

Monitoring genotoxicity in the occupational environment

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SORSA M, FALCK K, NORPPA H, VAINIO H. Monitoring genotoxicity in the occupational environment. *Scand j work environ health* 7 (1981): suppl 4, 61—65. New methods need to be developed for the biological monitoring and health surveillance of workers occupationally exposed to mutagenic and carcinogenic chemicals. Two potential approaches, the assay of urine for mutagenicity and the analysis of chromosomal changes in lymphocytes of workers, are discussed in this report with respect to experience in two occupational environments, the rubber industry and a hospital oncology unit. The urine of workers in the rubber industry has shown significantly increased mutagenic activity after the workweek in comparison to postvacation values. Handling cancer chemotherapeutic drugs in oncology units may cause exposure to the cytostatics, many of which are known mutagens and carcinogens. This exposure can be detected as an increased sister chromatid exchange frequency in cultured lymphocytes.

Key terms: biological monitoring, carcinogens, mutagens, sister chromatid exchanges, urinary mutagenicity.

Damage induced in cellular DNA (deoxy-ribonucleic acid) by genotoxic chemicals may be manifested as delayed carcinogenic, teratogenic or mutagenic effects (7). The latency period between the exposure and the final outcome varies from several months to decades.

The molecular basis of genotoxicity is unrepaired DNA damage that develops into a permanent heritable alteration, a mutation, when the damaged molecule is replicated. A mutation may affect the genetic code by altering the nucleotide sequence of DNA either through local misalignments (frameshift mutations) or through the mispairing of bases (base-pair mutations). In eucaryotic organisms, un-

repaired molecular breaks can also affect the linear coherence of the chromosome and thus induce microscopically visible changes in the chromosome structure (structural rearrangements and deletions). Finally, gross genomic alterations may be induced by agents affecting the spindle functions of the cell and may thus lead to malsegregation of the chromosomes and aneuploidy (13).

Before the preventive phase of estimating occupational genotoxic and, especially, carcinogenic hazards can be achieved, new methods are needed for biological monitoring and health surveillance. Short-term mutagenicity assays offer a promising possibility for monitoring exposure to genotoxic chemicals.

Some experience has been gained about testing ambient air for mutagenicity in short-term screening assays with either on-site methods or the off-site testing of air samples (8). Another approach is based

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on the biological monitoring of mutagenic substances in human body fluids and the detection of early mutational effects in populations exposed to genotoxic chemicals.

This presentation concentrates on recent experiences of the Institute of Occupational Health in Helsinki in research efforts in two very different occupational environments, the rubber industry and an oncology unit of a hospital.

Subjects and methods

Occupational groups

The rubber industry provides a multiexposure occupational environment in which several hundreds of different chemical entities are handled. Furthermore, the processing of the raw materials may generate new compounds and thermal degradation products.

The hygienic hospital environment represents quite different occupational conditions. In the oncology units, anticancer drugs are handled and prepared for chemotherapy by the personnel.

Mutagenic activity in urine

Two different approaches have been used for biological monitoring. The bacterial mutagenicity assay, which is able to show nonspecific mutagenicity in urine, has been utilized to detect exposure to mutagens. For a couple of years this method has been used in various occupational settings to show that mutagenic metabolites can be detected in the urine of workers supposedly exposed to genotoxic agents (14). Both the plate incorporation assay (Ames' test) (1) and the fluctuation test (5) have been utilized in detecting mutagenic activity in urine. In the fluctuation test, also used in the present studies, the bacteria mutate and grow in a liquid environment. The fluctuation assay has appeared to be more sensitive (5) than the conventional Ames' test on plates, and thus it seems especially suited for assaying mutagenicity in urine, in which the concentrations of mutagenic compounds are usually low.

The bacterial strains used have marker mutations in their genome, and these mutations make the strains dependent on growth factors in the medium, on histidine with the *Salmonella typhimurium* TA strains and on tryptophan with the *Escherichia coli* WP2 strains.

