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The Determination of N-Nitrosodiethanolamine in Cutting Fluids

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The formation of N-nitrosodiethanolamine (NDETA) from the reaction of triethanolamine with nitrite has been demonstrated by LIJINSKY et al. (1972) and also by ZINGMARK and RAPPE (1976) under simulated gastric conditions at pH 2-4. Subsequently, FAN et al. (1977) have shown that this reaction occurs in cutting fluids under alkaline conditions, pH 9-11, and have reported levels of NDETA as high as 3%. Little is known of the toxicity of NDETA, but DRUCKREY et al. (1967) have shown that it is carcinogenic in the rat.

In order to assess the magnitude of the problem of NDETA contamination in cutting fluids, a limited survey was made of cutting fluids used in Canada.

EXPERIMENTAL

Synthesis of N-nitrosodiethanolamine (PREUSSMAN, 1962)

Diethanolamine hydrochloride (283 mg, 2mM) was dissolved in water (1 ml) and sodium nitrite (200 mg, 2.8mM) in water (1 ml) added dropwise. The solution was left at room temperature for 24 hours and then evaporated to dryness using a rotary evaporator, bath temperature 30°C. The oily residue was transferred to a silica gel column, eluted with ethanol and 30 ml portions of the eluate were collected and checked by TLC. Appropriate eluate fractions were combined and concentrated to give a colourless oil (243 mg, 90% yield) which showed only one component on TLC and GLC and whose mass spectrum was consistent with NDETA.

Isolation of N-nitrosodiethanolamine

(a) Non-aqueous samples: Add sufficient anhydrous sodium sulfate to 0.5 - 1.0 g of cutting fluid to remove traces of water and transfer the mixture to a silica gel column (1 x 14 cm, 7 g Silica gel 60, E. Merck). Elute column

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with diethyl ether (75 ml), followed by 2% methanol in ethyl acetate (75 ml). Collect the ethyl acetate and concentrate to a small volume (1 ml) using a rotary evaporator, bath temperature 30°C, and finally evaporate using a stream of nitrogen.

(b) Aqueous samples: Add cutting fluid (0.5 g) to 0.1 N sodium hydroxide (10 ml) and saturate the solution with sodium chloride. Extract this solution with ethyl acetate (4 x 25 ml), combine the extracts and dry over anhydrous sodium sulfate. Filter, concentrate to ca. 0.5 ml and transfer to a silica gel column. Proceed as described above for non-aqueous samples.

Identification of N-nitrosodiethanolamine

(a) Thin layer chromatography: Spot a measured aliquot (1-10 ul) of the concentrated column eluate on a silica gel plate (0.25 mm) and run the plate in 10% methanol-ethyl acetate. Spray the plate with a freshly prepared mixture of equal volumes of (i) sulphanilic acid (0.5 g) in 30% acetic acid (150 ml) and (ii) N-(1-Naphthyl) ethylene-diamine dihydrochloride (0.1g) in 30% acetic acid (150 ml). Dry the plate and irradiate ca. 10 minutes with ultraviolet light (SEN and DALPE, 1972). NDETA shows up as a pink spot. Compare the intensity and R_f value with appropriate standard concentrations of NDETA (R_f 0.3-0.33) run on the same plate.

(b) Gas chromatography-mass spectrometry: A Finnigan 4000 gas chromatograph-mass spectrometer coupled with a data system was used. Operating conditions were:

(i) N-nitrosodiethanolamine analysis - A 6' x 2mm i.d. glass column packed with 3% OV-17 on Chromosorb WHP, 80-100 mesh. The injector temperature was 230°C and the column was programmed from 150 to 200°C at 6°/min. The carrier gas was helium at 25 ml/min. The mass spectrometer was operated in the methane chemical ionization mode with a methane pressure of 0.35 torr and a source temperature of 220°C. The retention time of NDETA under these conditions was 5.1 min.

(ii) N-nitrosodiethanolamine trimethylsilyl derivative - A 6' x 2mm i.d. glass column packed with 3% OV-1 on Chromosorb WHP, 80-100 mesh. The injector temperature was 230°C and the column was programmed from 150 to 200°C at 6°/min. The carrier gas was helium at 25 ml/min. The mass spectrometer was

operated in the electron impact mode (70 ev) and a source temperature of 220°C. The retention time under these conditions was 1.9 min.

