Reports

N-Nitroso Compounds: Detection in Ambient Air

Abstract. *By use of a new, highly selective detection technique for* N-*nitroso compounds, which is sensitive to picogram quantities and which is based on the catalytic cleavage of the N–NO bond and the subsequent detection of the nitrosyl radical, dimethylnitrosamine has been found in concentrations of 0.02 to 0.96 part per billion in three out of five air samples from Baltimore, Maryland, and 0.014 to 0.051 part per billion in five out of six air samples from Belle, West Virginia. The sensitivity of the analytical procedures used was 1 part in* 10^{12}. *The presence of dimethylnitrosamine has been confirmed by using the new detector in conjunction with both a gas-liquid chromatograph and a high-performance liquid chromatograph. In addition, between one and three as yet unidentified* N-*nitroso compounds were detected in both cities.* N-*Nitroso compounds were not found in air samples from Philadelphia, Pennsylvania; Wilmington, Delaware; and Waltham, Massachusetts.*

Since Magee and Barnes (*1*) demonstrated the carcinogenic effects of dimethylnitrosamine (DMN) in the rat, there has been growing concern about the significance of *N*-nitroso compounds in the environment. As noted by Lijinsky and Epstein (*2*), *N*-nitroso compounds seem to be a major candidate class of carcinogens that are likely to be causally related to human cancer. Of approximately 100 *N*-nitroso compounds tested, more than 75 percent have been shown to be carcinogenic (*3*). For example, DMN is carcinogenic in a wide range of species, including the mouse, hamster, mink, rat, guinea pig, rabbit, and rainbow trout. It is carcinogenic in mink at the lowest dose yet tested in rodents, 50 μg per kilogram body weight, when given in the diet (*4*).

Standard analytical methods based on gas-liquid chromatography (GLC) are sensitive to concentrations of parts per billion (ppb), but are usable only for the most volatile *N*-nitroso compounds (*5*). These complex and time-consuming procedures involve extensive cleanup and concentration by a factor of at least 1000, followed by GLC and detection by either the Coulson conductivity or the alkali flame ionization GLC detectors. Because the GLC detectors are not specific for *N*-nitroso compounds, confirmation by GLC–mass spectrometry is mandatory. However, although most *N*-nitroso compounds are expected to be derivatives of complex amines of high molecular weight, because of their thermal instability and high molecular weight they are not amenable to gas chromatographic procedures. Thus, although more than 100 papers have been published on the measurement of the environmental distribution of *N*-nitroso compounds, all the data have been restricted to about 14 compounds; the distribution of the majority of this important class of compounds is not known. In an attempt to overcome the limitations of previous techniques, we have developed (*6*) a new, specific method of detection for *N*-nitroso compounds based on the catalytic breaking of the N–NO bond and the subsequent detection of the nitrosyl radical. The new technique, called thermal energy analysis (TEA), is uniquely selective for the *N*-nitroso functional group in both volatile and nonvolatile *N*-nitroso compounds. In addition, TEA is sensitive to picogram quantities (*7*). The technique has been used in conjunction with both a gas-liquid chromatograph, TEA-GLC (*8*), and, more recently, with a high-performance liquid chromatograph, TEA-HPLC (*9*). Because of the selectivity and sensitivity characteristics of TEA, prior concentration and clean-up steps are unnecessary (*10*).

Using the TEA detector, we have identified DMN at the level of 1 ppb and detected up to three as yet unidentified *N*-nitroso compounds in the ambient air of Baltimore, Maryland, and Belle, West Virginia. Similar studies in three other cities, Waltham, Massachusetts; Philadelphia, Pennsylvania; and Wilmington, Delaware, did not reveal the presence of *N*-nitroso compounds even at the level of parts per trillion. The only previous reported finding of DMN in air is by Bretschneider and Matz (*11*), who reported the presence of the compound in the ambient air of a factory in Germany that was producing secondary amines. The concentrations they detected ranged from 0.001 to 0.43 ppb, with peak DMN values being dependent on the concentration of ambient nitrogen dioxide (NO_2) rather than on the amine levels. Their collection technique, however, did not exclude the possibility that *N*-nitrosation occurred inside the absorption tubes used to collect the samples.

