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Assessment of potential damage to DNA in urine of coke oven workers: an assay of unscheduled DNA synthesis

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Abstract

***Objectives*—A study was conducted in coke oven workers to evaluate the biological consequences of the exposure of these workers, particularly production of potential genotoxic factors.**

***Methods*—60 coke oven workers and 40 controls were recruited in the same iron and steel works. Exposure to polycyclic aromatic hydrocarbons (PAHs) was assessed by job and measurement of 1-hydroxypyrene (1OHP) in urine samples. An unscheduled DNA synthesis assay was performed on rat pleural mesothelial cells used as a test system to evaluate the effect of the workers' filtered urine on the DNA repair capacity of rat cells to determine whether DNA damaging agents are present in the urine of these workers.**

***Results*—Urinary concentrations of 1OHP ranged from 0.06 to 24.2 (mean (SD) 2.1 (3.6)) μmol/mol creatinine in exposed coke oven workers, and from 0.01 to 0.9 in controls (0.12 (0.15)). These high concentrations in coke oven workers reflected recent exposure to PAHs and were in agreement with the assessment of exposure by job. No significant difference was found between coke oven workers and controls in the DNA repair level of rat cells treated with urine samples. However, the rat cell repair capacity decreased with increasing 1OHP concentrations in the exposed population (r= -0.28, P< 0.05).**

***Conclusions*—As high concentrations of 1OHP were found in the urine of some workers, a more stringent control of exposures to PAHs in the workplace is required. Exposure to PAHs was not associated with a clear cut modification of the urinary excretion of DNA damaging factors in this test, as shown by the absence of increased unscheduled DNA synthesis in rat cells. However, impairment of some repair mechanisms by urinary constituents is suspected.**

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Coke production entails exposure of workers to high environmental concentrations of airborne pollutants including polycyclic aromatic hydrocarbons (PAHs). Both epidemiological and experimental evidence indicate that PAHs are carcinogenic in animals and possibly also in humans. Several PAHs—for example, dibenz(a,h)anthracene, benzo(a)pyrene—have been shown to induce tumours in numerous test species.[1] Exposure to PAHs has long been associated with an excess of various cancers in humans, mainly skin cancers and scrotal cancers, particularly among chimney sweeps.[1 2] An increased risk of lung cancer has been reported among workers involved in various industries such as chimney sweeping, aluminium refining, coal gasification, coke production, and in iron and steel foundries.[3 4] These compounds are produced during incomplete combustion of natural or synthetic organic materials; they are lipid soluble and can be absorbed into the body through all of the usual routes. Skin and lung routes are predominant in occupational exposure.[5 6] The level of exposure to PAHs in the workplace may be evaluated by atmospheric measurement of various PAHs or by measurement of PAH metabolites in urine.[7 8] Thus, the hydroxylated metabolite of pyrene in urine (1-hydroxypyrene, 1OHP) is considered to be a good biological indicator of exposure to PAHs which has the advantage of taking into account the various routes of penetration into the body.

Evaluation of the early consequences of human exposure to carcinogens in the workplace is an important issue. This has led to the development of various assays designed to identify early biological responses.[9] Recently, many studies have tried to evaluate the biologically effective dose by measuring various markers as surrogates for the target tissue dose—namely, urinary d-glucaric acid as an index of hepatic enzyme activity,[10 11] measurement of sister chromatid exchanges in peripheral lymphocytes,[11–14] and binding of PAH electrophilic metabolites to DNA in nucleated blood cells or to blood proteins.[13 15–20] Most of these studies were performed in coke oven workers,[10 12–14 18 19] graphite electrode plant workers,[10 12 14] workers exposed to PAHs from bitumen fumes,[11] and primary aluminium plant and foundry workers.[15 20] These studies showed contradictory results. Some of them did not show any difference between biological data obtained in subjects exposed to PAHs and in controls —for example, d-glucaric acid,[10 11] sister chromatid exchanges in peripheral lymphocytes,[13] antibody to benzo(a)pyrene

