage in peripheral lymphocytes of workers with low-level FA exposure at proposed the OEL. In order to assess the immune competence of workers occupationally exposed to FA, several subsets of peripheral blood mononuclear lymphocytes, for example, CD3+ (T cells), CD4+ (helper T cells), CD8+ (suppressor T cells), and CD20+ (B cells), and natural killer cells (NK) cells have been analyzed, and immunoglobulins (IgG, IgA, IgM), complement proteins, and tumor necrosis factor-alpha (TNF-α) levels have been determined. DNA damage in the lymphocytes of the workers was evaluated by single-cell gel electrophoresis (Comet) assay.

Materials and methods

Subjects

The study population consisted of 46 male workers occupationally exposed to FA from two medium density fibreboard (MDF) producing plants in Gebze, a town located in the Marmara region of Turkey, and 46 non-exposed male controls of comparable age, sex, lifestyles, and smoking habits living in the same area and with no history of occupational exposure to FA and other chemicals, employed in administrative government offices and in maintenance services (Table 1).

Health conditions, medical history, drug and alcohol usage, and smoking habits were inquired for each worker and control person using questionnaires. Use of protective measures and years of employment, any specific symptoms related to FA exposure, skin reactions, and respiratory diseases were also recorded.

All subjects participated voluntarily in the study and were fully informed about the procedures and the aim of the study. Prior to the study, each subjects signed an informed consent form. Ethical approval for this study was obtained from the local ethical commission of Hacettepe University. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Monitoring of occupational exposure of formaldehyde

Twenty-four samples were collected at stationary points at the workplaces. Also, air samples were collected from breathing zone of workers for the representative working periods (from 6 am to 2 pm, about 8 h) using a passive, personal air sampler. The 8-h time-weighted average (TWA_{8h}) was determined according to NIOSH method 3500 (NIOSH 1994).

Momentary values of FA concentration in the air samples were measured using an air-monitoring Formaldemeter 400^{TM} (PPM Technology limited Wales, UK), based on electrochemical sensing technology. The sampling time was about 3 min. The momentary values were measured just before the biological samplings.

Table 1 Characteristics of the study population

	Workers	Controls
Number of subjects	46	46
Age (years)	33.4 ± 0.9 (22–52)	38.4 ± 1.2 (24–53)
Years of employment	7.3 ± 0.8 (0.33–30)	–
Using protective mask	–	–
Yes	26 (56.5 %)	
No	20 (43.5 %)	
Smoking status		
Non-smokers	28 (60.9 %)	23 (50 %)
Smokers	18 (39.1 %)	23 (50 %)
Cigarettes/day	11.2 ± 1.2 cigarettes/day (3–20)	11.9 ± 1.1 cigarettes/day (4–20)

The values are given as mean ± standard error mean (range)

Blood sampling

A 10 ml peripheral blood sample was taken from each individual. All blood samples were stored at +4 °C and processed within 6 h. Five ml of each sample was collected in preservative-free heparin tubes and used for the analysis of peripheral blood mononuclear cells (PBMC) and DNA damage; 2 ml was collected in EDTA containing tubes for the analysis of total and differential blood cell counts; and 3 ml was allowed to clot for the measurement of serum immunoglobulins, complement proteins, and TNF-α concentrations. The serum samples were kept at −80 °C until the day of analysis.

Lymphocytes were separated using the Ficoll-Hypaque density gradient technique by centrifugation of heparinized peripheral blood samples (Boyum, 1976). After washing with phosphate-buffered saline (PBS) buffer twice, the cell concentrations were adjusted to approximately 2×10^5cells/ml PBS. Cell viability was determined by trypan blue and was found to be higher than 85 % in all cases. Lymphocytes were divided into two portions, one for the assessment of DNA damage and the other for the evaluation of mononuclear cells.

Alkaline single-cell gel electrophoresis (Comet assay)

The basic alkaline technique of the Comet assay of Singh et al. (1988), as described by Anderson et al. (1998) and Collins et al. (1997), was applied. A total of 50 µl of the cells, mixed with 75 µl of 0.65 % low melting point agarose (LMA), was embedded onto slides pre-coated with a layer of 1 % normal melting point agarose (NMA). Slides were allowed to solidify on ice for 5 min. Coverslips were then removed. The slides were immersed in fresh cold lysing solution (2.5 M NaCl,100 mM EDTA, 100 mM Tris,1 % sodium sarcosinate, pH 10), with 1 % Triton X-100 and 10 % DMSO added just before use for a minimum of 1 h at 4 °C. Then, they were removed from the lysing solution, drained, and left in the electrophoresis solution (1 mM sodium EDTA and 300 mM NaOH, pH 13) for 20 min at 4 °C to allow unwinding of the DNA and expression of alkali-labile damage. Electrophoresis was conducted at a low temperature (4 °C) for 20 min, using 24 V and adjusting the current to 300 mA by rising or lowering the buffer level. The slides were neutralized by washing 3 times in 0.4 M Tris-HCl (pH 7.5) for 5 min at room temperature. After neutralization, the slides were incubated in 50, 75, and 98 % alcohol for 5 min, successively.

