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Molecular markers of exposure to cadmium and nickel among alkaline battery workers

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The goal of the study was to evaluate the usefulness of metallothionein mRNA, anti-5-hydroxymethyl-2'-deoxyuridine antibodies titres (anti-HMdU Ab), and 8-hydroxy-deoxyguanosine (8OHdG) in urine as markers of the biologically active dose after exposure to airborne cadmium and nickel in human studies. Exposed persons (n = 38) were chosen from workers involved in the production and assembly, chemistry, and maintenance departments of a nickel–cadmium battery factory in Poland. Controls ($n = 52$) were chosen from administration personnel at the factory. Biological samples from workers were collected twice: once in the summer, after a month of vacation, and again in the winter, after 3 months of regular working activity within the plant. Controls were recruited during the second phase of the study. When exposure groups were defined on the basis of ambient air cadmium measurements, we found a two-fold increase in mean metallothionein mRNA values in the highest exposure group (air cadmium above 1000 $\mu g\ m^{-3}$) and a positive correlation of metallothionein mRNA with blood cadmium levels ($r = 0{\cdot}46$, $p < 0{\cdot}008$). Future studies can be designed to investigate further the inter- and intra-subject component of the variability and the possibility of the existence of MT gene polymorphisms, determining different responses and susceptibilities to cadmium exposure. We did not find any difference in the mean values of anti-HMdU Ab titres and 8OHdG in urine in any of the exposure groups analysed. Nickel exposure appeared to have greater impact on anti-HMdU Ab titres than cadmium.

Keywords: carcinogenic metals, metallothionein, 5-hydroxymethyl-2'-deoxyuridine, 8-hydroxydeoxyguanosine, biomarkers.

Abbreviations: Ab, antibodies; Cd, cadmium; HIS, homologous internal standard; HMU, 5-hydroxymethyl uracil; HMdU, 5-hydroxymethyl-2'-deoxyuridine; HMdU-BSA, 5-hydroxymethyl-2'-deoxyuridine coupled to bovine serum albumin; HPLC, high performance liquid chromatography; HRPO, horseradish peroxidase; M-BSA, mock-coupled BSA; MT, metallothionein; Ni, nickel; Pb, lead; PBL, peripheral blood lymphocytes; RT-PCR, reverse transcriptase polymerase chain reaction; TLV, threshold limit values; 8OHdG, 8-hydroxydeoxyguanosine.

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Introduction

Cadmium (Cd) and nickel (Ni) are very potent metallic toxicants of persisting environmental and occupational concern. The metals and their compounds are classified by IARC as Group 1 – carcinogenic to humans, (IARC 1993). Metallothioneins (MT) are low molecular-weight proteins that form complexes with toxic metals, the syntheses of which are induced by exposure to heavy metals such as cadmium, nickel, mercury, or zinc. *In vitro* studies have shown that peripheral blood lymphocytes are capable of induction of MT gene expression in response to cadmium exposure (Enger *et al.* 1983, Harley *et al.* 1989, Cosma *et al.* 1991). A study on workers exposed to high cadmium levels suggested that MT gene induction measured by a new quantitative reverse transcription polymerase chain reaction (RT-PCR) technique is a promising biomarker of biologically effective dose in small samples of frozen tissues or cells (Ganguly *et al.* 1996).

The ability of cadmium and nickel to induce DNA damage and inhibit its repair capacity has been established (Klein *et al.* 1991, Waalkes *et al.* 1992). Derivatives of cadmium and nickel were shown to induce oxidative stress, which is known to cause oxidation of various macromolecules, including DNA (Zhong *et al.* 1990, Frenkel 1992). Production of high titres of antioxidized DNA antibodies (Ab) as a result of exposure to metals was detected in humans (Frenkel *et al.* 1992, 1993, Sasson *et al.* 1993). In those studies, the oxidized thymidine 5-hydroxymethyl-2'-deoxyuridine (HMdU) coupled to bovine serum albumin (HMdU–BSA) was utilized as an antigen. The Ab that bind to HMdU–BSA were exclusively of the IgM isotype (Frenkel *et al.* 1994). Another established oxidative stress marker is 8-hydroxydeoxyguanosine (8OHdG) (Tagesson *et al.* 1992, 1995). Damaged DNA is repaired *in vivo* by exonucleases and the resulting free water-soluble 8OHdG is excreted without further metabolism in urine (Tagesson *et al.* 1996). Thus the urinary excretion of 8OHdG may be considered to reflect the current state of oxidative DNA damage and repair (Ames 1989, Fraga *et al.* 1990).

We report the results of a cross-sectional field study addressing the issue of the interrelationship between markers of external nickel and cadmium exposure, the internal dose, and the biologically active dose of the toxicants. The main goal of the study was to evaluate the relative efficacy of MT mRNA, anti-HMdU Ab titres, and 8OHdG in urine as markers of the biologically active dose after exposure to airborne cadmium and nickel in human studies. The study has the long-term goal of identifying sub-sets of the population at higher risk of cancer because they are more sensitive to the biological effects of cadmium and nickel from environmental exposure.

Materials and methods

Study population

Study subjects were recruited among the employees of the Centra Battery Factory in Poznan, Poland. Exposed persons ($n = 38$) were chosen from workers involved in the production and assembly of nickel–cadmium batteries, as well as from the chemistry and maintenance departments. Controls ($n = 52$) were chosen from administration personnel at the factory, not known to be directly exposed to cadmium and/or nickel. After obtaining informed consent, a brief standardized questionnaire was administered to each participant to collect demographic data and self-reported height and weight, occupational history, second jobs or hobbies, and current use of medications, vitamins, mineral supplements, tobacco, and alcohol.