DNA adducts,[19] and benzo(a)pyrene-albumin adducts.[15] Other studies reported a significant difference between exposed subjects and controls for some markers—for example, sister chromatid exchanges in peripheral lymphocytes,[11 12 14] benzo(a)pyrene-diol-epoxide (BPDE) adducts to haemoglobin,[12] PAH-DNA adducts,[13 17 20] and anti-BPDE((±)-*r*-7,*t*-8-dihydroxy-*t*-9, 10-oxy-7,8,9,10-tetrahydrobenzo(a) pyrene)-DNA adducts.[18] A few authors used urinary mutagenicity—for example, the Ames test, modified nucleosides—to investigate whether body fluids contain factors with a genotoxic potential as a result of occupational exposure.[10 12 21] In these studies, the urinary mutagenic activity was more likely dependent on smoking habits than on occupational exposure. Nevertheless, a significant effect of occupational exposure was also shown with such assays when tobacco smoking and diet were taken into account.

In the present work, we performed an unscheduled DNA synthesis assay on rat pleural mesothelial cells used as a test system, to evaluate the DNA damaging potency of urine of coke oven workers. Rat pleural mesothelial cells have been shown to be able to metabolise PAH, indicating *P*-450 metabolic activity of these cells.[22-24] The unscheduled DNA synthesis assay has been previously performed with the same cell type in chromium workers and coal miners.[25 26] In this assay, growth arrested rat cells showed a basal repair rate corresponding to the repair of spontaneously occurring lesions. An enhancement of DNA repair indicates the presence of genotoxic agents producing DNA damage. This repair can be detected by an increase in the amount of radiolabelled nucleoside incorporated into the DNA. By contrast, an impairment of repair mechanisms is associated with a decrease in [^{3}H] methyl thymidine ([^{3}H]dThd) incorporation into DNA.

Materials and methods

SUBJECTS

Urine samples were collected from 60 male coke oven workers (group 1) and 40 male controls recruited in the same steel foundry in Basse Normandie, France. Each subject was asked to fill in a questionnaire providing details on tobacco smoking, alcohol habits, drug consumption, medical history, and work conditions. For 40 of the 60 coke oven workers, a second urine sample was collected three months later, after the end of exposure once the factory was closed. These subjects are included in group 2. Two sets of data were obtained in this group; one provided by urine samples collected during the working period (group 2a), the other one supplied by samples collected after the end of exposure (group 2b). No subject had any known active disease at the time of the study. Subjects receiving chemotherapy, antibiotics, or known metabolic inducers were excluded. Blue collar controls with the same mean age (± 2 years) were selected on the basis of the absence of notable previous occupational exposure to carcinogens, as assessed by the occupational questionnaire. They had similar smoking habits to those of subjects of group 2.

URINE SAMPLES

Urine samples were collected in polyethylene flasks at the end of the workshift, after at least five consecutive days of work for group 1 subjects and during routine occupational medical survey for group 2b subjects and controls. The samples were frozen at - 20°C and kept in the dark until analysis.

EVALUATION OF EXPOSURE TO PAHS

Two methods were used: an assessment of exposure by job by the occupational physician; and measurement of urinary 1-hydroxypyrene (1OHP).

To assess PAH exposure by job, the occupational physician used a standardised questionnaire. Each worker was asked to list his successive jobs throughout his working life, with specific attention being paid to the tasks performed during the week preceding urine collection. This allowed the level of exposure to PAHs to be evaluated, based on concentrations of atmospheric PAHs registered between 1986 and 1990. Atmospheric measurements of PAHs were performed by gas chromatography after collection of individual dynamic samples (during a half shift of four hours) and static samples. This study showed that subjects working on top or near the door of the oven (atmospheric benzo(a)pyrene concentration generally >10 μg/m^3) presented a high level of exposure (class 1), subjects working on the side of the coke oven (atmospheric benzo(a)pyrene concentration generally 1–10 μg/m^3) were exposed to intermediate levels (class 2) and subjects with no direct contact with the oven area (atmospheric benzo(a)pyrene level <1 μg/m^3) were considered to be exposed to low levels (class 3). Two exposure indices were established for each subject: the "last day exposure", corresponding to the class of exposure of the last workshift preceding urine collection; the "week index", obtained by adding the individual scores of all workshifts of the week for a given subject (individual scores of class 1, class 2, and class 3 were 3, 2, and 1, respectively; the week index for a given coke oven worker therefore ranged from 5–15).

