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A Suruda, P Schulte, M Boeniger, et al.

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Cytogenetic Effects of Formaldehyde Exposure in Students of Mortuary Science[1]

Anthony Suruda,[2] Paul Schulte, Mark Boeniger, Richard B. Hayes, Gordon K. Livingston, Kyle Steenland, Patricia Stewart, Robert Herrick, Donald Douthit, and Marilyn A. Fingerhut

Division of Surveillance, Hazard Evaluation, and Field Studies, National Institute for Occupational Safety and Health [A. S., P. S., M. B., K. S., R. H., M. A. F.]; Occupational Studies Section, Environmental Epidemiology Branch, National Cancer Institute [R. B. H., P. S.]; Department of Environmental Health Sciences, University of Cincinnati School of Medicine [G. K. L.]; and Cincinnati College of Mortuary Science [D. D.]

Abstract

The effect of low-level exposure to formaldehyde on oral, nasal, and lymphocyte biological markers was studied prospectively in a group of 29 mortician students who were about to take a course in embalming. During the 85-day study period, the subjects performed an average of 6.9 embalmings and had average cumulative formaldehyde exposures of 14.8 ppm-h, with an average air concentration of 1.4 ppm during embalming. Since the average time spent embalming was 125 min, formaldehyde exposures calculated as an 8-h time-weighted average were 0.33 ppm on days when embalmings were done, which was less than the Occupational Safety and Health Administration permissible exposure limit of 0.75 ppm. Epithelial cells from the buccal area of the mouth showed a 12-fold increase in micronucleus frequency during the study period, from 0.046 ± 0.17/1000 cells preexposure to 0.60 ± 1.27/1000 cells at the end of the course ($P < 0.05$). Nasal epithelial micronuclei increased 22%, from 0.41 ± 0.52/1000 cells to 0.50 ± 0.67/1000 cells ($P = 0.26$). In blood cells, the frequency of micronucleated lymphocytes increased 28%, from 4.95 ± 1.72/1000 cells to 6.36 ± 2.03/1000 cells ($P < 0.05$), while sister chromatid exchanges decreased 7.5% ($P < 0.05$). A dose-response relationship was observed between cumulative exposure to formaldehyde and increases in buccal micronuclei in the 22 male subjects but not in the 7 female subjects. We conclude that low-level exposure to formaldehyde is associated with cytogenetic changes in epithelial cells of the mouth and in blood lymphocytes. These cytogenetic effects may be useful as markers of biologically effective dose.

Introduction

Formaldehyde is an important industrial chemical with many commercial uses and derivatives. It is also found in biological systems, where it is a metabolite in certain one-carbon reactions (1), and is normally present in human cells in low concentrations (2). Exposure to exogenous formaldehyde has been associated with a number of biological effects in humans such as skin sensitization (3) and eye and upper airway irritation (4). Exposure of rats to formaldehyde at 6 to 15 ppm caused development of nasal carcinomas (5, 6). The International Agency for Research on Cancer found limited evidence for the carcinogenicity of formaldehyde in humans (7). NIOSH[3] considers formaldehyde to be a potential human carcinogen (8). The OSHA PEL for formaldehyde is 0.75 ppm, calculated as an 8-h TWA (9). Formaldehyde exposure has been associated with nasal cancer (10, 11), nasopharyngeal cancer (12–14), buccal and pharyngeal cancer (15), and leukemia (16, 17).

Various biological markers indicating chromosomal change or damage have been studied in humans exposed to known or suspected carcinogens in an attempt to identify early effects (18–21). In this study we examined the effects of formaldehyde exposure on chromosomal micronuclei in nasal and buccal epithelial cells and on micronuclei and SCE in blood lymphocytes. Epithelial tissues of the nose, mouth, and upper respiratory system are the likely target organs for formaldehyde exposure in humans who breathe through both the nose and mouth (22). This study is part of ongoing investigations of cancer in the profession of embalming, which have included a proportionate mortality study (16) and a case-control study of brain cancer and leukemia (23).

Micronuclei are intracellular groupings of chromosomal material surrounded by a nuclear membrane, separate from and smaller than the main cell nucleus, which are formed when breakage produces fragments or when malfunction of the spindle apparatus leads to lagging intact chromosomes (24). The frequency of micronuclei in peripheral lymphocytes has been reported to increase with age (25), smoking (25), exposure to organic solvents (26), styrene (25), and mixtures of chemicals at a chemical plant (27) and to be higher in females (25).

