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# N-Nitroso Compounds in the Ambient Air of Metal Factories Using Metal-Working Fluids

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N-nitroso compounds are undesired industrial and environmental pollutants. They represent a major class of important chemical mutagens, carcinogens, teratogens, and immunotoxic agents, which have been described as a serious hazard to human health (Fuchs et al. 1995; Fadlallah et al. 1996; 1994a; 1994b; 1990a; Desjardins et al. 1992; Preussmann and Stewart 1984). In 1976, attention was focused on the occurrence of nitrosamines in the air and particularly in the workplace (Bretschneider and Matz 1976). In later studies, several populations were shown to be potentially exposed to volatile and non-volatile N-nitrosamines at significantly higher than background levels (Fadlahah et al. 1990b; Preussmann and Eisenbrand 1984; Fajen et al. 1980; 1982). Metal-working fluids (MWFs) are used for lubricating and cooling the cutting and grinding surfaces of metals in metallurgical industries. The presence of nitrosamines in MWFs has drawn considerable attention in recent years. Millions of gallons of MWFs are used annually in industrialized countries. N-nitrosodiethanolamine (NDELA) has been found to be present in several brands of industrial MWFs (Fadlallah et al. 1996., Keefer et al. 1990., Spiegelhalder et al. 1990). Workers handling NDELA contaminated products can be exposed to NDELA by direct contact or by inhalation of oil mists, during most machine shop operations. The dilute MWFs are sprayed, splashed and vaporized into the air particularly during milling and grinding operations. Since NDELA has been found to be carcinogenic in experimental animals, there is a great concern, that the workers can be exposed to this compound, if they use MWFs contaminated with NDELA. NDELA has been found to induce liver tumors and neoplasm in a variety of other organs in rats (Lijinsky and Kovatch 1985; Berger et al. 1987). Lung tumors were found in mice (Hecht et al. 1989) and tumors in the nasal cavity and trachea in hamsters (Hilfrich et al. 1978) when these animals were treated with NDELA. The Canadian government had moved in 1979 to ban the importation, sale, and advertisement of MWFs containing any nitrite when diethanolamine or triethanolamine is present (Brownstein 1979). In 1984, the United States Environmental Protection Agency (USEPA) passed a rule prohibiting the use of nitrites in MWFs containing alkanolamines. Despite the fact that NDELA is not sufficiently volatile to pose a problem as an air pollutant, sufficient exposure of workers can still occur by direct contact or by inhalation of oil mists

Because a limited survey is available on MWFs used in Canada, this study was performed to assess the magnitude of the problem of human exposure to these carcinogenic compounds. This study reports the detection and identification of NDELA in the ambient air of metal factories using gas liquid chromatography-mass spectrometry (GLC-MS). The volatile N-nitrosamines were detected and identified in the factory environment by gas liquid chromatography coupled with a thermal energy analyzer (GLC-TEA).

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## MATERIALS AND METHODS

The air samples of MWFs employed in this study were obtained from the Institut de Recherche en Santé et en Sécurité du Travail du Québec (Research Institute of Occupational Health and Safety of Québec) in Montreal, Quebec. They were analyzed as received from this institute. N-nitrosodiethanolamine (NDELA), N-nitrosodiisopropanolamine (NDiPLA), N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodibutylamine (NDBA), N-nitrosomorpholine (NMOR) and N-nitrosopyrrolidine (NPYR) were purchased from Sigma Chemical Co., St-Louis, Missouri, USA. N-nitrosopiperidine (NPIP), N-nitrosodipropylamine (NDPA) and ammonium sulfamate were obtained from Eastman Kodak Company, Rochester, New York, USA. Trifluoroacetic anhydride (TFAA) and all solvents ‘distilled in glass” grade were obtained from Caledon Laboratories Ltd., Georgetown, Ontario, Canada. Sodium sulfate anhydrous was purchased from J.T. Baker Chemical Co.. Phillipsburg, New Jersey, USA.