Sample collection

Biological samples from workers were collected twice: once in the summer, after a month of vacation, and again in the winter, after 3 months of regular working activity within the

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in the first phase of the study participated in the second one. Controls were recruited during the second phase of the study (winter) when measurements of air concentrations of cadmium, nickel and lead were conducted.

After an 8 h work-shift the study subjects were instructed to collect a small amount of midstream urine and peripheral venous blood was drawn into metal-free glass Vacutainer tubes containing EDTA as an anticoagulant. All specimens were coded and shipped to the Institute of Occupational Medicine in Lodz within 3 h of sampling and quickly frozen to –80°C. Frozen samples were subsequently shipped to New York University Medical Center for storage until assayed.

Quantitative RT-PCR

Titration and optimization of the HIS RT-PCR (homologous internal standard) assay were performed as described earlier (Ganguly *et al.* 1996). Total RNA from whole blood (1 μg) was mixed with 35 ng of Chinese hamster ovary RNA, and cDNA synthesis was initiated by addition of 1 μg of the antisense primer 5' TCAGGCGCAGCTGCACTT 3'. The reaction mixture, also containing 400 μg of all four dNTPs, 10 units AMV reverse transcriptase (United States Biochemical Corp.), and the RNase inhibitor RNAsin (United States Biochemical Corp.), was incubated for 2 h at 42°C. The sense primer 5' TGCAAATGCAAAGAGTGCAAA 3' was end-labelled by reaction with T4 polynucleotide kinase and [^{32}P]ATP and purified over Sephadex G-50 spin column. The PCR was initiated by adding 500 ng of the above end-labelled sense primer, 400 μg of dNTPs, and 2 units of Amplitaq (Perkin-Elmer Cetus) to the cDNA product. The PCR conditions were: denaturation at 94°C for 1 min, annealing at 62°C for 1 min, and extensions at 72°C for 1 min. After 30 cycles, there was a final extension at 72°C for 10 min. Because the human and hamster genes share exact homology in the primer sequences, both genes are simultaneously amplified with equivalent efficiency. The PCR product was digested with the restriction enzyme HgiA1 at 60°C for 2 h. Both human and hamster PCR products are 130 bp, but only the hamster sequence contains an HgiA1 site, allowing for cleavage of the product in half. Digested product was electrophoresed in a 7 % urea acrylamide gel. Radioactive bands at 130 (human) and 75 (hamster) bp were cut out and counted by liquid scintillation. Every experiment also included RNA from the cell line HEPG2 as a normalization standard. Quantitation of human MT message was calculated by dividing the ratio of the human to hamster band for each sample by the ratio for the HEPG2 standard.

In total, 58 samples from the workers (26 recruited in the summer and 32 in the winter phase of the study) and 36 samples from controls were analysed by quantitative RT-PCR. Sixteen samples from workers and 16 from controls were not included in the analysis because of poor RNA yield (21 samples), insufficient biological sample (5) or because the samples were lost (6) . Two of the workers refused to donate blood during the second part of the study.

Enzyme-linked immunosorbent assay

Preparation of antigens, consisting of the riboside of 5-hydroxymethyl uracil (HMU) coupled to bovine serum albumin (HMdU–BSA) and mock-coupled BSA (M-BSA), were prepared as previously described, (Frenkel *et al.* 1994). Assays were carried out in microtitre 96-well plates, which were coated with either HMdU–BSA or M-BSA (10 μg ml^{-1}). This allowed determination of specific and non-specific binding on the same plate. In addition to the sample sera and buffer (negative) control, a serum with a known high titre of anti-HMdU Ab was used as a positive control on all of the plates (at least three in HMdU–BSA- and three in M-BSA-coated wells). The presence of a positive control eliminates batch to batch variability of antigens and of the goat antihuman secondary Ab. Sera were diluted $2{\cdot}5 \times 10^3$ to 1×10^5 times and incubated in the antigen-coated wells at 37°C for 2 h. Wells were washed three times with PBS containing 0·05 % Tween-20, followed by incubation with goat antihuman IgM Ab (Sigma, St Louis, MO) labelled with horseradish peroxide (HRPO) for 1 h, and washed three times. Addition of H_2O_2 and the substrate (*o*-phenylenediamine) to the wells for HRPO-mediated oxidation during the 0·5-h incubation allows development of colour. The colour measured at 492 nm in the microplate reader (Anthos Labtec Instruments, Model 2001, Frederick, MD) is proportional to the amount of antihuman IgM Ab bound to the human serum that interacted with the antigen. Each serum was analysed four to eight times at different concentrations and the results are presented as mean values of A_{492} per μl undiluted serum. The reproducibility of this assay is 5·4 ±0·5 % (Sasson *et al.* 1993).

In total, 74 samples from the workers (38 recruited in the summer and 36 in the winter phase of the study) and 51 samples from controls were analysed by ELISA for anti-HMdU Ab. One control sample was lost.