Sister chromatid exchanges in lymphocytes

The other approach available for the biological monitoring of exposure to genotoxic chemicals is to study the direct damage of the genetic material in the lymphocytes in the peripheral blood of the exposed individuals. At present, two cytogenetic methods have been used. The more conventional one is to study structural chromosome changes in the cultured lymphocytes of the exposed population (13). Recently, great hopes have been directed towards the use of sister chromatid exchanges (SCEs) as a measure of genotoxic exposure (9). The SCE analysis has become possible after the invention of the harlequin staining of chromosomes by Wolff & Perry (12). Particularly studies on patients under cytostatic chemotherapy suggest that SCEs can be used as a sensitive indicator of acute mutagen exposure. Rather limited experience about various exposing agents as yet prevents the general use of SCE analysis as a biological monitoring method at the individual level.

Results and discussion

Workers in the rubber industry

Various job categories in the rubber industry differ both qualitatively and quantitatively with respect to exposure to genotoxic compounds. In a preliminary study with the fluctuation assay we have shown that workers in tire manufacturing show higher mutagenic activity in their urine than office personnel (4). The effect of chemical exposure in the rubber industry can be detected with the base-pair tester strain, *E. coli* WP2 *uvrA*, while the frameshift strain *S. typhimurium* TA98 additionally detects urinary mutagenicity caused by cigarette smoking (4).

Another approach was to define the extent of chemical exposure in different job categories. Mutagenic activity was determined for urine samples from workers representing different jobs both at the end of their normal workweek and after one month's vacation before entering the rubber factory. The results of the urine analyses for 30 rubber workers in different job categories are given in fig 1. The mutagenic activity, calculated in relation to the creatinine concentration of each urine sample [see Falck et al (4) for details], varied considerably between the groups, from $1,363 \pm 944$ (mean $\pm$ SD) among cleaners in the mixing department to 68 ± 42 among boot makers. Among all the groups of workers, except the boot makers, the urinary mutagenic activity was significantly higher in the samples taken after the work shift than after the vacation (fig 1).

Although the number of individuals in the different groups was small and the interindividual variation may have been quite high in the groups, the results point to the possibility that the urinary mutagenicity assay can be used in defining occupations and job categories with exposure to genotoxic substances. More research is needed to determine the restrictions of the method, especially to reveal the effects of "life-style," diet, etc, on the detectable mutagenicity in urine.

Cytogenetic methods can be used as another approach to define possible differences in biological response between individuals in the various job categories with deviant exposure loads. The analysis of structural chromosome aberrations and SCEs in various groups of rubber workers is presently under study.

Hospital personnel in oncology units

During the last decade, the use of chemotherapy has extensively increased in cancer treatment. Many of the antineoplastic drugs used are known mutagens and carcinogens (2, 6). Consequently, the handling of these cytostatics by hospital personnel requires special considerations to prevent unnecessary exposure.

We have used the fluctuation assay to detect mutagenicity in the urine of patients under chemotherapy, oncology nurses daily handling cytostatic drugs, and office personnel not known to be exposed to mutagenic chemicals (3). The results showed that exposure to the chemotherapeutics was probably the cause of increased mutagenicity in the urine of the oncology nurses. After the initial results became known, the work conditions of the nurses were improved (instructions for making solutions, masks, gloves, protective clothing, laminar flow chambers), and the mutagenicity in their urine dropped considerably, a result reflecting a decrease in exposure (14).

Many cytostatic drugs are known to increase SCEs in treated patients or in experimental systems (10). For a determination of whether the exposure of the oncology nurses to cytostatics could induce such biological responses, the SCEs were analyzed in the blood lymphocytes. Patients under chemotherapy were used as positive referents. Hospital nurses from other departments and office personnel formed the other reference groups. The results showed that the oncology nurses had a significantly higher ($p < 0.01$) mean SCE frequency than the office personnel (11). However, the difference between the oncology nurses and the other hospital nurses was not statistically significant. The SCE frequen-