The dried microscopic slides were stained with ethidium bromide (EtBr, 20 µg/ml in distilled water, 60 µl/slide) and covered with a cover-glass prior to analysis with a Leica® fluorescence microscope under green light. The microscope was connected to a charge-coupled device camera and a personal computer-based analysis system (Comet Analysis Software, version 3.0, Kinetic Imaging Ltd., Liverpool, UK) to determine the extent of DNA damage after electrophoretic migration of the DNA fragments in the agarose gel. Slides were examined at 40× in order to visualize DNA damage.

One-hundred cells from each of 2 replicate slides were counted, and results were expressed as tail intensity, tail moment (% of DNA in tail × tail length), and tail migration.

Flow-cytometric analysis of peripheral blood mononuclear cells (PBMC)

Lymphocytes were separated from heparinized peripheral blood as described before, and the cells were analyzed for cell surface phenotypes by the direct immunofluorescence technique (Hudson and Hay, 1991; Winchester and Ross 1986). Fluorescein-isothiocyanate (FITC)-conjugated monoclonal antibodies directed against human CD3+ (T cells), CD4+ (helper T cells), CD8+ (suppressor T cells), and CD20+ (B cells) cells were obtained from a Beckman Coulter. Natural killer (NK) cells were determined by indirect immunofluorescence using $CD16^+$/$CD56^+$ and FITC-conjugated antihuman polyclonal immunoglobulin antiserum (Beckman Coulter), respectively. The numbers of peripheral blood mononuclear cells (PBMC) were analyzed by flow cytometry (EPICS XLMCL-Coulter Electronics). The same batch of antibodies was used during the entire study, and the flow cytometry was carried out always by the same person.

Determination of immunoglobulins and complement proteins

Serum concentrations of immunoglobulins (IgG, IgA, IgM), and the C3 and C4 components of complement proteins were measured by turbidimetry using a Dade-Behring Turbitimer with reagents Turbiquant IgG (anti-human IgG), Turbiquant IgA (anti-human IgA), Turbiquant IgM (anti-human IgM), Turbiquant complement C3 (anti-human complement C3), and Turbiquant complement C4 (anti-human complement C4) from Dade-Behring (Marburg GmbH–A Siemens Company, Germany) (Hudson and Hay, 1991).

Determination of human tumor necrosis factor-alpha (TNF-α)

The quantitative determination of human TNF-α in serum was performed by Quantikine HS Immunoassay kit purchased from Quantikine®-HS. All reagents and samples were kept at room temperature before use. All samples, standards, and controls were assayed in duplicate. All

reagents, samples, and working standards were prepared according to the kit procedure. The optical density of each sample was determined within 30 min, using a micro-plate reader at 490 nm. The mean recovery of the kit was 93 % (85–98 %), and the detection limit was 0.106 pg/ml.

Statistical analysis

For statistical analysis, the "SPSS for Windows 10.0'' computer program was used. The results were expressed as mean ± standard error mean (SEM). The distribution of data was checked for normality by the Kolmogorov–Smirnov test. Depending on the distribution, the statistical analyses were carried out by the one was variances (ANOVA) test or Kruskal-Wallis *H* test, setting the probability level to $p < 0.05$. An analysis of linear regression was used to estimate the effects of the duration of occupational FA exposure and the smoking habits on the extent of DNA damage and immune parameters.

Results

The mean age of the workers and controls was 33.4 ± 0.9 years (range 22–52) and 38.4 ± 1.2 years (range 24–53 years), respectively. The mean work duration of the workers was 7.3 ± 0.8 years (range 0.33–30 years). Eighteen workers and 23 controls were smokers, and the average consumption of cigarettes in the workers' group and that in the controls were 11.2 ± 1.2 cigarettes/day and 11.9 ± 1.1 cigarettes/day, respectively (Table 1). The persons had not suffered from acute respiratory symptoms like bronchitis or coughing. Only 26 workers of the total 40 workers had used protective measures, such as masks.