NDELA in the ambient air of metal factories was detected and identified by gas liquid chromatography-mass spectrometry (GLC-MS). Mass spectra were recorded using a Finnigan GLC-MS TSQ 700. The analyses were performed using electron impact (EI) ionisation mode. The ion m/z 326 was selected for the quantitation of NDELA using SIM mode. Operating conditions were as follows: a DB-5 (J & W Scientific, Folsom, California, USA) fused-silica capillary column (30m x 0.25 mm I.D.; 0.25 mm film thickness was used. Helium was used as a carrier gas at a flow rate of 1 mL/min. The injection mode was splitless. The injector and transfer line temperature was 230°C. The initial oven temperature was maintained at 30°C for 2 min. then programmed at 20°C/min. to a final temperature of 230°C held for 10 min. Source temperature was 100°C, electron energy 70eV, emission current 0.30 mA, scan range 50 to 550 amu.

The volatile N-nitrosamines were detected and identified in the environment of different factories by gas liquid chromatography coupled with a thermal energy analyzcr (GLC-TEA). A Hewlett-Packard Model 5730A gas chromatograph with an integrator (Spectra-Physics, Model SP4000) coupled with a TEA detector (Thermedics Inc. Model 502) was used for this work. Operating conditions were as follows: a 2 m x 2 mm i.d. coiled glass column was packed with 10% Carbowax + 2% KOH on 80-100 mesh Chromosorb W (Chromatographic Specialties, Ontario, Canada). The initial temperature of the oven was 130°C. then programmed at 4°C/min. to a final temperature of 210°C held for 5 min The injector temperature was maintained at 200°C. The nitrogen carrier gas flow through the column was 15 mL/min. The detector furnace was operated at 550°C and the cold trap at -196°C while vacuum in the reaction chamber was maintained at 0.65 mm/Hg.

Air samples were collected by two methods. The sampling of non-volatile N-nitroso compounds (e.g. NDELA) was performed with impinger traps (Midget) (Supelco Ltd. Ontario. Canada), (185 mm x 24 mm, collecting solution capacity 25 mL) and their volatile analogues (e.g. NDMA, NDEA, NDPA, NDBA, NPIP, NPYR and NMOR) with Thermosorb/N cartridges (Thermedics Detection, Chelmsford, MA).

For the impinger trap method, the ambient air was drawn with an air-sampling pump (Model HFS 113A, Gillian Instrument Corp. N.J. USA) at a flow rate of 2 L/min for about 3 hr through a glass impinger containing 15 mL of 1 N KOH solution. N-nitroso compounds were collected in 1 N KOH solution. The extraction of non-volatile N-nitroso compounds was carried out as follows: To 10 mL of the sample in 1 N KOH solution were added 15 mg of ammonium sulfamate (nitrosation inhibitor). It was later extracted with 20 mL of ethyl acetate and the mixture was shaken mechanically on an Eberbach agitator for 10 min. After centrifuging at 600g for 5 min, the organic layer was separated and filtered. The extraction procedure was repeated a second and third time. The organic phases were combined, dried over anhydrous sodium sulfate, filtered and evaporated to dryness under vacuum (15-25mm/Hg) using a rotatory evaporator (Buchler Instruments Inc. Switzerland) at 30°C. The dried extract was reconstituted with 100 µL of the external standard corresponding to 0.2 µg of N-nitrosodiisopropanolamine (NDiPLA). The mixture was evaporated to dryness under nitrogen and later derivatized by adding 100 µL of TFAA. The mixture was vortexed for 30s and then kept at room temperature (20°C) in the dark for 2 hr. It was again evaporated to dryness at 30°C under a slow stream of nitrogen. The residue was then reconstituted with 100 µL of ethyl acetate and about 1 µL was injected into the gas chromatograph coupled to the mass spectrometer. Recoveries of NDELA and NDiPLA ranged from 82-86.5% (median 84.2%) and 80-82% (median 81%) respectively.