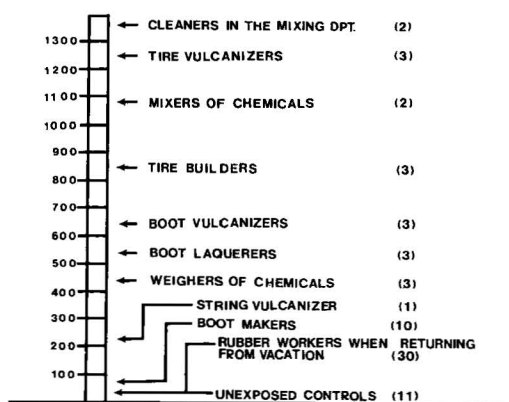


Fig 1. Mutagenic activity, detected by *Escherichia coli* WP2 uvrA, in urine samples of 11 nonexposed referents and of rubber workers (total of 30 persons) after a normal workweek and after vacation.

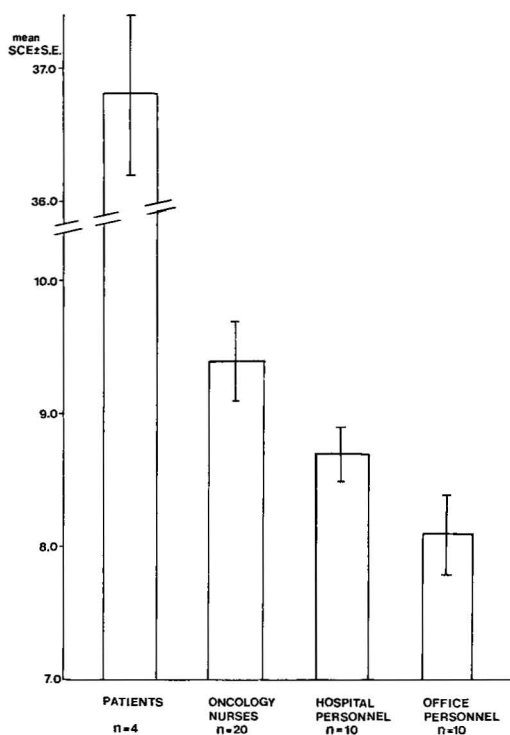


Fig 2. Mean number of sister chromatid exchanges ($\pm$ SE) detected per cell in cultured lymphocytes of patients on chemotherapy, hospital nurses in oncology units, nurses from other hospital departments and office personnel. All the subjects were nonsmokers in the same age range.

cy of the patients studied was about four to five times higher than that of the healthy subjects (fig 2).

Possibilities for the biological monitoring of exposure to genotoxic chemicals

The main obstacles to accurate estimations of occupational risks stem from the lack of knowledge about the chemical composition of the work environment, as well as from the inadequacies of available detection methods.

The need for the biological monitoring of exposure to genotoxicants is thus evident. These methods are needed to detect occupational risks and to monitor individuals in occupations in which exposure to mutagenic and carcinogenic chemicals is difficult to avoid.

This report discusses the use of urinary mutagenicity assay as a biological monitoring method to detect exposure to genotoxicants in various job categories in the rubber industry and in the oncology units of hospitals. This test is able to estimate the total exposure to mutagenic chemicals during a rather acute time period. An obvious limitation of the test is that it can only be used in cases in which the genotoxicants are excreted into urine in an active form or as conjugates which can be reactivated in the test procedure. Even with these limitations the assay seems to offer promising possibilities for biological monitoring in defined occupational environments.

The cytogenetic approaches discussed, ie, analysis of structural chromosome aberrations or of SCEs in cultured lymphocytes of exposed persons, represent a second stage of biological monitoring: the detection of early effects in the exposed occupational groups. Both chromosome aberrations and SCEs should be considered as adverse effects, even though the biological significance or health effects of these changes are not yet clear. At the group level there are, however, enough examples available to correlate chromosomal changes with cancer risk. This evidence warrants chromosomal changes being regarded as a warning of potentially serious effects, and their determination should lead to a decrease in the exposure to the causative genotoxicants.

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