DNA adducts in coal miners: association with exposures to diesel engine emissions

Shu-Xin Qu, James Leigh, Hamilton Koelmeyer and Neill H. Stacey

The potential carcinogenic effects of exposure to diesel engine emissions (DEE) are of growing concern. Due to the use of diesel equipment in underground mines, DNA adducts in peripheral blood mononuclear cells have been measured using the ^{32}P-postlabelling technique in workers from two coal mines (A, B) in NSW, Australia, before and after a period of more intense exposure (long wall change out, LWCO). DNA adducts were readily detected in all workers. At Mine A, in the 89 participants before LWCO, no significant difference was found among the groups categorized by exposure levels. However, significantly higher concentrations of total DNA adducts were observed in the specific job categories, 'miners and loadmen', and 'machinemen, drivers and shiftmen' and in the smoking group. On comparing total DNA adducts before and after LWCO in a small number of workers, a significant increase was also found. At Mine B, before or after LWCO, the total DNA adduct levels showed no significant difference among groups categorized by exposure conditions, smoking status, job categories and job time length. However, the total DNA adducts for the 61 subjects were significantly increased (geometric means) from 297 to 389 amol μg^{-1} DNA after LWCO ($p < 0.0001$, paired t test). Some individual adducts were also elevated to a greater extent ($p < 0.05$, paired non-parametric test, Wilcoxon signed rank test). Furthermore, using generalized estimating equations for adjusting all factors across the observation period, no particular factor showed any significant interactive effects. Given the association of exposure to DEE with lung cancer and the apparent increase in adducts during a period of intense DEE exposures it would be prudent to pay particular attention to keeping exposures as low as possible, especially during LWCO operations.

Keywords: *DNA adducts, human peripheral blood mononuclear cells, diesel engine emissions, ^{32}P-postlabelling, coal miners.*

Introduction

Diesel engine emissions (DEE) contain a number of aromatic compounds including polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs, both of which are well-documented classes of genotoxic carcinogens in experimental animals (IARC 1989, Gallagher *et al.* 1991). Human exposure to PAHs, such as benzo[*a*]pyrene (B[*a*]P), has been and continues to be of occupational and environmental concern. Although considerable occupational data have been reported, more studies are required to clearly evaluate the hazards and risks of DEE exposures. Diesel-powered equipment is used extensively in mining and the underground use is considered to be associated with higher exposures. Since DNA is the cellular macromolecule believed to be the most closely involved in carcinogenesis, modification (adduct formation) of DNA by carcinogens appears to be directly relevant to potential tumour formation in both experimental animals and humans (Gallagher *et al.* 1991, Beach and Gupta 1992). Therefore, quantitative indicators of underground miners' exposure to DEE would be extremely useful in risk assessment studies.

In humans the lung is the primary target organ for cancer from DEE exposure (IARC 1989). In experimental animals the carcinogenic effects of B[*a*]P have been intensively studied and shown to induce a wide range of tumours that depend to a certain extent on the route of administration. However, the target tissues for many chemical carcinogens are not readily accessible for monitoring exposure in humans, so some other tissues, such as peripheral white blood cells, have been investigated as possible surrogates. The available data from previous publications and our studies have demonstrated the applicability of peripheral blood mononuclear cells (PBMNs) for estimating exposure in the target organs (Gupta and Randerath 1988, Ross *et al.* 1990, Nesnow *et al.* 1993). It was therefore decided to investigate the levels of DNA adducts in PBMNs of underground miners exposed to DEE to determine levels of DNA adducts in their white blood cells.

Specifically, it was decided to determine (1) if DNA adducts are present in PBMNs from coal miners exposed to DEE; (2) if there is a dose–response relationship between exposure and adduct levels; (3) if adducts are detected, which jobs are most associated with high levels; (4) if DNA adduct levels are altered with changes in workplace conditions that change levels of DEE exposure.

The change in workplace conditions that was selected for investigation was the long wall change out procedure. When mining at one site is completed the long wall mining equipment is transferred to the next site. This involves intensive use of heavy equipment, diesel powered in these mines, over a 2–3 week period to move to the new site. It is widely and readily accepted in the industry that this operation results in a period of considerably increased exposure of miners to DEE. As it was a routine operation at the mines it provided the opportunity to examine changes in adducts in the same miners before and immediately after this period of increased exposure.

S.-X. Qu, J. Leigh *and* ***N. H. Stacey*** *(author for correspondence) are at the National Institute of Occupational Health and Safety (Worksafe Australia), GPO Box 58, Sydney, NSW 2001, Australia, and* ***H. Koelmeyer*** *is at the Joint Coal Board, Warners Bay, NSW 2282, Australia.*

MATERIALS AND METHODS

Chemicals and reagents

Proteinase K, RNase A, T1, micrococcal nuclease (MN), spleen phosphodiesterase (SP) and redistilled butanol were purchased from Sigma Chemical Co. (St Louis, MO). T4 polynucleotide kinase (PNK) (3′-phosphatase free) was provided by Boehringer Mannheim Biochemicals (Indianapolis, IN). Ficoll-Paque was supplied by Pharmacia LKB (Uppsala, Sweden). Machery Negel PEI–cellulose thin layer chromatography (TLC) plates were purchased from Alltech

1354–750X/97 $12·00

Associates (Australia) Pty Ltd (Sydney, Australia). [γ-^{32}P]ATP was purchased from Du Pont (with specific activity 3000 Ci mmol^{-1}). All other chemicals were of analytical grade.

Worker characteristics and exposures

Male miners from two mines (Mine A and Mine B) located in New South Wales, Australia, were invited to participate in the study after agreement with management and unions. Workers were divided into non, low, medium, and higher exposure categories (for DEE), on the basis of discussions with the workers and the relevant safety officer and worker representatives. The participants included a variety of personnel from different locations in the mines. Before and after long wall change out (LWCO) were defined as time 0 and time 1, respectively. To enable further statistical analyses, based on a similarity of working conditions and the estimated exposure levels, all participants were classified into five specific job categories, job 1 (fitters), job 2 (loadmen and miners), job 3 (deputy, underground managers, shift managers, engineers, electricians and surveyors), job 4 (machinemen, driver, shiftmen and mechanical unit) and job 5 (clerk, surface, lamp cabin attendants, planning coordinator, safety training coordinators and boiler markers who were normally working in non-DEE exposure conditions). The job length for both mines was classified in 5-year intervals. Other relevant personal information, such as smoking history and medication, was also collected via questionnaires.

