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Note

Urinary dimethylbenzoic acid excretion as an indicator of occupational exposure to white spirit

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Distillates of crude oil are also used as solvents marketed under names such as Stoddard solvent, white spirit, mineral turpentine or solvent naphtha. Their compositions vary widely depending on the boiling ranges of the fractions. However, aliphatic alkanes dominate in the most versatile mixtures with boiling ranges of 150°C to 200°C. The complex composition of the mixtures — over 200 compounds are frequently encountered — does not allow the identification of the causative agents of the occupational diseases related to the exposure [1]. Our own experimental studies with rats implicate the aliphatic alkanes [2, 3].

Virtually all commercially available white spirit in Finland originates from a single refining company. Its own quality control has shown that the product with a boiling range of 152°C to 182°C has remained very stable in its composition over recent years. Typically, it contains 11% aromatics with 1% trimethylbenzene isomers by weight [2]. When absorbed in the animal body, the

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relative concentrations of individual compounds remain comparable to those in the original liquid without an accumulation. Specifically, the total body burden in experimentally exposed rats is correlated to the inhaled dose [2].

Exogenous lipophilic chemicals are oxidized in the body to render them water-soluble for the excretion in the urine or faeces. The metabolites of aliphatic and alicyclic molecules have been characterized to some extent [4, 5]. Their analysis presents, however, many technical problems while the demonstration of aromatics is relatively simple because of their ultraviolet absorbing properties. Thus, trimethylbenzene-derived metabolites in the rabbit have shown to include dimethylbenzoic acid isomers [6]. Analogous to this, excretion of the same acid isomers by white spirit-exposed rats correlates to the absorbed dose despite the fact that the parent trimethylbenzene isomers represent a minor fraction of the mixture [3].

This communication describes a novel liquid chromatographic method for the quantitation of occupational exposure to white spirit vapour by the analysis of the dimethylbenzoic acid isomers.

SUBJECTS AND METHODS

Ten car washers participated in the study. They were divided into three groups according to their exposure to white spirit. Five people were exposed to 118—150 mg/m^3, three to 152—234 mg/m^3 and two to 420—500 mg/m^3 white spirit vapour calculated as a 6-h time-weighted average concentration in their breathing zone (Niemelä et al. [7]). All were exposed to vapour evaporated from the mist applied by air guns to remove organic stain from the car chassis. The hygienic measurements were carried out on Thursdays, and the urine specimens were collected after the shift in the afternoon of the same days.

The urine samples (5 ml) were boiled in stoppered tubes for 1 h after the addition of 5 ml of 50% sodium hydroxide to hydrolyse the dimethylbenzoic acid glycine conjugates [6]. The hydrolysates were neutralized with sulphuric acid and extracted three times with dichloromethane. The extracts were combined, dried with sodium sulphate and evaporated under vacuum. The residue was dissolved in an acetic acid—water—methanol mixture (2 ml, 1:39:60, v/v/v). 2,3-, 2,4-, 2,5-, 3,4- and 3,5-dimethylbenzoic acids served as standards in the liquid chromatographic analysis. The apparatus (Pye Unicam LC 3-XP) was equipped with a 20-μl sample loop and a 15-cm column (5 μm ODS Hypersil, Shandon). An isocratic separation mode was used with the acetic acid—water—methanol (1:39:60, v/v/v) mixture as an eluent. A UV detector (Pye Unicam LC) was empoyed at 238 nm. This procedure yielded a good separation of 2,3- and 3,5-dimethylbenzoic acids while the other isomers migrated as a combined peak (see Fig. 1). The recovery of added isomers (2.5 μg/ml, six experiments) in the extraction and analysis was 95 ± 1%. The coefficient of variation at the same concentration level was 0.11 ± 0.01.

To find out the correlation with the vapour exposure, all isomer concentrations were added together, and the sum was corrected for the creatinine excretion determined by the alkaline picric acid method. The least-squares method was used to find out the mathematical correlation.

RESULTS AND DISCUSSION

No dimethylbenzoic acid was detected in the unexposed controls (Fig. 1) while a good separation of the acid isomers from the solvent front and other aromatic constituents was found in the exposed subjects. The mass spectral identification of the peaks revealed configurations reported for the isomers [6].

The mean excretion rates by the exposed workers were linearly correlated to the total hydrocarbon exposure (Fig. 2). The ranges in the excretion are very likely caused by the biological variables as the accuracy of the method is good. The hygienic correlations were done to the time-weighted average which tends to smooth out the effect of the solvent peaks associated with the spray gun operations. On the other hand, the body functions in a way as an integrator [8].

The biological monitoring method offers several advantages in comparison to the hygienic surveys. The exposure is difficult to characterize because the hydrocarbons evaporate rapidly from the sprayed mist [9] causing sharp peaks in the exposure profile. The urine test is often acceptable to the workers, and it relates better to the individual exposure than hygienic measurements so that the exposure may be more accurately evaluated.

