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EXPOSURE TO *N*-NITROSODIMETHYLAMINE IN A LEATHER TANNERY

D. P. ROUNBEHLER, I. S. KRULL, E. U. GOFF, K. M. MILLS, J. MORRISON, G. S. EDWARDS and D. H. FINE

New England Institute for Life Sciences, Waltham, MA 02154

J. M. FAJEN and G. A. CARSON

National Institute of Occupational Safety and Health, Division of Surveillance, Hazard Evaluation and Field Studies, Cincinnati, OH 45226

and

V. RHEINHOLD

Massachusetts Institute of Technology, Chemistry Department, Cambridge, MA 02139, USA

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Abstract—*N*-Nitrosodimethylamine (NDMA) was detected in the atmosphere of a leather tannery at levels of up to 47 $\mu g/m^3$. As far as we know this is the highest occupational exposure to this potent animal carcinogen yet discovered. NDMA was detected in all the air samples taken at different sites in the tannery on three visits in 1978. The highest level was in the re-tanning, colouring and fat-liquoring area. The average atmospheric level of NDMA on the first and second visits was 19 $\mu g/m^3$. The levels were considerably reduced on the third visit, when the tannery had been thoroughly cleaned, and ranged from 0·1 to 3·4 $\mu g/m^3$. Small amounts of NDMA were also found in process and waste water. The tannery examined uses basic processes that are standard within the industry; therefore, NDMA may also contaminate the atmosphere of other tanneries. Exposure of tannery workers to NDMA suggests an area for epidemiological studies, as well as for further analytical work, which could contribute to a knowledge of the possible carcinogenic effects of NDMA in man.

INTRODUCTION

Since 1956 when Magee and Barnes demonstrated the carcinogenic effects of *N*-nitrosodimethylamine (NDMA) in the rat, NDMA has become the most extensively studied member of this class of carcinogens. It has been shown to cause cancer in a variety of organs in virtually every species in which it has been tested (Magee, Montesano & Preussmann, 1976). A recent study by the International Agency for Research on Cancer (1978) concludes that: "the general (human) population may be exposed to low levels of *N*-nitrosodimethylamine; however, no exposed group suitable for an epidemiological investigation has yet been identified". We report here on the possibility that leather tannery workers may constitute just such a group. We are unaware of any epidemiological studies that have specifically focused on such workers, although there have been studies on bootmakers (Acheson, 1976) and "leather operatives" (Viadana, Bross & Houten, 1976).

As part of our study of human exposure to *N*-nitrosamines, we have been examining work environments in which amines are used. Leather tanning was chosen because dimethylamine sulphate is used as a depilatory agent in some tanneries (Walker, Gordon, Thomas & Ouellette, 1976). For the initial study we chose a tannery in New England employing about 300 workers and processing about 2000 hides per day in which the method of tanning used was characteristic of the industry (New England Tanner Club, 1977).

EXPERIMENTAL

Tannery visits. The selected tannery was in a one-storey building, divided into wet- and dry-operations areas. Some sites were used for several processes; for instance, at the re-tanning site, colouring, dyeing and fat-liquoring were also carried out. Three visits were made to the factory and the entire leather tanning and finishing processes were studied. On the first visit (11 April 1978) six air samples representing a cross section of the air in the tannery were collected and on the following visit (13 April 1978) 20 air samples were taken. In addition, on the second visit, numerous bulk samples were taken. The third visit was made on 1 June 1978 when 10 air samples and numerous bulk samples were collected. A total of 27 bulk samples were examined for *N*-nitroso compounds and these consisted of 11 of chemicals or chemical mixes, two of hide and leather, two of waste water, and 12 of process water from the wet-operations area of the tannery.