Exfoliated cells from the buccal surface of the oral cavity can be sampled easily by gentle scraping with a blade or soft plastic brush, similar to the technique for obtaining Pap smears (28). Micronucleus frequency in buccal epithelial cells has been reported to be increased by cigarette smoke

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[2] To whom requests for reprints should be addressed, at Rocky Mountain Center for Occupational and Environmental Health, Building 512, University of Utah, Salt Lake City, UT 84112.

[3] The abbreviations used are: NIOSH, National Institute for Occupational Safety and Health; OSHA, U.S. Occupational Safety and Health Administration; PEL, permissible exposure limit; TWA, time-weighted average; SCE, sister chromatid exchange;

(29), tobacco chewing (28, 30), betel nut chewing (25), and ionizing radiation (25) and to be higher in females (31) and in paint industry workers exposed to lead and to organic solvents (32). In prospective studies of patients undergoing radiation therapy, the increased frequency of micronuclei in buccal cells was of short duration and returned to baseline within 1 or 2 months following cessation of the exposure (33) consistent with a turnover time for buccal cells of approximately 25 days (34). Fontham *et al.* (29) reported a frequency of 0.24% in the buccal cells of smokers compared to 0.07% in controls. Stich *et al.* (28) reported buccal micronucleus frequencies of 2.2% in tobacco chewers and 4.7% in betel nut chewers, compared to 0.47% in controls. Livingston *et al.* (30) reported a frequency of 2.2% in tobacco chewers compared to 0.27% in controls.

Epithelial cells in the anterior portion of the nose can be sampled with a cotton swab or brush and analyzed in a manner similar to oral cells. Sarto *et al.* (35) reported mean nasal micronucleus frequencies of 0.44% ± 0.59 in a control group, 0.50% ± 0.78 in a group of workers exposed to chromic acid fumes, and 0.77% ± 0.53 in workers exposed to ethylene oxide. In three workers exposed to an accidental leak of ethylene oxide, serial nasal swabs showed that micronucleus frequency increased from 0.73% at 3 days to 2.32% at 16 days following exposure.

SCE involves breakage of double-stranded DNA in both chromosomes followed by an exchange of DNA duplexes. The exact mechanism of SCE formation is unknown, but SCEs are not considered to represent mutations (18), although they may indicate exposure to a mutagen (36). The frequency of SCE has been reported to be increased in smokers (18, 36) and women (36) and to increase with age (18) and following measles or smallpox vaccinations (36). In a prospective study of eight medical students exposed to formaldehyde from embalmed bodies used in an anatomy class, Yager (37) reported an increase in SCE frequency of 13% ($P = 0.02$). However, cross-sectional studies of occupational populations chronically exposed to formaldehyde have not found changes in SCE frequency (38, 39) or in chromosomal aberrations (40).

Materials and Methods

Study Population. Students enrolled in associate or bachelor's degree programs at a college of mortuary science who were about to take an initial course in embalming were studied to test the hypothesis that exposure to formaldehyde could result in cytogenetic effects in oral and nasal tissue and in blood cells. As a requirement for graduation, these students must participate in at least 25 embalmings. NIOSH and National Cancer Institute investigators had previously characterized the embalming laboratory at this college as having levels of airborne formaldehyde of approximately 1 ppm (23, 41). Because some incoming students have done little or no previous embalming work, this population was chosen for a prospective study of the short-term effects of formaldehyde on cells.

Subjects were enrolled in the study during the first quarter of school, 3 weeks prior to their first embalming course. Each subject completed a questionnaire concerning past occupational activity, past embalming experience, smoking, diet, medication, and X-ray exposure. Swabs of the nose and mouth and blood samples were taken as described below. The blood, oral, and nasal sampling was repeated after the first 9 weeks in the embalming laboratory, and an interim questionnaire was administered.

Exposure Monitoring. Exposures to formaldehyde were assessed by personal sampling using a passive monitoring device (PF-20 short-term exposure limt monitor; Air Quality Research, Berkeley, CA). Prior to use the PF-20 passive monitor was evaluated in a laboratory exposure chamber and under field conditions in the embalming area of the mortuary college. In the laboratory, the PF-20 performed essentially the same as an active sampling method (NIOSH method 2502; Ref. 42) using a glass tube containing a solid sorbent medium connected to a personal sampling pump (43). In field use, the PF-20 had an average negative bias of 25%. The cause of this bias is unknown but may be due to the slower diffusion of nonmonomeric species of formaldehyde across the monitor's diffusion membrane (43).