Urinary 1OHP was determined by an adaptation of the method of Jongeneelen *et al.*[7] The technique included enzymatic hydrolysis of the conjugates with a mixture of glucuronidase and sulphatase followed by chromatography on octadecyl silica cartridges (Bond-Elut 500 mg, Analytichem, Harbor City, CA, USA) performed with a Vac Elut SPS 24 from Varian previously activated with methanol and distilled water. Samples were then extracted with methanol, dried with an evaporator, and then resuspended in 200 μl acetonitrile. A second chromatography was performed on a Nucleosil 10 C18 column with an isocratic elution (distilled water: acetonitrile; 60:40 v/v) at a flow rate of 1 ml/min. The high performance liquid chromatography system was 9000 Varian including two 9010 Varian pumps. The retention time of 1OHP was found to be six

minutes. 1-Hydroxypyrene concentrations were measured by fluorimetry (excitation 240 nm, emission 390 nm, 821-FP JASCO spectrofluorometer). The procedure was regularly calibrated with aliquots of 0.1 μg/l to 20.0 μg/l of 1OHP in the elution medium (Aldrich, Paris). An external standard was regularly chromatographed to assess the validity of the procedure. The detection limit was 0.1 μg/l and the results were standardised to urinary creatinine.

UNSCHEDULED DNA SYNTHESIS ASSAY

Rat pleural mesothelial cells were cultured in 24 well cluster culture dishes (Costar).[24] Eighty thousand cells were seeded per well in complete medium. The cells grew as a monolayer and reached confluence after four days of incubation; the medium was then replaced with Roswell Park Memorial Institute (RPMI) medium containing 1% fetal calf cells (5 mM , hydroxy urea (HU) 100 U/ml penicillin, and 50 μg/ml streptomycin. The cells were incubated for 24 hours at 37°C in a humidified atmosphere of 5% CO_2 in air then incubated for 24 hours in RPMI medium containing 1% fetal calf cells (5 mM HU) and 4 μCi/ml [^{3}H]dThd (specific activity: 50 to 60 Ci/mmol), with or without urine. A laboratory reference (an RPMI medium containing 500 μg/ml crocidolite fibres) was used to check the response of rat cells. Crocidolite fibres produce a considerable enhancement of DNA repair in rat cells due to DNA damage.[24] The urine samples were previously filtered through Amicon membrane filters YM 10 according to Emerit *et al*[27] and the volume added to each well was adjusted to initial urinary creatinine (maximum volume 20 μl). The cells were then washed three times with phosphate buffered saline. Acid soluble material was removed by rinsing the cell layer with 10% cold trichloroacetic acid for 10 minutes, followed by incubation in a mixture of 0.2 M NaOH and 1% sodium dodecyl sulphate. Aliquots (200 μl) were placed in vials and scintillation fluid (Ultima Gold MV, Packard) was added. Radioactivity was determined on a Beckman LS 6000SC apparatus. The DNA cell content was assessed by fluorometric assay.[28] Fluorescence was measured with a Millipore Cytofluor 2350. The cell repair capacity was evaluated from the ratio of [^{3}H]dThd incorporation (dpm)/μg DNA in each well. Results of the unscheduled DNA synthesis assay were expressed as the ratio of the repair capacity of rat cells treated with urine to that of untreated cells in RPMI medium.

MTT ASSAY

The purpose of this test was to control the viability of rat cells after treatment with urine samples. The 3-[4,5-dimethylthiazol-2-YL] -2,5-diphenyltetrazolium bromide (MTT) is reduced into formazan by mitochondrial dehydrogenases. This reduction can only occur in active cells. The quantity of formazan is directly proportional to the number of viable cells.[29] Rat pleural mesothelial cells were cultured in 96 well cluster culture dishes (Costar) in complete medium. After confluence, the cells were treated with the filtered urine samples, or crocidolite, or cultured in RPMI medium, as in the unscheduled DNA synthesis assay. Five wells were used for each treatment. The cells were incubated for 24 hours under the same conditions as for the unscheduled DNA synthesis assay. To each well MTT solution (40 μl, 2 mg/ml) was then added. After three hours of incubation (37°C in a humidified atmosphere, 5% CO_2), the supernatant was carefully removed and the formazan crystals were dissolved by addition of 200 μl dimethyl sulphoxide. The cluster culture dishes were agitated for two minutes with a multidish agitator and the optical density was measured by spectrophotometry at 540 nm. The mean optical density of the five replicate wells was calculated for each treatment. Results were expressed as the ratio of the mean obtained for each group to the mean obtained for samples in the same dish in RPMI medium (optical density in urine treated rat cells/ optical density in untreated rat cells).

