

Biological monitoring of occupational exposure to mutagenic chemicals in the rubber industry

Use of the bacterial urinary mutagenicity assay

by Kai Falck, PhD¹

FALCK K. Biological monitoring of occupational exposure to mutagenic chemicals in the rubber industry: Use of the bacterial urinary mutagenicity assay. *Scand j work environ health* 9 (1983): suppl 2, 39-42. The assay of mutagenicity in the urine with the bacterial fluctuation test was used as an approach to monitor the exposure to mutagenic chemicals among 129 rubber workers. Especially those workers exposed to chemical dusts or fumes showed high mutagenicity in their urine. Smoking was also found to increase mutagenicity in the urine, but this effect could be detected only with the frame-shift detector strain *Salmonella typhimurium* TA 98. *Escherichia coli* WP 2 uvrA could thus be used to monitor the occupational exposure to base-pair substitution mutagens, without the interfering effect of smoking.

Key terms: bacterial mutagenicity tests, carcinogenesis, occupational safety, urine analysis.

The manufacture of rubber requires several different work phases and a large number of chemicals. The more than 500 chemicals used for the manufacture of rubber products include several known or suspected mutagens and carcinogens (9, 10). In addition rubber products vulcanized at high temperatures and high pressures may emit biologically active reaction products (7, 8). Because of the high number of exposing chemicals, the conventional methods of biological monitoring are not always useful in assessing exposure to genotoxic agents in the rubber industry.

Several epidemiologic studies have shown that the workers in the rubber industry have an increased cancer risk. [For a review see volume 28 of the International

Agency for Research on Cancer's monograph series (11).] Mutagenicity is a general characteristic of carcinogenic chemicals (1), and therefore the monitoring of mutagenicity in the ambient air or in the biological samples of workers, eg, in urine, can be used as an approach to detect hazardous exposures and persons exposed to mutagenic chemicals.

Bacterial assays using urine samples from exposed persons as sources for the determination of mutagenicity can show whether the persons in question have been exposed to chemical mutagens that are excreted in the urine in an active form or as biotransformed metabolites that can be reactivated again with exogenous activation systems, eg, with the S-9 mix (1).

Because the bacterial fluctuation test was found to detect smaller amounts of mutagenic chemicals than the Ames' plate incorporation test (5, 6), it was used to determine the potentially mutagenic substances that are excreted in the urine due to occupational exposure to mutagenic chemicals (2, 3, 4, 12, 13, 15).

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Subjects, materials and methods

The mutagenic activity of the urine from 129 exposed workers from one Swedish and two Finnish rubber factories was analyzed. Fifty-four office clerks in the same factories served as the referents. In addition 45 of the 129 exposed workers gave in additional urine sample after a four-week annual vacation and thus served as their own referents. Of the workers, 67 were smokers and 62 were nonsmokers. Among the referents there were 20 smokers and 34 nonsmokers. According to the medical reports and the workers' and referents' own information all the persons who participated in these studies were "healthy."

The urine samples of the exposed workers and nonexposed referents were collected at the end of their workshift, usually on Thursday or Friday afternoon. If the workers served as their own referent (15), the samples were collected also in the morning of their first workday after their annual vacation of four weeks. Immediately after the urine samples (100–200 ml) were collected, the bottles were cooled with crushed ice and taken to the laboratory, where an aliquot (5 ml) was taken from which the concentration of creatinine in the sample was determined. Both the actual sample and the aliquot were frozen at -70°C . Immediately before preparation the urine samples were thawed at room temperature. The urine samples were concentrated using the XAD-2 adsorption method described by Yamasaki & Ames (16).

The bacterial fluctuation test was performed according to Falck et al (4). The bacterial strains used in the mutagenicity assay were *Salmonella typhimurium* TA 98 (a histidine-auxotrophic strain that is able to detect frameshift mutagens) and *Escherichia coli* WP 2 *uvrA* (a tryptophan-auxotrophic strain able to detect base-pair substitution mutagens). All the assays were carried out using the S-9 mix for metabolic activation.

Results and discussion

Mutagenicity in the urine of the exposed rubber workers

In general the urine samples of the rubber workers had a higher mutagenic activity

than the urine samples of the clerks. When the samples were collected after a four-week annual vacation, the mutagenicity in the urine samples of workers was decreased, and then no difference could be seen in the mutagenicity of the samples of the workers and the referents. This decrease of mutagenic activity in the urine samples of workers taken before beginning work after a vacation indicates that, at least partly, the mutagenicity detected in the urine when the workers actually participated in rubber manufacturing was of occupational origin.