N-Nitrosodiethanolamine trimethylsilyl derivative

N-Nitrosodiethanolamine (1 mg) was dissolved in dry ethyl acetate (1 ml) and n-trimethylsilylimidazole (25 µl) was added. The mixture was left overnight at room temperature and then blown to dryness under a stream of nitrogen. The residue was dissolved in a known volume of hexane and analysed by gas chromatography-mass spectrometry.

Nitrite determination

The cutting fluid (1 g) was dissolved in water (100 ml) and nitrite was then determined using the standard method for nitrite in water or waste waters (STANDARD METHODS, 1975).

RESULTS AND DISCUSSION

In order to provide a rapid and simple method of analysis for N-nitrosodiethanolamine in cutting fluids, a screening procedure based on thin-layer chromatography was developed. After an initial silica gel column clean-up, the samples were analysed by thin-layer chromatography using silica gel plates and a specific spray reagent which gives a pink colour with the nitrite ion formed from the nitrosamine by irradiation of the chromatogram with ultra-violet light. A semi-quantitive estimation of NDETA in the samples can be made by visual estimation of the intensity of the colour of the spot as compared to standard aliquots of NDETA run on the same plate.

Twenty-four samples of cutting fluid concentrates were collected from commercial outlets or were obtained directly from the manufacturer. Analysis of these samples for nitrite showed that nine of the samples contained nitrite with levels up to 9.8% (Table 1).

The cutting fluid concentrates were analysed for NDETA by thin-layer chromatography after a silica gel column clean-up. Recoveries of controls spiked at 0.1 and 1.0 mg/g were greater than 90%. As would be expected only those cutting fluids which contained nitrite were found to contain NDETA; at levels from 0.4 to 4.2 mg/g (Table 1). The cutting fluid concentrates are normally diluted with water before use and

analysis of these aqueous solutions requires an extraction of NDETA with ethyl acetate before the column clean-up. Recovery yields will be dependent on the dilution and the nature of the co-extractives and should be determined for each type of cutting fluid.

TABLE I

N-Nitrosodiethanolamine in Cutting Fluids

Sample	N-Nitrosodiethanolamine (mg/g)		% Nitrite
	TLC	GC/MS	
A	0.55	0.36	8.0
B	0.41	0.23	7.2
C	4.15	5.53	8.2
D	0.69	0.40	3.4
E	0.83	0.99	9.8
F	trace	--	0.2
G	0.83	0.62	8.6
H	0.42	0.35	3.8
I	--	--	1.7

Those samples containing NDETA were further analysed by gas chromatography-mass spectrometry. The electron-impact mass spectrum of NDETA showed no molecular ion but the chemical ionization, methane reagent gas mode, mass spectrum was more distinctive (Table II) showing MH^+ at m/e 135, $MC_2H_5^+$ at m/e 163 and $MC_3H_5^+$ at m/e 175.

TABLE II

Chemical Ionization (Methane) Mass Spectrum of N-Nitrosodiethanolamine

m/e	% abundance
175	11.4
163	41.9
135	16.4
117	56.8
104	100
74	100
57	87.5

The presence of NDETA in the sample extracts was confirmed by comparison of the CI-mass spectrum with that of standard NDETA; quantitation was by measurement of the total ion current compared to standard injections of NDETA (Table I). The results obtained were in good agreement with those estimated by TLC. Further confirmation was obtained by formation of the trimethylsilyl derivative of NDETA and comparison of the mass spectrum, electron impact mode (Table III), of this derivative with that obtained from GC-MS of the trimethylsilyl derivatives obtained from the sample extracts.

TABLE III

Electron Impact Mass Spectrum of N-Nitrosodiethanolamine Trimethylsilyl Derivative

m/e	% abundance
263	17.8
248	3.2
218	14.3
130	20.1
117	9.6
116	24.8
103	21.0
73	100

The results obtained in this study on cutting fluids available in Canada are in agreement with those values (0.2-29.9 mg/g) reported by FAN et al. (1977) for cutting fluids available in the U.S.A. and indicate the widespread nature of the problem. A recent symposium (1977) on the problem of NDETA in cutting fluids indicated that the industry is seeking to reformulate the cutting fluids where possible to avoid this problem and that animal studies are in progress to evaluate the toxicity of NDETA.

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