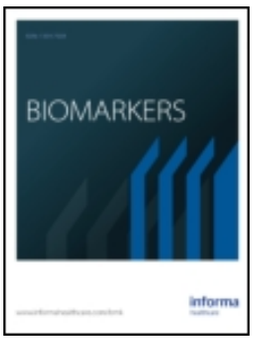

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SHORT COMMUNICATION

The utility of epithelial-cell micronuclei in the assessment of intermittent exposures

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Epithelial-cell micronuclei (MN) are potentially useful markers of occupational exposure to genotoxicants. With intermittent exposures, cells sampled either before or after a specific time interval, reflecting the time it takes for damaged cells to become available at the epithelial surface, are unlikely to be exposure-related. It may then be important to conduct an exposure-window analysis, with the goal of identifying the relevant exposures. We re-analysed individual exposure data from a previous study (Suruda *et al.* 1993) of MN formation in 22 male mortuary science students exposed to formaldehyde during a 90-day embalming class. We conducted an exposure-window analysis and compared the results with those obtained with 90-day cumulative exposure. The window widths varied between 7 and 25 days, in 1 day increments, assuming a constant 7-day cell-cycle. We assessed the fit (likelihood-ratio test) of a linear regression model, regressing the change in buccal MN prevalence on formaldehyde exposure, using both asymptotic and non-asymptotic methods. Exposures defined from 7–15 to 7–18 days before specimen collection provided a slightly better fit than the 90-day cumulative exposure, with a doubling of the regression coefficient for the exposure effect (for the 7–16-days window LR = 5.32, $p = 0.032$, coefficient = 0.088 MN per 1000 cells per ppm-hr; 95% CI = 0.014, 0.16; for the 90-day cumulative exposure LR = 4.44, $p = 0.048$, coefficient = 0.045 MN per 1000 cells per ppm-hr, 95% CI = 0.0038, 0.086). Although hampered by the small number of subjects, these results reinforce the potential importance of exposure timing.

Keywords: *micronuclei, occupational markers, exposure windows.*

Abbreviations: *CI, 95% confidence interval; LR, likelihood ratio; MN, micronuclei; ppm-hr, parts per million *hours of exposure.*

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Introduction

Micronuclei (MN) are DNA-containing fragments of the main nucleus, formed when chromosomes are not incorporated into the nucleus during mitosis (Vine 1990). Epithelial-cell MN have been reported to occur in response to both acute and chronic genotoxic exposures such as tobacco and high dose radiation (Stich *et al.* 1982, Stich and Rosin 1984, Rosin 1992) and have been considered as potential intermediate markers of occupational exposure to genotoxic agents (Diaz *et al.* 1990, Loomis *et al.* 1990, Sarto *et al.* 1990, Anwar and Gabal 1991, Gonzalez *et al.* 1991, Ballarin *et al.* 1992).

Suruda *et al.* (1993) previously reported an increased prevalence of micronucleated buccal, but not nasal, epithelial-cells in a small cohort of mortuary science students exposed to formaldehyde. The authors assessed the potential for a dose–response relationship between the change in micronuclei (MN) prevalence and total formaldehyde exposure cumulated across the entire 90-day embalming course, even though each student was exposed to formaldehyde only intermittently during that time. Since epithelial-cell MN can only be sampled for a short time period after a single genotoxic exposure (Vine 1990, Rosin 1992), determined by the cell-cycle kinetics of the epithelium, the 90-day cumulative exposure may have misclassified the biologically-relevant exposure.

In this paper we report on a re-analysis of the original Suruda *et al.* (1993) data using each subject's daily exposure log to redefine cumulative exposure within a set of different window periods. Our objective was to determine if there was an optimal exposure definition for this cohort, in which the exposure was highly intermittent.

MATERIALS AND METHODS

The study population is described in Suruda *et al.* (1993). Briefly, 22 male and seven female students enrolled in the associate or bachelor's degree programme at a college of mortuary science, and who were about to take an initial course in embalming, were enrolled in this study. The methods for collecting and analysing individual formaldehyde samples, are also presented in detail in Boeniger and Stewart (1992) and the original Suruda *et al.* (1993) study, but are described briefly here. Each subject was supplied with a personal passive air monitoring device (PF-20 STEL monitor; Air Quality Research, Berkeley, CA) that sampled air from their breathing zone. The personal monitor was worn during each formaldehyde exposure so that we had individual exposure measurements for each embalming. The same procedures were followed to estimate the few formaldehyde exposures occurring outside the embalming laboratory. All monitors were analysed by the manufacturer using the chromotropic acid method (Boeniger and Stewart 1992).