Human PBMNs isolation

Human peripheral blood was obtained from the antecubital vein of the study participants. The PBMNs were isolated following the method described previously (Boyum 1977). The isolated cell pellets were quickly frozen in liquid nitrogen and kept at –80 °C until DNA isolation.

DNA isolation

PBMNs were homogenized in 2 ml of 50 mM Tris–HCl/10 mM EDTA buffer (pH 8.0) to prepare RNA and protein-free DNA as described previously (Gupta 1984, Beach and Gupta 1992). The concentration and purity of DNA were measured by spectrophotomer using the A_{260} value (1 A_{260} unit = 50 μg DNA), A_{230}/A_{260} ratio (0.40–0.45) and A_{260}/A_{280} ratio (1.80–1.85).

^{32}P-Postlabelling analysis of DNA adducts

^{32}P-Postlabelling analysis of DNA adducts was performed by previously developed methods (Gupta 1985, Reddy and Randerath 1986, 1987, Gupta and Randerath 1988) but with some modifications as follows (Qu and Stacey 1996).

MN and SP digestion

Ten μg of isolated DNA was dissolved in 10 μl MNSP 1 × buffer (20 mM sodium succinate, 10 mM $CaCl_2$, pH 6.0) followed by addition of 2.5 μl MNSP enzyme (1 μg μl^{-1} each enzyme). After 3 h incubation at 37 °C, the digest was diluted to 25 μl with double distilled water (0.4 μg DNA μl^{-1}).

Butanol extraction

Twenty μl of the above diluted digest was mixed with 20 μl of 100 mM ammonium formate, pH 3.5, 20 μl of 10 mM tetrabutylammonium chloride, and 140 μl of water. The mixture was extracted twice with 200 μl of 1-butanol. The combined butanol extracts were washed three times with 350 μl of water (butanol saturated). After adding 5 μl of 200 mM Tris (pH 9.5) the butanol extract was dried using a Dynavac freeze dryer (Sydney, Australia).

PNK reaction

Dried residue was redissolved in 10 μl double distilled water to obtain a final DNA concentration of 8 μg/10 μl. Half of this was used for the PNK reaction. After adding 5 μl 'hot mix' (consisting of [γ-^{32}P] ATP 3 μl, 30 μCi; PNK 0.3 μl, 3 units; 10 × kinase buffer 1.0 μl and double distilled water 0.7 μl), incubation was carried out at 22 °C for 1 h.

Mapping of adducts

PNK reaction mixture (8 μl) was spotted on the TLC plate that had been prewashed with double distilled water. The TLC plate was developed in different solvent systems, named D1, D3, D4 and D5 as detailed in the relevant figures. The same TLC plate was used to run the two samples simultaneously (before and after LWCO) for each worker.

Mapping of total nucleotides

The diluted post-MNSP DNA digestion (0.4 μg μl^{-1}) was serially diluted with distilled water (final concentration was 0.2 ng μl^{-1}). An aliquot (6 μl) of this diluted solution was labelled with 3.0 μl of 'hot mix' (described as above) and was further diluted to 60 μl (0.02 ng μl^{-1}) with 10 mM Tris/5 mM EDTA, pH 9.5. The diluted labelled solution (5 μl) was then spotted on a TLC plate (5 × 10 cm size) and developed with 0.3 M lithium chloride to the top. After soaking in a container supplied with running water for 5 min, the sheets were dried, and developed with 4.5 M ammonium formate, pH 3.5, to 5 cm from the bottom edge (Gupta and Randerath 1988).

TLC chromatography and measurement

DNA adducts and total nucleotides on the TLC plates were visualized using a Bio-Imaging Analyser with BAS 1000 workstation and quantified by MacBas version 1.0 software from Fuji Photo Film Co (Tokyo, Japan). Relative adduct labelling (RAL) values were calculated via the total nucleotides, and then converted to amol μg^{-1} DNA adducts (Gupta 1985, Gupta and Randerath 1988).

Statistical methods

Total DNA adducts, the sum of the individual adduct levels, was used as the main outcome variable. Descriptive statistics for the distribution of total adducts were computed overall and also for categories of each of the explanatory variables: job category, exposure level at time 0 and time 1, smoking status and length of time in job.

Linear regression was used to test for an association between $\log_e$ (total DNA adducts) at time 0 and each of the explanatory variables. Because the distribution of total adducts was very skewed, the logarithmic transformation was required to meet the normality assumption underlying the regression method. A difference in $\log_e$ (total DNA adducts) between groups would indicate a multiplicative effect between geometric means for those groups. All explanatory variables except 'length of time in job' were treated as categorical, with 'non-smokers', 'non-exposure' and 'job 5' being the referent category for each variable. For each categorical variable, the model estimates give the difference in $\log_e$ (total DNA adducts) between the category and the referent group. For instance, an estimate of 0.27 would represent a 31% higher level in that group relative to the referent (based on $e^{0.27} = 1.31$).

Multiple linear regression was used to examine the simultaneous effect of the explanatory variables on $\log_e$ (total DNA adducts) and to derive the most parsimonious model through backward elimination of non-significant variables. A significance level of $p < 0.1$ was used as the criterion for retaining variables in the model to identify possible associations. In the multiple linear regression analyses, the exposure variable was treated as a trend (continuous) variable to avoid mathematical difficulties of collinearity with the job category variable.

Changes in $\log_e$ (total DNA adducts) between time 0 and time 1 were analysed at Mine B using a paired *t*-test. Regression models of the difference in $\log_e$ (total DNA adducts) (time 1–time 0) were also fitted to test for an association between changes in adduct levels and smoking status, job category, time in job and reported exposure level at time 0. Because reported exposure levels varied with

time and some reported exposures were in fact lower on the second assessment, Generalized Estimating Equations (Zeger and Liang 1986) were also used. These models are designed to take into account correlation between observations for the same individual over time and are hence appropriate for the analysis of longitudinal data such as that obtained in this study. One of the major advantages of this approach is that covariates, such as level of exposure, can take on different values at different time points. For two time points, this approach reduces to a regression model of the difference in $\log_e$ (total DNA adducts) between time 0 and time 1 when the covariates do not change with time. These models were fitted using the SPIDA statistical package (Gebski *et al.* 1992).

Results

For the 89 workers at Mine A who participated in the study at time 0, seven workers were followed up at time 1 (after LWCO). At Mine B, data were available at time 0 for 75 workers, of whom 61 were followed up after LWCO. Data for the two mines have been analysed separately.