Determination of urinary 8-hydroxydeoxyguanosine

The 8OHdG was synthesized and handled as described earlier (Simic *et al.* 1989). All chemicals were of reagent grade quality and methanol was of HPLC quality. The pH of the urine samples was adjusted to 6–7, then centrifuged, and the clear supernatant used for analysis. The samples were then further processed by a Gilson ASPEC sample processor (Gilson Medical Electroni

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USA) and analysed by coupled-column HPLC as described in detail earlier (Tagesson *et al.* 1995) The mobile phase in dimension I was phosphoric acid, 20 mmol l^{-1}, EDTA, 0·2 mmol l^{-1}, and heptanesulphonic acid sodium salt, 5 mmol l^{-1}, adjusted to pH 4·0 by the addition of sodium hydroxide, 5 mol l^{-1}. In dimension II, the mobile phase was a mixture of phosphate, 50 mmol l^{-1}, EDTA, 0·2 mmol l^{-1}, pH 7·0, and methanol 19:1 (v/v). The solutions were prepared in 5 l batches and used within 14 days. The flow was 0·75 ml min^{-1} in dimension I and 1 ml min^{-1} in dimension II. Washing solutions for the columns were prepared by addition of methanol to the solutions above to make a concentration of methanol of 50 % (v/v). At the beginning of every working session, a 1000 nmol l^{-1} standard was injected al least twice to check the retention time of the 8OHdG on the dimension I column. The valve station was programmed to inject the 2 ml fraction from column I appearing up to 1 min after the 8OHdG retention time on the dimension I column. To avoid appearance of 'ghost' peaks both columns were washed with the solutions with 50 % methanol after each run. Standard curves were linear up to at least 500 nmol l^{-1} and calibration was carried out using a 32 nmol l^{-1} standard. A quality control programme was made up of three control samples (high, medium, and low levels) analysed at the start, middle, and end of each series. These control samples were prepared by addition of 8OHdG to a 24 h urine collection from a healthy individual. Based on this quality control programme, no corrections for drift in the method was found to be necessary. Each value was adjusted by the degree of dilution of the urine.

The levels of urinary 8OHdG were measured in samples from all subjects recruited in the first and second phase of the study – 76 from the workers (38 sampled in the summer and repeated in the winter) and 52 from controls.

Analysis of cadmium, nickel and lead in ambient air

Ambient exposures to cadmium, nickel and lead were measured for 36 of the workers and in a sample of controls (9 workers). Two of the workers did not have ambient air cadmium, nickel and lead measurements because of technical reasons (not enough pumps available). For the duration of an 8 h work shift, workers from all job categories were fitted with a closed-face polystyrene battery-charged personal breathing air sampling pump (Casella BGI Inc. AFC123), set at a sampling flow of 2 l min^{-1}, and equipped with 37 mm (inlet diameter: 4 mm) millipore AA cellulose ester membrane filters (Millipore Corp., Bedford, MA). The pore size was 0·8 μm. Flow rates were checked at the beginning and end of each of the sampling periods. Total airborne dust was sampled. The sampling cassettes were replaced when visual inspection revealed that the filters were heavily loaded. Experimental filters as well as filter and control samples (spikes, blanks, and standards of the National Institute of Standards and Technology) were wet ashed, the solutions filtered, and the metal content measured by atomic absorption spectrometry. Residues contained negligible amounts of nickel, cadmium and lead as determined by X-ray fluorescence spectrometry. The lower limit of detection, calculated as three times the standard deviation of the blanks, was 2·5 μm for nickel, 2·8 μm for cadmium, and 0·3 micrograms for lead.

Analysis of cadmium in whole blood samples

Whole blood cadmium levels were measured by electrothermal atomic absorption spectrometry using Zeeman background correction. All samples were run at least two times, and the average of all runs was used as the final value. The coefficient of variation between runs was 15 % on average. A standard curve was run each day using control samples from Kaulson Laboratories (West Caldwell, NJ), or UTAK Laboratories (Valencia, CA). The cadmium concentrations (in blood or serum) of the standard controls ranged from 0·4 to 15 μg l^{-1}. The lower limit of detection was 0·5 μg l^{-1}. A sample blank and a standard reference control were re-run after every five samples to ensure machine accuracy.

Measurements of cadmium in whole blood were performed on 73 samples from the workers (38 recruited in the summer and 35 in the winter) and on 50 samples from the controls. Three blood samples (one from a worker and two from controls) were not analysed for cadmium levels because insufficient amounts of blood were obtained from these subjects. Two workers refused to donate blood during the second phase of the study.

Statistical analysis

Differences in means between groups in normally distributed data were evaluated by Student's *t*-test; the Mann–Whitney test was used to evaluate differences in medians between groups in non-normally distributed data (such as MT mRNA and 8 OHdG), and Wilcoxon signed ranks test was used for paired data. Analysis of variance with Duncan's multiple-range test was applied to investigate differences in MT mRNA levels among categories of air cadmium exposure. Chi square and Fisher's exact test were utilized to compare proportions. Linear association between variables were evaluated with correlation analysis (Spearman). The level of significance for each test was set at $p < 0{\cdot}05$.

Cadmium and nickel measurements in the air samples of four workers were compared with those performed 2 years ago on the same subjects. The intraclass correlations yielded were very strong for both metals–$R = 0{\cdot}99$ for Cd and $R = 0{\cdot}82$ for Ni (table 1A). Four samples

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Table 1A. Reliability of cadmium and nickel measurements in ambient air.

Subject number	Air Cd ($\mu g\ m^{-3}$)	Air Cd (2 years later)	Air Ni ($\mu g\ m^{-3}$)	Air Ni (2 years later)
1	9·5	0·0	31·7	0·0
2	27·2	53·9	104·6	24·9
3	881·7	748·1	35·5	44·9
4	1302·1	2174·9	310·1	204·1
ICC	$R = 0·99$		$R = 0·82$	

Key: ICC, intraclass correlation coefficient; R, estimator of ICC.

Table 1B. Reliability of MT mRNA measurements.

