Environment International, Vol. 15, pp. 49 – 56, 1989
Printed in the U.S.A.

0160-4120/89 $3.00 +.00

BIOMONITORING OF EXPOSURE TO POTENTIALLY GENOTOXIC SUBSTANCES FROM ENVIRONMENTAL TOBACCO SMOKE

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EI 87-481 (Received 5 November 1987; Accepted 5 May 1987)

In two experimental studies, 10 volunteer nonsmokers were put on a controlled diet and exposed to two different ETS concentrations for 8 hours. During exposure in Experiment 1, the average indoor air levels of CO, NO, NO_2, formaldehyde, and nicotine reached 10 μL/L, 160 nL/L, 20 nL/L, 40 μg/m³ and 60 μg/m³, respectively. During Experiment 2, the respective concentrations were 20 to 25 μL/L, 310 nL/L, 150 nL/L, 50 μg/m³ and 120 μg/m³. On the average, carboxyhemoglobin increased by 0.7% after exposure in Experiment 1 and by 2.0% in Experiment 2. The serum cotinine concentration increased by 1 ng/mL and 5 ng/mL in Experiment 1 and 2, respectively. On the average, the subjects excreted 24 μg (Experiment 1) and 70 μg cotinine (Experiment 2) in the 24-h urine after ETS exposure. No statistically significant increase was found in the urinary mutagenicity after either of the two exposure regimens, whereas thioether excretion was significantly elevated. The data suggest that nonsmokers in real-life situations take up very low doses of ETS constituents, and detoxification of the genotoxic substances inhaled is effective.

INTRODUCTION

Environmental tobacco smoke (ETS) is a complex mixture of several thousand chemical compounds. These compounds are found in mainstream smoke as well, although its quantitative composition is quite different (Klus and Kuhn 1982). Dosimetry of ETS exposure should be based on biochemical markers that should be specific and representative. The commonly used markers, such as nicotine, cotinine, carboxyhemoglobin (COHb), and thiocyanate, do not meet all these requirements (for extensive review, see U.S. Department of Health and Human Services 1986). Carboxyhemoglobin and thiocyanate (a metabolite of tobacco-smoke-derived hydrogen cyanide) were found to be inadequate biomarkers in field studies mainly because of interference with sources other than ETS. Because of its stability and specificity for tobacco smoke, cotinine in body fluids appears to be the short-term marker of choice in population studies. However, since carbon monoxide, hydrogen cyanide, and nicotine are constituents of the gas phase of ETS (Eudy et al. 1985), an adequate marker for quantitating particulate-phase exposure is lacking. Additionally, these substances are not related to the genotoxic properties of ETS, an attribute that is of highest concern with respect to

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long-term health effects of ETS exposure (IARC 1986). Solid risk assessments and comparisons to other risk factors such as smoking, diet, and ambient air pollution require dosimetric data on the uptake of toxic and genotoxic substances during ETS exposure.

The objective of the investigation was to measure urinary excretion of mutagenicity and thioethers in addition to COHb and cotinine in serum and urine after high but controlled exposures of nonsmokers to ETS. Urinary mutagenicity, which can be regarded as a measure of exposure to genotoxic substances, has been found to increase in three experimental settings (Bos et al. 1983; Sorsa et al. 1985; Mohtashamipur et al. 1987) but not in another experiment (Scherer et al. 1987) nor in a real-life situation (Husgafvel-Pursiainen et al. 1987). Thioether excretion, which can be regarded as an indicator of exposure to electrophilic substances, has been reported to increase after smoking (Van Doorn et al. 1979) but to be unchanged after ETS exposure (Sorsa et al. 1985). Interest was focused on the relationship between excretion of thioethers and mutagenicity in the urine after controlled ETS exposure, since both urinary thioethers and mutagenicity might at least partly be caused by exposure to the same substances.

SUBJECTS AND METHODS

Subjects

Twenty-four healthy male subjects (14 nonsmokers and 10 smokers) aged 18 to 44 years (mean age 22.0 years) volunteered to take part in either one or both experiments. After admission to the laboratory on a Friday evening, they completed a questionnaire on socioeconomic and life-style factors as well as on their ETS exposure during the past 48 h.