Apparatus. Laboratory equipment, including gas- and high-pressure liquid chromatographs (GC, HPLC), thermal energy analyser (TEA), fume hood and detectors, was located inside a fully equipped mobile laboratory (Krull, Fan, Fine, Young & Egan, 1978), which was parked near the tannery. An HPLC–TEA was constructed (Fine, Rounbehler, Pellizzari, Bunch, Berkley, McCrae, Bursey, Sawicki, Krost & DeMarrais, 1976) by combining a high-pressure pump (Altex model 110, Altex Corp., Berkeley, CA) with an injector (Rheodyne model 7120, Altex Corp.), a stain-

less steel column (4 × 39 mm) packed with 10 μ Lichrosorb Si60 (E. Merck Labs, Elmsford, NY) and a TEA detector (TEA model 502LC, Thermo Electron Corp., Waltham, MA). For GC–TEA, a single column isothermal GC (Thermo Electron Corp.) was constructed from 14 ft of stainless steel tubing (outer diameter 1/8 in.) packed with 10% Carbowax 20M containing 0·5% KOH on Chromosorb W HP (80–100 mesh) interfaced to a TEA (Fine & Rounbehler, 1975). The column temperature was 160°C, and the carrier gas was argon (flow-rate 15 ml/min).

The GC–flame ionization detector (FID) used was a Hewlett-Packard 5710 (Palo Alto, CA) equipped with the same GC column used for GC–TEA.

Chemicals. Dilute standard solutions of NDMA and *N*-nitrosomorpholine (NMOR) were obtained from the Analytical Services Laboratory of Thermo Electron Corp. Dichloromethane, isooctane, acetone and diethyl ether, of a grade distilled in glass, were obtained from Burdick and Jackson (Muskegon, NJ). AnalaR grade potassium hydroxide (KOH), sodium sulphate, sulphuric acid and dimethylamine hydrochloride were used.

Collection and analysis. Air samples were collected (Fine, Rounbehler, Sawicki & Krost, 1977) in 1 N-KOH traps by passing air at a flow rate of between 1 and 2 litres/min through a glass impinger containing 45 ml of 1 N-KOH. For analysis, the KOH solutions were extracted with dichloromethane and concentrated at 52°C in a Kuderna-Danish evaporator (Kontes, Vineland, NJ) to a volume of 0·5–1·0 ml. All of the concentrates were analysed by GC–TEA and/or HPLC–TEA (Fine, Rounbehler, Silvergleid & Ross, 1977). For NDMA and NMOR, the HPLC–TEA was operated isocratically using 2 ml/min of *n*-hexane–acetone (85:15, v/v). HPLC–TEA screening for other *N*-nitrosamines (Fan, Krull, Ross, Wolf & Fine, 1978) was carried out using the following solvent systems: acetone–isoctane in the ratios 50:50, 25:75 and 85:15 v/v.

All quantitative data on atmospheric NDMA were obtained from air samples collected in 1 N-KOH traps. In order to demonstrate that NDMA was not being formed in the traps, three other trapping techniques were also used. Two air samples were collected on solid dry Tenax GC material (obtained from Applied Science, State College, PA; Fine *et al.* 1976). The 10 × 1·5 cm cartridges were eluted with 75 ml of ether, dried over sodium sulphate, filtered and concentrated to 0·5 ml on a Kuderna-Danish evaporator. Two samples each were also collected on a PREPTUBE (model 117 Thermo Electron Corp.) and on a cellulose based material (Tampax tampon, Tampax, Inc., Palmer, MA) both of which had been soaked in 1 N-KOH. All four cartridges were eluted with dichloromethane and the eluate concentrated on a Kuderna-Danish evaporator. The three alternative techniques, after correcting for low efficiencies and recoveries, gave data that were indistinguishable from those obtained with the KOH traps.