Subject	First measurement	Second measurement
1	0·25	0·63
2	0·36	0·41
3	5·32	5·58
4	0·69	0·46
ICC	$R = 0·99$	

ICC, intraclass correlation coefficient; R, estimator of ICC.

Table 2. Selected characteristics of the study population (mean ±sd).

Characteristic	Controls ($n = 52$)	Workers ($n = 38$)
Age	40 ±8	41 ±8
Gender – males	27 (53 %)	15 (40 %)
Quetelet Index[a]	25·6 ±4·4	25·3 ±3·9
Months of employment	125 ±116	121 ±80
Current smokers (%)	32 (63 %)	24 (63 %)
Number of cigarettes per day	15 ±7	17 ±9
Current drinkers	43 (83 %)	33 (87 %)
Beer	24 (46 %)	18 (47 %)
Wine	20 (38 %)	11 (29 %)
Hard liquor	30 (58 %)	28 (74 %)

[a] Weight (kg) divided by height (m^2).

analysis were split and assigned different codes. The intraclass correlation between MT mRNA values of the split samples was very strong – $R = 0·99$, $p < 0·0001$ (table 1B).

Results

Selected characteristics of the study population are summarized in table 2. The workers and control groups were very similar in age, Quetelet Index, number of smokers, and duration of employment. The subjects from both groups had comparable sex distribution, and daily tobacco use; exposed persons were more likely to be drinkers of hard liquor and less likely to drink wine than controls.

Measurements of ambient air and internal dose of exposure

The ambient air measurements of cadmium, nickel and lead of workers ($n = 36$) and controls ($n = 9$) are reported in table 3. The range of the air exposure, as measured by the personal monitors, was very broad, with s

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Table 3. Ambient air measurements of cadmium, nickel, and blood cadmium levels among controls and workers – winter and summer (mean ± sd, median, and range; for blood cadmium measurements geometric mean ± gsd and range, [*N*].)

Marker of exposure	Controls	Workers (winter)	Workers (summer)
Air Cd (μg m^{-3})	1·74 ± 1·97, 0·70 (0–4·8) [9]	678·2 ± 873·8, 468·7 (2·3–3562·4) [36]	—
Air Ni (μg m^{-3})	1·38 ± 2·14, 0 (0–6·3) [9]	309·5 ± 517·6, 133·3 (8·5–2811·7) [36]	—
Blood Cd (μg L^{-1})	2·8 ± 3·2, 2·5 (0–28·6) [50]	22·0 ± 1·9, 20·5 (3·4–85·2) [35]	19·5 ± 2·5, 22·0 (0·3–80·0) [38]

Key: *N*, number of subjects.

levels of cadmium being much higher (over 100-fold) than that recommended by the WHO threshold limit value (TLV) (20 μg m^{-3}). (IARC 1993). Cadmium and nickel concentrations in ambient air samples of the workers were orders of magnitude higher than corresponding values in controls. The ambient air measurements of cadmium showed positive correlations with those of nickel and lead ($r = 0{\cdot}64$, $p < 0{\cdot}0001$ and $r = 0{\cdot}54$, $p < 0{\cdot}0001$, respectively). There was a weak positive correlation between ambient air levels of nickel and lead ($r = 0{\cdot}25$, $p < 0{\cdot}09$). Mean blood cadmium levels were eight times higher in the winter measurements of the workers ($p < 0{\cdot}0001$) and seven-fold in the summer ones ($p < 0{\cdot}0001$) as compared with controls (Table 3). No statistically significant difference was observed between the mean blood cadmium levels of workers sampled in the summer and winter. We observed a strong positive correlation between the summer and winter measurements of workers' blood cadmium levels ($r = 0{\cdot}72$, $p < 0{\cdot}0001$). As shown in Figure 1, cadmium in ambient air was weakly

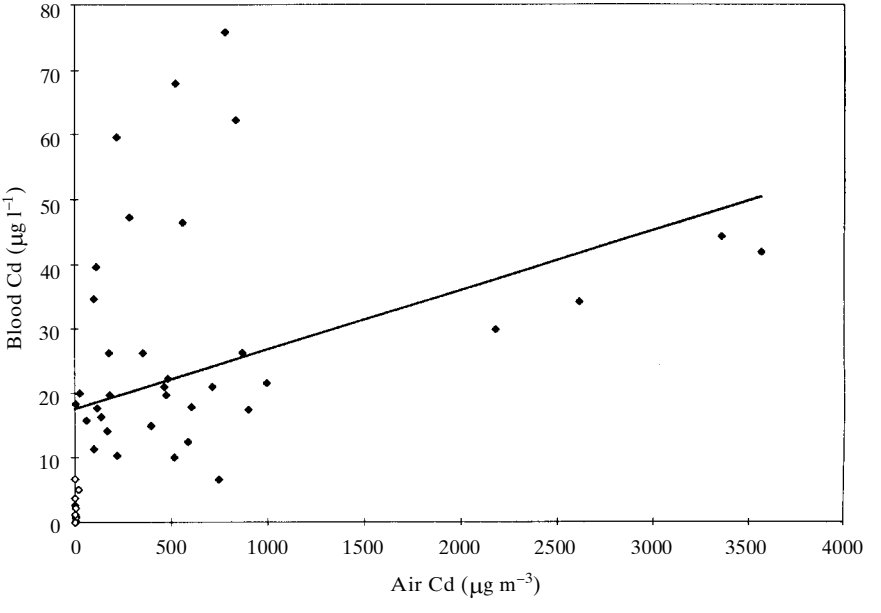

Figure 1. Spearman correlation between air and blood cadmium in the study population – among workers ($r = 0{\cdot}36$, $p < 0{\cdot}03$) and among controls ($r = -0{\cdot}43$, $p < 0{\cdot}25$).