Mine A—time 0

General information on miners

Of the 89 participants at this mine, 26% were smokers and 35% were ex-smokers. The nine 'surfacemen' (10% of workers) were classified as having 'no exposure'. One-third (34%) was classified as 'low' exposure, 32% as 'medium' exposure and 24% 'high' exposure. Two of the job categories, 'miners and loadmen' (job 2) and 'machinemen, driver and shiftmen' (job 4) included 55% of the total participants. These jobs were generally considered to be associated with higher exposures. Over half of the participants (56%) had worked in the mine for more than 10 years.

Total DNA adduct levels

The patterns of DNA adducts on the TLC sheet are shown in Figure 1. Overall, the mean $\log_e$ (total DNA adducts) for the 89 workers was 5.1 (SE 0.06) corresponding to a geometric mean of 165.5 with 95% CI (147.3–185.9). Univariable linear regression models showed a significant positive association between total adducts and smoking status ($p = 0.04$), job category ($p = 0.03$) and a negative association with length of time in the job ($p = 0.03$) (Table 1). Adduct levels for smokers were on average 31% higher than for non-smokers, but levels in ex-smokers were approximately 90% of the levels in non-smokers. Workers in job categories 2 and 4 had levels approximately 60% higher than surfacemen (job 5). Total DNA adducts decreased with length of time in the job. There was no significant association between total DNA adducts and

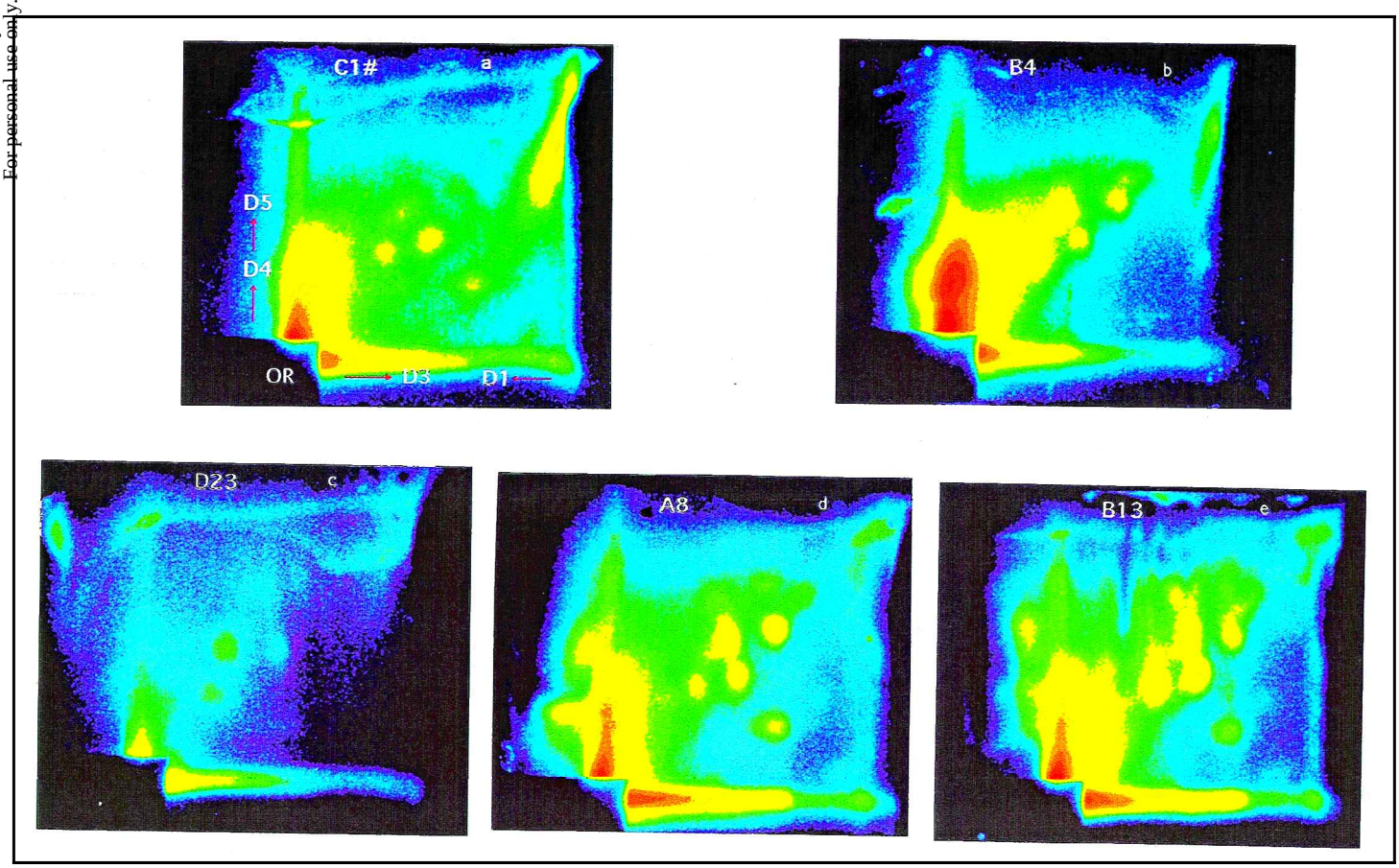

Figure 1. ^{32}P-postlabelling chromatograms of DNA adducts in human PBMNs from Mine A. (a) Non-smoker, (b) smoker, (c) job 5, (d) job 2 and (e) job 4. # Sample ID number. The origin of each chromatogram is at the lower left corner. TLC developing solvents were D1 (1.0 M sodium phosphate, pH 6.8), D3 (3.6 M formic lithium and 8.5 M urea, pH 3.5), D4 (0.8 M lithium chloride, 0.5 M Tris and 8.5 M urea, pH 8.0) and D5 (1.7 M sodium phosphate, pH 6.0). Chromatography was carried out on 10 × 12 cm PEI–cellulose plates. Exposure times were 3 h at room temperature.

	Regression coefficient	SE	
Model 1			
Smoking status			$F_{2,86} = 3.3$, $p = 0.04$, $R^2 = 0.07$
Intercept	5.08	0.09	
Ex-smokers	–0.11	0.13	
Smokers	0.27	0.15	
Model 2			
Exposure status			$F_{3,85} = 1.1$, $p = 0.33$, $R^2 = 0.04$
Intercept	4.79	0.19	
Low	0.33	0.21	
Medium	0.38	0.21	
High	0.37	0.22	
Model 3			
Job category			$F_{4,84} = 2.9$, $p = 0.03$, $R^2 = 0.12$
Intercept	4.79	0.18	
Job 1	0.26	0.25	
Job 2	0.50	0.20	
Job 3	0.10	0.21	
Job 4	0.48	0.23	
Model 4			
Length of time in job			$F_{1,87} = 5.0$, $p = 0.03$, $R^2 = 0.05$
Intercept	5.33	0.11	
Time in years	0.02	0.01	

Table 1. Univariable linear regression models of $\log_e$ (total adducts) for Mine A at time 0.

reported exposure level, either fitted as a categorical variable ($p = 0.33$) or a trend ($p = 0.19$) (Figure 2).