DETERMINATION OF COTININE IN URINE SAMPLES

Cotinine is a metabolite of nicotine, which can be used to evaluate the last two days of individual nicotine intake in smokers, taking into account various parameters, such as tobacco strength, duration of inhalation, filtered or unfiltered cigarettes. Cotinine was evaluated by an automated colorimetric method on COBAS-BIO with 1% barbituric acid derived from the manual method of Peach et al.[30] Results were expressed in relation to urinary creatinine.

STATISTICAL ANALYSIS OF RESULTS

The relation between PAH exposure and cell repair capacity was examined by two methods: comparison of the [^{3}H]dThd incorporation / DNA ratio between exposure groups; and correlation of this ratio with urinary 1OHP.

Three types of comparisons were carried out between exposure groups: coke oven workers during exposure versus controls (group 1 *v* controls); coke oven workers after the end of exposure versus controls (group 2b *v* controls); coke oven workers during exposure versus coke oven workers after cessation of exposure (groups 2a *v* 2b). Coke oven workers and controls were compared by χ^2 test and Student's *t* test for univariate analysis. A paired *t* test was used to examine the variables of coke oven workers before and after the end of exposure.

Similar comparisons were carried out for the MTT test.

Relations between urinary 1OHP and other biological variables were examined by Pearson's correlation coefficients and adjustment for tobacco smoking was made with urinary cotinine by multiple linear regression.

All statistical analyses were performed with the SAS package.[31]

Results

Table 1 shows the characteristics of the subjects included in the study (age, smoking habits) and the urinary cotinine and creatinine of these subjects. Coke oven workers and con-

Table 1 Sociodemographic characteristics of the study population and biological indices of tobacco smoking

Variables	Coke oven workers			Controls
	Group 1 (n=60)	Group 2a (n=40)	Group 2b (n=40)	(n=40)
Age (y):				
Mean (SD)	40.9 (8.6)	43.2 (6.3)		41.7 (8.8)
Median (range)	43 (19–51)	44.5 (28–51)		44.5 (21–51)
Smoking habits:				
Current smokers (n (%))	30 (50)	16 (40)		18 (45)
Former smokers (n (%))	16 (26.7)	13 (32.5)		11 (27.5)
Non-smokers (n (%))	14 (23.3)	11 (27.5)		11 (27.5)
Urinary creatinine (mmol/l):				
Mean (SD)	14.1 (6.7)	13.8 (6.7)	16.5(8.9)	11.7 (0.6)
Median(range)	13.9 (1.2–32.4)	13.5 (3.5–32.4)	15.9(5.8–45.1)	11.3 (2.7–24.7)
Urinary cotinine (mmol/mol creatinine):				
Mean (SD)	2.7 (3.1)	2.0 (2.5)	2.0(2.6)	3.2 (4.3)
Median (range)	1.1 (0–10.9)	0.5 (0–8.8)	0.5(0–8.6)	0.9 (0–18.3)

trols were similar for age, smoking, and alcohol habits. Twenty per cent of subjects consumed drugs. No significant difference was found between coke oven workers and controls for urinary variables, except in group 2b, in which urinary creatinine was higher than in controls.

The urinary cotinine concentration was considered to reflect current smoking consumption, whereas 1OHP was considered to be representative of exposure to PAHs. Coke oven workers had higher urinary 1OHP concentrations than controls, even after adjustment for urinary cotinine (fig 1). After three months without occupational exposure, the urinary 1OHP concentrations were similar in coke oven workers and in controls. There was a good agreement between urinary 1OHP values and PAH exposure levels as defined by the assessment of exposure by job. Coke oven workers with high exposure (class 1) during the last shift before urine collection had higher 1OHP concentrations than less exposed subjects (class 2 and class 3 subjects), even after taking urinary cotinine concentration into account (median values, mean (SD) 3.7 (5.6 (6.5)), 1.3 (1.7 (1.2)) and 0.6 (1 (0.8)) μmol 1OHP/mol creatinine for class 1, class 2, and class 3 subjects, respectively; P<0.001). The urinary 1OHP value was strongly correlated with the week index (r=0.52; P=0.001), even when smoking habits were taken into account.