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Table 4. Molecular markers of inner exposure among controls and workers – winter and summer (mean ±sd, median, [*N*]).

Molecular marker	Controls	Workers (winter)	Workers (summer)
mRNA (RT-PCR), (%)	1·98 ±2·35, 0·74 [36]	1·34 ±1·30, 0·68 [32]	1·77 ±2·22, 0·59 [26]
Anti HMdU Ab (A_{492} per µl)	29·0 ±22·4, 21·3 [51]	27·0 ±20.7, 18·6 [36]	28·8 ±21·2, 21·8 [38]
Urinary 8OHdG(nmol l^{-1})	28·9 ±17·6, 24·0 [52]*	22·0 ±12·3, 17·1 [38]	36·4 ±52·1, 23·4 [38]**

* $p < 0·01$ [controls vs workers using Mann–Whitney test];
** $p < 0·04$ (Wilcoxon rank test for paired data); *N*, number of subjects.

positively correlated with blood cadmium ($r = 0·36$, $p < 0·03$) in exposed workers, but not among the controls ($r = –0·43$, $p < 0·25$).

Molecular markers of exposure

In table 4 are presented the results from the three assays tested as markers of the biologically effective dose of cadmium and nickel exposure in the control and workers (winter and summer) groups.

Quantitative RT-PCR

The mean values of MT mRNA as determined by the quantitative HIS RT-PCR procedure did not show any significant differences between controls and workers or when comparing the summer and winter measurements from the workers. The frequency distribution of MT mRNA in controls and workers (summer) followed a trimodal pattern with cut off points at 2·0 and 6·0. No correlation was found between the summer and winter values of MT mRNA measurements from the workers. Among workers, MT mRNA levels were positively correlated with blood cadmium levels ($r = 0·46$, $p < 0·008$, figure 2), but not with cadmium in ambient air ($r = 0·31$, $p < 0·09$, figure 3). No association was observed between MT mRNA and nickel ambient air measurements.

To investigate further the effect of cadmium, all the subjects (exposed and controls) were classified on the basis of external exposure (air) and internal dose (blood) measurements of the metal. We observed more than a two fold elevation of MT mRNA values ($R^2 = 0·31$, $p < 0·001$) in the group with the highest air cadmium exposure (air Cd > 1000 µg m^{-3}) in comparison with the low (up to TLV) and medium exposure groups as measured by the personal monitors (3·78, 1·38, and 0·99 respectively) (figure 4). No significant differences in MT mRNA levels between exposure groups based on blood cadmium levels (up to TLV, up to 50 µg l^{-1} and above 50 µg l^{-1}) were observed.

Enzyme-linked immunosorbent assay

The values of anti-HMdU Ab titres did not show any significant difference between controls and workers or when comparing the summer and winter measurements from the workers. We observed extremely strong positive correlation between the summer and winter measurements of anti-HMdU Ab titres ($r = 0·98$, $p < 0·0001$), from the workers. From the ambient air measurements performed, neither cadmium nor nickel showed any signifi

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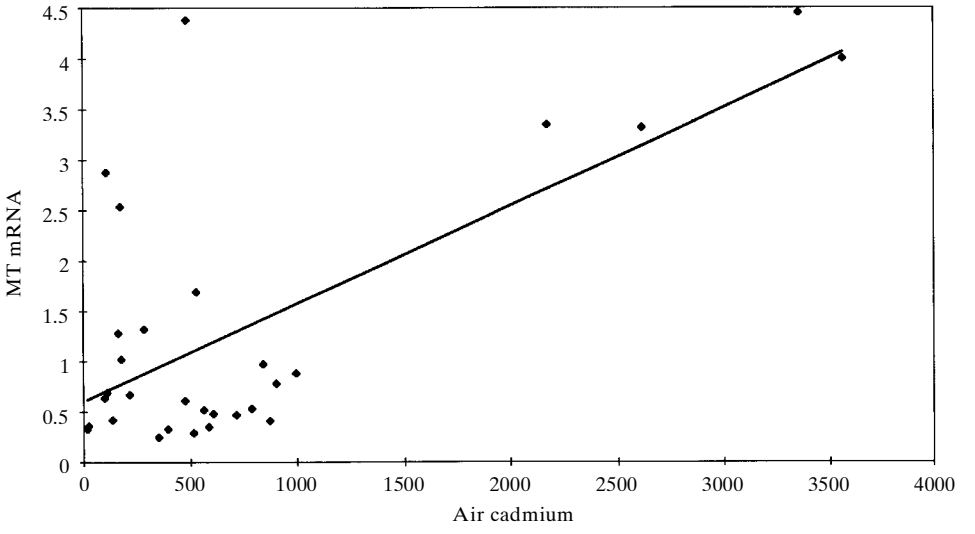

Figure 2. Spearman correlation between MT mRNA levels and blood cadmium in workers ($r = 0{\cdot}46$, $p < 0{\cdot}008$).