Protocol

Experiment 1. After admission to the laboratory at 8:00 PM, 10 nonsmokers aged 18 to 29 (mean age 23.8 ± 3.6) years were put on a defined diet low in polycyclic aromatic hydrocarbons during the course of the experiment. The following night and day (control day) any exposure to ETS was avoided. On the first day, the subjects spent 8 h in an unventilated, ordinarily furnished room of 45 m^3 in order to simulate exposure conditions. On the second day (exposure day), the subjects were exposed to ETS at a level of approximately 10 µL/L CO in the unventilated room for 8 h. The exposure session started at 8:30 AM and was finished at 5:00 PM with a 30-minute lunch break at noon. The subjects were only allowed to leave the room to go to the lavatory. The smoke was generated by two smokers smoking cigarettes, so that a CO level of about 10 µL/L was maintained. Blood samples were taken before the subjects entered the room at 8:00 AM and after they had left the room at 5:00 PM on both the control and exposure days. Each subject sampled his 24-h urines on two consecutive days. Sampling began after discarding the first morning urine at approximately 8:00 AM on the control day. The subjects were dismissed from the laboratory on the morning after the exposure day.

Experiment 2. Experiment 2 was carried out in the same way as Experiment 1 except for the following changes: It was performed in two separate runs, each of them comprising 5 nonsmokers and 5 smokers. Six of the nonsmokers had participated in Experiment 1. The age of the subjects ranged from 19 to 28 (mean age 23.7 ± 2.7) and from 24 to 44 (mean age 32.4 ± 7.0) years for the 10 nonsmokers and the 10 smokers, respectively. The smokers had to refrain from smoking after admission to the laboratory until entering the exposure room on the exposure day. After this they were free to smoke cigarettes of their own brand. The CO level on the exposure day of Experiment 2 varied between 20 and 25 µL/L. The 10 smokers served as positive controls for the biological monitoring.

Room monitoring

The air sampling tubes were installed in breathing height of a sitting person at the end of the room, which was opposite to where the smokers sat. Carbon monoxide and nitrogen oxides were measured continuously by a Carbon-Monoxide Analyzer, Model 8310 (Monitor Labs Inc., USA) and a Nitrogen-Oxide Analyzer, Model 8840 (Monitor Labs Inc., USA), respectively. Nicotine was sampled on Extrelut-filled tubes for 0.5 to 2 h (flow rate 2.4 L/min). The loaded tube was alkalized by ammonia, and the alkaloid was eluated with 20 mL ethyl acetate. In the dried and concentrated eluate, nicotine was determined by capillary gas chromatography (Klus et al. 1987). Formaldehyde was measured according to the method of Kennedy and Hill (1982). The aldehyde was derivatized with N-benzylethanolamine to form N-benzyloxazolidine that was detected by capillary gas chromatography.

Biomonitoring

Carboxyhemoglobin (COHb) was measured by means of a CO-Oximeter, Model 182 (Instrumentation Laboratories Ltd., USA) immediately after drawing the blood sample. Cotinine in serum and urine was detected by a radioimmunoassay as described by Langone

et al. (1973) and modified by Haley et al. (1983). Thioethers in urine were determined by measuring sulfhydryl groups with Ellman's reagent after alkaline hydrolysis of the thioether bonds. Corrections were made for free sulfhydryl compounds (Van Doorn et al. 1979; Heinonen et al 1983).

Extraction of urine samples for mutagenicity testing was performed according to the original method of Yamasaki and Ames (1977) modified by Mohtashamipur et al. (1985). Briefly, aliquots of 400 mL filtered urine samples were adjusted to pH 8.0 (NaOH) and loaded on an XAD-2 column (0.7 x 8 cm). After washing with 3 mL of water, the column was extracted with 40 mL methanol. The first drops were discarded until the brownish eluate appeared. The eluate was evaporated to dryness under reduced pressure at 65 to 70°C. The residue was dissolved in 2 mL DMSO. This procedure is reported to lower the histidine concentration in the extract to undetectable levels (Mohtashamipur et al. 1985). The urine concentrates were tested for mutagenicity by the Salmonella (TA98) microsome (S9-mix derived from Aroclor-treated rats) assay. Each sample was determined in triplicate using 10, 25, 50 and 75 µL urine concentrate, corresponding to 2, 5, 10 and 15 mL of original urine. The slope of the linear part of the dose-response curve obtained by linear regression technique was used to calculate the mutagenic activity in the 24-h urine. In each case, the steepest of the three slopes was used for further analyses.

Statistical analysis

The one sample t-test for differences (exposed minus nonexposed) was applied.