We used the TEA detector with a single-column (Chromosorb) GLC (*8*, *12*)

Table 1. Summary of air data collected from five U.S. cities. Abbreviations: ND, not detectable; *N*, number of unknowns.

Location	Date (1975)	Time	DMN (ppb)		TEA-GLC unknowns (ppb)		TEA-HPLC unknowns (ppb)
			TEA-GLC	TEA-HPLC	No. 1	No. 2	
Philadelphia	15 Aug.	9–11 a.m.	ND		Trace	ND	
Philadelphia	15 Aug.	1–3 p.m.	ND			ND	
Philadelphia	16 Aug.	7–9 p.m.	ND		ND	ND	
Philadelphia	16 Aug.	9–11 a.m.	ND		ND	ND	
Philadelphia	16 Aug.	1–3 p.m.	ND		ND	ND	
Philadelphia	18 Aug.	7–9 p.m.	0.025	Trace	0.053	ND	ND
Wilmington	19 Aug.	9–11 a.m.	ND		ND	ND	
Wilmington	19 Aug.	1–3 p.m.	ND		ND	ND	
Wilmington	19 Aug.	8–10 p.m.	ND		ND	ND	
Wilmington	20 Aug.	9–11 a.m.	ND		ND	ND	ND
Wilmington	20 Aug.	1–3 p.m.	ND		ND	ND	
Wilmington	20 Aug.	7–9 p.m.	ND		ND	ND	
Baltimore	22 Aug.	9–11 a.m.	ND		ND	ND	
Baltimore	22 Aug.	1–3 p.m.	ND		ND	ND	
Baltimore	22 Aug.	7–9 p.m.	0.10	0.011	ND	ND	
Baltimore	23 Aug.	1–3 p.m.	0.96	0.84	ND	0.28	0.054 (N = 3)
Baltimore	23 Aug.	7–9 p.m.	0.033	0.022	ND	0.015	0.012 (N = 3)
Belle	25 Aug.	1–3 p.m.	0.022	0.022	ND	0.084	(N = 3)
Belle	25 Aug.	7–9 p.m.	0.015	*	0.060	0.066	(N = 1)
Belle	26 Aug.	Morning	Trace		Trace	ND	
Belle	26 Aug.	9–11 a.m.	0.014		ND	Trace	
Belle	27 Aug.	9–11 a.m	0.051		ND	Trace	
Belle	26 Aug.	1–3 p.m.	0.016		ND	0.012	0.041 (N = 1)
Waltham	28 July	2–4 p.m.	ND	ND	ND	ND	ND
Waltham	29 July	2–4 p.m.	ND	ND	ND	ND	ND

*Sample had partially evaporated from leaking vial before TEA-HPLC analysis; DMN was found but could not be quantitated.

operated isothermally at 185°C. The same detector was also used with a high-performance liquid chromatograph in a TEA-HPLC system (*9, 13*) operated isocratically with a solvent system comprising 2.5 percent acetonitrile and 98.5 percent isooctane and a μ Bondapak CN column.

Sites in the five cities selected for analysis were as follows: in Baltimore, near both a sewage treatment facility and a chemical plant known to manufacture unsymmetrical dimethylhydrazine, for which DMN is generally used as an intermediate; in Belle, near a chemical plant manufacturing dimethylamine; in Philadelphia, near a dimethylamine plant; on and around the Delaware Bridge in New Jersey and Delaware; and in a suburban industrial park in Waltham (see Table 1 for details).

