Bull. Environ. Contam. Toxicol. (1994) 52:681–686

Environmental Contamination and Toxicology

Effects of Ozonation on Mutagenic Activity of Nitrosamines

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Received: 21 June 1993/Accepted: 10 August 1993

Since Magee and Barnes (1956) reported on the carcinogenic effects of nitrosodimethylamine in rats, there has been growing concern as to the significance of N-nitrosocompounds in the environment (Linjinsky and Epstein 1970).

Many naturally occuring and man-made precursors of N-nitrosocompounds, amines and nitrites, are present in water where nitrosamines may be formed. So, a source for nitrosamines was traced to a chemical factory that was manufacturing unsymmetrical hydrazines for which N-nitrosocompounds were produced as intermediates (Fine *et al.* 1977).

Contamination of water supplies by organic pollutants, including nitrosocompounds become an important environmental problem. Purification of water containing organic residues by chlorination is thought to contribute to this problem due to the generation of potentially carcinogenic halogenated hydrocarbons (Shih and Lederberg 1976, Dolora *et al.* 1981, Zoeteman *et al.* 1982). Ozonation appears to be a leading alternative to chlorination in water purification treatment (Rice *et al.* 1981). The reaction of ozone with nitrosamines in aqueous media has been relatively unknown. However Caufield *et al.* 1979 have shown that N-methyl-N'-nitro-N-nitrosoguanidine is unaffected by ozone treatment. Moreover dimethylnitrosamine and its ozonation intermediates were not mutagenic in usual method of Ame's test.

The purpose of our study is to evaluate the effectiveness of ozonation on two N-nitrosocompounds, i.e. nitrosodibutylamine (NDBA) and nitrosomorpholine (NMOR), in aqueous solution as measured by GLC-TEA analysis and the Ame's liquid preincubation assay (Yahagi *et al.* 1977, Maron and Ames 1983).

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MATERIALS AND METHODS

Nitrosodibutylamine and nitrosomorpholine (Sigma) were solubilized in water at the concentration of 200 mg/L.
Ozone was produced with a Trailigaz air-fed unit, model labo 76 with a production of 10 mg O_3/liter of air. 100 ml of nitrosamine solutions were ozonated in a bubble chamber. Several ozonations were carried out for nitrosamines (Table 1).

Table 1. Ozonation of Nitrosamines

	Ozone mg	Volume of air L
Nitrosamines	50	5
	100	10
	500	50
	1000	100
	2500	250

After ozonation, 100 mL nitrosamine solutions were extracted two times with 50 ml dichloromethane (SDS). The pooled extracts were dried over sodium sulphate and concentrated using a Kuderna Danish evaporator. The extracts were analysed by gas chromatography in which a Thermal Energy Analyser (TEA) was used. A sigma 3B Perkin Elmer chromatograph was used coupled to a TEA detector (Thermoelectron). A 2 m x 6.3 mm o.d. stainless steel column packed with 15% FFAP on 80-100 mesh Chromosorb X was used. The oven temperature was kept at 140°C for NDBA and 150°C for NMOR. The injector temperature was 250°C, the carrier gas (Ar-CH_4) flow was 60 mL/min. The GC-TEA interface and pyrolyser temperatures were 250 and 500°C respectively.

The mutagenic activities of nitrosamines and ozonated nitrosamines were tested using *Salmonella Typhimurium* TA 100. This strain was kindly supplied by Dr B.N. Ames, Biochemistry department, University of California, Berkeley. CA (USA). The mutagenicity test was performed according to the modified preincubation method (Yahagi *et al.* 1977, Maron and Ames 1983).
Liver excised from rats (Sprague Dawley), pretreated with Aroclor 1254 (Monsanto) at 500mg/kg, intraperitoneally were homogenized. Liver homogenate (S_9) and S_9 mix were prepared according to the method described by Ames *et al.* 1975.

To a tube containing 0.1 mL of appropriately diluted nitrosamine solution was added 0.5 mL of S_9 mix (0.3 mL S_9, 33 μM KCl, 8 μM MgCl2, 4 μM glucose-6-phosphate, 5 μM NADP and 100 μM citrate buffer pH 5 per mL) and

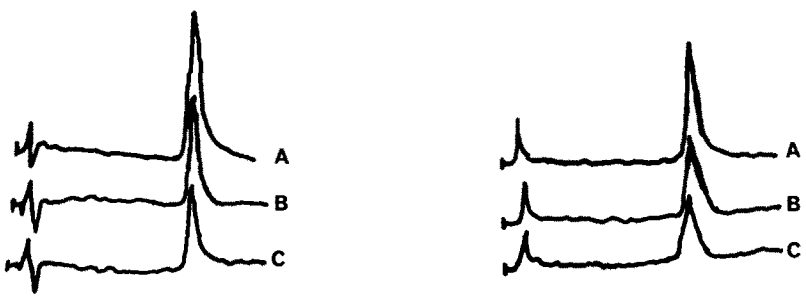
then 0.1 mL of overnight cultured bacterial suspension. This mixture was pre-incubated at 25°C for 30 min, mixed with 2.5 mL of Top Agar at 45°C and poured onto a minimal glucose agar plate containing a limited amount of L-histidine.