RESULTS

Room monitoring

In both experiments the ventilation conditions were rather poor, which is reflected by high room temperatures (27 to 30°C), high relative humidities (70 to 90%) and an unpleasant odor. The time patterns of CO during the control and exposure days of both experiments are shown in Fig. 1. The distribution of cigarettes smoked by time in both experiments is indicated at the bottom of Fig. 1. During Experiment 1, a total number of 42 cigarettes was smoked. During each session of Experiment 2, the total number of cigarettes smoked amounted to 100.

The CO levels hardly exceeded 2 µL/L on the control day. During the exposure day of Experiment 1, the CO concentration fluctuated around 10 µL/L. For Experiment 2, the CO level was between 20 and 25 µL/L and reached 27 µL/L at the end of the session. The CO concentration decreased in the 30 min lunch break during which smoking was stopped and the room was kept closed.

Approximately the same time pattern was observed for NO. Control-day levels amounted to 30 nL/L, whereas the NO concentrations increased approximately to 160 nL/L in Experiment 1 and to 300 nL/L during Experiment 2. The NO_2 levels measured during the exposure day of Experiment 1 hardly exceeded the control-day levels amounting to 10 nL/L and were even lower in the afternoon. Interestingly, the peak concentration was observed during lunch. The reasons for this are not known yet. During the exposure day of Experiment 2, the NO_2 concentration amounted to about 150 nL/L and showed a slight increase during the course of the session. The NO_2 level in Experiment 2 is more than proportionately elevated when compared to Experiment 1.

The formaldehyde level, which was 15 to 20 $\mu g/m^3$ on the control day, rose to about 40 $\mu g/m^3$ during Experiment 1 and approximated 60 $\mu g/m^3$ in the afternoon of Experiment 2. The formaldehyde concentrations are only weakly associated with the number of cigarettes smoked in the test room.

The nicotine concentration increased from a background level of 15 to 20 $\mu g/m^3$ up to 100 $\mu g/m^3$ during the first experiment and up to 180 $\mu g/m^3$ during the second. It is worth mentioning that the nicotine level follows more strictly the number of cigarettes smoked than the other ETS components.

Biomonitoring

The results of the biomonitoring are summarized in Table 1. The COHb remained constant in nonsmokers on both control days and increased by 0.7% on the average after the first exposure regimen and by 2.0% after the second exposure regimen. For smokers, the COHb decreased to nonsmoker levels during the phase of smoking abstinence and increased by 7.0% after smoking cigarettes for 8 h. On the average, the smokers smoked 14.7 ±7.8 (range 5 to 34) cigarettes in the 8-h exposure period and 8.8 ±4.3 (4 to 15) cigarettes during the following evening.

The ETS-related increase in serum cotinine amounted to about 1 ng/mL after exposure in Experiment 1 and to about 5 ng/mL in Experiment 2. Due to the long half-life of cotinine, the smokers' serum cotinine levels remained more than 100 times higher than those of the nonsmokers. The cotinine concentrations measured in smokers during the control day and before the start of smoking on the exposure day reflect the well-known slow cotinine elimination kinetics.

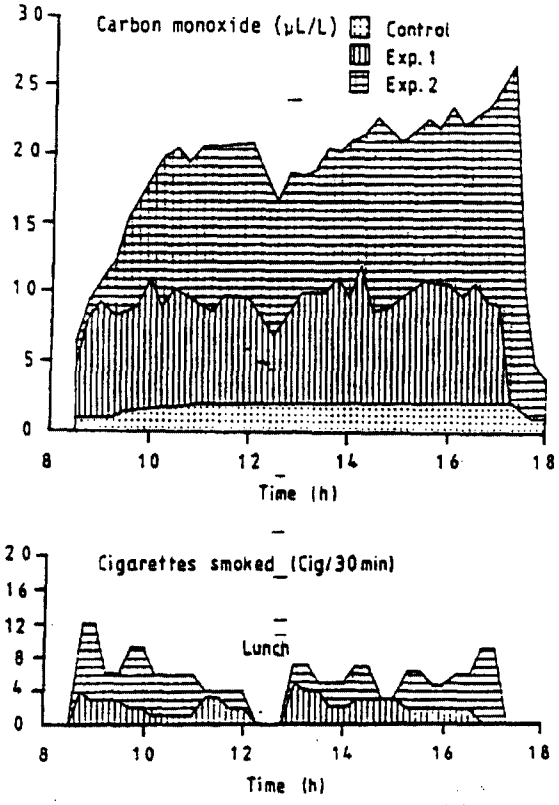

Fig.1. Indoor air concentration of carbon monoxide and number of cigarettes smoked during Experiment 1 and Experiment 2.