The extraction of volatile N-nitroso compounds was performed as follows: The Thermosorb/N cartridge (20 mm long; 15 mm id.) containing a suitable sorbent and an amine trapping (complexing) agent, in addition to a nitrosating inhibitor was used. The air samples were collected by drawing air through the cartridges at a constant rate of 2 L/min. for about 3 hr using a pump. After sampling, the sorbent was eluted with 2 mL of a mixture containing 25% methanol in 75% dichloromethane. A precise volume of about 5 µL was injected into the gas chromatograph coupled with TEA detector. Recoveries of NDMA, NDEA and NDBA ranged from 90-94% (median 92%) 92-96% (median 94%) and 94-98% (median 96%) respectively.

## RESULTS AND DISCUSSION

The airborne levels of NDELA, NDMA, NDEA and NDBA in the factories using different brands of MWFs are given in Table 1. The levels of NDELA range from trace amounts to 0.193 $\mu g/m^3$. These levels were determined by GLC-MS. A chromatogram with specific mass of the trifluoroacetyl derivative of standard NDELA is shown in Figure 1 using the EI mode. A chromatogram with specific mass of an ambient air sample is shown as an example (Figure 2). It can be seen that it is identical to that of authentic NDELA, confirming the presence of this compound in ambient air samples. The results in Table 1 show, that the levels of NDMA and NDBA vary from 0.011-0.076 and 0.031-0.167 $\mu g/m^3$ respectively. NDEA was found only in one air sample at concentration of 0.025 $\mu g/m^3$. These levels were determined by GLC-TEA. Figure 3 shows the chromatogram obtained by GLC-TEA analysis from volatile nitrosamine standards. Figure 4 illustrates the chromatogram obtained from an air sample containing NDMA, NDEA and NDEA using GLC-TEA. NDMA, NDEA and NDBA were the only volatile N-nitroso compounds, which could be found in air samples using different MWFs. Other volatile N-nitroso compounds (NDPA, NPIP, NPYR and NMOR) could not be detected in any sample and their concentrations were below the detection limits of the described method.

The data in Table 1 indicate, that there is no correlation between the NDELA concentration in MWFs and the levels of NDELA, NDMA, NDEA and NDBA in the corresponding air samples. There is a great variation in NDELA content in different MWFs studied in this investigation. Some manufacturers might have intentionally made the formation of NDELA less probable in their formulation, for example, by adding antioxidants (Järvholm et al. 1991). Williams et al. 1978 found NDELA levels between 230 and 5,530 ppm in the MWFs used in Canada. Our previous study (Fadlahah et al. 1990a) showed the concentrations of NDELA between 36 and 95 ppm in synthetic fluids and between 5 and 35 ppm in soluble oils. By comparing the studies reported by two different Canadian groups in 1978 and 1990,

Table 1. Airborne levels of NDELA, NDMA, NDEA and NDBA in the surveyed factories using different brands of MWFs

| Sample | NDELA concentration in MWFs (ppm*) | Airborne levels ($\mu g/m^3$) | | | |
|---|---|---|---|---|---|
| | | NDELA | NDMA | NDEA | NDBA |
| | | GLC-MS | GLC-TEA | | |
| Control | N.D. | N.D. | N.D.$^+$ | N.D. | N.D. |
| 1 | 0.25 | N.D. | 0.012 | N.D. | N.D. |
| 2 | 1.28 | 0.128 | 0.050 | N.D. | N.D. |
| 3 | 0.46 | 0.193 | 0.102 | 0.025 | 0.167 |
| 4 | 0.03 | 0.046 | 0.053 | N.D. | N.D. |
| 5 | 0.01 | Trace | 0.076 | N.D. | N.D. |
| 6 | 0.95 | N.D. | 0.033 | N.D. | N.D. |
| 7 | 1.38 | N.D. | 0.031 | N.D. | 0.067 |
| 8 | 7.53 | Trace | N.D. | N.D. | N.D. |
| 9 | 0.46 | 0.044 | 0.011 | N.D. | N.D. |
| 10 | 0.10 | 0.111 | 0.011 | N.D. | N.D. |
| 11 | 0.43 | Trace | 0.031 | N.D. | N.D. |
| 12 | 0.05 | N.D. | 0.044 | N.D. | N.D. |
| 13 | 0.02 | Trace | 0.028 | N.D. | 0.111 |
| 14 | Trace | Trace | N.D. | N.D. | 0.031 |