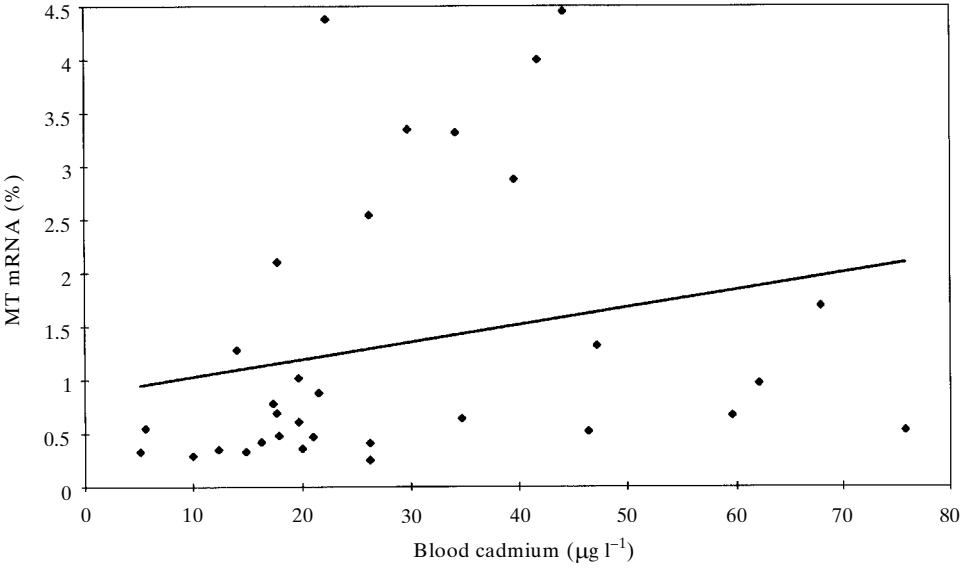

Figure 3. Spearman correlation between MT mRNA levels and air cadmium in workers ($r = 0{\cdot}31$, $p < 0{\cdot}009$).

anti-HMdU Ab titres. Blood cadmium levels from the winter samples of the workers showed no association with this oxidative stress marker. No significant differences between mean anti-HMdU Ab titres in exposure groups defined on the basis of ambient air and blood cadmium measurements were found.

Urinary 8-hydroxydeoxyguanosine

The 8OHdG excretion among workers was higher in the summer measurements ($p < 0{\cdot}04$) as compared with the winter values.

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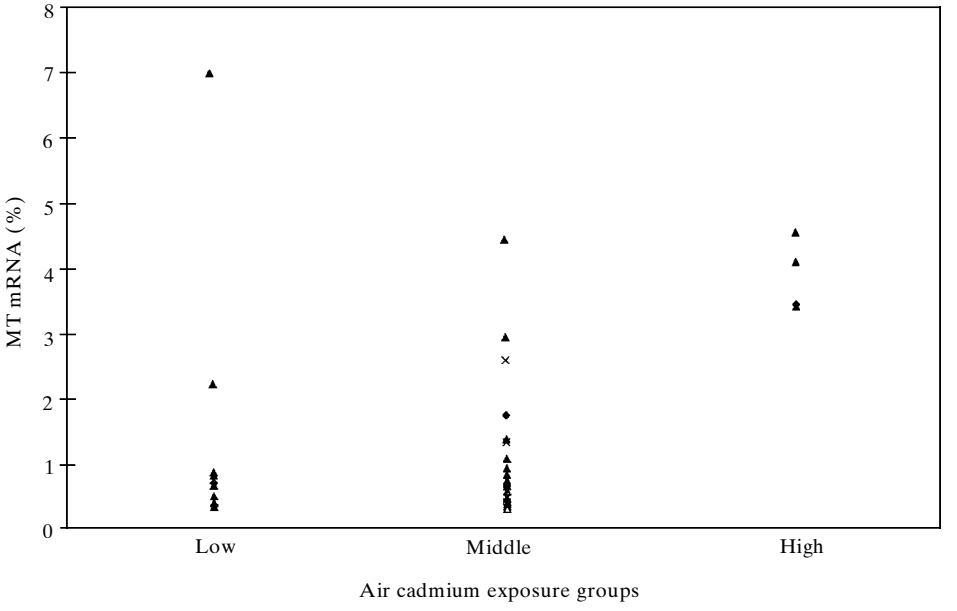

Figure 4. Distribution of MT mRNA levels according to air cadmium exposure categories: low (< 20 μg m^{-3}), middle (20–1000 μg m^{-3}), and high (above 1000 μg m^{-3}) Data analysed with analysis of variance with Duncan's multiple range test.

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urine were elevated in the control group when compared with the winter measurements of workers ($p < 0{\cdot}01$). We observed a positive correlation between the summer and winter measurements of 8OHdG levels in urine from workers ($r = 0{\cdot}39$, $p < 0{\cdot}02$). Air and blood cadmium levels from the winter samples did not show an association with the 8OHdG levels in urine. No significant differences between mean 8OHdG levels in urine in exposed groups defined on the basis of ambient air and blood cadmium measurements were found.

No correlation was found between the three markers: MT mRNA, anti-HMdU Ab titres, and 8OHdG levels in urine.

Discussion

This study reports the results of measurement of several biomarkers of biologically effective dose in a population of workers exposed to carcinogenic metals (Ni and Cd). Biomarker measurements were done both in winter, after a 3 month period of occupational exposure, and in summer, after a mandatory 1-month vacation. We observed a strong correlation between anti-HMdU Ab titres in each individual measured in the summer and winter, consistent with other studies (Frenkel *et al.*, 1998). Similarly, among the workers the urinary 8OHdG measured in the winter was correlated with the measurement taken in the summer.

If a particular biomarker is to be considered a good measure of the biological effect of a certain exposure, then changing the level of exposure should result in a corresponding change in the biomarker level, after taking into account pharmacodynamic factors such as half life or induction period. If instead the biomarker value remains stable regardless of exposure conditions, other factors, such as the individual genetic profile, may play a more significant role for that biomarker.