The exposure levels of FA in the MDF producing plants were given in Table 2. The mean levels of FA exposure ($TWA_{8\ h}$) in the 1st and the 2nd MDF producing plant were 0.19 ± 0.07 ppm (0.11–0.33 ppm) and 0.20 ± 0.05 ppm (0.10–0.28 ppm), respectively. The mean level of FA exposure ($TWA_{8\ h}$) of the 46 individuals in the MDF producing plants was 0.20 ± 0.06 ppm (0.10–0.33 ppm), which was in compliance with permitted occupational exposure limit (OEL) of 0.2 ppm in Turkey and the OEL recommended by SCOEL (2008). The exposure levels of FA (TWA_{8h}) in 37.5 % of studied areas in the plants (stationary measurements) were higher than 0.2 ppm. Momentary FA levels in 16.6 % of study areas were higher than the value of 0.3 ppm recommended by ACGIH (2003). The highest momentary value (0.35 ppm) was detected in the MDF pressing section of the 1st MDF producing plant. This was still in compliance with a STEL of 0.4 ppm.

The DNA damage in the blood cells of workers exposed to FA was not higher than in the control group, as seen in Table 3 and Fig. 1. DNA damage in the lymphocytes of the workers was even significantly lower than the controls ($p < 0.05$). Smokers, both in the worker and control groups, had significantly higher DNA damage than the non-smoker workers and non-smoker controls. There was no statistically significant difference on DNA damage in the peripheral lymphocytes between the workers using protective masks and the workers taking no protective measures.

No significant differences in peripheral blood cells in terms of white blood cells (WBC), red blood cells (RBC), hemoglobins (Hb), neutrophils, and monocytes were observed between workers and controls (Table 4). However, the percentage of lymphocytes was increased significantly in the workers ($p < 0.05$). Also, the absolute numbers and percentages of T lymphocytes and NK cells were higher in the workers, compared to controls ($p < 0.05$). No significant differences were found in the levels of helper T, suppressor T, and B lymphocytes in the workers, compared to controls.

The levels of IgG and IgM in exposed workers were statistically lower than in the controls ($p < 0.05$). The levels of IgA and complements C3 and C4 were not different between the groups. The levels of TNF-α were also statistically higher in the workers than in the controls ($p < 0.05$) (Table 5). No relationship between the duration of exposure and the studied immune parameters was observed.

No significant differences were found in the levels of the parameters studied such as numbers of hematologic cells, lymphocyte subpopulations, IgG, IgA, IgM, complement C3 and C4 except TNF-α levels between the workers using protective measures and the workers without protective measures. It was only found that the levels of TNF-α in the workers using protective measures were statistically lower in than the workers using no protective measures ($p < 0.05$) (Table 6).

Discussion

Formaldehyde (FA) is a ubiquitous potentially toxic compound, and many countries (e.g., the U.S., Germany, the Netherlands, Scandinavian countries) have adopted or proposed permissible concentration levels (ACGIH 2003; DECOS 2003; DFG 2000; Nordic Expert Group 2003). The carcinogenicity of FA and the derivation of a safe occupational exposure limit have been matters of documentations by a number of official bodies and scientific expert panels (Bolt et al. 2010). The Scientific Committee on Occupational Exposure Limits of the EU (SCOEL 2008) has considered FA to be a "genotoxic carcinogen, for which a practical threshold is supported" and has recommended a

Table 2 FA exposure in the MDF producing plants

Characteristics of works	N	Use of protective measures (±)	FA momentary (ppm)	FA exposure level (TWA_{8h}) (ppm)[b]
1st MDF producing plant				
"Impregnated" section	2	+	0.21	0.19
Glue production area	2	+	0.17	0.15
Glue warehouse	2	−	0.15	0.14
MDF gluing section(outer part)	2	−	0.13	0.13
MDF gluing section(inner part)	2	+	0.25	0.22
MDF production section	2	+	0.31	0.30
MDF pressing section	1	+	0.35	0.33
MDF partition section	2	−	0.19	0.19
Semi-finished MDF warehouse	1	+	0.31	0.28
MDF warehouse	2	−	0.19	0.20
Semi-finished hardwood warehouse	2	−	0.09	0.12
Hardwood warehouse	2	−	0.12	0.11
Consignment section	2	−	0.18	0.17
	24	10/24	0.20 ± 0.08[a] (0.09–0.35)	0.19 ± 0.07[a] (0.11–0.33)
2nd MDF producing plant				
"Impregnated" section	2	+	0.11	0.10
Glue production area	2	+	0.24	0.22
MDF gluing section (inner part)	2	+	0.24	0.24
MDF production section	2	+	0.31	0.28
MDF pressing section	2	−	0.25	0.21
MDF partition section	2	−	0.17	0.17
Semi-finished MDF warehouse	2	−	0.26	0.25
MDF warehouse	2	−	0.17	0.17
Melamine coating area	2	+	0.19	0.18
Melamine pressing section	2	−	0.18	0.17
Consignment section	2	−	0.17	0.17
	22	10/22	0.21 ± 0.06[a] (0.11–0.31)	0.20 ± 0.05[a] (0.10–0.28)
Overall MDF producing plants	46	20/46	0.21 ± 0.07[a] (0.09–0.35)	0.20 ± 0.06[a] (0.10–0.33)