Table 2 shows the results of DNA repair capacity and viability (MTT test) of rat cells treated with urinary filtrates. No significant difference was found for dpm/DNA values between group 1 workers and controls, or between coke oven workers before (group 2a) and after the end of exposure (group 2b) regardless of smoking status (fig 2). Similar results were found when analysis was restricted to comparison between subjects with the highest level of exposure and controls. This lack of significant change was not due to a dysfunction of rat cells used as a test system, as [^{3}H]dThd incorporation was higher in the cells treated with crocidolite (mean (SD) = 8420 (751) dpm/μg) than in the untreated cells (mean (SD) = 7687 (1079) dpm/μg) (P<0.001), as expected.

The viability of rat cells treated with urine samples from coke oven workers was slightly altered (although not significantly; P=0.08) in comparison with urine from controls. This phenomenon was found both for samples collected during working activity and after three months without occupational exposure. Viability of rat cells treated with crocidolite was decreased to 89.9% compared with untreated rat cells (P<0.001).

The correlation between DNA repair capacity of rat cells and individual biological variables showed a reduction of incorporation of [^{3}H]dThd which depended on the urinary 1OHP concentration in G1 workers (r= - 0.28, P= 0.05). This reduction cannot be attributed to a loss of cell viability, as the viability of rat cells was not significantly modified by urine treatment. Moreover, crocidolite reduced the

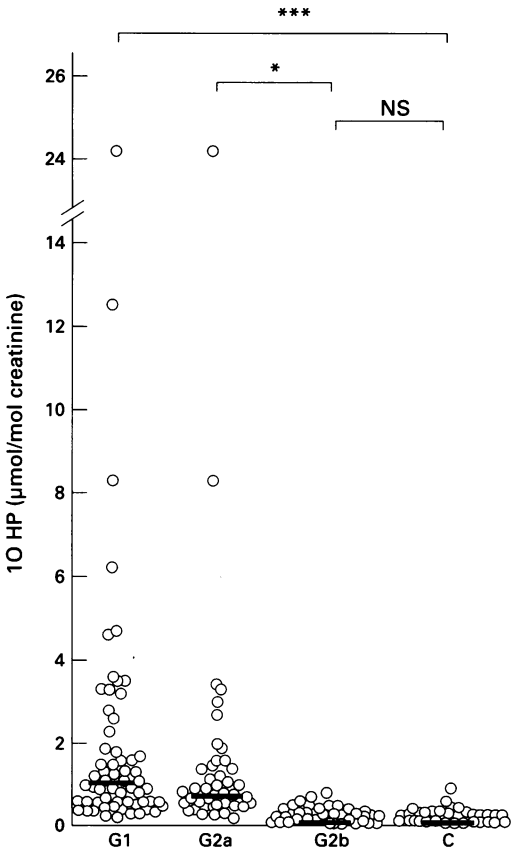

*Figure 1 Determination of urinary 1OHP. Each open circle represents the individual value of urinary 1OHP of a given subject. Black horizontal bar represents median for each group of workers. 1OHP = 1 hydroxypyrene. G1=60 coke oven workers, urine collection during the working period; G2=a second urine sample was collected three months after closure of the factory in 40 of the 60 coke oven workers; G2a=samples collected during the working period; G2b=samples collected after the end of exposure; C=blue collar controls. * p<0.05; *** p<0.001.*