For mass-spectral analysis of NDMA in the air samples, the contents of several traps were combined, concentrated and quantitated by HPLC–TEA, GC–TEA and GC–FID. The structures of these chromatographically-purified TEA-positive fractions were confirmed by one of two mass-spectral methods, depending on the amount of sample available. For microgram quantities of sample, low resolution gas-liquid chromatography–mass spectrometry (GC–MS) was used and the entire mass range of each eluting GC peak was magnetically scanned at 4 sec intervals using a Hitachi RMU6 instrument (Perkin-Elmer, Norwalk, CT). The combination of GC retention time and mass spectral identity was used to confirm structure. Nanogram quantities of sample were analysed by high resolution GC–MS (Gough, Webb, Pringeur & Wood, 1977) using a Varian MAT Ch-7 mass spectrometer (Bremen, Federal Republic of Germany). The magnetic analyser was set to resolve the exact mass of NDMA ($C_2H_6N_2O$–74·0480; resolution ~9000, peak-matched against a perfluoroalkane standard), from other background materials. Repetitive scanning over the *m/e* 74 nominal mass region resolved four minor components only one of which increased coincident with GC elution time of NDMA. Sample analysis and the detection of an exact mass fitting the composition for the molecular-ion of NDMA combined with coincident GC retention time, were used to confirm the presence of NDMA.

For the solid and chemical samples, 1 g was dissolved or soaked (for 30 min) in 5 ml of acetone and then filtered through approximately 2 g of sodium sulphate. The filtrate was analysed by GC–TEA and HPLC–TEA. For the water samples, 15 ml was loaded onto a PREPTUBE which had been presoaked with 15 ml of dichloromethane. The PREPTUBE was eluted with 30 ml (3 × 10 ml) dichloromethane, and the eluate concentrated in a Kuderna-Danish evaporator. The concentrate was analysed by GC–TEA and HPLC–TEA. The detection limit was 0·1 μg/g for solid and chemical samples and 0·001 μg/ml for the water samples.

RESULTS AND DISCUSSION

On the first visit to the tannery NDMA was found in all six air samples at levels ranging from 6 $\mu g/m^3$ in the spray finishing area to 33 $\mu g/m^3$ near the chrome-tanning operation. All the air samples taken inside the tannery on the second visit contained NDMA, including those collected in the lunch room (0·1 $\mu g/m^3$) and in the shipping room (1·4 $\mu g/m^3$), although the levels in the grading and staking areas were not significant. The combined data from both visits are shown in Fig. 1. The highest NDMA level of 47 $\mu g/m^3$ was found in the re-tanning, colouring and fat-liquoring area. The average amount of airborne NDMA found on the two visits was 19 $\mu g/m^3$. On both the first and second visits, the floor was partially covered with decaying animal flesh and fat. The odour of putrified flesh was overpowering to the uninitiated. In addition, parts of the floor were wet and slippery with process water. Because of the cool weather, ventilation was by only a few fans mounted near the ceiling.

In addition to NDMA, NMOR at a level of 2·0 $\mu g/m^3$ was found in three air samples taken in the chemical doping area on the second visit. Two of the samples at this location were collected using a Tenax GC dry trap (Fine *et al.* 1976). The identity of the NMOR was confirmed by its retention time on both GC–TEA and HPLC–TEA.