Buccal and nasal epithelial cells ($n = 6$ per area) were sampled from each student twice, once before the start of the embalming course and once 90 days later, at the end of the embalming course. Methods for sampling buccal epithelial cells and scoring them for micronuclei are described in detail in Suruda *et al.* (1993). Briefly, buccal epithelial cells were obtained from each student by gently scraping the epithelium with a cytobrush (Surgipath C-E Brush; Surgipath Medical Industries, Grayslake, IL). Brushes were suspended in 5 cm^3 of Hanks' basic salt solution, the vials were vortexed to suspend the epithelial cells, and the resulting suspension 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

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Fast Green. Micronuclei were scored by the method of Livingston *et al.* (1990). Nasal epithelial cells, collected with cytobrushes from the inferior turbinate of each nostril, were processed in exactly the same manner. A total of 1500 buccal and nasal cells were scored for each sample.

An appropriate window period for buccal-cell MN has two components, each with potential inter- and intra-subject variability. First, there is a minimum period reflecting the time it takes for damaged stem cells to reach the epithelial surface and then there is a window period during which we assume damaged cells will remain available for sampling. Since the outcome of interest is assessed at the final post-exposure sampling, each exposure window is defined relative to that day. Stich *et al.* (1983) observed the first radiation-induced MN in oral mucosa 7–10 days after the onset of radiation therapy. We defined a period of 7 days prior to the post-exposure sampling (i.e. 83 days after the baseline cell sample was taken) as the minimum for all possible windows. Thus the first exposure-window was the 1-day interval 7 days before the final cell sample was taken. Each additional window was 1 day longer than the previous one: the second window included the 2-day interval 7 and 8 days before the final cell sample was taken, the third estimated exposure window cumulated exposure over the 3-day interval 7, 8, and 9 days before the final cell sample was taken, and so forth. Exposure in each window was calculated by cumulating all formaldehyde exposures occurring during the interval defining the window. The last window was the 19 day interval 7–25 days before the final cell sample was taken. Thus our exposure-window analysis included 19 estimated windows to be contrasted among themselves and with the 90-day cumulative exposure used by Suruda *et al.* (1993).

In each window we used a simple linear regression model to relate exposure (ppm-hr) to the change in MN prevalence between the pre- and post-exposure period. Since important confounding variables such as age, gender and smoking, were identical at the baseline and post-exposure samplings (due to the within subject design), these variables should not be considered as potential confounders in this study and were not controlled for in the analysis. Thus from each regression we obtained two parameter estimates, the intercept and the regression coefficient for the exposure term. For each window, we used the likelihood ratio statistic to compare a model with only the intercept term to a model that included both the intercept and the exposure term. Higher values of the likelihood ratio indicate stronger support for the corresponding parameter estimates. In this case, the likelihood ratio test is equal to the *F*-test for the model (Afifi and Azen 1979). Windows were then contrasted based on their associated likelihood-ratio statistic with the goal of choosing the window that maximized the observed statistic (Salvan *et al.* 1995).

Due to the small number of people in our sample, and because MN data are not normally distributed, we supplemented the analyses based on the linear regression models with analyses based on exact non-parametric methods (Cytel Software 1992). For each window, we conducted a linear-by-linear association test (Agresti 1990), using the numeric values of the exposure variables as scores. The test statistic and its associated p-value were used to contrast windows.

Results

Our initial analysis involved both nasal and buccal epithelial cells in males and females. There was no association between change in MN prevalence in the nasal cells and any exposure measure and this result confirms the findings by Suruda *et al.* (1993) and it is not discussed further. Our analysis of buccal cell MN is restricted to the 22 male students in the cohort, since the seven female subjects were too few for an exposure-window analysis.

As reported in Suruda *et al.* (1993), subjects had intermittent exposures to formaldehyde over a 90-day period. For the 22 male subjects, 90-day cumulative exposures ranged from 4.3 to 33.6 ppm-hr, with a mean of 15.01 ppm-hr. Table 1 shows the individual exposure data for the last 28 days of monitoring, as well as the 90-day cumulative exposure for each subject and his change in MN per 1000 cells per ppm-hr. Where no exposure measure is noted, no exposure occurred (i.e. the student did not perform any embalming at all).