NIOSH staff were present during the first 2 weeks of the embalming course to instruct each participant as to the proper use of and recording of pertinent data for the passive monitors. After the second week, participants monitored themselves. During each laboratory session, participants took a dosimeter from a supply placed near the embalming area, activated the sampling device by removing its protective cover, and recorded pertinent data such as their name, start and stop times, and type of embalming during that laboratory session. Sampling was ended by replacing the cover at the end of the session. Compliance by the subjects was evaluated by comparison with the instructor's log, which lists the duration and type of each embalming, names of participants, and the amount of solution used.

Several students lived at funeral homes or had part-time jobs in funeral homes. These students were asked to follow the same procedures for measuring exposures during embalmings outside the college laboratory. There was, however, no way to determine compliance for these embalmings.

Used monitors and a log of outside embalming activities were collected frequently by the investigators. The monitors were analyzed by the manufacturer using the chromotropic acid method (44). Information from the dosimeters and from the embalming log was used to estimate cumulative exposures to formaldehyde during the study period.

In addition to the personal monitoring, short-term (peak) exposure measurements were performed using a continuous reading instrument (model 4160 SP; Interscan, Chatsworth, CA). Teflon tubing attached to the instrument inlet was located at about head height of the embalmers and directly over the embalming tables. The monitoring instrument was connected to an external logging device, and the measurement data were later down-loaded to a personal computer. This system provided measurements of formaldehyde concentration approximately every 0.6 s.

A study of the material safety data sheets for the embalming solutions used at the college indicated that many contained other potentially toxic compounds besides formaldehyde. Compounds in these various embalming solutions that had relatively high volatility included glutaraldehyde, methanol, isopropyl alcohol, and phenol. NIOSH air sampling methods 2531, 2000, 1400, and 3502, respectively, were used to determine these compounds in the air from both personal and area samples (42).

Although formaldehyde has been reported to penetrate latex gloves of the type used at this college of mortuary science (45), it was not feasible to measure skin absorption of formaldehyde during this study. Students in the laboratory usually wore double sets of latex gloves and changed these frequently.

Cytogenetic Assays. All blood and tissue specimens were obtained in the morning, prior to any embalming, and were processed by the cytogenetic laboratory on the same day that they were obtained from the subjects.

Assay for micronucleus frequency in buccal cells was performed as described by Stich (46), except that cytopathology brushes (Surgipath C-E Brush; Surgipath Medical Industries, Grayslake, IL) rather than spatulas were used for sampling. Specimens were obtained from separate brushings from the left and right cheek of each individual. Each brush was immediately immersed in a vial containing 5 cc of Hanks' basic salt solution and delivered within 2 h to the cytogenetics laboratory. The vials were then vortexed to suspend the epithelial cells, and the resulting suspension from each brush was centrifuged directly onto a glass microscope slide using a cytocentrifuge. The slides were fixed in methanol, stained with the Feulgen reaction, and counterstained with Fast Green.

Nasal cells were processed and assayed for micronuclei in a manner identical to that for buccal cells. Swabs were taken from the inferior turbinate of each nostril using a cytopathology brush, and the brush was placed in a vial of 5 cc of Hanks' basic salt solution and processed as described above.

Lymphocytes were isolated using standard methods and prepared for micronucleus assay by the method of Fenech and Morley (47).

Counting of micronuclei was done by the method of Livingston *et al.* (30), in which cells are assigned to one of five classes on the basis of nuclear features, designated as 0, 25, 50, 75, and 100. The zero class is defined as structurally normal, and the 100 class is totally devoid of any Feulgen-positive (DNA) nuclear material, *i.e.*, the cell is anucleated. Intermediate categories of 25, 50, and 75 reflect progressive degradation of nuclear structure. Micronuclei were scored only in cells with a nuclear classification of 0, 25, or 50; 1500 cells were counted per sample for epithelial cells, and 2000 cells per sample for lymphocytes.

Lymphocyte cultures for SCE assay were set up using standard cytogenetic methods (48). Briefly, aliquots of 0.3 ml of blood were placed in 10 ml of culture medium (RPMI-1640) containing phytohemagglutinin-M (100 mg/ml), 5-bromodeoxyuridine (20 μm), fetal calf serum (17%), and penicillin/streptomycin and cultured at 37°C. Cells were arrested in mitosis with a Colemid block from 66–68 h, harvested using standard methods, and stained with fluorescein plus Giemsa. A minimum of 50-s division metaphases were scored for each sample.