Results from the multivariable linear regression model (Table 2) were consistent with the linear regression results given above. Given the other variables in the model, the estimated total adduct levels for smokers were 37% higher than for non-smokers. No significant difference between ex-smokers and non-smokers was found in this model. Total adduct levels for job categories 2 and 4 were estimated to be approximately 50% higher than for surfacemen, and total adducts again showed a negative association with length of time in job. Inclusion of the exposure variable in the model resulted in no change to the other coefficients and the incremental F value for the exposure variable of 0.0003 was clearly non-significant ($p = 0.99$).

Mine A—comparison of time 1 and time 0

There were just seven participants at time 1, with six of them showing an increase in total DNA adducts. In a preliminary estimate with a paired non-parametric test for these seven workers, the geometric mean at time 1 is significantly different from time 0 (456 vs 330 amol μg^{-1} DNA, $n = 7$, $p = 0.0469$).

Mine B—time 0

General information

Approximately one-third of the participants were smokers and 21% were ex-smokers. Four workers (5%) were classified as

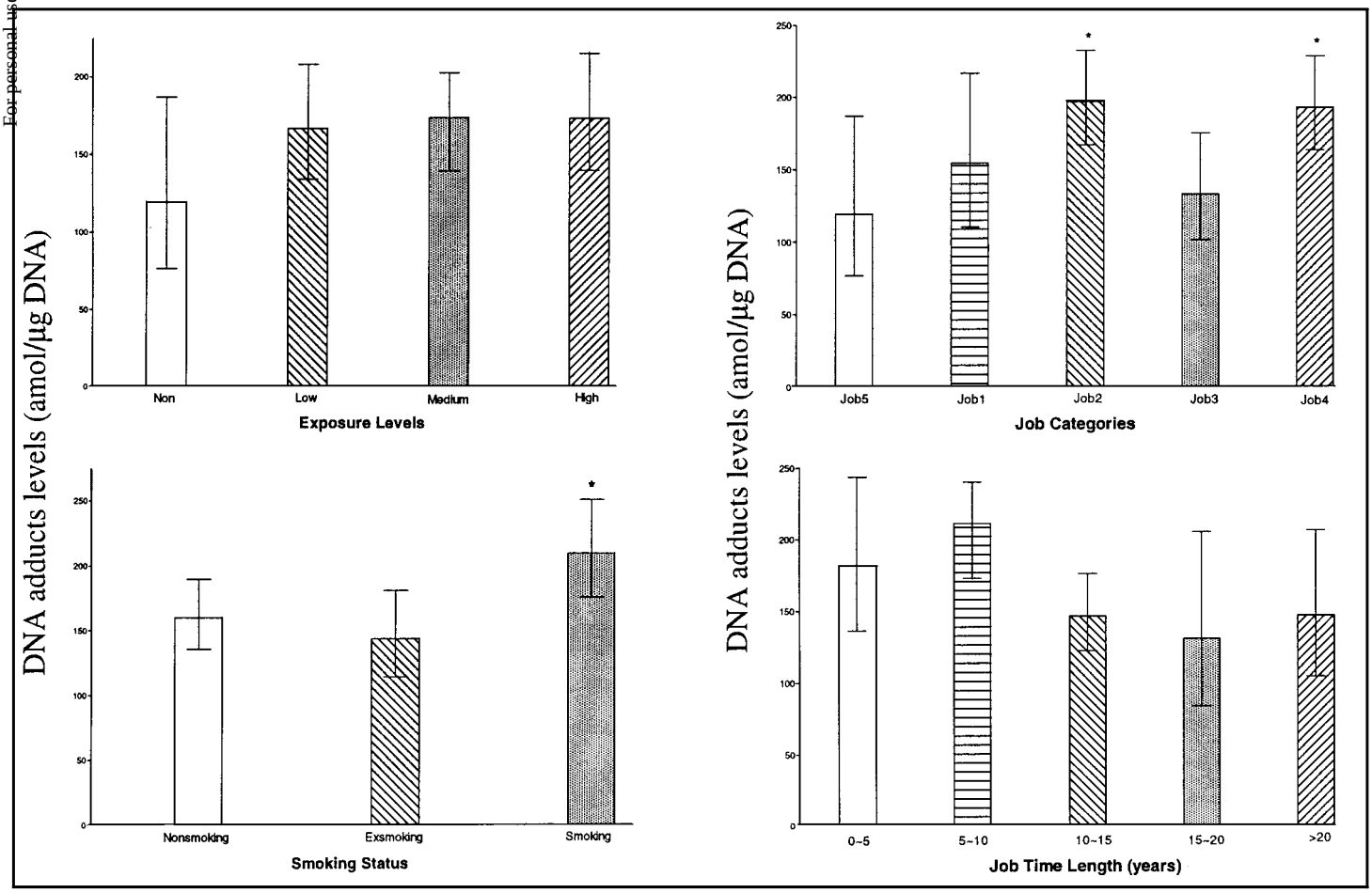

Figure 2. Total DNA adducts in PBMNs of miners from Mine A before LWCO (time 0). Each bar shows the geometric mean and 95% CI.

	Regression coefficient	SE	
Intercept	4.99	0.21	
Smoking status			$F_{2,81} = 3.1$, $p = 0.05$
Ex-smokers	–0.04	0.13	
Smokers	0.31	0.15	
Job category			$F_{4,81} = 2.0$, $p = 0.10$
Job 1	0.06	0.25	
Job 2	0.41	0.20	
Job 3	0.21	0.21	
Job 4	0.43	0.23	
Length of time in job			$F_{1,81} = 5.15$, $p = 0.03$
Time in job (years)	–0.02	0.01	

Table 2. Multiple linear regression models of $\log_e$ (total adducts) including all independent variables for Mine A at time 0.

Note: each of the *F* statistics is conditional on the other variables being in the model.