N number of workers, *FA* formaldehyde, *MDF* medium density fiberboard, TWA_{8h} 8-h time-weighted average

[a] The values are given as mean ± standard deviation (range)

[b] NIOSH method 3500

health-based Occupational Exposure Limit of 0.2 ppm. Regarding indoor exposures, Nielsen and Wolkoff (2010) have provided a new documentation on FA concluding that the guideline value of WHO (World Health Organization) (2000) of 0.08 ppm FA was preventive of carcinogenic effects.

Available data on the immunotoxicity and the hematoxicity of FA in exposed populations are sparse and inconsistent. No detailed and comprehensive immunotoxicity study has been performed in workers occupationally exposed to FA. Our study, in which immune parameters in workers with long-term low levels exposure to FA, is a contribution to the assessment of immunotoxicity of FA in humans. In the present study, the percentages of lymphocytes, the absolute number and the percentages of T lymphocytes (CD3+) and of NK (CD56 +) cells, and the levels of TNF-α were found significantly higher, but the blood levels of IgG and IgM were significantly lower in the workers compared to their controls ($p < 0.05$). The findings of increased numbers and the percentages of NK

Table 3 Assessment of DNA damage in peripheral lymphocytes of formaldehyde-exposed workers and controls by the Comet assay

	Tail intensity	Tail moment	Tail migration (μm)
Workers ($n = 46$)	4.25 ± 0.29 (1.48–10.31)[a]	0.624 ± 0.003 (0.24–1.92)[a]	1.68 ± 0.005 (0.48–4.80)[a]
Smoker workers ($n = 18$)	6.03 ± 0.44 (2.68–10.31)[c]	0.96 ± 0.005 (0.24–1.92)[c]	2.16 ± 0.009 (1.44–4.80)[c]
Non-smoker workers ($n = 28$)	3.27 ± 0.22 (1.48–5.44)	0.384 ± 0.001 (0.24–0.72)	1.20 ± 0.005 (0.48–3.12)
Controls ($n = 46$)	5.28 ± 0.22 (2.30–8.63)[a]	0.816 ± 0.002 (0.24–1.68)[a]	2.16 ± 0.007 (0.72–5.76)[a]
Smoker controls ($n = 23$)	6.08 ± 0.24 (4.46–8.63)[b]	1.08 ± 0.003 (0.48–1.68)[b]	2.64 ± 0.011 (1.44–5.76)[b]
Non-smoker controls ($n = 23$)	4.47 ± 0.27 (2.30–8.14)	0.552 ± 0.002 (0.24–1.2)	1.68 ± 0.009 (0.72–5.04)

The values are given as mean ± standard error mean (range)