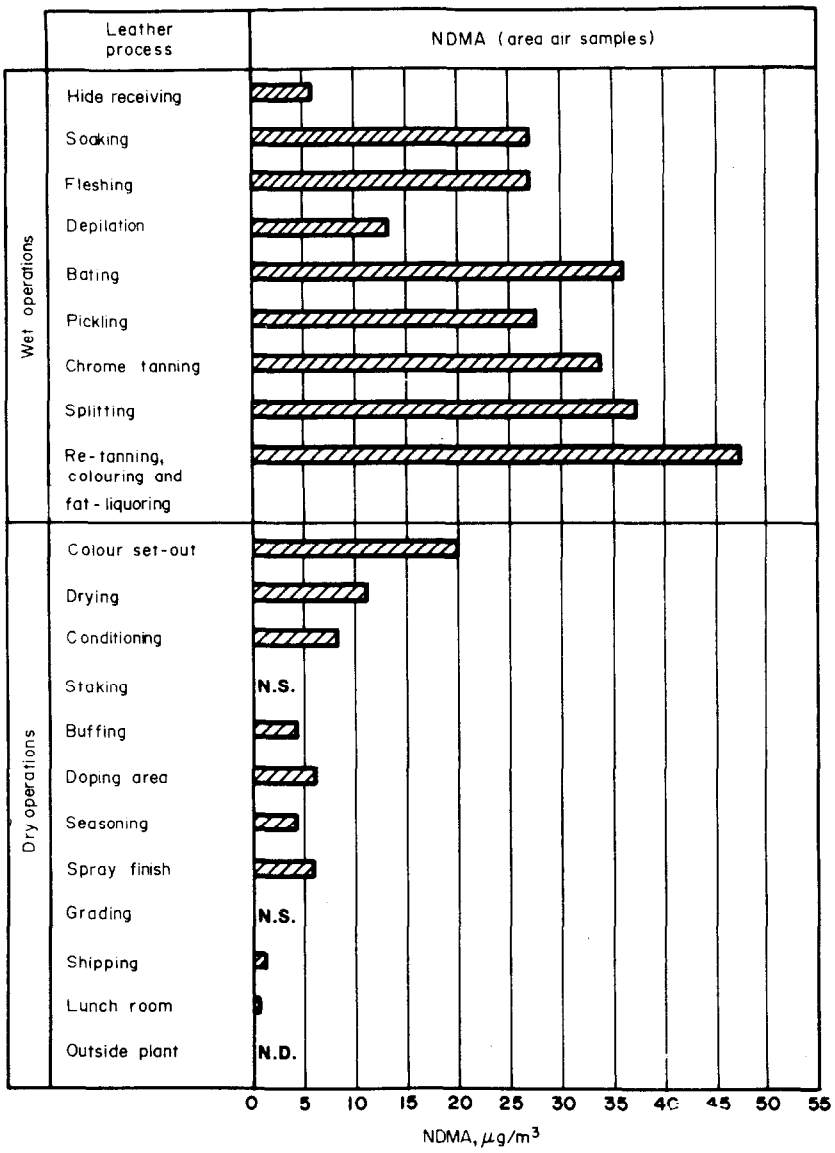

Fig. 1. The highest levels of NDMA in the atmosphere at various stages of the tanning process (combined data from the first two visits): ND = not detected; NS = not significant.

By the third visit the tannery had been thoroughly cleaned. The floors were clean and dry, there was no sign of decaying flesh or fat and no odour of putrified flesh. In addition, the hot weather necessitated the opening of all the ventilation windows and doors. On this visit NDMA was again found in the atmosphere at all sites, but the levels were considerably reduced, ranging from 1·1 to 3·4 $\mu g/m^3$.

Only four of the 27 bulk samples contained NDMA (Table 1). The highest level of NDMA (0·5 $\mu g/g$) was found in a sample from a 36·5% aqueous solution of dimethylamine sulphate. According to plant personnel, 117 litres of dimethylamine sulphate are used each day in the depilation process. This would amount to less than 60 mg NDMA/day from the pre-contaminated amine. NDMA (0·0014 $\mu g/ml$) was also found in the process water of the re-lime pit. This process water contained 5·7 litres of dimethylamine sulphate in 8000 kg of lime-saturated water and is used to remove hair from the raw hides. The other samples which contained NDMA (0·004 $\mu g/ml$ and 0·006 $\mu g/ml$) were waste-water samples from the tannery outlet pipe collected at the local municipal waste-water treatment plant. We were informed that the amount of waste water entering the waste-water treatment plant from the tannery was about $3·8 \times 10^6$ litres/day. Approximately 20 g of NDMA would be required to produce 0·006 $\mu g/ml$ in this amount of water (assuming no losses by evaporation, breakdown, etc.). A sample of the salted hide was also examined for NDMA, but none was detected (detection limit 0·5 $\mu g/g$). This salted cowhide was further examined by placing a 1 g piece in 5 ml of sulphuric acid (pH 3) containing 50 mg of dimethylamine hydrochloride for 4 hr at 25°C. This test was carried out to determine if the raw cowhide itself contained any material which could possibly nitrosate dimethylamine, even under ideal conditions. At a detection