	Regression coefficient	SE	
Model 1			
Smoking status			$F_{2,72} = 1.5$, $p = 0.24$, $R^2 = 0.04$
Intercept	5.71	0.07	
Ex-smokers	–0.17	0.11	
Smokers	–0.001	0.12	
Model 2			
Exposure status			$F_{3,71} = 0.9$, $p = 0.47$, $R^2 = 0.04$
Intercept	5.42	0.20	
Low	0.34	0.22	
Medium	0.24	0.21	
High	0.20	0.22	
Model 3			
Job category			$F_{4,70} = 1.4$, $p = 0.25$, $R^2 = 0.07$
Intercept	5.42	0.19	
Job 1	0.32	0.21	
Job 2	0.12	0.22	
Job 3	0.21	0.21	
Job 4	0.36	0.22	
Model 4			
Length of time in job			$F_{1,70} = 0.02$, $p = 0.88$, $R^2 = 0.0003$
Intercept	5.63	0.09	
Time in years	0.001	0.01	

Table 3. Univariable linear regression models of $\log_e$ (total adducts) for Mine B at time 0.

'non-exposure', 22% as 'low', 48% 'medium' and 25% 'high'. Job categories 2 and 4, which would tend to have higher exposures, accounted for 23% and 20% of participants, respectively. Over 60% of participants had been in the job for at least 10 years.

Total DNA adducts

The overall geometric mean total adducts for the 75 participants at Mine B was 285.6 with 95% CI (260.90–312.80). No significant associations were found between total adducts and the explanatory variables for workers at this mine (Table 3).

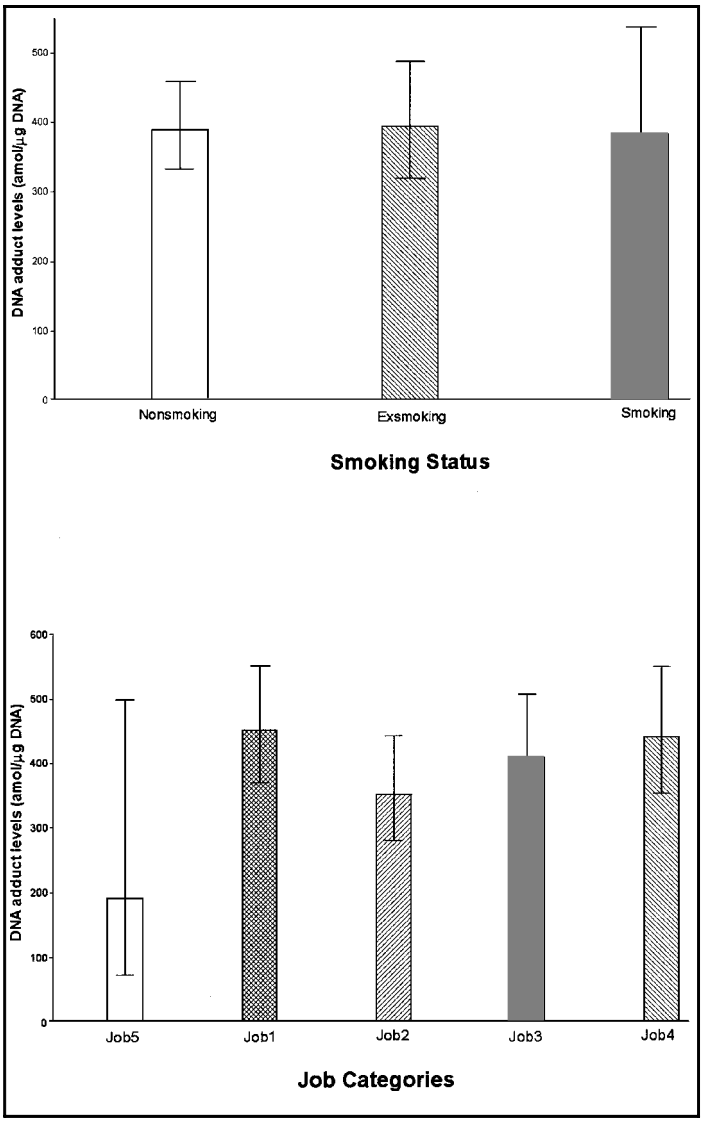

Figure 3. Total DNA adducts in PBMNs of miners from Mine B after LWCO (time 1). Each bar shows the geometric mean and 95% CI.

Mine B—time 1

General information

There were 61 subjects followed up after LWCO (time 1). No significant changes in smoking and job category distributions were found at the two time points. For these 61 subjects, the percentage of miners who were exposed to the high DEE increased from 25% (time 0) to 54% (time 1).

Total DNA adducts

The total DNA adduct data are shown in Figure 3 for time 1 where it is clear that there is an increase after the period of more intense exposure to DEE.

Mine B—Changes between time 0 and time 1

The average difference in $\log_e$ (total adducts) was 0.271 (SE 0.05), representing an overall increase of 31% with 95% CI (18–46%) (Table 4, Figures 4 and 5). Only smoking status showed an association with change in levels ($p = 0.07$) with ex-smokers showing a greater increase than non-smokers. The

Status		Number	Minimum	Maximum	Mean	SE	Median	25th percentile	75th percentile
Overall		61	–1.35	1.3	0.27	0.05	0.27	–0.01	0.5
Smoking	1	28	–0.7	0.9	0.19	0.06	0.19	0.001	0.4
	2	18	–0.04	1.3	0.46	0.10	0.39	0.15	0.67
	3	15	–1.35	0.91	0.19	0.14	0.22	–0.05	0.57
Job category	1	15	–0.24	0.91	0.32	0.09	0.32	0.14	0.48
	2	15	–0.09	1.3	0.31	0.10	0.25	–0.01	0.57
	3	15	–0.7	0.9	0.33	0.11	0.31	0.12	0.62
	4	12	–0.11	1.29	0.25	0.12	0.12	–0.04	0.39
	5	4	–1.35	0.39	–0.17	0.40	0.14	–0.69	0.35

Table 4. Changes in $\log_e$ total adducts ($\log_e$ $total_{time1}$–$\log_e$ $total_{time0}$) at Mine B after LWCO (time 1).

Key: Smoking: 1 = Non-smokers; 2 = ex-smokers; 3 = smokers. Job category: 1 = Fitter; 2 = Miner, Transport Driver, etc; 3 = Engineer and manager, etc; 4 = machineman, mechanical unit; 5 = surface man.

change for smokers and non-smokers was similar. After adjusting the job category, the association with smoking status became less marked ($p = 0.13$).

Because some workers experienced a decrease in reported exposure over time, GEEs were used to model $\log_e$ (total adducts) as a function of time of measurement, reported exposure level, smoking status, job category and time in job. The last three variables did not vary with time. After adjusting for these variables, the estimated difference was 0.278 (SE 0.07) representing an overall 32% increase with 95% CI (15%–42%). Adjusting for these variables had very little effect on the estimated effect.