[a] $p < 0.05$, workers compared to controls

[b] $p < 0.05$, smoker controls compared to non-smoker controls

[c] $p < 0.001$, smoker workers compared to non-smoker workers

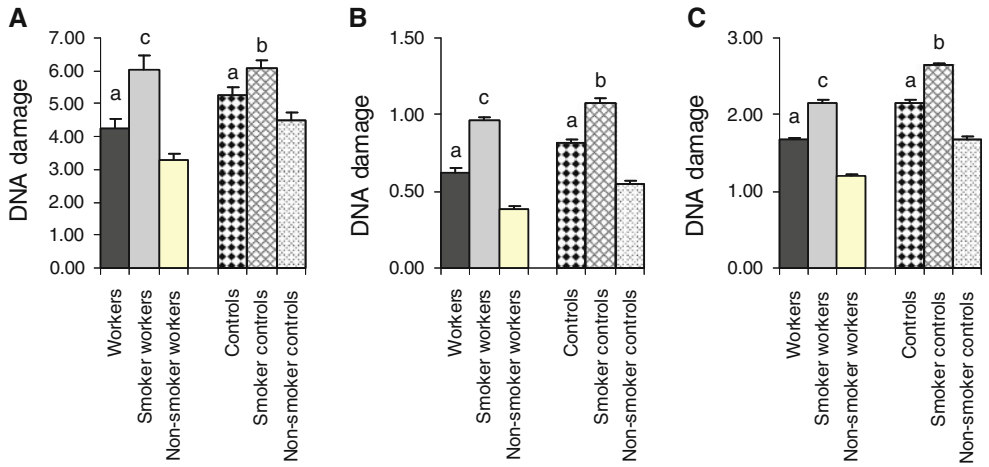

Fig. 1 DNA damage in peripheral lymphocytes of workers exposed to low levels of formaldehyde and controls, expressed as (**a**) tail migration, (**b**) tail moment, and (**c**) tail intensity. The results are given as mean ± standard error mean. [a]$p < 0.05$, workers ($n = 46$) compared to controls ($n = 46$), [b]$p < 0.05$, smoker controls ($n = 23$) compared to non-smoker controls ($n = 23$), [c]$p < 0.001$, smoker workers ($n = 18$) compared to non-smoker workers ($n = 28$)

cells and the levels of TNF-α might suggest an immune activation and potentially an increased susceptibility to inflammation of these subjects exposed to FA levels at current OELs. A more detailed account on the immunotoxicity of FA can be found in the Electronic Supplementary Material (Annex I) accompanying this article.

Although chromosomal damage by FA exposure in human peripheral blood cells was been claimed to occur (Ladeira et al. 2011; Santovito et al. 2011), in our study an increase in DNA damage, as determined by the Comet assay, was not found in long-term FA-exposed workers. This data are consistent with the results of others. It had been suggested that DNA–protein cross-links could arrest DNA replication, but such an arrest would not be detectable at low FA concentrations (Heck and Casanova 1999; Merk and Speit 1998). Several studies have shown that short-term (8 weeks) exposure to higher levels of FA (0.41–0.8 ppm) increased micronuclei (MN) frequency in nasal epithelial cells (Knasmueller et al. 2011; Ye et al. 2005). Significant increases in the frequency of MN in peripheral lymphocytes and in buccal cells of workers from pathology and anatomy laboratories who had been exposed to very high levels of FA were also reported (Costa et al. 2008; Ladeira et al. 2011; Orsiere et al. 2006). For instance, Shaham et al. (2002) found higher of SCE in peripheral lymphocytes of 90 workers from 14 hospital pathology departments who were exposed to FA for 15 or more years. Also chromosomal damage leading to micronucleated lymphocytes was found to be more frequent in highly exposed pathology and anatomy laboratory workers than in controls. The difference was suggested to be due to a higher frequency of chromosome loss, suggesting FA-induced defects in the mitotic apparatus (Orsiere et al. 2006). It is also being debated whether FA-induced genetic alterations, in conjunction with proliferation of immune cells, are the key components for the proposed mode of

Table 4 Values of WBC, RBC, Hb, neutrophils, monocytes, and lymphocytes and the numbers of the lymphocytes and lymphocytes subpopulations in formaldehyde-exposed workers and controls