Table 1. *NDMA levels in liquid and solid bulk samples**

Sample description	NDMA (μg/g)
Chemicals	
Azo Rubine Dye	ND
Penetrator L-219	ND
Nigrosine Blue L	ND
Polar Sol 5	ND
Betz Formula NA-6	ND
Boiler rust inhibitor mix	ND
Ammonia paste wash	ND
P-Nitrophenol	ND
KITO-40 (fungicide)	ND
Fresh brine	ND
Aqueous dimethylamine sulphate (36·5%)	0·5
Leather samples	
Chrome-tanned leather	ND
Raw-salted cowhide	ND
Waste water	
Beam-house waste water	0·004
Tanning-house waste water	0·006
Process water	
Re-lime pit	0·0014
Bating solution (two samples)	ND
Pickling solution (two samples)	ND
Chrome-tanning solution (two samples)	ND
Final rinse from chrome tanning (two samples)	ND
Wash out of colouring	ND
First rinse from fat-liquoring	ND
Final rinse from fat-liquoring	ND

ND = Not detected

*Combined data from visits 2 and 3.

limit of 0·04 μg/g neither NDMA, nor any other TEA-responsive compounds (*N*-, *C*- and *S*-nitro and nitroso) were found.

The NDMA impurity in the dimethylamine sulphate used in the depilation step is insufficient to account for either the atmospheric or aqueous NDMA levels. If the dimethylamine moiety were somehow nitrosated in the tannery, then sufficient dimethylamine sulphate is used daily to account for the observed NDMA. However, a possible nitrosating agent has not been identified. Analysis of the various process solutions used in the wet area where the NDMA levels are highest did not reveal the source of the NDMA. We do not know how the NDMA in the tannery is being produced, and are unable to speculate as to a possible mechanism of production. Much further work is needed to determine the source of the NDMA in the tannery.

Although atmospheric NDMA was not found outside the original tannery, the most recent field studies, carried out in August 1978 did show NDMA to be present in the air immediately adjacent to tanneries in New Hampshire (0·10 μg/m^3) and in New Jersey (0·15 μg/m^3). It was concluded from these very limited studies that NDMA is probably present in and near most tanning facilities.

The significance of these findings may be inferred from the results of a recent study by Moiseev & Benemansky (1975). They reported that 30 male Wistar rats breathing air containing 220 μg NDMA/m^3 continuously for 25 months showed an increase in malignant tumours from 13% in the controls to at least 83% in the treated animals. These tumours were mainly of the liver and kidney. In the same experiment, female rats were exposed for 25 months and Balb/C mice (both male and female) for 17 months. These groups showed significant increases in benign tumours, especially of the lung and kidney. We estimate that the male rats in this study were exposed to NDMA at 525 μg/m^2 of body surface area/day (the rats were assumed to have an average weight over the experimental period of 400 g, a body surface area of 0·054 m^2 and to breathe 0·09 litres/min). This represents a total exposure to 400 mg/m^2 (54 mg/kg) over the course of the experiment, an NDMA dose similar to those reported by other laboratories to cause tumours in several species when administered both by the oral and inhalation routes (Clapp & Toya, 1970; Druckrey, Preussman, Ivankovic & Schmahl, 1967; Terracini, Magee & Barnes, 1967; Tomatis & Cefis, 1967).

Assuming that an average worker weighs 70 kg (1·85 m^2 body surface area) and breathes 20 litre/min for an 8-hr working day, then a worker breathing airborne NDMA at 47 μg/m^3 in the wet process area, will be exposed to NDMA at a level of 240 μg/m^2 of body surface area/day. Thus the highest exposure level found corresponds to only slightly less than half the exposure which caused an increase in the incidence of malignant tumours in male rats from 13 to 83%. Even in the shipping room the exposure is 7 μg/m^2/day or approximately 1% of the dose used in the rat study.

As far as we know, this is the highest level of

chronic exposure to NDMA yet reported. Since certain categories of skilled workers (e.g., master tanners and hide splitters) have usually been employed in tanneries, especially the wet process area, for many years, we believe that a strong case can be made for further monitoring in other tanneries and for the initiation of corrective measures to reduce worker exposure. It also seems likely that these workers may be the first population in which the potential hazards of NDMA in humans can be assessed.

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