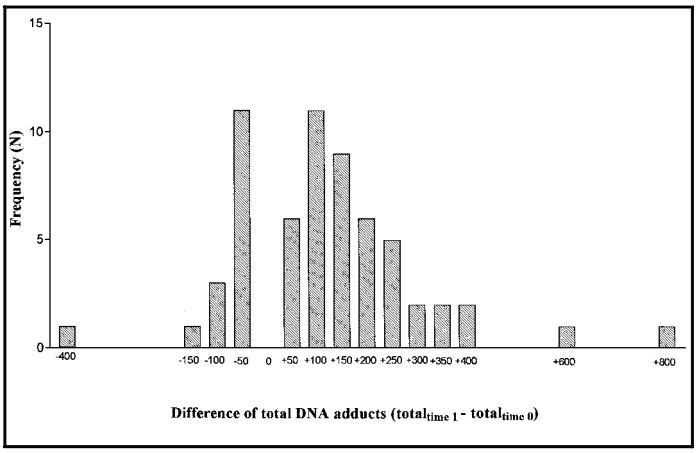

Figure 5. Frequency distribution of differences of total DNA adducts between time 1 and time 0 at Mine B.

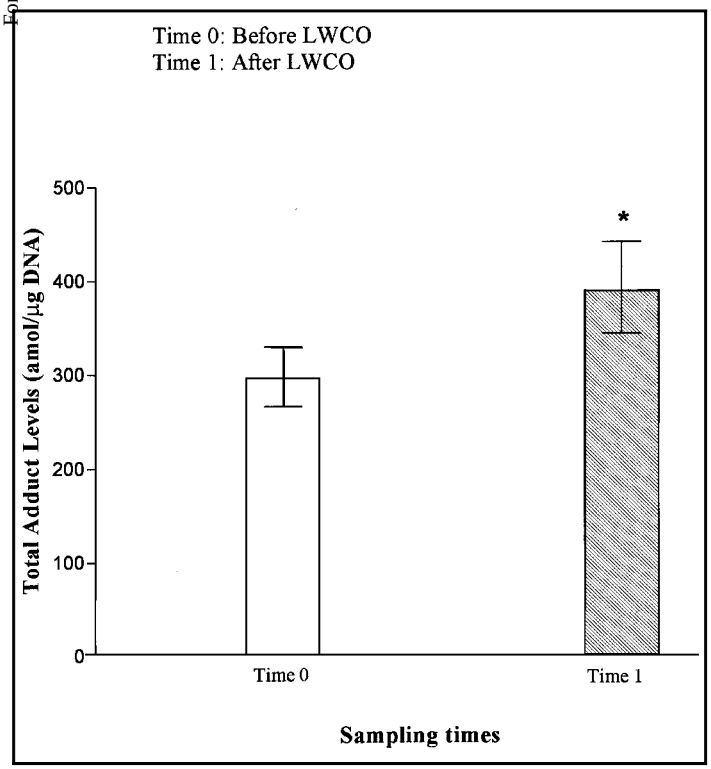

Figure 4. Differences of total DNA adducts between time 0 and time 1 from Mine B miners. Each bar shows the geometric mean and 95% CI. * Shows significant difference, paired *t*-test.

Comparison of individual DNA adducts

At Mine B the individual DNA adducts among 61 miners have been analysed for both time 0 and time 1 (Figure 6). It can be seen that after LWCO some of the individual DNA adducts have been significantly increased, especially adduct numbers 1, 4, 7, 9 and 10.

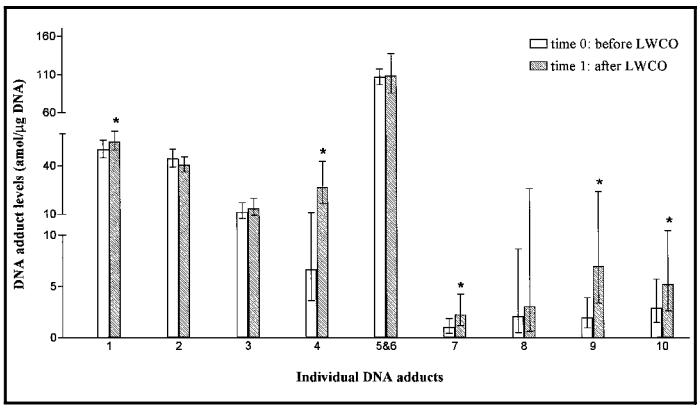

Figure 6. Differences of some individual DNA adducts between time 0 and time 1 from Mine B miners. Each bar shows the geometric mean and 95% CI. * Indicates significant difference, paired non-parametric test.

Discussion

Due to potential carcinogenic effects, DEE have been classified as a 'potential occupational carcinogen' (NIOSH 1988) or as a 'probable human carcinogen' (IARC 1989, EPA 1990). It is interesting to note that the genotoxic properties of DEE are supported by the detection of DNA adducts in lung tissues of experimental animals exposed to DEE in both short-term (Hsieh *et al.* 1986, Bond *et al.* 1990) and long-term (Wong *et al.* 1986) inhalation studies. As there is a strong relationship between DNA adduct formation and subsequent tumour formation (Hsieh *et al.* 1986, Bond *et al.* 1988), the determination of DNA adducts in peripheral white blood cells, which are readily accessible in humans, has been recommended to assist risk assessment for possible cancer in workers exposed to DEE (Gallagher *et al.* 1991, Gryzbowska *et al.* 1993). In a case-control study and retrospective cohort study by Garshick and colleagues (Garshick *et al.* 1987, 1988), a statistically significant relationship was found between diesel exhaust exposure and lung cancer, where tobacco smoking had been allowed for as a potential confounder.

At Mine B, no significant relationships were found between the exposure status, smoking status, job categories or job time at time 0. For total DNA adducts at Mine B, an increase of 31% (95% CI, 18–46%) was found after LWCO, a period of intensive use of diesel powered equipment. Furthermore, at Mine A, the level of DNA adducts in seven miners also increased significantly after LWCO although this was predominantly due to much higher levels in just two of the workers. It is worth highlighting that, in the present study, the same TLC plate was used for the two samples (before and after LWCO) for each worker. The use of the same TLC plate to analyse samples from the same worker offered the considerable advantage of removing plate to plate virtually in the assay. In our laboratory this is of significant concern because the level of variability reduces confidence in comparisons across exposure groups. It seems that other laboratories may experience similar difficulties but this is not readily apparent in the literature. Hence examination of before and after samples on the same plate provides results in which the authors have a high level of confidence. This elevation of total DNA adducts after LWCO is consistent with the greater exposures experienced during this operation and consistent with the recent publication by Hemminki *et al.* (1994).