Ambient air at a flow rate of 1.8 liter/min was drawn through three successive cold traps for a period of 2 hours. The total volume of air per sample was about 200 liters (278 g). The temperature of the first cold trap (salt and ice) was −12°C, and that of the remaining two traps (Dry Ice and acetone) was −80°C. Moisture in the air was frozen out in the cold trap at −12°C and only small amounts of water were collected in the traps at −80°C. The third cold trap was used to ensure efficient collection of the more volatile compounds. After the collection, the traps were momentarily thawed and rinsed with distilled water. The contents of the three traps plus washings were combined (total volume about 50 ml) and refrozen at −80°C in plastic bottles. Before reuse, the traps were rinsed with water and the washings were used for the analytical blank. The samples were stored and shipped frozen. In the laboratory, the samples were thawed and extracted with three 8-ml portions of redistilled dichloromethane. The combined dichloromethane extracts were concentrated on a Kuderna Danish evaporator to a final volume of 0.5 ml. The recovery efficiency of the extraction from the thawed water was determined by spiking several of the water blanks with 0.05 μg/liter of a mixture of four *N*-nitrosamines: DMN, diethylnitrosamine (DEN), dipropylnitrosamine (DPN), and *N*-nitrosopyrrolidine (PYRN). In order to check on artifact formation during analysis, dimethylamine hydrochloride and sodium nitrite were added at 10 ppm to one water blank from each sampling site. The samples were then extracted and analyzed for DMN. Several samples were split in two parts and analyzed both with and without added KOH.

The recovery efficiency of the extraction process was typically 55 to 60 percent for DMN and 80 to 90 percent for DEN, DPN, and PYRN. We did not find DMN in the blanks to which amine and nitrite had been added at 10 ppm. Identical results were obtained with and without added KOH. The TEA-GLC trace (Fig. 1) shows two peaks, one corresponding in retention time to DMN at 0.96 ppb in the air and the other corresponding to an unidentified *N*-nitroso compound at about 0.28 ppb. The TEA-HPLC chromatogram shows a peak corresponding in retention time to DMN at 0.84 ppb together with peaks for three unidentified *N*-nitroso compounds. The data for all the air samples are summarized in Table 1. It is to be noted that wherever DMN was found by TEA-GLC, it was also found by TEA-HPLC. In addition, two *N*-nitroso compounds of unknown identity were found by TEA-GLC in several air samples from different sites. The TEA-HPLC showed, in addition to the DMN, the likely presence of up to three unknown *N*-nitroso compounds.

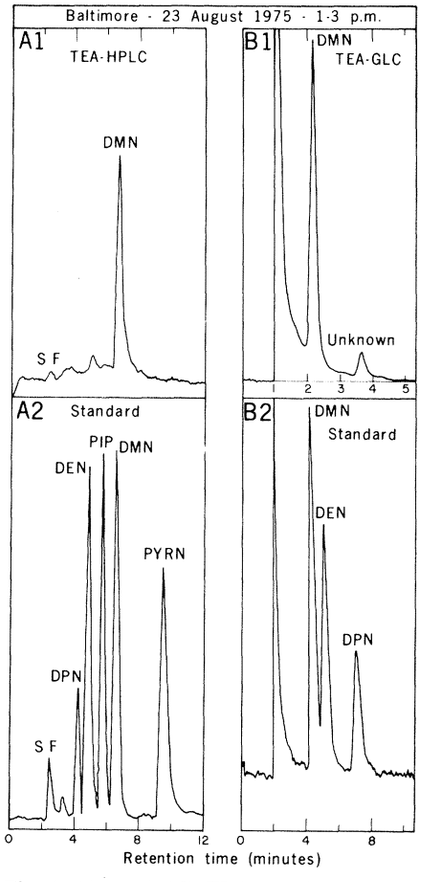

Fig. 1. (A1 and A2) TEA-HPLC chromatograms for (A1) 10 μl of an air sample extract collected from an industrial area in Baltimore and (A2) 10 μl of a standard solution containing dipropylnitrosamine (DPN), diethylnitrosamine (DEN), *N*-nitrosopiperidine (PIP), dimethylnitrosamine (DMN), and *N*-nitrosopyrrolidine (PYRN) in a total concentration of 1.0 μg/ml. The attenuation (range setting) was 16; *SF*, solvent front. (B1 and B2) TEA-GLC chromatograms for (B1) 20 μl of an air sample extract from an industrial area in Baltimore (attenuation 32) and (B2) 20 μl of a standard solution of DMN, DEN, and DPN in a total concentration of 0.1 μg/ml (attenuation 4).