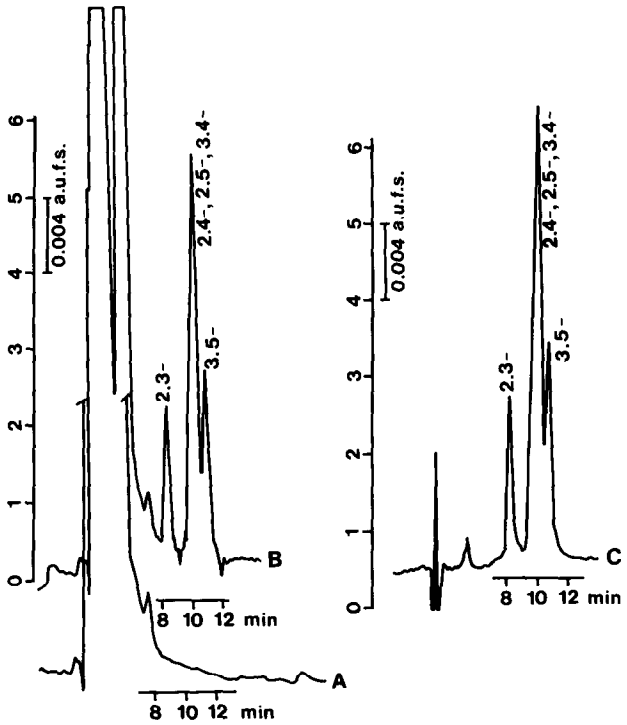

Fig. 1. Liquid chromatographic analysis of dimethylbenzoic acid isomers in urine of a white spirit vapour-exposed worker (B) and of unexposed control (A). 2,3- (2.3 μg/ml) and 3,5-dimethylbenzoic acid (2.2 μg/ml) elute at 8.1 min and 10.5 min, respectively, while 2,4-, 2,5- and 3,4-dimethyl isomers (2.2, 2.4 and 2.0 μg/ml, respectively) at 10.0 min in the standard preparation (C) migrate combined. Note that the control urine does not contain dimethylbenzoic acid.

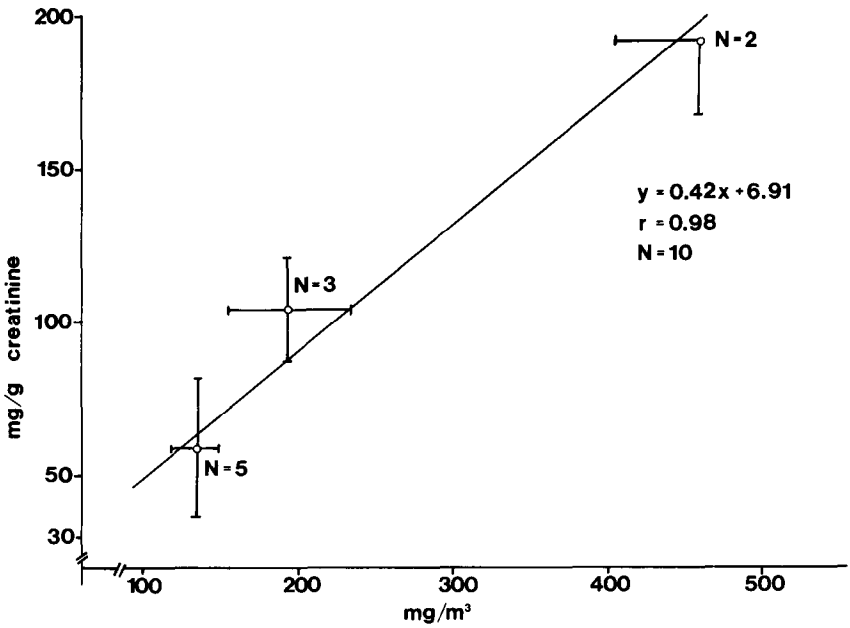

Fig. 2. The white spirit vapour exposure (x) is correlated to the sum concentration of urinary dimethylbenzoic acid isomers (y) in a linear fashion given by the equation. Bars indicate ranges and N the number of subjects. The correlation coefficient to the mean concentration is given by r.

It is likely that the trimethylbenzene isomers in the white spirit mixture have a negligible role in its toxicity while the aliphatic *n*-nonane may be significant in this respect [3]. The analysis of its metabolites is hampered by the fact that they are in all probability fatty acids and alcohols whose pharmacokinetics and participation in the intermediary metabolism are unknown. Therefore, the dimethylbenzoic acid isomers offer a versatile alternative despite their minor share of the total mixture.

As discussed above, the trimethylbenzene shows similar kinetics to those of the general white spirit as a whole. Therefore, the excretion is dependent on the concentration and composition of the inhaled vapour. If the white spirit originates always from a single producer there is no practical limitation on the reliance on the biological monitoring method. The usage of aromatic-free mixtures, of course, abolishes this possibility. The result is the same when employing products with different boiling ranges possibly originating from different refineries. Therefore, the analysis of excretion of the metabolites with a particular white spirit brand should be carried out before the adoption of the biological exposure test.

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