All slides were coded and marked by one of the investigators to blind the reader to the exposure status of any individual. To avoid reader variation caused by analyzing pre- and postexposure slides at different times, staining of epithelial cell and lymphocyte micronuclei specimens was deferred until both pre- and postexposure samples had been obtained, and then both sets of slides from each subject were stained together on the same day. Slides from any one individual were then read on the same day by the same reader.

A 10% sample of all slides was chosen for quality control analysis. These slides were recoded and then rescored for micronuclei or SCE in the same manner and by the same reader.

Data Analysis. Multivariate analysis of baseline lymphocyte SCE and micronuclei was done with a multiple linear regression model using the "maximum r^2" technique (PC SAS version 6.04) (49) to identify variables contributing the most improvement to r^2 when added to the model. Variables statistically significant at the $P = 0.15$ level using this method were then included in the final model along with *a priori* variables such as previous embalming experience and cigarette smoking. Baseline levels of nasal micronuclei could not be fitted to a linear regression model and were analyzed by Poisson regression. Baseline buccal micronuclei were too few for multivariate analysis. Evaluation of environmental sampling data for outlier values was done using Grubb's statistic (50).

Each subject was used as his or her own control in the analysis of change in levels of the various cytogenetic markers. Differences in mean pre- and postexposure marker values were assessed by using a matched Student's t test for values that were normally distributed (SCE) or by the Wilcoxon sign-rank test, a nonparametric test, for values that were not normally distributed (micronuclei) (51). Comparison of the change in cytogenetic markers with cumulative formaldehyde exposure was done using Spearman's rank correlation coefficient and by linear regression if the regression residuals were normally distributed.

Results

Study Population

Students of mortuary science with little or no prior embalming experience were encouraged to enroll in the study. Baseline specimens were obtained from 34 students. Three students left school during the period of study, and so postcourse specimens were obtained on 31 subjects. One subject who, as a teaching assistant, had performed 85 embalmings in the 90-day period prior to the study, and one subject who took up chewing tobacco during the study period were dropped, leaving 29 subjects for analysis. Eight of the subjects had never participated in an embalming prior to the study, six had assisted with from one to four embalmings, and 15 had assisted with five or more embalmings in their lifetimes. The average age of the subjects was 23.6 years. Seven of the 29 subjects were women, and five of the 29 (two of whom were women) were current smokers. Incoming students were routinely offered vaccination against hepatitis B by the school health office; 14 of the 29 subjects reported receiving hepatitis vaccine during the 12 months prior to entering the study.

Exposure to Formaldehyde

During the study period the 29 subjects performed 144 embalmings in the college laboratory. Embalmings of autopsied bodies made up 36% of all cases, "normal" embalmings of intact bodies, 21%; embalming of bodies donated to a local medical school for anatomic examination, 36%; and other categories, 7%. Exposure measurements were obtained for 121 of these 144 embalmings (84%). The average air concentration was 1.4 ppm of formaldehyde, with a range of 0.15–4.3 ppm. The mean length of an embalming was 125 min. The highest exposures to formaldehyde were encountered when autopsied cases were embalmed, followed by anatomical and normal cases (Table 1).

Short-term exposure monitoring during 11 embalmings found peak exposures from 3 to 9 times that of the corresponding TWA. Peak exposures were associated with leaks of embalming fluid occurring during injection into vessels and body cavities, application of embalming gels to hands, feet, or facial parts, and leaks of embalming fluid from tubing and other appliances (Fig. 1). Instantaneous peak exposures

Table 1 Exposure measurements during embalming course

Case type	Number	Mean formaldehyde concentration[a] (ppm)	Range (ppm)
Normal bodies	25	0.9	0.2–2.1
Bodies for anatomical donation	44	1.3	0.5–4.3
Autopsied bodies	44	1.5	0.4–3.8
Other cases	8	0.2	0.15–0.9
All cases	121	1.4	0.15–4.3

[a] Mean exposure duration was 125 min.