Until now changes of individual DNA adducts have not been evaluated for human exposures to DEE. Some comparison work between the adducts in humans and animals may provide more information to help clarify the relative importance of each adduct. In the present study it has been found that the individual DNA adducts, 1, 4, 7, 9 and 10 in our system, were increased. The significance of the specific individual adducts in the miners requires further investigation.

Smoking is an important factor for lung cancer. At Mine A, smokers had higher DNA adducts than non-smokers, but no significant differences were found at Mine B at time 0. After adjustment for smoking, there was still a significant increase in adduct level after LWCO at Mine B.

It is interesting to note here that some controversial findings of cigarette smoking effects have been published within the last few years. In a human study, smoking-related DNA adducts have been detected in specimens from lung tissues (Randerath *et al.* 1989), and a linear relationship was demonstrated between lung (Phillips *et al.* 1988a) or bronchial (Phillips *et al.* 1988b) adduct levels and indices of active smoking. Mustonen *et al.* (1993) also reported that the bronchial DNA adduct levels in smokers were statistically higher than those in non-smokers. Among five smokers, for whom both bronchial and lymphocyte DNA were available, 7-methylguanine levels correlated in the two tissues ($r = 0.77$). Lymphocytes plus monocytes from smokers have elevated levels of DNA adducts compared with non-smokers. However, DNA adducts in smokers were not correlated with cigarettes per day, pack-years and plasma cotinine, indicating large interindividual variations in DNA adduct formation (Santella *et al.* 1992).

On the other hand, data concerning the effects of cigarette smoking on DNA adducts in peripheral blood cells are very conflicting. An *in vitro* study showed no significant difference between smokers and non-smokers in the formation of DNA adducts in lymphocytes following incubation with B[*a*]P (Jahnke *et al.* 1990). In human studies, most epidemiological investigations have not detected any effect of smoking on the concentrations of DNA adducts determined in blood cells from workers, nor has it been shown that passive smoking increases the levels of aromatic adducts in lymphocytes (Hemminki *et al.* 1994). Even in active smokers the level of aromatic adducts is only moderately increased (Savela and Hemminki 1991). Phillips *et al.* (1988b) also found no significant influence of smoking habits on the occurrence of DNA adducts. Furthermore, from 31 heavy smokers and 20 non-smokers Phillips *et al.* (1990) reported no difference in mean levels of aromatic hydrophobic DNA adducts in workers' white blood cells. Overall, the available evidence seems to indicate that smoking has a limited effect on DNA adducts measured in peripheral blood cells, and that the contribution of occupational exposure to PAHs probably overshadows that of smoking effects.

Regarding the DNA adduct changes in different job categories, there was no significant difference found at Mine B either before or after LWCO, though job 1 and job 4 had higher values than others at both time 0 and time 1. At Mine A, however, a higher level of DNA adducts was suggested at time 0 for the two job categories, which are basically constituted by the miners and loadmen (job 2) and the machinemen, drivers and shiftmen (job 4). This result would be consistent with a higher exposure of DEE for workers belonging to these two job categories. Furthermore, these two job categories form more than 50% of the composition of workers at both the medium and high exposure group at Mine A.

Further work to relate the source of the increased adducts to DEE exposures would provide support to the association suggested by our data. This would preferably be done using *in vitro* human tissues with appropriate metabolic activation or possibly with experimental animals, although the increased adducts may vary in comparison to h

In conclusion, there was some evidence for increased DNA adducts in smokers and in two job categories, but this was not consistent between the two mines studied. When samples after LWCO, a period of intense exposure to DEE, were compared with those taken during normal operations, an increase in DNA adducts was observed. These samples were assayed on the same TLC plate, this removing a major source of variability in the technique and thereby giving greater confidence in the data. Given the association of exposure to DEE with lung cancer and the apparent increase in adducts during a period of intense DEE exposures it is suggested that particular attention be paid to keeping exposures as low as possible, especially during LWCO operations.

Acknowledgements

This work was sponsored by the Joint Coal Board Health and Safety Trust, NSW, Australia. The authors wish to acknowledge Petra Macaskill, Epidemiology and Surveillance Unit, Worksafe Australia, for her kind assistance with the statistical analysis.

References

BEACH, A. C. AND GUPTA, R. C. (1992) Human biomonitoring and the ^{32}P-postlabelling assay. *Carcinogenesis*, **13**, 1053–1074.

BOND, J. A., WOLFF, R. K., HARKEMA, J. R., MAUDERLY, J. L., HENDERSON, R. F., GRIFFITH, W. C. AND MCCLELLAN, R. O. (1988) Distribution of DNA adducts in the respiratory tract of rats exposed to diesel exhaust. *Toxicology and Applied Pharmacology*, **96**, 336–346.

BOND, J. A., JOHNSO, N. F., SNIPES, M. B. AND MAUDERLY, J. L. (1990) DNA adduct formation in rat alveolar type II cells potentially at risk for inhaled diesel exhaust. *Environmental and Molecular Mutagenesis*, **16**, 64–69.

BOYUM, A. (1977) Separation of lymphocytes, lymphocytes subgroups and monocytes: a review. *Lymphology*, **10**, 71–76.

ENVIRONMENTAL PROTECTION AGENCY (EPA) (1990) Health Assessment Document for Diesel Emissions, Workshop Review Draft, EPA-600/8-90/057A (Office of Research and Development, Office of Health and Environmental Assessment, Washington, DC).

GALLAGHER, J. E., KOHAN, M. J., GEORGE, M. H. AND LEWTAS, J. (1991) Improvement in the diagnostic potential of ^{32}P-postlabelling analysis demonstrated by the selective formation and comparative analysis of nitrated-PAH-derived adducts arising from diesel particle extracts. *Carcinogenesis*, **12**, 1685–1691.

GARSHICK, E., SCHENKER, M. B., MUNOZ, A., SEGAL, M., SMITH, T. J., WOSKIE, S. R., HAMMOND, K. S. AND SPEIZER, F. E. (1987) A case-control study of lung cancer and diesel exhaust exposure in railroad workers. *American Review of Respiratory Diseases*, **135**, 1242–1248.