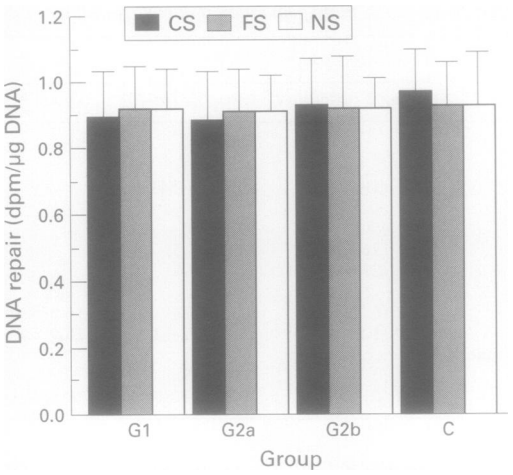

Figure 2 DNA repair in rat pleural mesothelial cells treated with urine from coke oven workers or blue collar controls according to smoking habits. Results of the unscheduled DNA synthesis assay were expressed as the ratio of the repair capacity of rat cells treated with urine from coke oven workers or controls to that of untreated cells (RPMI medium control). G1=60 coke oven workers, urine collection during the working period; G2=a second urine sample was collected three months after closure of the factory in 40 of the 60 coke oven workers; G2a=samples collected during the working period; G2b=samples collected after the end of exposure; C=blue collar controls; CS=current smokers; FS=former smokers; NS=non-smokers. There was no significant difference for dpm/DNA values between groups of workers, whatever the smoking status.

cell viability but enhanced DNA repair. By contrast, there was no correlation between DNA repair capacity of rat cells and urinary cotinine (r= - 0.20, NS), or between DNA repair capacity and results of viability of rat cells (r= - 0.06, NS) in group 1 subjects. The DNA repair capacity of rat cells was not correlated with any of these variables in group 2b subjects or in controls.

No correlation between rat cells viability and urinary cotinine or 1OHP concentrations was found in any of the groups.

Discussion

This study was designed to assess whether urinary filtrates from subjects with various levels of occupational exposure to PAH were able to induce DNA damage or modify DNA repair processes in rat cells used as a test system. An enhancement of DNA repair would indicate that the urine filtrates contain clastogenic molecules; a decrease in DNA repair would suggest the presence of compounds able to impair repair processes.

Exposure to PAHs was evaluated according to the levels of an internal dose indicator, urinary 1OHP, and by an assessment of exposure by job by the occupational physician. 1-Hydroxypyrene is a non-mutagenic metabolite which can be found in the urine of humans exposed to PAHs. Its concentration in biological fluids cannot be used to evaluate the carcinogenic risk for exposed workers, which probably depends on the various PAH profiles of pitch and tar volatiles polluting the work environment. However, because of the very good correlation between PAH concentrations in the work environment and urinary 1OHP concentrations,[32-34] this metabolite has been proposed as a biological indicator of occupational exposure to PAHs.[8 35] Moreover, this metabolite shows exposure to pyrene independently of the route of exposure.[32 36] The values of urinary 1OHP found in the present study were in the range 0.01–24.2 µmol/mol creatinine and are consistent with those reported by other authors in workers with similar occupations.[8 12 21 32] Moreover, a fairly good correlation was found between 1OHP and the week exposure index, confirming that this variable is a good marker of recent exposure. The urinary 1OHP concentrations were also lower three months after the end of occupational exposure and were similar to control concentrations. This is in agreement with the half life of this metabolite, which is about 18 hours.[8] The urinary 1OHP concentration is considered to be influenced by smoking habits only in the case of low occupational exposure to PAHs.[8 36-38] Thus, as some of the exposed workers had a low exposure during the week preceding collection of the urine sample and as smoking habit is a confounding factor, we selected controls with similar smoking habits. This allowed evaluation of the true effect of occupational exposure on the DNA repair capacity of rat cells treated with urine from these workers. The effect of diet, particularly fried or grilled meat, may affect the urinary excretion of 1OHP.[36 39] Although eating behaviour was not controlled in our study, results of 1OHP were consistent with the evaluation of the level of exposure of the last workshift, as assessed by job by the occupational physician. We therefore consider that diet had a minimal influence in our population compared with that of occupation.