	Workers	Controls
WBC (10^3/mm^3)	7.81 ± 0.29 (5.10–13.60)	8.37 ± 0.38 (4.30–14.90)
RBC (10^6/mm^3)	4.92 ± 0.05 (4.35–5.67)	4.76 ± 0.07 (4.00–5.91)
Hb (g/dl)	14.79 ± 0.13 (13.40–16.70)	14.43 ± 0.17 (12.20–16.70)
Neutrophils (%)	55.78 ± 1.29 (34.90–74.30)	51.82 ± 2.37 (10.30–81.00)
Neutrophils (10^3/mm^3)	4.38 ± 0.21 (2.00–8.20)	4.42 ± 0.32 (1.10–10.90)
Monocytes (%)	5.73 ± 0.29 (1.30–10.10)	6.26 ± 0.52 (2.40–22.60)
Monocytes (10^3/mm^3)	0.56 ± 0.11 (0.10–5.10)	0.54 ± 0.05 (0.20–2.30)
Lymphocytes (%)	36.1 ± 1.1(17.3–51.1)[a]	31.8 ± 0.9 (16.5–42.3)
Lymphocytes (10^3/mm^3)	2.78 ± 0.12 (1.30–5.10)	2.62 ± 0.09 (1.50–4.50)
T cell (%)	67.5 ± 1.21 (40.0–80.0)[a]	63.8 ± 1.1 (50.0–76.0)
Absolute T cells (10^3/mm$^{3)}$	19243 ± 1061 (7541–33915)[a]	16489 ± 725 (9183–27968)
Th cells (%)	40.7 ± 0.9 (31.0–54.0	42.4 ± 1.1 (23.0–57.0)
Absolute Th cells (10^3/mm^3)	11597 ± 639 (4763–23374)	10784 ± 499 (5235–18044)
Ts cells (%)	24.5 ± 0.9 (15.0–42.0)	22.6 ± 9.2 (10.0–70.0)
Absolute Ts cells (10^3/mm^3)	6837 ± 485 (2746–16800)	6055 ± 557 (2329–22865)
B cells (%)	11.3 ± 0.7 (4.0–20.0)	10.2 ± 0.6 (3.0–22.0)
Absolute B cells (10^3/mm^3)	3307 ± 273 (1126–6853)	2665 ± 233 (609–7282)
NK cells (%)	13.9 ± 0.9 (2.0–29.0)[a]	10.4 ± 0.8 (2.0–25.0)
Absolute NK cells (10^3/mm^3)	3899 ± 253 (686–9122)[a]	2631 ± 221 (662–5797)

The values are given as mean ± standard error mean (range)

WBC white blood cells, *RBC* red blood cells, *Hb* hemoglobulin, *T cells* T lymphocytes, *Th cells* helper T lymphocytes, *Ts cells* suppressor T lymphocytes, *NK cells* natural killer cells, *B cells* B lymphocytes

[a] $p < 0.05$, workers compared to controls

Table 5 Levels of the immunoglobins (Ig G, Ig A, and Ig M) and complement C_3 and C_4 proteins and TNF alpha in formaldehyde-exposed workers and controls

	Ig G (mg/dl)	Ig A (mg/dl)	Ig M (mg/dl)	C_3 (mg/dl)	C_4 (mg/dl)	TNF α (pg/ml)
Workers ($n = 46$)	847 ± 41[a] (178–1530)	166 ± 12 (39–388)	86 ± 5[a] (43–181)	117 ± 3 (82–169)	24.5 ± 0.9 (11.4–40.3)	15.1 ± 1.9 (2.1–76.0)
Controls ($n = 46$)	1106 ± 32 (639–1530)	184 ± 9 (92–338)	117 ± 6 (42–176)	113 ± 3 (74–171)	26.9 ± 1.2 (7.6–50.8)	4.9 ± 0.8 (0.0–23.0)

The results are given as mean ± standard error mean (range)

[a] $p < 0.05$, workers compared to controls

Table 6 Levels of the immunoglobins (Ig G, Ig A, and Ig M), complement C_3 and C_4 proteins, and TNF alpha in workers exposed to formaldehyde, related to the use of protective measures

	Workers with protective measures ($n = 26$)	Workers without protective measures ($n = 20$)
Ig G (mg/dl)	818 ± 62 (178–1530)	883 ± 50 (521–1420)
Ig A (mg/dl)	150 ± 15 (39.4–388)	187 ± 18 (70.9–352)
Ig M (mg/dl)	88 ± 6 (42.5–168)	84 ± 9 (43.4–181)
C_3 (mg/dl)	116 ± 4 (81.8–169)	117 ± 4 (88.6–165)
C_4 (mg/dl)	23.4 ± 0.9 (11.4–30.6)	26.1 ± 1.8 (13.5–40.3)
TNF α (pg/ml)	11.44 ± 1.72 (2.1–38)[a]	19.88 ± 3.61 (3.6–76)[a]

The values were given as mean ± standard error mean (range)

[a] $p < 0.05$, the workers using protective measures compared to the workers using no protective measures

action for FA-induced lymphohematopoietic malignancies (DeVoney et al. 2006). In general, positive genotoxicity results were reported in studies at exposure levels much higher than currently proposed OELs. In our study, there were no other confounding work-places chemicals. The study, therefore, confirms that the currently proposed OEL (SCOEL 2008) is safe with regard to genotoxicity. On the other hand, our study also suggests that additional studies on possible immunotoxic effects of FA in exposed populations are warranted. It has been claimed that the likelihood for the development of allergic asthma increases proportionately with indoor FA concentration, when levels exceed 0.08 ppm (Kim et al. 2011). In general, it appears that the aspect of immunotoxicity of formaldehyde at low-level exposures should be further investigated.