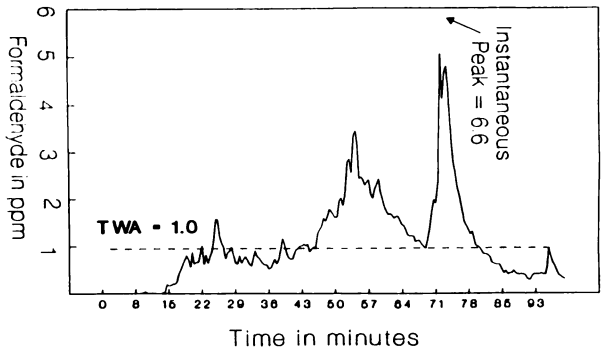

Fig. 1. Short-term exposure measurements during an embalming (peak exposure occurred during a leak of fluid while injecting a vessel).

ranged up to 6.6 ppm, and some exposures exceeded the OSHA short-term exposure limit of 3 ppm for a 15-min period. Ten of the subjects worked part time at outside funeral homes during the study period and participated in a total of 56 embalmings. Exposure monitors were worn for only 8 of these 56 embalmings because participants were reluctant to wear the dosimeters while working outside of the college. Formaldehyde exposures during outside work ranged from 0.58 to 3.32 ppm, with an average of 1.06 ppm. These measurements were similar to formaldehyde exposures at funeral homes reported in the literature (52, 53).

When both the embalmings performed at the college and at private funeral homes were considered together, the group as a whole completed 200 embalmings, an average of 6.9 per participant (range, 2–15). Unmeasured exposures at the school were estimated from the mean of the samples taken for that type of case. Unmeasured exposures outside the school were estimated using any prior measurements at that location or from the published mean levels for this trade (52, 53).

For each subject, cumulative exposure to formaldehyde during the 85-day study period was the sum of the measured or estimated concentration (ppm) during an embalming multiplied by the total duration (h) to give ppm-h. The average was 14.8 ppm-h, with a range of 4.3–33.6 ppm-h (Fig. 2). Average time spent embalming was 10.6 h, with a range of 3.8–24 h. The calculated 8-h TWA exposures for days on which embalming was done ranged from 0.10 to 0.96 ppm, with a mean of 0.33 ppm. Male and female subjects performed similar numbers and types of embalmings and had similar average cumulative exposures and TWA exposures.

Of 16 samples collected for glutaraldehyde, none contained detectable analyte. The limit of detection for glutar-

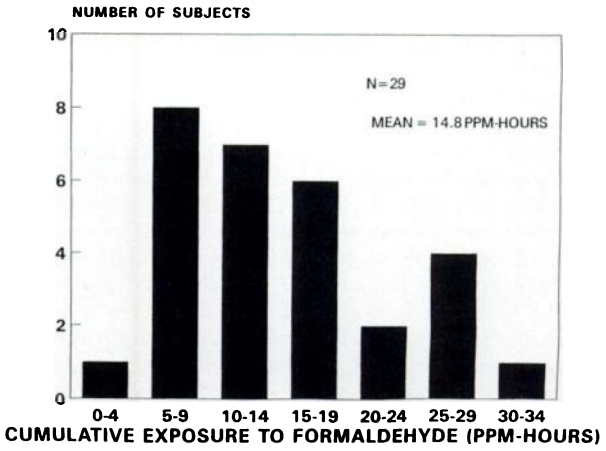

Fig. 2. Formaldehyde exposure during embalming course.

aldehyde was approximately 0.15 ppm. The OSHA PEL for glutaraldehyde is 0.2 ppm as a ceiling concentration (54). Eight area samples were taken for phenol on separate occasions, and none of these contained detectable analyte. Some of these samples were taken during embalming of anatomical cases where the embalming fluid contained phenol. The limit of detection for phenol was approximately 0.1 ppm. The OSHA PEL for phenol is 5 ppm as an 8-h TWA (54). Ten personal and area samples for isopropyl alcohol were collected. The concentrations measured ranged from nondetectable (Level of Detection) to 12 ppm. The OSHA PEL for isopropyl alcohol is 400 ppm measured as an 8-h TWA (54). Seven area air samples were collected in the embalming laboratory for methanol. All results were below the least detectable amount of about 0.8 ppm.

Cytogenetic Effects

Table 2 shows the baseline, postexposure levels, and percentage change of the various cytogenetic markers as well as the correlation of change with cumulative formaldehyde exposure. Because females in this study tended to have higher baseline levels and because increases in some markers were seen mainly in subjects with low baseline levels, additional analyses are shown by sex.

Buccal (Oral) Cells

The baseline levels of micronuclei per 1000 buccal epithelial cells were 0.046 ± 0.17 for the entire study group and 0.00 ± 0.00 for males (Table 2). Females had baseline levels of 0.19 ± 0.30. Since only two subjects, both female, had any baseline buccal micronuclei, multivariate analysis of baseline buccal values was not done.