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Preliminary studies have shown that anti-HMdU Ab titres are markers of future development of certain cancers and are particularly high in subjects with a positive family history of cancer (Frenkel *et al.* 1998). Given this fact and the data presented here, it is therefore likely that this marker reflects more the individual genetic ability to respond to oxidative stress than the influence of heavy metal exposure.

Urinary 8OHdG did not correlate significantly with any of the other markers investigated, neither was there any difference in mean values among the exposure groups analysed. Although a number of previous investigations have suggested that 8OHdG is formed as a result of free radical reactions, it does not appear to be sensitive to the oxidative effect of cadmium and nickel in the population studied here.

In this study, the ambient air cadmium measurements in workers (a marker of external exposure) showed a positive correlation with the index of internal dose – blood levels of the metal (0·36, $p < 0·03$). These two markers reflect different aspects of exposure. The air measurements represent a snap shot and do not quantify the day-to-day exposure variation, nor the actual amount of the xenobiotic absorbed by the organism. In contrast, blood cadmium levels are influenced by the accumulated exposure at least over the previous few months, since the decline of cadmium concentrations in blood has a half-time of 3 months (Shaikh *et al.* 1990, Alessio *et al.* 1993, Lauwerys *et al.* 1994). A portion of the blood cadmium levels reflects the body burden in view of the known transport of the metal via blood from the liver to the kidneys and other tissues (WHO 1992).

Although blood cadmium levels of the workers were more than seven-fold higher than those of controls, there was no statistically significant difference between the summer (after 1 month of vacation) and winter (after 3 months of normal working activity within the plant) measurements (correlation coefficient: $r = 0·72$, $p < 0·0001$). Considering the half time of 3 months for clearance of blood cadmium, we would expect that blood cadmium levels would be reduced to approximately 80 % of winter values after a 1 month vacation.We observed in our study that the mean summer value for blood cadmium was 86 % of the winter mean value.

We found a significant correlation between blood cadmium levels and MT mRNA in the winter measurements of the workers ($r = 0·46$, $p < 0·008$), but not among the summer measurements. This is consistent with the idea that MT mRNA levels (as opposed to blood cadmium) are sensitive to a 1 month cessation of exposure (summer). After 3 months of exposure (winter) the steady state MT mRNA reflects and correlates both with the ongoing exposure and blood cadmium.

When all the subjects (exposed and controls) were reclassified on the basis of ambient air cadmium measurements rather than on job classification, we found a two fold increase in mean MT mRNA values in the highest exposure group (air cadmium above 1000 $\mu g\ m^{-3}$) as compared with the medium and low exposure groups. No differences in MT mRNA levels between exposure groups based on blood cadmium levels were observed. This observation suggests that MT mRNA is sensitive to daily changes of air cadmium exposure, independently from the classical job exposure classification. Previous studies conducted in the same plant have yielded similar results (Ganguly *et al.* 1996).

The mean levels of anti-HMdU Ab titres did not differ when exposure groups were formed on the basis of cadmium exposure. An in

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oxidative stress following induced MT synthesis by metals has been observed *in vitro* (Klaassen and Lehman-McKeeman 1989). There was a weak correlation between the ambient air measurements of nickel and anti-HMdU Ab titres ($r = 0{\cdot}32$, $p < 0{\cdot}06$). Nickel exposure appeared to have greater impact on anti-HMdU Ab titres than cadmium, and may be a more potent inducer of oxidative stress. Previous investigations in the same plant have also yielded stronger correlations between anti-HMdU Ab titres and air nickel measurements as compared with air cadmium (Frenkel *et al*. 1994).

Among the limitations of our study, we cannot rule out that the control group, constituted of employees of the cadmium–nickel battery factory, may have experienced some secondary cadmium exposure (Lauwerys *et al*. 1976). In addition, the workers were exposed to several metals, including lead. Little is known about the complex mechanisms of interaction between these metals *in vivo*. It is not uncommon to find saturation of molecular markers at high occupational exposure because of toxicity and changes in basic cell activities such as in the anion transport system (Hulka and Margolin 1992, Waalkes *et al*. 1992).

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References

ALESSIO, L., APOSTOLI, P., FORNI, A. and TOFFOLETTO, F. 1993, Biological monitoring of cadmium exposure – an Italian experience. *Scandinavian Journal of Work Environment and Health*, **19**, (suppl. 1), 27–33.

AMES, B.N. 1989, Endogenous oxidative DNA-damage, aging, and cancer. *Free Radical Research Communication*, **7**, 121–128.

COSMA, G.N., CURRIE, D., SQUIBB, K.S., SNYDER, C.A. and GARTE, S.J. (1991), Detection of cadmium exposure in rats by induction of lymphocyte metallothionein gene expression. *Journal of Toxicology and Environmental Health*, **34**, 39–49.

ENGER, M. D., HILDEBRAND, C. E. and STEWARD, C.C. (1983), Cd^{2+} responses of cultured human blood cells. *Toxicology and Applied Pharmacology*, **69**, 214–224.

FRAGA, C. G., SHIGENAGA, M.K., PARK, J.W. DEGAN, P. and MES, B. N. (1990), Oxidative damage to DNA during aging: 8-hydroxydeoxy-2'deoxyguanosine in rat organ DNA and urine. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 4533–4537.

FRENKEL, K. (1992), Carcinogen-mediated oxidant formation and oxidative DNA damage. *Pharmacology and Therapeutics*, **53** (1), 127–166.