Acknowledgments This study was supported by the grants from Hacettepe University Scientific Research Units (HUBAB 0401103/004 and HUBAB 09 T09 102 004).

Conflicts of interest The authors declare that there are no conflicts of interest.

References

ACGIH (American Conference of Governmental Industrial Hygienists) (2003) Documentation of the TLVs® and BEIs® with other worldwide occupational values. CD-ROM,ACGIH, Cincinnati, OH

Anderson D, Yu TW, McGregor DB (1998) Comet assay responses as indicators of carcinogen exposure. Mutagenesis 13:539–555

Benyajati C, Place AR, Sofer W (1983) Formaldehyde mutagenesis in Drosophila. molecular analysis of ADH-negative mutants. Mutat Res 111:1–7

Bolt HM, Degen GH, Hengstler JG (2010) The carcinogenicity debate on formaldehyde: how to derive safe exposure limits? Arch Toxicol 84:421–422

Boyum A (1976) Isolation of lymphocytes, granulocytes and macrophages. Scand J Immunol 5:9–15

Chanet R, von Borstel RC (1979) Genetic effects of formaldehyde in yeast. III. nuclear and cytoplasmic mutagenic effects. Mutat Res 62:239–253

Collins AR, Dobson VL, Dusinka M, Kennedy G, Stetina R (1997) The comet assay: what can it really tell us? Mutat Res 375:183–193

Costa S, Coelho P, Costa C, Silva S, Mayan O, Santos LS et al (2008) Genotoxic damage in pathology anatomy laboratory workers exposed to formaldehyde. Toxicology 252:40–48

DECOS [Health Council of the Netherlands: Dutch Expert Committee on Occupational Standards] (2003) Formaldehyde. Health-based recommended occupational exposure limit. Health Council of the Netherlands, The Hague publication no. 2003/02OSH

DeVoney D, Jinot J, Keshava C, Hsu C, Whalan J, Vandenberg J (2006) A hypothesized mode of action in support of the biological plausibility of formaldehyde-induced lymphohematopoietic malignancies. Society for Risk Analysis, Baltimore, MD

DFG (Deutsche Forschungsgemeinschaft of the Federal Republic of Germany) (2000) Formaldehyde. Occupational Toxicants 17:163–201

Heck H, Casanova M (1999) Pharmacodynamics of formaldehyde: applications of a model for the arrest of DNA replication by DNA-protein cross-links. Toxicol Appl Pharmacol 160:86–100

Heck HA, Casanova M (2004) The implausibility of leukemia induction by formaldehyde: a critical review of the biological evidence of distant-side toxicity. Regul Toxicol Pharmacol 40:92–106

Hudson L, Hay FC (1991) Practical Immunology, 3rd edn. Blackwell, Oxford

IARC (International Agency for Research on Cancer) (2006) Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropanol-2-ol. IARC monographs on the evaluation of carcinogenic risks to humans, vol 88. World Health Organization, Lyon, pp. 39–325

IPCS (The International Programme on Chemical Safety) (1989) Environmental health criteria monographs: formaldehyde. http://www.inchem.org/documents/ehc/ehc/ehc89.htm

Kim KH, Jahan SA, Lee JT (2011) Exposure to formaldehyde and its potential human health hazards. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 29:277–299

Knasmueller S, Holland N, Wultsch G, Jandl B, Burgaz S, Misík M, Nersesyan A (2011) Use of nasal cells in micronucleus assays and other genotoxicity studies. Mutagenesis 26:231–238

Kreiger RA, Garry VF (1983) Formaldehyde-induced cytotoxicity and sister chromatid exchanges in human lymphocyte cultures. Mutat Res 120:51–55

Ladeira C, Viegas S, Carolino E, Prista J, Gomes MC, Brito M (2011) Genotoxicity biomarkers in occupational exposure to formaldehyde—the case of histopathology laboratories. Mutat Res 721:15–20

Lu K, Collins LB, Ru H, Bermudez E, Swenberg JA (2010) Distribution of DNA adducts caused by inhaled formaldehyde is consistent with induction of nasal carcinoma but not leukemia. Toxicol Sci 116:441–451

McGregor D, Bolt H, Cogliano V, Richter-Reichelm HB (2006) Formaldehyde and glutaraldehyde and nasal cytotoxicity: case study within he context of the 2006 IPCS human framework for the analysis of a cancer mode of action for humans. Crit Rev Toxicol 36:821–835