There was a 12-fold increase in frequency in buccal cell micronuclei after the embalming course, to 0.60 ± 1.27 per 1000 cells (Table 2), which was statistically significant ($P < .05$), and the increase was seen in both male and female subjects. In the postexposure sampling, 13 subjects had buccal micronuclei. For the 22 male subjects only, there was a dose-response relationship between cumulative exposure to formaldehyde and increases in buccal epithelial micronuclei ($r = 0.50$, $P = 0.01$). A plot of cumulative formaldehyde exposure *versus* change in buccal cells is shown in Fig. 3.

Table 2 Baseline values, change in cytogenetic markers after embalming course, and correlation with cumulative formaldehyde exposure (ppm-h)

	Buccal micronuclei/ 1000 cells	Nasal micronuclei/ 1000 cells	Micronucleated lymphocytes/ 1000 cells	SCEs/cell
All subjects (29)				
Baseline value	0.046 ± 0.17	0.41 ± 0.52	4.95 ± 1.72	7.72 ± 1.26
After embalming course	0.60 ± 1.27	0.50 ± 0.67	6.36 ± 2.03	7.14 ± 0.89
Change	+1200%[a]	+22%	+28%[a]	−7.5%[a]
Spearman coefficient	0.22	−0.19	−0.06	−0.15
	(P = 0.25)	(P = 0.33)	(P = 0.76)	(P = 0.42)
Males only[b] (22)				
Baseline value	0.00 ± 0.00	0.38 ± 0.45	4.34 ± 0.73	7.31 ± 1.03
After embalming course	0.49 ± 0.57	0.43 ± 0.57	6.09 ± 1.90	7.10 ± 0.92
Change	(∞)[a]	+13%	+40%[a]	−3%
Spearman coefficient	0.50[a]	−0.28	0.21	−0.25
	(P = 0.01)	(P = 0.21)	(P = 0.34)	(P = 0.26)
Females[b] (7)				
Baseline value	0.19 ± 0.30	0.52 ± 0.69	6.85 ± 2.35	9.01 ± 1.16
After embalming course	1.14 ± 2.30	0.71 ± 1.38	7.20 ± 2.15	7.26 ± 0.79
Change	+505%	+37%	+5%	−19%[a]
Spearman coefficient	−0.22	0.43	−0.61	−0.25
	(P = 0.64)	(P = 0.33)	(P = 0.15)	(P = 0.59)

[a] $P < 0.05$.
[b] Three male subjects and two female subjects were current smokers.

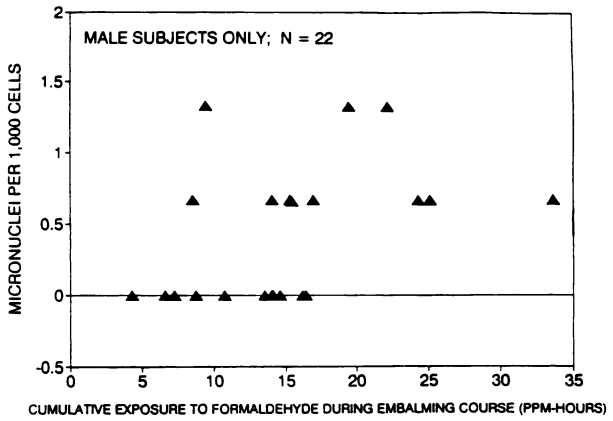

Fig. 3. Change in oral micronuclei with exposure to formaldehyde.

When parametric methods (linear regression) were used to analyze the dose response, there was a statistically significant relationship between exposures to formaldehyde in the final 10 to 21 days of the study and the changes in buccal cells for the entire group. However, nonparametric methods (Spearman's correlation) did not show a statistically significant dose response for the final 10 to 21 days.

Nasal Cells

The baseline levels of micronuclei per 1000 nasal epithelial cells were 0.41 ± 0.52 for the entire study group (0.38 ± 0.45 for males, 0.52 ± 0.69 for females; Table 2). The variables of age, sex, smoking status, and prior embalming experience were not significantly predictive of baseline nasal micronucleus frequency in a Poisson regression model.

There was a 22% increase in nasal micronucleus frequency after the embalming course, but this was not statistically significant (P = 0.26). No dose-response relationship was seen between changes in nasal cells and cumulative formaldehyde exposure for the entire study (r = −0.19, P = 0.33).