The DNA repair assay has been previously performed on rat cells with concentrated urine samples from subjects exposed to suspected or known genotoxic agents.[25 26] A clastogenic activity was shown in rat cells treated with urine from chromium workers and patients receiving radiotherapy with or without chemotherapy for cancer.[25] Emerit *et al*[27 40 41] showed the presence of clastogenic factors in various biological samples (plasma, synovial fluid, cerebrospinal fluid) from patients with congenital

Table 2 Mean (SD) values of DNA repair and viability of rat cells treated with urine filtrates

Variable	Coke oven workers			Controls
	Group 1 (n=60)	Group 2a (n=40)	Group 2b (n=40)	(n=40)
DNA repair:				
[^{3}H]dThd incorporation (dpm)	0.89 (0.10)	0.89 (0.10)	0.90 (0.09)	0.91 (0.11)
DNA (µg)	0.99 (0.07)	1.0 (0.07)	0.98 (0.07)	0.97 (0.07)
[^{3}H]dThd incorporation/DNA (dpm/µg DNA)	0.90 (0.13)	0.90 (0.13)	0.92 (0.13)	0.95 (0.14)
Viability (MTT test) OD (540 nm)	0.94 (0.12)	0.93 (0.14)	0.92 (0.12)	0.99 (0.13)

breakage syndromes such as ataxia telangiectasia and Bloom's syndrome, as well as chronic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, and progressive systemic sclerosis. Most of the clastogenic factors isolated to date have a molecular weight lower than 10 000 Da—such as 4-hydroxynonenal (150 Da) or inosine triphosphate (388 Da). Our urine filtration procedure allowed collection of these factors in our filtrates. Incorporation of [^{3}H]dThd was significantly enhanced in cells treated with crocidolite, compared with untreated cells, attesting that our test system responded accordingly. Results obtained with urine samples from coke oven workers were unexpected, as the results obtained by Pillière *et al*[25] with urine samples from chromium workers suggested that this urine enhanced DNA repair in rat cells indicated the presence of clastogenic factors in urine as in other fluids.[40] By contrast, in our study, we found a tendency for less incorporation of [^{3}H]dThd in the cells treated with urine samples from exposed coke oven workers when 1OHP was increased. These results cannot be explained by smoking habits (as assessed by urinary cotinine), or by urinary concentration, as the volume was adjusted in each well according to the initial concentration. An impairment of cell viability is also unlikely.

The different results obtained for DNA repair capacity of rat cells treated with urine from chromium workers and from coke oven workers suggests that subjects exposed to different types of carcinogens excrete different compounds in urine. Urine samples from populations exposed to chromium derivatives might therefore enhance DNA repair of rat cells as a result of DNA breakage, whereas other urine samples might decrease the cell's ability to repair DNA. This tendency was suggested by the results obtained by Knudsen *et al*.[42] These authors showed that unscheduled DNA synthesis in peripheral lymphocytes induced by N-acetoxy-N-acetylaminofluorene was decreased in welders compared with a reference population. Exposure to mutagenic or carcinogenic agents may therefore lead to the formation of factors inhibiting DNA repair, an effect that might account for the accumulation of errors in DNA integrity.

Although the biological response found in the DNA repair assay performed on rat cells treated with urine filtrates from coke oven workers was only moderate, urinary 1OHP concentrations indicated that exposure to PAHs was excessive in this population. Urine from coke oven workers also exhibited some inhibitory effect on the DNA repair capacity of rat cells. More stringent control of exposure is therefore required in the workplace.

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All manuscripts submitted to *Occup Environ Med* should conform to the uniform requirements for manuscripts submitted to biomedical journals (known as the Vancouver style.)

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References should be numbered consecutively in the order in which they are first mentioned in the text by Arabic numerals on the line in square brackets on each occasion the reference is cited (Manson[1] confirmed other reports[2][3][4][5]). In future references to papers submitted to *Occup Environ Med* should include: the names of all authors if there are seven or less or, if there are more, the first six followed by *et al*; the title of journal articles or book chapters; the titles of journals abbreviated according to the style of *Index Medicus*; and the first and final page numbers of the article or chapter. Titles not in *Index Medicus* should be given in full.

Examples of common forms of references are:

1 International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomed journals. *JAMA* 1993;**269**:2282-6.
2 Soter NA, Wasserman SI, Austen KF. Cold urticaria: release into the circulation of histmaine and eosinophil chemotactic factor of anaphylaxis during cold challenge. *N Engl J Med 1976*;**294**:687-90.
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