Table 1. COHb and cotinine in serum and urine of ETS-exposed nonsmokers and cigarette smokers. Means (Standard deviation).

	Experiment 1		Experiment 2			
	Nonsmokers (N=10)		Nonsmokers (N=10)		Smokers (N=10)	
	Control day	Exposure day	Control day	Exposure day	No smoking	Smoking
COHb (%)						
8.00 a.m.	0.34 (0.15)	0.18 (0.02)	0.63 (0.19)	0.65 (0.32)	2.44 (1.10)	0.81 (0.44)
5.00 p.m.	0.32 (0.13)	0.87 (0.04)***	0.62 (0.13)	2.69 (0.13)***	1.24 (0.40)***	7.87 (2.20)***
Serum cotinine (ng/mL)						
8.00 a.m.	0 (0)	0 (0)	1.2 (1.7)	0.4 (1.0)	378 (130)	146 (51)
5.00 p.m.	0 (0)	1.1 (0.3)***	0.9 (1.6)	4.9 (0.9)***	242 (84)	244 (84)***
Urine cotinine (µg/24h)	8 (8)	23 (8)**	21 (13)	67 (26)***	4490 (1800)	3580 (1280)*

Statistical comparisons: COHb and serum cotinine: 5.00 pm vs 8.00 am; urine cotinine : exposure vs control day
Levels of significance: * : $p < 0.05$; ** : $p < 0.01$; *** : $p < 0.001$

Basically, the same pattern is to be seen for the urinary cotinine excretion. The nonsmokers excreted on the average 15 µg/24 h (Experiment 1) and 46 µg/24 h (Experiment 2) more cotinine than on the corresponding control days.

After ETS exposure in Experiment 1, the thioether excretion was elevated by 13.9 µmol/24 h ($p=0.07$) and in Experiment 2 by 21.4 µmol/24 h ($p < 0.01$) (Fig. 2). The average amount of thioethers excreted by the 10 smokers on the control day was still elevated (89.1 µmol/24 h) as compared with nonsmokers and increased to 136.1 µmol/24 h ($p < 0.01$) after smoking (Fig. 2).

Urinary mutagenicity shows a high interindividual variability under all experimental conditions (Fig. 3). In nonsmokers, no increase was found in Experiment 1, whereas a slight but statistically not significant increase ($p = 0.15$) was observed in Experiment 2 (Fig. 3). After smoking, the mutagenic activity of the urine was significantly increased ($p < 0.01$) (Fig. 3).

DISCUSSION

The indoor air data reveal that the exposure level in Experiment 1 may correspond to real-life situations (Klus et al. 1987; Triebig and Zober 1984; Sterling et al. 1982), although the daily exposure time is usually less than 8 h; on average, it is about 3 h (Letzel and Johnson 1984; Ministerium für Arbeit, Gesundheit und Soziales NRW, 1987). The exposure level and duration in Experiment 2 bears no relation to a real-life situation. These considerations are also supported by the COHb measurements. An increase by 0.7% in COHb as observed after ETS exposure in Experiment 1 is rarely reached by nonsmokers in normal indoor environments where smoking is permitted (Szadkowski et al. 1976; Jarvis et al. 1983). The cotinine levels in serum and urine as measured in the experiments are not suitable for comparison with results of field studies on ETS exposure (Jarvis et al. 1983; Wald et al. 1984). This is due to the protocol of the study, which includes only a single