The mutagenic activity due to occupational exposure was especially clearly seen when the samples were tested with the base-pair substitution strain *E coli* WP 2 *uvrA*. When the samples were tested with the frameshift detector strain *S typhimurium* TA 98, smoking was found to increase the mutagenicity (2, 4) in both the urine of the workers and the clerks.

Regardless of their smoking habits, the chemical mixers, the cleaners working in the same department, the vulcanizers of tires or boots, and the tire builders had higher mutagenic activity in their urine than the other groups of workers. This higher level may indicate that the exposure to mutagenic (and probably carcinogenic) chemicals is higher or that more potent mutagens are dealt with in these jobs than on the average in the rubber industry.

No difference could be seen between most of the groups of smokers, but the urine of chemical mixers and tire builders who were smokers showed significantly higher activities as compared to the urine of nonexposed smokers.

Worker exposure to genotoxic substances in the rubber industry

The finding that there is both frameshift, as well as base-pair, substituting mutagenic activity in the mixers' urine is in agreement with the fact that they handle virtually all the chemicals used in rubber factories. The mixers may inhale the chemical dusts when they add chemicals to the mixing machine, and they sometimes handle the mixed rubber without gloves, when the rubber is removed from the mixing machine (10, 15).

The cleaners working without protective devices in the weighing and mixing departments could be exposed to spilled or leftover chemicals (13, 14, 15).

The tire builders usually make and handle unfinished tires without protective gloves because of the need for accuracy. All the chemicals that are present in the uncured rubber of tires may come into contact with the tire builders' skin. In addition many solvents are used to seam the different parts of tires. These lipid-soluble solvents may enhance the skin penetration of the rubber chemicals.

The high mutagenicity in the urine of vulcanizers (13, 14, 15) is probably due to exposure to different curing fumes in the factory because Hedenstedt et al (8) and Hedenstedt (7) showed that air samples collected near the curing ovens contained mutagenic substances.

Of the vulcanizers, the light tire vulcanizers had the highest level of mutagenicity in their urine (15). The vulcanizers of boots had lower but still significant mutagenic activity in their urine, but the urine of men involved in the curing of heavy truck tires showed only marginal mutagenic activity.

Because the light tires are removed manually, one by one, from the curing press, the light tire vulcanizers are in the closest contact with the product and its emissions. The average production rate per worker is 200–300 tires per workshift; therefore the workers may come into contact with the curing fumes nearly every 2 min.

Rubber boots are cured in large ovens, the carriages of which are pulled by the vulcanizers. Although the ovens are ventilated, there could be small emissions from the cured products.

The curing of heavy truck tires is the most automated process. The workers only need to assemble the tire within the press and pick it up at the end of the conveyor line, so the curing fumes have possibly dissolved into the ambient air before reaching the vulcanizers.

in a form that is potentially capable of reaction with bacterial deoxyribonucleic acid. The potentially mutagenic metabolites of chemicals are excreted according to their toxicokinetic properties. Most of the metabolites of mutagens in urine are detoxified conjugates or biotransformed molecules. Before the mutagenicity of these potentially mutagenic molecules can be determined, they must be reactivated. The exogenous metabolic activation system must have deconjugating enzymes, as well as microsomal monooxygenases. The most frequently used metabolic activation system, the S-9 mix, contains both of these activities.

Occupational exposures can be evaluated in a comparison of the mutagenic activities in the urine of exposed and nonexposed groups, or, more preferably, exposed workers can be used as their own referents who give two urine samples, one during the work period and another after an exposure-free period (usually after the annual vacation) (15).

The confounding effect of smoking can be avoided if all the urine samples are tested with both types of bacterial tester strains, one, eg, *S typhimurium* TA 98, detecting frameshift mutagens caused by exposure to industrial chemicals and smoking and the other, eg, *E coli* WP 2 uvrA, detecting base-pair substitution mutagens caused by exposure to occupational chemicals but not by smoking (2, 3).

Because some mutagenic chemicals do not have potentially mutagenic urinary metabolites, the lack of mutagenic activity in the urine does not necessarily indicate that no exposure to genotoxic agents has occurred. Therefore the assay of mutagenic activity in the urine is the most suitable as a component test of a battery of genotoxicologic assays for the detection of exposures to mutagens and the early biological responses caused by such exposures (13, 14, 15).

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The applicability of bacterial urinary mutagenicity assays in the monitoring of occupational exposures

The bacterial urinary assay can detect the mutagenic activity caused by those chemicals and metabolites excreted in the urine

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