Enhanced excretion of thioethers in urine of operators of chemical waste incinerators

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ABSTRACT Thioether concentrations were determined in urine samples obtained from ten workers in the despatch department ($n = 69$), three chemical waste incinerator operators ($n = 67$), and an analyst ($n = 21$), all working in the same chemical plant. Urine samples ($n = 196$) obtained from non-exposed men, including smokers, served as controls. Enhanced excretion of thioethers was found in urine samples taken from incinerator workers at the end of work. A regular pattern in the time course of the urinary thioether excretion was shown by a non-smoking incinerator worker; end-of-work values were always higher than prework values. This phenomenon was not found in samples obtained from the analyst. These findings suggest that incinerator workers inhale or otherwise absorb electrophilic compounds or their precursors, which are subsequently metabolised to, and excreted as, thioethers in urine.

In assessing the risks to health from chemical substances exposure data as well as toxicity data are required. Recently much progress has been made in detecting toxic properties of chemicals by introducing short-term screening methods for mutagenic and carcinogenic compounds.¹⁻⁵

In the near future the aim must be to investigate and evaluate the toxic properties of all compounds before their large-scale production and use. Toxic compounds must be eliminated from the environment wherever and whenever possible.

Reduction of industrial exposure levels contributes to the safety and health of workers exposed to toxic chemicals. Reliable parameters of exposure are thus of great importance. When the air in the working environment is contaminated with certain known chemicals the most obvious approach is to measure concentrations in the air.

More directly related to health risks, however, is biological monitoring—that is, the measurement of internal exposure through analysis of a biological specimen that contains polluting compounds or their metabolites.⁶ Examples are the determination of hippuric acid in urine after exposure to toluene^{7,8} and the determination of urinary mandelic and phenylglyoxylic acids in exposure to styrene.^{9,10} Whenever possible, selective methods must be

preferred in biological monitoring. Thus far, however, only a limited number of exposure tests have been described and evaluated. In addition, in many instances workers are exposed to a mixture of chemicals. Therefore, non-selective assays also have their value—for instance, as a first step in developing selective tests.

Non-selective assays for determining exposure to toxic chemicals include the determination of mutagens in urine^{11,12} and the determination of mercapturic acids in urine.^{13,14} Mercapturic acids and other thioether compounds are the end products in the detoxication of electrophilic agents through conjugation with glutathione.^{15,16} Recently it was shown that thioethers as well as mutagens are excreted in the urine of cigarette smokers.¹⁷

In the present study we describe the application of a thioether assay to urine samples from operators of chemical waste incinerators. These workers are exposed to a mixture of chemicals including benzene, ethylbenzene, styrene, and many polymeric compounds and their combustion products.

Materials and methods

SUBJECTS

Thirty-four prework and 33 end-of-work urine samples were collected from three operators occupied with chemical waste incineration. A further 69 urine samples from 10 people working in the despatch

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department and 21 samples from a chemical analyst working in the same chemical plant were obtained.

The samples were stored for up to three weeks at 4°C until brought to our laboratory. On arrival they were frozen at -20°C until required. If there are facilities to store urine at -20°C we recommend that urine samples be frozen after collection. Otherwise the samples should be assayed as soon as possible, because a decrease in the urinary thioether concentration of up to 15% (depending on the thioethers present in the samples) may occur during storage for two weeks at 4°C.

To establish normal values for urinary thioether concentrations 196 urine samples were obtained from non-exposed men, including cigarette smokers. These urine samples were collected at random during the day.

CREATININE ASSAY

The creatinine content of the urine samples was measured with an automated analyser method based on the reaction of creatinine with alkaline picrate, as described by Gorter and De Graaff.¹⁸ Urinary thioether concentrations were determined in samples with a creatinine content of 5.0 mmol/l or higher.

THIOETHER ASSAY

Thawed urine samples were centrifuged for five minutes at 3000 g before processing. Aliquot samples of 5.0 ml of clear urine were transferred into glass-stoppered tubes, and the pH was adjusted to 1.5-2.0 with 4 N HCl. After the addition of 8.0 ml of ethyl acetate the layers were shaken vigorously for 15 minutes using a shaking apparatus. The layers were separated by centrifugation at 3000 g for five minutes. After removal of the ethyl acetate layer the extraction procedure was repeated with another 8.0 ml of ethyl acetate.