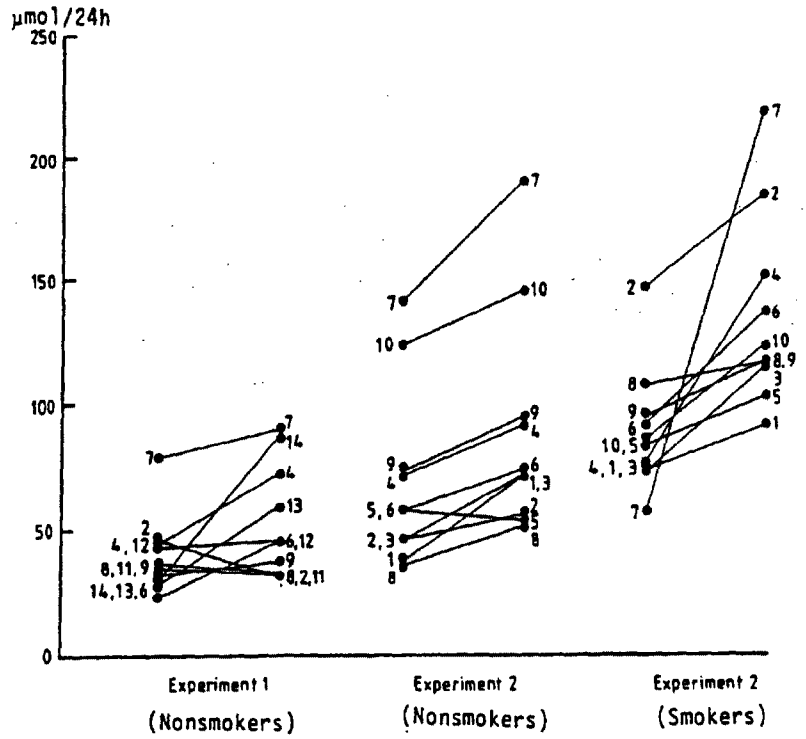

Fig.2. Individual changes of urinary thioether excretion after ETS exposure and cigarette smoking. (The figures refer to individual subjects.)

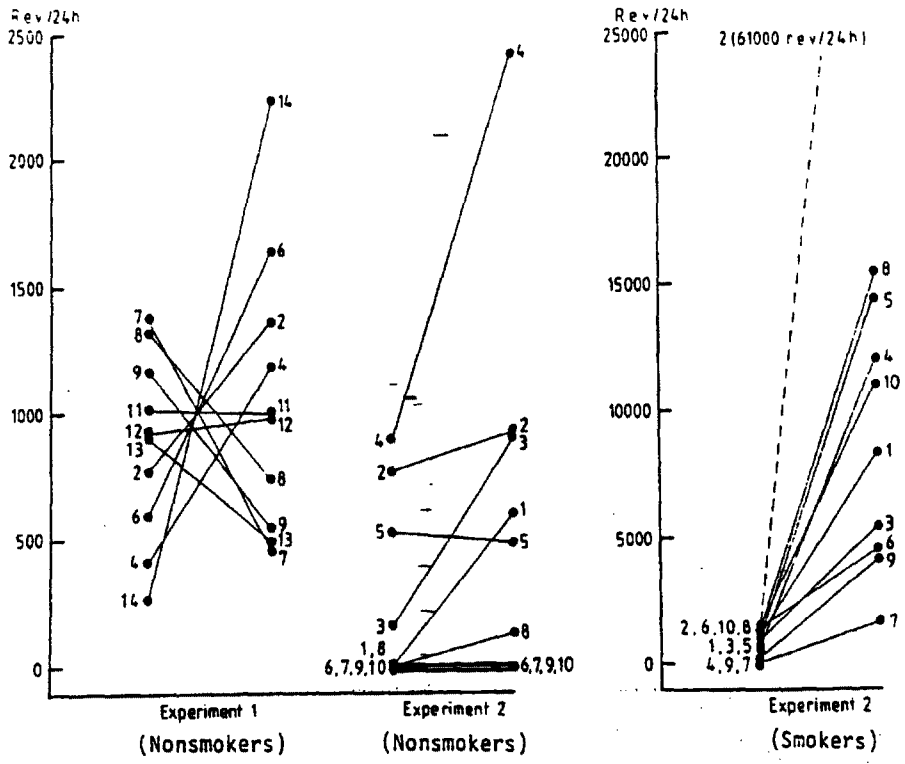

Fig.3. Individual changes of urinary urinary mutagenecity after ETS exposure and cigarette smoking. (The figures refer to individual subjects.)

ETS exposure period of 8 h and to the relatively long half-life of cotinine of about 20 h. Both facts prevent cotinine from attaining steady-state levels in the subjects.

While an increase in thioether excretion after smoking is well documented (Van Doorn et al. 1979), an increase in thioether excretion after high ETS exposure has been described for the first time. Recently, Sorsa et al. (1985) were unable to find a change in thioether excretion after moderate ETS exposure (4 µL/L CO, 5 h/d, for 2 days) of 6 volunteers in a chamber experiment. The relatively low exposure level, insufficient dietary control, and the fact that habitual smokers were used in the study might explain the discrepancy with the present study. According to the findings, smoking does not lead to a substantially higher increase in thioether excretion when compared to ETS exposure. The smokers, however, show higher base levels in thioether excretion, which could be due to their previous smoking and/or dietary factors. The latter might also be the reason for the somewhat elevated thioether level in the nonsmokers on the control day of Experiment 2. Preliminary data obtained in the laboratory show that smokers have to refrain from smoking and be kept under controlled dietary conditions for at least 3 to 5 days before a steady state in the daily amounts of thioethers excreted is attained.