The absence of possible artifacts was established by the fact that DMN could not be detected when water to which both sodium nitrite and dimethylamine hydrochloride had been added at 10 ppm was analyzed. Thus, even if NO_2 and amine had been present in the air at 2 ppm [a value many times greater (*11, 14*) than would be expected], DMN could not have been formed during collection or analysis. The coincidence of the retention times found for DMN with both GLC (where the elution order depends on solubility and vapor pressure) and HPLC (where the elution order depends on polarity) may be taken as confirmation of the presence of DMN, particularly because the TEA detector used has been shown to be selective to *N*-nitroso compounds (*7, 10, 15*). The only compounds known to cause interference with the TEA detector are nitrites, but they are readily distinguished. In TEA-GLC, nitrites are mainly eluted in the solvent peak with a column temperature of 185°C; in TEA-HPLC, the nitrites are too polar to elute under the conditions used.

Although we detected several *N*-nitroso compounds with TEA-GLC and TEA-HPLC, we were only able to confirm the presence of DMN by both techniques. Because of the limited number of samples taken, there is no basis for determining how representative the data are. We can only speculate about the origin of the DMN found. In Baltimore, the most likely source is either leakage of DMN from the chemical plant or nitrosation by NO_x of amines released from the sewage treatment facility. In Belle the DMN could have resulted from the combination of leaking dimethylamine with atmospheric NO_x. The site in Philadelphia was also in the neighborhood of a dimethylamine plant, but *N*-nitroso compounds were not detected there. The Delaware Bridge is in the midst of a large chemical industrial center. Other sources for the amines could be combustion products from both stationary and nonstationary sources. *N*-Nitroso compounds have been found in exhausts from gasoline and diesel engines (*16*). The role of oxides of nitrogen in *N*-nitrosation of secondary amines needs to be examined.

The high DMN concentration at the Baltimore site (0.96 ppb) is of interest. Continuous exposure to this concentration of DMN in the air would be equivalent to a daily dose for humans of about 14 μg (*17*) or approximately 1/150 of the

lowest dose that has been tested and found to be carcinogenic in rodents. Such an exposure is greater than the known DMN exposures from nitrite preserved foodstuffs (*18*). The public health effects of exposure to such concentrations of carcinogens remain to be assessed. Of possible interest in this connection are recent epidemiological findings (*19*) which, despite their possible limitations, suggest an association between ambient community NO_x levels and cancer.

Note added in proof: The experiments reported here have since been repeated by several independent workers (*20, 21*), including scientists from the chemical companies involved (*22, 23*). They have all confirmed the presence of DMN by GLC–mass spectrometry techniques. In Baltimore, the source was found to be the chemical plant which was using DMN as an intermediate; the plant was ordered closed as of April 1976 (*21*). In Belle, the DMN was traced to the amine manufacturing facility. This company has now reported that they have found a point-source discharge of DMN (*23*).

D. H. FINE
D. P. ROUNBEHLER, N. M. BELCHER
Thermo Electron Research Center, Waltham, Massachusetts 02154
S. S. EPSTEIN
Case Western Reserve University Medical School, Cleveland, Ohio 44106