GARSHICK, E., SCHENKER, M. B., MUNOZ, A., SEGAL, M., SMITH, T. J., WOSKIE, S. R., HAMMOND, K. S. AND SPEIZER, F. E. (1988) A retrospective cohort study of lung cancer and diesel exhaust exposure in railroad workers. *American Review of Respiratory Diseases*, **137**, 820–825.

GEBSKI, V., LEUNG, O., MCNEIL, D. AND LUNG, D. (1992) *SPIDA User's Manual version 6* (Statistical Computing Laboratory, Eastwood, NSW, Australia).

GRZYBOWSKA, E., HEMMINKI, K., SZELIGA, J. AND CHORAZY, M. (1993) Seasonal variation of aromatic DNA adducts in human lymphocytes and granulocytes. *Carcinogenesis*, **14**, 2523–2526.

GUPTA, R. C. (1984) Nonrandom binding of the carcinogen *N*-hydroxy-2-acetylaminofluorene to repetitive sequences of rat liver DNA *in vivo*. *Proceedings of the National Academy of Sciences USA*, **81**, 6943–6947.

GUPTA, R. C. (1985) Enhanced sensitivity of ^{32}P-postlabeling analysis of aromatic carcinogen: DNA adducts. *Cancer Research*, **45**, 5656–5662.

GUPTA, R. C. AND RANDERATH, K. (1988) Analysis of DNA adducts by ^{32}P-postlabeling and thin layer chromatography. In *DNA Repair—A Laboratory Manual of Research Procedures*, Vol. 3, E. C. Friedberg and P. C. Hanawalt, eds (Marcel Dekker, Inc., New York and Basel) pp. 399–418.

HEMMINKI, K., SOEDERLING, J., ERICSON, P., NORBECK, H. E. AND SEGERBAECK, D. (1994) DNA adducts among personnel servicing and loading diesel vehicles. *Carcinogenesis*, **15**, 767–769.

HSIEH, L. L., WONG, D. AND HEISIG, V. (1986) Analysis of genotoxic compounds in diesel engine emissions. In *Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust*, N. Ishinishi, A. Koizumi, R. O. McClellan and W. Stoeber, eds (Elsevier Science, Amsterdam), pp. 223–234.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC) (1989) *IARC Monographs on Evaluation of Carcinogenic Risk of Chemicals to Humans*, Vol. 46 (IARC Publications, Lyon).

JAHNKE, G. A., THOMPSON, C. L., WALKER, M. P., GALLAGHER, J. E., LUCIER, G. W. AND DIAUGUSTINE, R. P. (1990) Multiple DNA adducts in lymphocytes of smokers and nonsmokers determined by ^{32}P-postlabelling analysis. *Carcinogenesis*, **11**, 205–211.

MUSTONEN, R., SCHOKET, B. AND HEMMINKI, K. (1993) Smoking-related DNA adducts: ^{32}P-postlabelling analysis of 7-methylguanine in human bronchial and lymphocyte DNA. *Carcinogenesis*, **14**, 151–154.

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (NIOSH) (1988) NIOSH Current Intelligence Bulletin 50.

NESNOW, S., ROSS, J., NELSON, G., HOLDEN, K., EREXSON, G., KLIGERMAN, A., EARLEY, K. AND GUPTA, R. (1993) Quantitative and temporal relationships between DNA adduct formation in target and surrogate tissues: implication for biomonitoring. *Environmental Health Perspectives*, **101 (Suppl.)**, 37–42.

PHILLIPS, D., HEWER, A., MARTIN, C. N., GARNER, R. C. AND KING, M. M. (1988a) Correlation of DNA adducts levels in human lung with cigarette smoking. *Nature*, **336**, 790–792.

PHILLIPS, D., HEMMINKI, K., ALHONEN, A., HEWER, A. AND GROVER, P. L. (1988b) Monitoring occupational exposure to carcinogens: detected by ^{32}P-postlabelling of aromatic DNA adducts in white blood cells from iron foundry workers. *Mutation Research*, **204**, 531–541.

PHILLIPS, D., SCHOKET, B., HEWER, A., BAILEY, E., KOSTIC, S. AND VINCZE, I. (1990) Influence of cigarette smoking on the levels of DNA adducts in human bronchial epithelium and white blood cells. *International Journal of Cancer*, **46**, 569–575.

QU, S. X. and STACEY N. H. (1996) Formation and persistence of DNA adducts in different target tissues of rats after multiple administration of benzo[a]pyrene. *Carcinogenesis*, **17**, 53–59.

RANDERATH, E., MILLER, R. H., MITTAL, D., AVITTS, T. A., DUNSFORD, H. A. AND RANDERATH, K. (1989) Covalent DNA damage in tissues of cigarette smokers as determined by ^{32}P-postlabelling assay. *Journal of the National Cancer Institute*, **81**, 341–347.

REDDY, M. V. AND RANDERATH, K. (1986) Nuclease P1-mediated enhancement of sensitivity of ^{32}P-postlabelling test for structurally diverse DNA adducts. *Carcinogenesis*, **7**, 1543–1551.

REDDY, M.V. AND RANDERATH, K. (1987) ^{32}P-postlabeling assay for carcinogen–DNA adducts: nuclease P_1-mediated enhancement of its sensitivity and applications. *Environmental Health Perspectives*, **76**, 41–47.

ROSS, J., NELSON, G., HOLDEN, K., KLIGERMAN, A., EREXSON, G., BRYANT, M., EARLEY, K., GUPTA, R. AND NESNOW, S. (1990) Formation and persistence of novel benzo(*a*)pyrene adducts in rat lung, liver, and peripheral blood lymphocyte DNA. *Cancer Research*, **50**, 5088–5094.

SANTELLA, R. M., GRINBERG-FUNES, R. A., YOUNG, T. L., DICKEY, C., SINGH, V. N., WANG, L. W. AND PERERA, F. P. (1992) Cigarette smoking related polycyclic aromatic hydrocarbon–DNA adducts in peripheral mononuclear cells. *Carcinogenesis*, **13**, 2041–2045.

SAVELA, K. AND HEMMINKI, K. (1991) DNA adducts in lymphocytes and graunlocytes of smokers and nonsmokers detected by the ^{32}P-postlabelling assay. *Carcinogenesis*, **12**, 503–508.

WONG, D., MITCHELL, C. E., WOLFF, R. K., MAUDERLY, J. L. AND JEFFREY, A. M. (1986) Identification of DNA damage as a result of exposure of rats to diesel engine exhaust. *Carcinogenesis*, **7**, 1595–1597.

ZEGER, S. AND LIANG, K. Y. (1986) Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*, **42**, 121–130.

Received 3 October 1996, revised form accepted 19 November 1996