The collected ethyl acetate layers were evaporated to dryness using a rotary evaporator. The residue was taken up in 2.0 ml of aqua dest. Alkaline hydrolysis was performed on 1.0 ml samples in brown-glass screw-capped tubes by the addition of 0.5 ml of 4 N NaOH, saturation with nitrogen, and keeping the closed tubes in a boiling waterbath for 50 minutes. Then the tubes were cooled in ice for 10 minutes. Under mixing 0.5 ml of 4 N HCl was added. Exactly five minutes later the SH-concentration was determined according to Ellman¹⁹ with slight modifications. A 0.25 ml aliquot sample of the aqueous solution was added to a freshly made mixture consisting of 2.0 ml of 0.5 M phosphate buffer (pH = 7.1) and 0.3 ml of a 5,5'-dithiobis-(2-nitrobenzoic acid)-solution (0.4 mg DTNB per ml of 1% sodium citrate solution). Absorbances were read at 412 nm on a Pye Unicam SP 1750 spectro-

photometer. Corrections were made for the contribution of the extract and of the DTNB-solution to the absorbance. The SH-concentration was calculated from the corrected absorbance and the molar absorbance of the reference compound N-acetyl-L-cysteine in the SH-determination.

Thioether concentrations of urine samples were expressed in mmol SH/mole creatinine. No corrections were made for the presence of thiols, disulphides, or thioesters in the extracts.

Results and discussion

Application of the thioether assay to urine samples of the non-exposed people showed the presence of a background, as is shown in figure 1. These samples were collected at random during the day and included samples obtained from smokers.

The limits of the normal values were determined by application of the procedure described by Rümke and Bezemer.²⁰ The mean value of the urinary thioether concentration was 3.8 mmol SH/mole creatinine and the outer limit of the percentile P₉₅ ($\gamma = 90\%$) was calculated to be 5.9 mmol SH/mole creatinine. The latter should be regarded as a warning limit; by this method of statistical analysis it can be constituted with 90% confidence that at most 5% of a non-exposed population have urinary thioether values above 5.9 mmol SH/mole creatinine.

Figure 2(a) shows the prework and end-of-work distribution pattern of thioether values in urine samples of chemical waste incinerator operators. The mean value in prework urine samples was

Fig 1 Distribution of thioether concentrations in a group of 196 urine samples obtained from non-exposed men (including cigarette smokers).

Fig 2 Thioether concentrations in prework and end-of-work urine samples obtained from (a) three operators of chemical waste incinerators ($n = 67$) and (b) ten people working in the despatch department ($n = 69$).

3.9 mmoles SH/mole creatinine with 3% of the values exceeding the percentile P_{95} mentioned above. The mean value in the end-of-work samples was 5.1 mmoles SH/mole creatinine, and 24% crossed the warning limit.

Such a difference was not found in samples from workers of the despatch department (fig 2(b)). The mean values of pre- and end-of-work urines were

Fig 3 Time course in urinary excretion of thioethers by workers of a chemical plant. (a) Samples from a non-smoking incinerator worker. (b) Samples from a non-smoking analyst. Urinary thioether concentrations are in mmoles SH/mole creatinine.

3.8 and 4.0 mmoles SH/mole creatinine respectively with 6% and 9% of the values exceeding the P_{95} limit. Enhanced thioether excretion in the end-of-work urine of incinerator workers is unlikely to be caused by cigarette smoking; only one of the three incinerator workers is a smoker whereas four out of ten despatch workers are smokers.

Figure 3(a) shows the time course of the thioether excretion in urine of one of the non-smoking incinerator workers during a 12-day period. Each day the postwork thioether value was higher than the values at the beginning of a work period. Such regular patterns were not found in urine samples obtained from a chemical analyst (fig 3(b)). We conclude, therefore, that the enhanced thioether excretion is due to a diurnal rhythm. These findings indicate that there is a positive relation between the exposure of the operators during incineration work and the excretion of thioethers in urine. On the other hand, the exposure of the analyst and the workers of the despatch department to toxic chemicals cannot be excluded. From these results no conclusions can be drawn as to which compound(s) caused the enhanced excretion of thioethers in the urine of the chemical waste incinerator operators. Most probably, however, electrophilic compounds or their precursors are inhaled or absorbed through the skin and subsequently metabolised to and excreted as thioethers. Identification of the excreted thioethers would open the possibility to develop a selective assay, allowing a more quantitative estimation of internal exposure.

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