Excretion of N-mononitrosopiperazine in urine in workers manufacturing piperazine*

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Summary. Piperazine has been shown to nitrosate in vivo to N-mononitrosopiperazine (MNPZ) by oral intake. Urine from workers exposed to piperazine in a chemical plant was analysed for nitrosamines by gas chromatography-thermal energy analysis. In five out of 11 exposed cases, MNPZ excretion in urine was 0.3 to 4.7 μg/24 h (during and after a work shift). In four cases, MNPZ was detected in some urine samples, and in two cases MNPZ was not detected (< 0.1 ng/ml). The individual excretion was strongly dependent on piperazine exposure, which ranged from 0.06 to 1.7 mg/m³ (time-weighted average; Spearman's rank correlation 0.78, \( P = 0.01 \)). The MNPZ excretion showed no significant correlation with nitrite or nitrate in saliva (both: \( r = 0.50, P = 0.10 \)).

Key words: N-mononitrosopiperazine – Endogenous formation – Occupational/work environment – Piperazine – Urinary excretion

Introduction

Most N-nitroso compounds are mutagenic in plate assays and carcinogenic in experimental animals. Positive evidence of carcinogenic effects in man is, however, still lacking; a major problem in this field is the characterization of N-nitroso compound exposure, which is very complex and emanates not only from foodstuffs and the general environment, but also from such disparate sources as baby pacifiers, drugs and the work environment.

The secondary heterocyclic aliphatic amine piperazine (1,4-diazacyclohexane) and its salts have a wide range of industrial applications, but are mostly used as anthelmintic drugs in veterinary and human medicine.

Piperazine nitrosates rapidly in vitro to N-mononitrosopiperazine (MNPZ), which in turn can nitrosate to \( N,N' \)-dinitrosopiperazine (DNPZ). The latter is mutagenic and carcinogenic. MNPZ has also shown mutagenic (Braun et al. 1977) and carcinogenic effects, which, however, might be attributed to in vitro or in vivo formation of DNPZ (Love et al. 1977).

Piperazine has been shown to nitrosate in vivo in fasting volunteers taking therapeutic doses of the drug (Bellander et al. 1985). MNPZ is formed in the stomach and, to some extent, excreted in the urine.

The aim of the present study was to investigate if occupational exposure to piperazine can also lead to in vivo nitrosation, in spite of the intake being airborne and the dose much smaller than the therapeutic one.

Materials and methods

The plant. The plant employs 85 shift workers and manufactures a variety of chemicals, mainly pharmaceutical and veterinary products, surface-active agents, organic chelating agents, and micronutrients for agriculture. Piperazine and its salts are quantitatively dominating.

Study group. Nine male workers (Nos. 1 to 9), aged 22 to 52 years, were monitored during a 12-h exposure during day or night. Two of these (Nos. 7, 9) were also monitored during another 12-h exposure (called 7', 9'). Two of the other nine workers were also monitored during a shorter exposure period (called 1', 2'). They were all occupied in the manufacturing of either anhydrous piperazine or piperazine hexahydrate, where exposure to piperazine is predominantly as vapour. At these two sites there was no other production at that time.

Two of the exposure cases were omitted (9 and 9'), the first because the analysis of MNPZ was performed by Laboratory B only and in that case without recovery control. The second
case was omitted because there was no exposure (<0.03 mg/m³ piperazine as a time-weighted average).

Routine clinical tests for liver and kidney functions were all within the normal range. The subjects were on free diets and five were current smokers (Nos. 1, 2, 3, 5, 7).

Sampling schedules. Breathing zone samples for determination of piperazine in air were taken over the whole working day, as a rule in 2-h intervals.

Immediately before exposure, a urine sample was collected from the workers for determination of MNPZ, DNPZ, piperazine, nitrite and nitrate. During exposure all urine was sampled at 4-h intervals. The collection was continued after the end of exposure and samples were taken after 4 and 12 h.

Saliva. Samples for analysis of nitrite and nitrate were obtained from both exposed and controls before work, and in addition, just before and 1 h after each meal and at the end of the work shift.

Sampling procedures, storage, transportation and analysis

MNPZ in urine. Solid sodium hydroxide was added in advance to polyethylene sample bottles (aiming at 2 g/l) to prevent artifactual formation of nitrosamines, with the exception of the pre-exposure sample and the last sample during the workshift. Immediately after sampling a urine specimen, the pH was, when necessary, adjusted to 10 to 12 with solid NaOH. Four aliquots of 20 ml were drawn from each sample. After coking, and less than 24-h storage at 4°C, two aliquots were sent to our laboratory (A) and the remaining two to another laboratory (B). The samples were shipped overnight in ice which had not melted completely on arrival.