Merk O, Speit G (1998) Significance of formaldehyde-induced DNA-protein crosslinks for mutagenesis. Environ Mol Mutagen 32:260–268

Natarajan AT, Darroudi F, Bussman CJM, van Kesteren-van Leeuwen AC (1983) Evaluation of the mutagenicity of formaldehyde in mammalian cytogenetic assays in vivo and in vitro. Mutat Res 122:355–360

Nielsen GD, Wolkoff P (2010) Cancer effects of formaldehyde: a proposal for an indoor air guideline value. Arch Toxicol 84:423–446

NIOSH (The National Institute for Occupational Safety and Health) (1994) Formaldehyde: method 3500. NIOSH Manual of Analytical Methods (NMAM), U.S. Department of Health and Human Services, Fourth Edition, 2, 1–5, Cincinnati, OH

Nordic Expert Group (2003) The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and The Dutch Expert Committee on Occupational Standards. 132. In: Wibiwo A, Arbete och Hälsa (eds), Formaldehyde. Vetenskaplig Skriftserie No. 11: 2003

Orsiere T, Sari-Minodier I, Iarmarcovai G, Botta A (2006) Genotoxic risk assessment of pathology and anatomy laboratory workers exposed to formaldehyde by use of personal air sampling and analysis of DNA damage in peripheral lymphocytes. Mutat Res 605:30–41

Ren X, Ji Z, McHale CM, Yuh J, Bersonda J, Tang M, Smith MT, Zhang L (2012) The impact of FANCD2 deficiency on formaldehyde-induced toxicity in human lymphoblastoid cell lines. Arch Toxicol [Epub ahead of print]. 10.1007/s00204-012-0911-6

Salonen H, Pasanen AL, Lappalainen S, Riuttala H, Tuomi T, Pasanen P (2009) Volatile organic compounds and formaldehyde as explaining factors for sensory irritation in office environments. J Occup Environ Hyg 6:239–247

Santovito A, Schilirò T, Castellano S, Cervella P, Bigatti MP, Gilli G, Bono R, DelPero M (2011) Combined analysis of chromosomal aberrations and glutathione S-transferase M1 and T1 polymorphisms in pathologists occupationally exposed to formaldehyde. Arch Toxicol 85:1295–1302

SCOEL (Scientific Committee on Occupational Exposure Limits) (2008) Recommendation from the Scientific Committee on Occupational Exposure Limits for formaldehyde. SCOEL/SUM/125, March 2008. European Commission, Luxemburg. Available via http://ec.europa.eu/social/home.jsp?langId=en. Accessed 4 September 2009

Shaham J, Gurvich R, Kaufman Z (2002) Sister chromatid exchange in pathology staff occupationally exposed to formaldehyde. Mutat Res 514:115–123

Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191

Speit G, Zeller J, Schmid O, Elhajouji A, Ma-Hock L, Neuss S (2009) Inhalation of formaldehyde does not induce systemic genotoxic effects in rats. Mutat Res 677:76–85

Sul D, Kim H, Oh E, Phark S, Cho E, Choi S, Kang HS, Kim EM, Hwang KW, Jung WW (2007) Gene expression profiling in lung tissues from rats exposed to formaldehyde. Arch Toxicol 81:589–597

Takahashi K, Morita T, Kawazoe Y (1985) Mutagenic characteristics of formaldehyde on bacterial systems. Mutat Res 156:153–161

Thompson CM, Grafström RC (2011) Considerations for the implausibility of leukemia induction by formaldehyde. Toxicol Sci 120:230–232

WHO (World Health Organization) (2000) Air quality guidelines for Europe, 2nd edn. World Health Organization, Regional Office for Europe. Copenhagen pp. 87–91

Winchester RJ, Ross GD (1986) Methods for enumerating cell populations by surface markers with conventional microscopy. In: Rose NR, Friedman J, Fahey JL (eds) Manual of clinical laboratory immunology, 3rd edn. American Society for Microbiology, Washington, pp 212–225

Ye X, Yan W, Xie H, Zhao M, Ying C (2005) Cytogenetic analysis of nasal mucosa cells and lymphocytes from high-level long-term formaldehyde exposed workers and lowlevel short-term exposed waiters. Mutat Res 588:22–27

Zeller J, Högel J, Linsenmeyer R, Teller C, Speit G (2012) Investigations of potential susceptibility toward formaldehyde-induced genotoxicity. Arch Toxicol 86:1465–1473