ppm* Parts per million
N.D.$^+$ Not detectable

Detection limit : 0.01 ppm for NDELA
: 0.009 $\mu g/m^3$ for NDMA
: 0.014 $\mu g/m^3$ for NDEA
: 0.029 $\mu g/m^3$ for NDBA

concentrations of NDELA in the MWFs used in Canada were significantly reduced after a period of 12 years. The present study shows an additional significant decline in the concentrations of NDELA in Canadian MWFs. There is a great variation in the airborne levels of NDELA using different brands of MWFs. Workers staying in rooms with a mean concentration of NDELA in the air of about 1 $\mu g/m^3$ revealed two times more DNA strand breaks than workers staying in an environment with less than 50 $ng/m^3$ of NDELA (Fuchs et al. 1995). This means the concentrations of NDELA in the air samples found in this study can

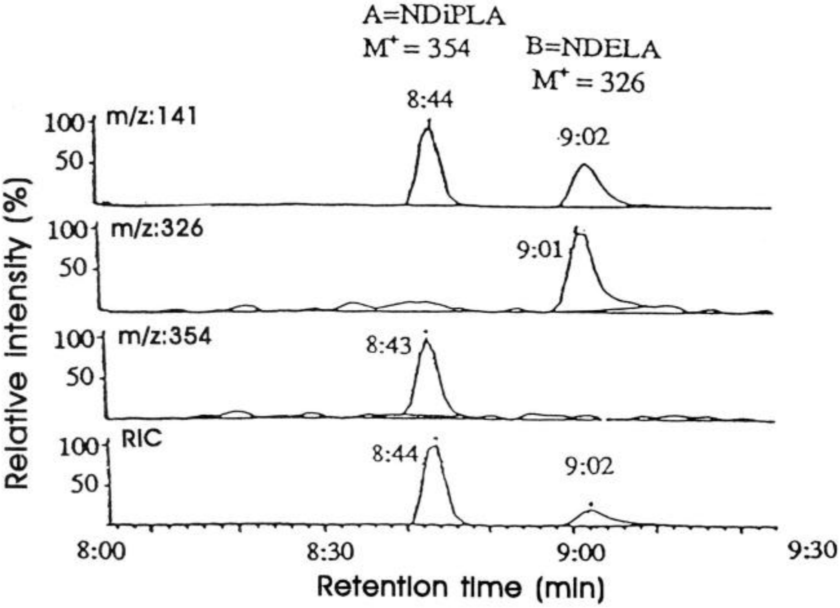

**Figure 1.** Chromatogram of standard NDELA derivatized with TFAA using the electron impact mode.

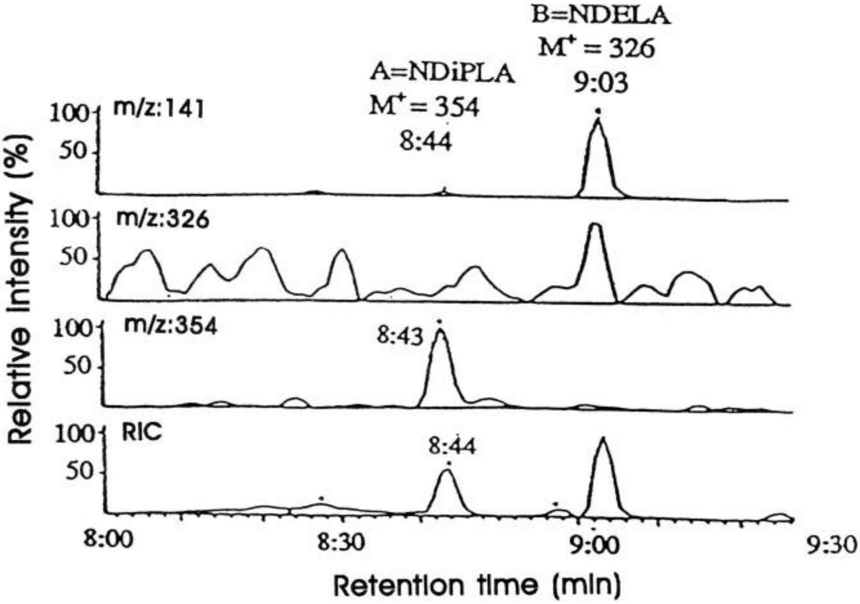

**Figure 2.** Chromatogram of an air sample extract derivatized with TFAA using the electron impact mode.

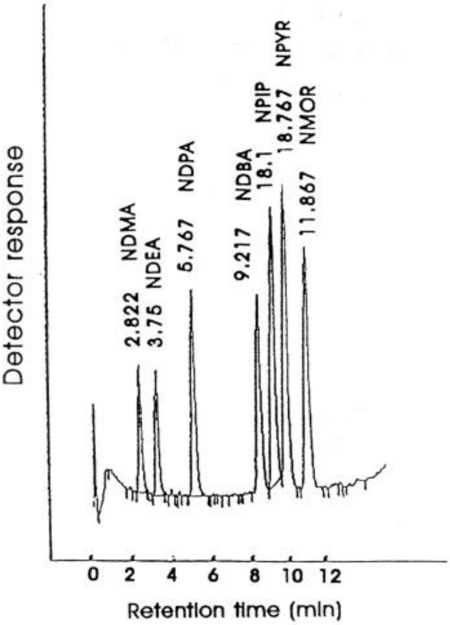

Figure 3. Chromatogram obtained from volatile nitrosamine standards using GLC-TEA.

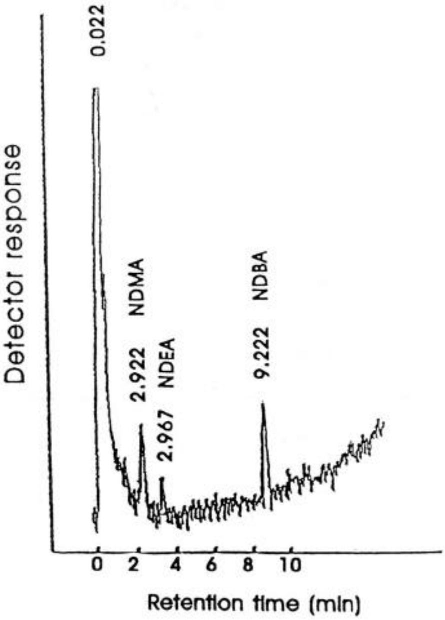

Figure 4. Chromatogram obtained from an air sample containing NDMA, NDEA and NDBA using GLC-TEA.

cause damage to DNA of metalworkers. Fuchs et al. 1995 reported in their study, that the workers exposed to NDELA by inhalation is probably more relevant for genotoxic damage than NDELA resorbed by skin. An increased level of DNA damage was found in metal workers depending on the concentration of NDELA in the air of the work places. However, without further investigations, it cannot be excluded that other concomitant agents in the environment may be responsible for the observed genotoxic effect.

The volatile nitrosamines like NDMA, NDEA and NDMA in the ambient air of metal factories can alkylate DNA (Fadlallah et al. 1994a; 1994b; Lachapelle et al. 1992; Hofe et al. 1987). The presence of the volatile nitrosamines in our study is of particular interest because these nitrosamines are listed in a circular published by the French Government (Circular No.7, 1988) as substances with sufficient evidence of carcinogenic effects in animals. It further recommends that suitable preventive measures be applied in order to avoid their exposition during metallurgical operations. Despite the low levels found during this survey, it seems useful, as stated by NIOSH (Roundbehler and Fajen 1983) to encourage industries producing or using nitrosatable amines to control their processes regarding potential nitrosamine formation. Although we do know the human risks of exposure to NDELA, it seems reasonable to keep exposure as low as possible, because the absorption of non-volatile NDELA by metal workers mainly occurs by inhalation of mist or by penetrating the skin. Minor absorption may also occur by oral intake. The protective masks may partly solve the absorption of NDELA and other volatile nitrosamines by inhalation, if the workers strictly use the protective masks under rigorous supervision. Absorption by skin can be avoided in part by using hand gloves, if the major exposition is due to the handling of MWFs.

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