The absence of a significant increase in urinary mutagenicity after ETS exposure as found in this study is in accordance with results reported by Sorsa et al. (1985) and Husgafvel-Pursiainen et al. (1987), but at variance with those published by Bos et al. (1983) and Mohtashamipur et al. (1987). The increase in mutation rates as found by Bos et al. (1983) was just significant and amounted to about 4% of that measured in smokers, an increase that is rather similar to the rise by 2% that was observed in Experiment 2. Mohtashamipur et al. (1987) reported an increase in urinary mutagenicity after experimental ETS exposure that is comparable to smoking 4 or 5 cigarettes actively and that would correspond to an ETS-related rise in urinary mutagenicity of 15% relative to that found in smokers of 30 cig/d. This is clearly in con-

trast to the findings of Bos et al. (1983) and the present study's results. Differences in the urine extraction methods might have contributed to this discrepancy. Mohtashamipur et al. (1987) speculate that the urine volume tested (15 mL in the present experiment vs. 50 mL in their study) might have caused this discrepancy. While this might play a role in this study, it is not relevant to the study of Bos et al. (1983), who also tested urine aliquots of about 50 mL. On the other hand, increasing cytotoxicity that interferes with the Ames test when testing urine volumes considerably higher than 15 mL was observed. With the exception of the study by Husgafvel-Pursiainen (1986) from work-related exposure to ETS, all studies, including this one, were performed under experimental conditions. The time- integrated ETS exposure doses are estimated to be similar or somewhat lower in the studies of Bos et al. (1983) and Mohtashamipur et al.(1987), while they are substantially lower in the study of Sorsa et al. (1985) when compared to Experiment 2. Sorsa et al. (1985) reported a nonsignificant elevation of mutagenic activity after ETS exposure that amounts to 70% of that found after smoking, despite the relatively low ETS exposure level. This discrepancy could partly be attributed to the low mutation rates found after smoking and partly to the fact that abstinent smokers (instead of nonsmokers) were used for this experiment. Except for smokers when smoking, it was found that the number of revertants induced by the urine extracts were low and usually less than twice the spontaneous mutation rate. Additionally, no dose-response relationship was observed with urine concentrates of ETS-exposed nonsmokers. This indicates that the criteria of a positive test result as established by Yamasaki and Ames (1977) were not met. Furthermore, the high variability in the urinary mutagenicity, which was observed despite strictly controlled conditions, suggests that yet unknown methodological and endogenous factors clearly outweigh marginal increases in urinary mutagenicity after ETS exposure. Moreover, the relevance of an increased urinary mutagenicity with respect to cancer risk is unclear as of yet. Rüdiger and Lehnert (1988) stated that an increased urinary mutagenicity does not necessarily indicate a higher exposure with genotoxic substances, since the compounds active in the Ames test may be formed from inactive urinary precursors by the in vitro metabolizing system. On the other hand, a negative Ames test does not exclude a genotoxic burden of the organism, since active metabolites may react with cellular constituents in the body and are thus not excreted in the urine. These considerations lead to the conclusion that measuring the urinary mutagenic activity, at least in passive smokers, is not an appropriate method of predicting an increased risk to human health.

Another aspect of the mutagenicity data attracted interest: the relationship of urinary excretion of thioethers and mutagenicity. The intracellular glutathione-S-alkyl-transferase/glutathione system efficiently protects the organism from destructive effects of electrophilic substances. Thioethers (mercapturic acids) are excreted in the urine as final products of this detoxifying pathway (Chasseaud 1979). The mutagenic activity in urine as detected by the S. typhimurium microsome assay may at least partly be caused by electrophilic intermediates or their precursors that escape from reactions in the organism and appear in the urine. Van Doorn et al. (1979) observed rather parallel time courses of urinary thioethers and mutagenicity in smokers who were advised to stop smoking and to start again. No measurable increase in urinary mutagenicity after extreme ETS exposure, despite a significant increase in thioether excretion, was seen. On the other hand, both parameters are significantly increased after smoking. The data are compatible with the assumption that either the mutagenicity in smokers' urines may be caused by compounds that ETS-exposed nonsmokers do not take up in measurable amounts or that the cellular glutathione-S-alkyl-transferase/glutathione system in nonsmokers may detoxify substances that otherwise would reach the urine and could be detected as mutagens by the Ames test.

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