The results of the exposure-window analysis are presented in Figure 1. For each window, the figure displays the point estimate and 95% confidence interval (CI) for the coefficient of the exposure term in increments of MN per 1000 cells per ppm-hr and the corresponding likelihood-ratio statistic. Although results do not vary greatly across most windows, the likelihood-ratio statistic shows a maximum for the window 7–16 days before the sample of buccal cells was taken (LR = 5.32, $p = 0.032$). The corresponding coefficient estimate for the exposure term is 0.088 (95% CI = 0.014, 0.16). This should be contrasted with the results for the 90-day cumulative exposure in which LR = 4.44, $p = 0.048$, coefficient estimate = 0.045 (95% CI = 0.0038, 0.086). Thus, there was a small improvement in fit with the restricted exposure definition based on the 7–16 days window over the 90-day cumulative exposure. This was accompanied by an approximate doubling in size of the estimated exposure effect. Results based on exact non-parametric methods (not shown) identified the strongest association between exposure and change in buccal MN prevalence for the same 7–16 days window.

Discussion

The limitations of epithelial MN, an acute response to injury in a tissue with a short half-life, are well known (Vine 1990, Rosin 1992). With highly intermittent exposures, we do not expect MN prevalence to reach a steady state. If we want to be confident in attributing changes in MN prevalence to the exposure of interest, we need to establish biologically appropriate sampling windows for various epithelial tissues.

We conducted an exposure-window analysis of a cohort of male mortuary science students for whom Suruda *et al.* (1993) had already demonstrated an association between cumulative formaldehyde exposure and increased buccal-cell MN. The first window was defined as 7 days before the post-exposure sampling, based on considerations of average cell kinetics. Additional windows were defined in increments of 1 day and they were inclusive of the previous windows. The last window was 7–25 days prior to the post-exposure sampling. The data indicated a slightly better fit using the 10-day wide window 7–16 days before the final sample of buccal cells was taken than using the 90-day cumulative exposure as the estimated relevant exposure. For this window, the estimate of the exposure effect was approximately twice that obtained with the 90-day cumulative exposure. However, the results did not vary greatly across several exposure-windows, due in large part to the limited number of exposures experienced by the students in this cohort, as well as the small sample size.

Although the interpretation is hampered by the small number of subjects, these results reinforce the potential importance of exposure timing and suggest that an exposure-window analysis may be an important consideration for future

	Window period in: Number of days before the post-exposure sample[b]																			
90-day exposure[a]	7 days	7 to 8	7 to 9	7 to 10	7 to 11	7 to 12	7 to 13	7 to 14	7 to 15	7 to 16	7 to 17	7 to 18	7 to 19	7 to 20	7 to 21	7 to 22	7 to 23	7 to 24	7 to 25	MN[c]
6.3	0	0	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	6.3	6.3	6.3	0.67
3	0	1	1	1	1	1	1	1	3	3	3	3	3	3	3	3	3	3	3	0
3.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0.67
11.1	0	4.5	4.5	4.5	4.5	4.5	4.5	4.5	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	11.1	1.33
1.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.4	1.4	1.4	1.4	1.4	0
24.3	1.3	1.3	5.9	5.9	5.9	5.9	5.9	5.9	5.9	6.7	6.7	6.7	6.7	6.7	10.6	10.6	10.6	10.6	10.6	0.67
1.9	0	0	0	0	0	0	0	0	0	0	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	0.67
2.2	0	0	0	0	0	0	0	0	0	0	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	2.2	0
12.5	0	0	0	0	0	0	0	0	0	0	8.2	8.2	8.2	8.2	8.2	8.2	8.2	12.5	12.5	0.67
4.4	0	0	0	0	0	0	0	0	0	0	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	0
1.9	0	0	0	0	0	0	0	0	0	0	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	0.67
15.8	0	0	0	0	0	0	0	0	0	2.7	2.7	2.7	2.7	2.7	2.7	9.2	15.7	15.7	15.7	0.67
2.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.2	1.2	1.2	1.2	1.2	0
4.6	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.9	3.5	3.5	3.5	3.5	3.5	0
6.7	2.9	2.9	2.9	2.9	2.9	2.9	5.7	5.7	5.7	5.7	5.7	5.7	5.7	6.7	6.7	6.7	6.7	6.7	6.7	0
1.8	0	0	0	0	0	0	0	0	0	0	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	0
13.1	0	0	5.8	5.8	5.8	5.8	5.8	5.8	5.8	7	7	7	7	7	7	7	13.1	13.1	13.1	0.67
11.6	0	6.1	6.1	6.1	6.1	6.1	10.1	10.1	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	11.6	1.33
11.5	0	0	0	0	0	0	0	0	0	0	2.7	2.7	2.7	2.7	2.7	5	5	11.5	11.5	0
7.3	0	2	2	2	2	2	2	2	7.3.	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	0
3.4	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	3.4	3.4	3.4	3.4	3.4	3.4	3.4	0
1.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.3	1.3	1.3	1.3	1.3	1.33