At Laboratory A, the urine was analysed according to a previously described method (Österdahl and Bellander 1983): Extraction to dichloromethane was from a kieselguhr column, concentration and gas chromatography-thermal energy analysis (GC-TEA). External MNPZ standards were applied. GC-TEA was also used at Laboratory B, but after derivatization with trifluoroacetic anhydride (J.R.A. Pollock personal communication). In this case N-nitrosodipropylamine was used as the internal standard in the GC-TEA determination.

Sample aliquots spiked with MNPZ (5 μg/l) for recovery control, or nitrite (2 mg/l) for artifact control, were randomly intermingled in each shipment (except exposure case 9). The recovery of standard additions of MNPZ was established to 58 ± 13 (n = 22) and 106 ± 50% (n = 21) for Laboratories A and B, respectively, during the study. No artifactual formation of MNPZ could be detected in samples with added nitrite.

Sampling and analysis of piperazine in air (Hagmar et al. 1982) and urine (Bellander et al. 1985) were as previously described, with minor modifications.

Saliva for determination of nitrite and nitrate was sampled, without stimulation, in plastic vials containing 1 ml 0.5 mol/l ferric chloride and 2 ml 0.6 mol/l sodium carbonate for protein denaturation. The samples were immediately frozen, and analysed within a week. After thawing, dilution and filtering, an aliquot was drawn for nitrite analysis by a modified colorimetric method (Sen 1978). Another aliquot was reduced on a Cd column and analysed for nitrite, which was the sum of nitrite and nitrate in the sample. Recovery of additions of nitrite and nitrate to saliva, before freezing, was close to 100%.

Urine samples for analysis of nitrite and nitrate were drawn from the alkalized urine and stored at 4°C until analysis. Urine samples spiked with nitrate were prepared from each participating individual on each occasion, for calculation of recovery after storage. The samples were analysed within three weeks and the storage losses were less than 15% (n = 69). The analysis was performed in a way similar to that for saliva, but with control for individual sample recovery of added nitrate. Nitrite was never detected in urine (<0.5 mg/l; <7 μmol/l).

Creatinine in urine. To verify that the exposed workers had collected all urine during and after exposure, creatinine was analysed according to the kinetic Jaffé method, in all urine samples with enough volume (n = 48 of 51). The calculated 24-h excretion was normal (≥12 mmol) in 12 exposure occasions. In exposure case 3, the urine sampling is suspected of being incomplete (creatinine was only 10.2 mmol/24 h).

Bacteria in urine. A part of the non-alkalized pre-exposure sample was stored at 4°C for up to 33 h. A 10-μl aliquot and 10 μl of diluted urine (1:100) were cultivated on blood agar plates containing 4% (w/v) of defibrinated horse blood. Significant growth was never noted (<100 colonies for undiluted urine and <10 colonies for diluted).

Chemicals

MNPZ stock standard solution was obtained from Thermo Electron Corporation (Waltham, MA, USA). DNPZ stock standard solution was made from synthesized DNPZ. Dichromomethane, methanol and kieselguhr (Extrelut) were analytical grade (E. Merck AG; Darmstadt, FRG). Hydrochloric acid, ferric chloride, sodium carbonate, ammonia, sulfanilic acid, N-(1-naphthyl)ethylenediamine dihydrochloride, acetic acid, sodium nitrite, sodium nitrate, and sodium hydroxide were all analytical grade. Anhydrous piperazine was obtained from a local supplier. Water was deionized or distilled. Blood agar base was from Oxoid (Basingstoke, Great Britain).

Statistical methods. The arithmetic means and the standard deviations were calculated for variables that were approximately normally distributed. For others, the median and range are given. In correlations where the independent variable was approximately normally distributed, Pearson’s linear correlation coefficient (r) was calculated. When the independent variable was judged not to be normally distributed, Spearman’s rank correlation (rₛ) was calculated. When appropriate, correction for split ranks (Siegel 1956) was applied. Unless otherwise stated, the computations of correlations are based on individuals, i.e. for Subjects 1, 2 and 7 the means of the two exposure occasions are used. Analysis of variance with a covariate was used for nitrate and nitrite in saliva. P-values of confidence are one-tailed. Significant correlations refer to P < 0.05.

Results

Nitrosamines in urine

In five out of 11 exposed cases, MNPZ excretion in urine could be followed during and after exposure (Fig. 1). In four of these the level was still above the detection limit in the last sample. The total excretion over 24 h was 0.3 to 4.7 μg/person. In four other cases MNPZ was detected (0.1 to 0.6 μg/l) in some urine