The presence of R factor in this strain was checked by seeding bacteria on agar containing Ampicillin. Culture was verified for crystal violet sensitivity and mutability using methyl methane sulfonate (MMS) and amino anthracene (AA) in the presence of S9 metabolic activating system ; 0.1 μL MMS per plate induced 470 revertants ; 2 μg AA in the presence of 150 μL S9 per plate induced 850 revertants.

The assays were performed in duplicate for each concentration with and without metabolic activation (S9 mix) and repeated three time with a Relative Standard Deviation of less than 15%.

RESULTS AND DISCUSSION

Typical chromatograms of nitrosamines (NDBA and NMOR) and ozonated nitrosamines are represented Figure 1.



Nitrosodibutylamine Nitrosomorpholine

Figure 1. TEA chromatograms of nitrosamines before and after ozonation. Nitrosamines standard (A), nitrosamines ozonated by 1 g (B), 2.5 g (C) of ozone.

Nitrosamines are poorly eliminated when they are ozonated with a great amount of ozone (1 and 2.5 g) (Table 2). No peaks of ozone byproducts like nitramines was observed. Nitramines are often byproducts formed during some nitrosamines oxydation processes (Emmons 1954). Moreover nitramines can be detected in the thermal energy analyser (Walker and Castegnaro 1980).

Table 2. Elimination of Nitrosodibutylamine and Nitrosomorpholine

mg O3	Percentage of elimination	
	NDBA	NMOR
0	0	0
50	0	1
100	8	8
500	11	15
1000	27	20
2500	39	26

So the elimination percentage of nitrosamines ozonated with 100 and 250 liters ozonated air has been compared with the elimination percentage of nitrosamines treated with 100 and 250 liters air. The results are shown in Table 3. The difference was not significant for NDBA at room temperature and for NMOR after treatment at +4°C. It leads us to predict that volatile nitrosamines were partially carried by the gaseous flow.

Table 3. Effects of treatment (air or ozone) on the Nitrosamine elimination

Nitrosamines	Treatment	% of elimination	Difference
Nitrosodibutylamine	1g ozone	27	**3**
	100 L air	24	
	2,5 g ozone	39	**4**
	250 L air	35	
Nitrosomorpholine	1 g ozone	20	**11**
	100 L air	9	
	2,5 g ozone	26 (11)*	**12 (3)***
	250 L air	14 (8)*	

* ( ) treatment at +4°C

These results were confirmed by mutagenicity test (Figure 2). Without metabolic activation system, no mutagenic activity was observed after NDBA and NMOR ozonation. In addition, with metabolic activation (S_9 mix), the same mutagen effects were shown between nitrosamines and ozonated nitrosamines.

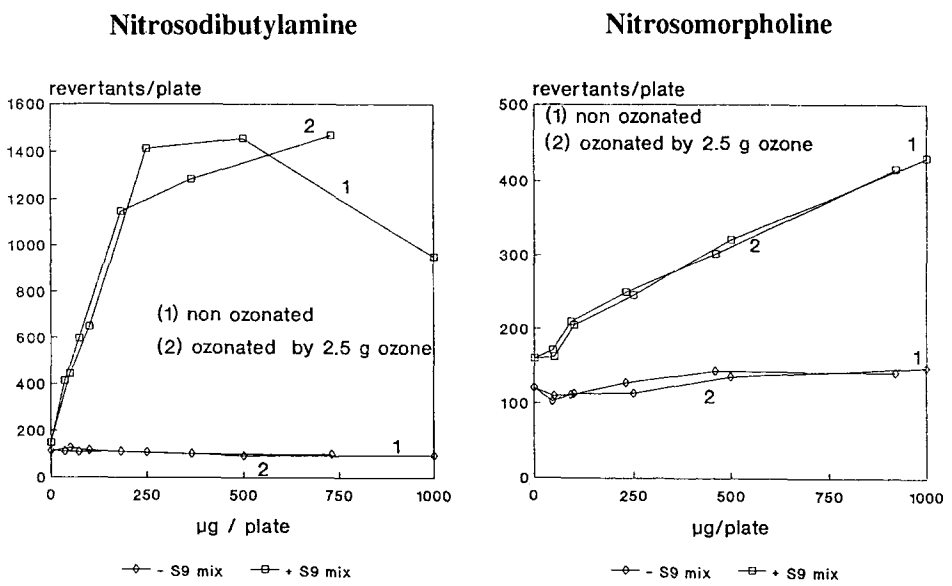


Figure 2. Dose-response curves of mutagenicity toward *Salmonella typhimurium* TA 100 of nitrosamines (NDBA and NMOR) before and after ozonation.

We have previously described that ozonation represents an interesting alternative method for the destruction of various water contaminants (Fouillet *et al.* 1991). However, concerning the nitrosamines (specially NDBA and NMOR) our study show that they are not degraded by ozone.

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