Table 1. Cumulative formaldehyde exposures and change in buccal-cell micronuclei per 1000 cells for 22 male mortuary science students taking their first embalming course.

[a] Formaldehyde exposure cumulated over the entire 90-day period.

[b] Column headings refer to the size of the window period over which formaldehyde exposures were cumulated so that '7 days' is the 1-day window period 7 days before the post-exposure sample, '7 to 8' is the 2-day window period 7–8 days before the post-exposure sample, '7 to 9' is the 3-day window period 7–9 days before the post exposure sampling, etc.

[c] The change in the number of buccal-cell micronuclei per 1000 cells in the pre-exposure sample to the number of buccal-cell micronuclei per 1000 cells in the post-exposure sample.

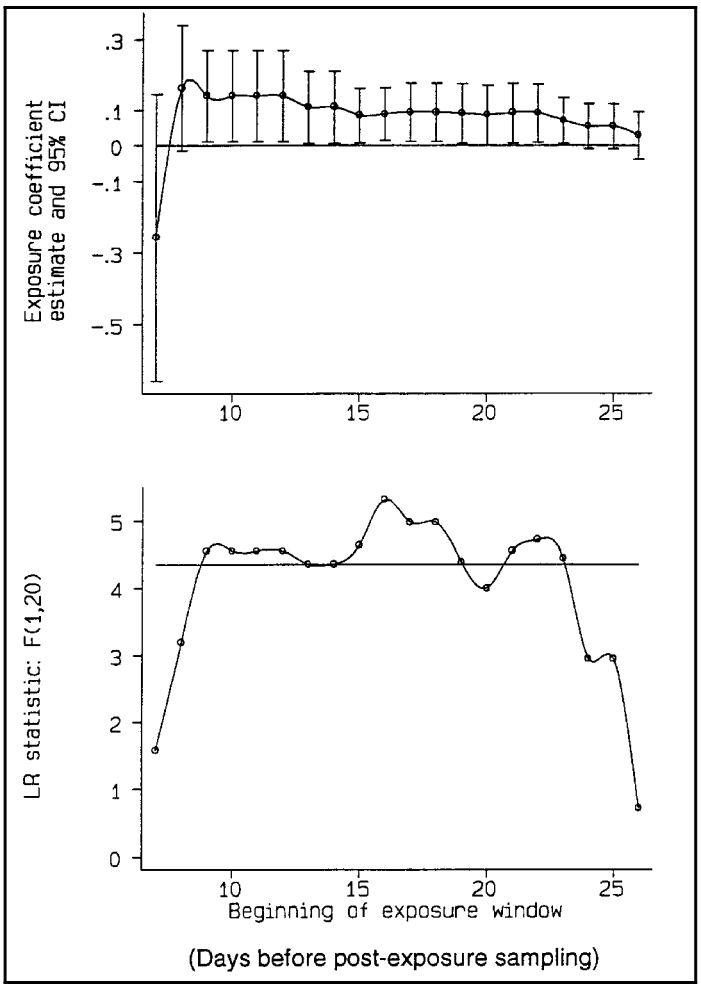

Figure 1. Exposure window analysis. Linear regression model. Males, $n = 22$.

studies of changes in MN prevalence in relation to many occupational exposures. In the future, studies involving epithelial-cell micronuclei as intermediate exposure markers may benefit from taking multiple cell samples over time as well as daily exposure logs. Such designs should be more informative than cumulative designs in which samples are taken only at the end of a fixed observation period.

Finally, we would note that where the critical biological parameters are well understood, an acute response measure such as epithelial-cell micronuclei should provide a distinct advantage. When the response develops and disappears quickly, the window period for the response can be used to predict when exposure-induced responses should be noted and when they should disappear. Studies using repeated sampling designed around these predicted intervals should prove to be a powerful means for attributing the response to a specific type of exposure (M. Rosin, personal communication).

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