FRENKEL, K., KHASAK, D., KARKOSZKA, J., SCHUPACK, J. and STILLER, M. (1992), Enhanced antibody titers to an oxidized DNA base in inflammatory and neoplastic diseases. *Experimental Dermatology*, **1**, 242–247.

FRENKEL, K., KARKOSZKA, J., KIM, E. and TAIOLI, E. (1993) Recognition of oxidized DNA bases by sera of patients with inflammatory diseases. *Free Radical Biology and Medicine*, **14**, 483–494.

FRENKEL, K., KARKOSZKA, J., COHEN, B., BARANSKI, B., JAKUBOWSKI, M., COSMA, G. and TAIOLI, E., TONIOLO, P. (1994), Occupational exposure to Cd, Ni, and Cr modulate titers of antioxidized DNA base antibodies. *Environmental Health Perspectives*, **102**, (suppl. 3), 221–225.

FRENKEL, K., KARKOSZKA, J., GLASSMAN, T., DUBIN, N., TONIOLO, P., TAIOLI, E., HOONEY, L. and KATO, I. (1998). Serum auto-antibodies recognizing 5-hydroxymethyl-2′-deoxyuridine, an oxidized DNA base, as biomarkers of cancer risk in women. *Cancer Epidemiology, Biomarkers and Prevention*, **7**, 49–57.

GANGULY, S., TAIOLI, E., BARANSKI, B., COHEN, B., TONIOLO, P. and G

RIGHTSLINK

metallothionein gene expression determined by quantitative reverse transcription-polymerase chain reaction as a biomarker of cadmium exposure. *Cancer Epidemiology, Biomarkers and Prevention*, **5**, 297–301.

HAMER, D. (1986), Metallothionein. *Annual Review of Biochemistry*, **55**, 913–951.

HARLEY, C. B., MENON, C.R., RACHUBINSKI, R.A. and NIEBOER, E. (1989), Metallothionein mRNA and protein induction by cadmium in peripheral-blood leukocytes. *Biochemical Journal*, **262**, 873–879.

HULKA, B. S. and Margolin, B.H. (1992), Methodological issues in epidemiologic studies using biologic markers. *American Journal of Epidemiology*, **135**, 200–209.

IARC (1993), Beryllium, cadmium, mercury and exposures in the glass manufacturing industry. IARC vol. 58 *Monographs* (Lyon: IARC), pp. 143, 210.

KLAASSEN, C. D. and LEHMAN-MCKEEMAN, L. D. (1989), Induction of metallothionein. *Journal of the American College of Toxicology*, **8**, 7.

KLEIN, C. B., FRENKEL, K. and COSTA, M. (1991), The role of oxidative processes in metal carcinogenesis. *Chemical Research in Toxicology*, **4**, 592–604.

LAUWERYS, R. R., BUCHET, J. P. and ROELS, H.A. (1976), The relationship between cadmium exposure or body burden and the concentration of cadmium in blood and urine in man. *International Archives of Occupational and Environmental Health*, **36** (4), 275–285.

LAUWERYS, R. R., BERNARD, A.M., ROELS, H.A. and BUCHET, J. P. (1994), Cadmium: Exposure markers as predictors of nephrotoxic Effects. *Clinical Chemistry*, **40** (7), 1391–1394.

SASSON, M., STILLER, M.J., SHUPACK, J.K., KHASAK, D., KARKOSZKA, J. and FRENKEL, K. (1993) Antibody titers to an oxidized thymidine moiety are altered by systemic pharmacotherapy and by ultraviolet B phototherapy. *Archives of Dermatological Research*, **285**, 227–229.

SHAIKH, Z. A., ELLIS, K. L., SUBRAMANIAN, K. S. and GREENBERG, A. (1990), Biological monitoring for occupational cadmium exposure: the urinary metallothionein. *Toxicology*, **63**, 53–62.

SIMIC, M. G., BERGTOLD, D. S. and KARAM, L.R. (1989), Generation of oxy radicals in biosystems. *Mutation Research*, **214**, 3–12.

TAGESSON, C., KALLBERG, M. and LEANDERSON, P. (1992), Determination of urinary 8-hydroxy-deoxyguanosine by coupled-column HPLC with electrochemical detection: a noninvasive assay for *in vivo* oxidative DNA damage in humans. *Toxicology Methods*, **1**, (4), 242–251.

TAGESSON, C., KALLBERG, M., KLINTENBERG, C. and STARKHAMMAR, H. (1995), Determination of urinary 8-hydroxydeoxyguanosine by automated coupled-column HPLC: a powerful technique for assaying *in vivo* oxidative DNA damage in cancer patients. *European Journal of Cancer*, **31A**, (6), 934–940.

TAGESSON, C., KALLBERG, M. and WINGREN, G. (1996), Urinary malondialdehyde and 8-hydroxy-deoxyguanosine as potential markers of oxidative stress in industrial art glass workers. *International Archives of Occupational and Environmental Health*, **69**, 5–13.

WAALKES, M.P., Coogan, T.P. and Barter, R.A. (1992), Toxicological principles of metal carcinogenesis with special emphasis on cadmium. *Critical Reviews in Toxicology*, **22** (3, 4), 175–201.

WHO 1992, cadmium. *Environmental Health Criteria*, No. 134, (Geneva: WHO), p. 90.

ZHONG, Z., TROLL, W., KOENIG, Kl., and FRENKEL, K. (1990), Carcinogenic sulfide salts of nickel and cadmium induce H_2O_2 formation by human polymorphonuclear leukocytes. *Cancer Research*, **50**, 7564–7570.

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