Blood Cells

Micronucleated Lymphocytes. The baseline levels of micronucleated lymphocytes per 1000 cells were 4.95 ± 1.72 for the entire study group and 4.34 ± 0.73 for males (Table 2). Females had baseline levels of 6.85 ± 2.35. Multivariate modeling of baseline levels showed that age, gender (female), and smoking were associated with higher values, while recent prior embalming experience did not significantly influence baseline levels of micronuclei (Table 3).

The frequency of micronucleated lymphocytes increased 28% after exposure to formaldehyde during the embalming course, which was statistically significant ($P < 0.05$; Table 2). Male subjects showed a much greater increase (40%) than females (5%).

There was no correlation between cumulative formaldehyde exposure and the change in micronucleated lymphocytes (r = −0.06, P = 0.76). In a multivariate model, a dose-response relationship between exposure to formaldehyde and the increase in micronucleated lymphocytes was seen only in the 22 male subjects and only if current smoking status and coffee drinking were included in the model (P = 0.02).

Lymphocyte Sister Chromatid Exchange. The baseline levels of SCEs per cell were 7.72 ± 1.26 for the entire study group (7.31 ± 1.03 for males, 9.01 ± 1.16 for females; Table 2). Multivariate modeling of baseline levels showed that both gender (female) and smoking were associated with higher baseline SCE levels, but recent prior embalming experience was not (Table 3). There was a 7.5% drop in SCE frequency after the embalming course (Table 2), which was statistically significant (P = 0.01). The seven female subjects showed a decrease of 19%, with a mean postexposure SCE value of 7.26 ± 0.79.

Table 3 Baseline levels of lymphocyte biomarkers and results of multivariate modeling

Variable	Micronucleated lymphocytes/ 1000 cells	SCEs/cell	Micronucleated nasal cells/ 1000 cells
No. of subjects	29	29	29
Mean value	4.95 ± 1.72	7.72 ± 1.26	0.41 ± 0.52
Coefficients from multiple regression models[a]			
Intercept	1.99 ($P = 0.28$)	7.40 ($P = 0.001$)	
Age (yrs)	0.18 ($P = 0.01$)	0.04 ($P = 0.43$)	−0.021 ($P = 0.79$)
Sex (males = 1)	−1.95 ($P = 0.001$)	−1.42 ($P = 0.001$)	−0.34 ($P = 0.53$)
Smoking (categorical)	1.00 ($P = 0.12$)	1.45 ($P = 0.01$)	0.37 ($P = 0.63$)
Performed embalming in 90 days prior to study (categorical)	−0.01 ($P = 0.98$)	0.19 ($P = 0.68$)	0.50 ($P = 0.48$)
R^2 for linear regression	0.64 ($P = 0.001$)	0.53 ($P = 0.001$)	

[a] Terms for lymphocyte markers were derived by linear regression; terms for nasal cells were derived by Poisson regression.

Multivariate modeling of the change in SCE showed that sex was a significant predictor of change, while cumulative formaldehyde exposure was not. The change in SCE frequency did not appear to be related to hepatitis vaccination status within the past year or to personal factors other than sex.

Quality Control

A recount of 10% of slides showed that 6 of 6 SCE and 11 of 12 lymphocyte micronucleus preparations were scored within ±15% of the initial value when the blinded, coded slides were reread by the same reader. Evaluation of the recount of the epithelial cell preparations was made difficult by the low micronucleus frequencies, since specimens which were originally read as having no micronuclei were found to have a single micronucleus when reread, or vice versa. For 18 buccal slides (9000 cells counted), three micronuclei were found in toto on the recount where only one micronucleus had been found initially. For 18 nasal slides (9000 cells counted) from the same subjects, three micronuclei in toto were also found on the recount where there had been one initially.

The buccal and nasal preparations showed little cell disintegration. Over 98% of cells counted fell into the three highest categories for scoring nuclear artifacts; that is, the cells had little or no nuclear distortion.

Discussion

Some of the strengths of this study are the prospective design, which used each subject as his or her own control, the detailed day-by-day measurements of exposure, and the ability to monitor individual exposures through the use of passive dosimeters. Air sampling measurements indicated little or no exposure to substances in the laboratory other than formaldehyde. The limitations include the small number of measurements for outside embalming exposures and the prior embalming experience of many of the subjects.

The total exposure to formaldehyde that the subjects received was low, with an average 8-h TWA of O.33 ppm, 44% of the OSHA PEL of 0.75 ppm. However, measurements of short-term exposures indicated that peak exposures during embalming were likely to have exceeded the OSHA short-term exposure limit of 3 ppm for a 15-min period, a finding supported by the study of Stewart *et al.* (23), who reported peak exposures during embalming of over 12 ppm. For the group as a whole, work in the embalming laboratory was infrequent, averaging less than one embalming per week during the study period. In designing the study we had anticipated that students would perform one to two embalmings per week, but other class work and the lack of bodies presented to the college during the winter of 1989–90 limited embalming opportunities for students.

The most interesting finding of this study was the large increase in mean micronucleus frequency in cells of the mouth. However, a dose-response relationship with formaldehyde exposure was seen only in the 22 male subjects. The low initial micronucleus frequencies in the oral cells was due to the large number of subjects (27 of 29) who had no micronuclei in 1500 cells counted for the preexposure sampling. It is not rare to have zero micronuclei per 1500 cells in oral cells; Sarto *et al.* reported zero per 1500 in 10 of 16 electroplating workers (35). Because of the way the slide reader was blinded as to subject exposure status, and because a recount of 10% of the slides showed frequencies in the same range, we believe it is unlikely that reader bias accounted for the low value. The same reader scored the lymphocyte micronuclei, and these values were within the range reported in the literature. The young age of our study group and the relatively low smoking prevalence (5 of 29) may have contributed to the low oral micronucleus frequency.

Our sampling method for buccal cells differed from that reported in the literature in that cytobrushes rather than wooden spatulas were used to collect specimens. Cytobrush sampling for Pap smears has been shown to produce a "toothpick" effect in which epithelial fragments are sampled in addition to surface epithelial cells (55, 56). Whether cytobrush, swab, and spatula techniques can produce samples with varying micronucleus frequencies is unknown.

Recent work by Tolbert *et al.* (57) has indicated that the criteria often used for scoring micronuclei might include other nuclear anomalies and extraneous objects that might be read as micronuclei. Many of these objects may be the result of cytotoxic exposures. The method of scoring used in

our study reduces the likelihood of false positive readings by assigning candidate cells to five categories based on nuclear features. Only cells in the top three categories (those with the least distortion of features) are scored for micronuclei.

The increase in nasal epithelial micronucleus frequencies (22%) was small compared that in buccal cells (1200%). Possible explanations are that breathing patterns (mouth *versus* nose breathing) depended somewhat on formaldehyde air levels and favored mouth breathing in these subjects, or that the effects of formaldehyde in the human nose lie beyond the anterior 3/4 inch of the nose where sampling was done. In the rat, lesions of the nose induced by formaldehyde are limited to a small area, due to regional airflow patterns influencing deposition (58). In the rhesus monkey, cellular changes induced by formaldehyde are found in several areas of the nose (59). In human volunteers, formaldehyde inhibits mucous flow in the anterior two-thirds of the nose only (60).

The 28% increase seen in micronucleated lymphocytes was unexpected. Formaldehyde is a highly reactive substance, and it is believed that there is little likelihood that formaldehyde can induce toxicity at sites distant from the respiratory tract (61). A dose-response relationship was not seen between cumulative formaldehyde exposure and the increase in lymphocyte micronuclei. However, the frequency of lymphocyte micronuclei in a given subject has been reported to be relatively stable with repeated measurement over time (62, 63), and the changes seen in the present study are thus unlikely to be artifacts.

The route by which formaldehyde could have affected blood lymphocytes is unclear. The anterior portion of the human nose is highly vascular and contains numerous small blood vessels just beneath the mucosal surface (64), which could provide a means of absorbing formaldehyde into the blood. It is also possible that formaldehyde in embalming solutions penetrated the latex gloves of the subjects and was absorbed through the skin (45).

In contrast to the findings with micronuclei, SCEs decreased significantly (7.5%), and this was largely due to the average 19% drop seen in the female subjects. Studies of other toxins have reported that SCE and micronucleus findings are not always linked. For example, in workers exposed to ethylene oxide SCEs have been reported to be a sensitive indicator of exposure, while micronuclei do not change (65–67).

Formaldehyde is known to be genotoxic to insects and to human cells *in vitro* (68–71). *In vivo* cytogenetic changes in humans due to formaldehyde exposure are of great interest because it is a suspected human carcinogen (7, 69, 72). The findings of changes in oral and nasal epithelial cells and peripheral blood cells do not indicate a direct mechanism for carcinogenesis but do indicate that DNA alteration has occurred. The changes seen in peripheral lymphocytes indicate that cytogenetic effects can be seen in tissues distant from the area of initial contact. The association of these cytogenetic effects with formaldehyde exposure indicates that they may be useful as markers of biologically effective dose (19–21).

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