References and Notes

1. P. N. Magee and J. M. Barnes, *Br. J. Cancer* **10**, 114 (1956).
2. W. Lijinsky and S. S. Epstein, *Nature (London)* **225**, 21 (1970).
3. P. N. Magee and J. M. Barnes, *Adv. Cancer Res.* **10**, 164 (1967); H. Druckrey, R. Preussmann, S. Ivankovic, D. Schmahl, *Z. Krebsforsch.* **69**, 103 (1967).
4. N. Koppang and H. Rimslatten, paper presented at the Fourth Meeting on the Analysis and Formation of *N*-Nitroso Compounds, International Agency for Research on Cancer, Tallinn, Soviet Union, October 1975.
5. G. M. Telling, T. A. Bryce, J. Althorpe, *J. Agric. Food Chem.* **19**, 937 (1971); T. A. Gough and K. S. Webb, *J. Chromatogr.* **79**, 57 (1973); P. Bogovski, E. A. Walker, W. Davis, Eds., *N-Nitroso Compounds in the Environment* (International Agency for Research on Cancer, Lyon, France, No. 3, 1972; No. 9, 1974).
6. D. H. Fine, F. Rufeh, D. Lieb, *Nature (London)* **247**, 309 (1974).
7. ———, D. P. Rounbehler, *Anal. Chem.* **47**, 1188 (1975).
8. D. H. Fine and D. P. Rounbehler, *J. Chromatogr.* **109**, 271 (1975).
9. P. E. Oettinger, F. Huffman, D. H. Fine, D. Lieb, *Anal. Lett.* **8**, 411 (1975); D. H. Fine, F. Huffman, D. P. Rounbehler, N. M. Belcher, paper presented at the Fourth Meeting on the Analysis and Formation of *N*-Nitroso Compounds, International Agency for Research on Cancer, Tallinn, Soviet Union, October 1975.
10. D. H. Fine, D. P. Rounbehler, P. E. Oettinger, *Anal. Chim. Acta* **78**, 383 (1975).
11. K. Bretschneider and J. Matz, *Arch. Geschwulstforsch.* **42**, 36 (1974).
12. The TEA-GLC furnace temperature was 300°C; cold trap temperature, −150°C; chamber pressure, 3.6 mm-Hg; and nitrogen carrier gas, 30 ml/min. The GLC column was stainless steel, 21 feet by ⅛ inch (outer diameter), with the first 8 feet packed with 2 percent KOH and 5 percent free fatty acid phase (FFAP) on Chromosorb WHP 80/100 mesh and the remainder packed with 10 percent FFAP on Chromosorb WHP 80/100 mesh. The GLC temperature was 185°C. A Thermo Electron TEA model 502 was used.
13. The TEA-HPLC furnace temperature was 400°C; cold trap temperature, −150°C; chamber pressure, 3.5 mm-Hg; and HPLC flow rate, 2.0 ml/min.
14. U.S. Environmental Protection Agency, Air Pollution Control Office, *Air Quality Criteria for Nitrogen Oxides* (Publication AP84, Environmental Protection Agency, Washington, D.C., 1971).
15. E. A. Walker and M. Castegnaro, paper presented at the Fourth Meeting on the Analysis and Formation of *N*-Nitroso Compounds, International Agency for Research on Cancer, Tallinn, Soviet Union, October 1975.
16. Using TEA-HPLC and an analytical technique similar to that described here, D. H. Fine (unpublished data) detected several *N*-nitroso compounds in the exhaust gases from a truck diesel engine and an automobile internal combustion engine. The identity of the compounds has not yet been established.
17. An average person takes 16 breaths per minute, with a volume of 0.45 liter each. In the course of a 24-hour day, a person inhales 10,400 liters of air weighing 14,000 g. If the air had a DMN content of 1 ppb, the daily DMN intake would be 14 μg, or 0.35 μg/kg for a 40-kg person. This is to be compared with 50 μg/kg, which has been shown to be carcinogenic in rodents (*4*).
18. Cooked bacon, bologna, and smoked ham have a DMN concentration of approximately 1 to 5 μg/kg. If 100 g of these foods were eaten daily, the daily intake of DMN could only be as high as 0.5 μg.
19. P. M. Sprey, I. Takacs, J. Morton, J. K. Allison, *A Study of Photochemical Pollutants and Their Health Effects* (Enviro Control Inc., Rockville, Md., 1973); R. J. Hickey, D. E. Boyce, E. B. Harner, R. C. Clelland, *Ecological Statistical Studies on Environmental Pollution and Chronic Disease in Metropolitan Areas of the United States* (Philadelphia Regional Science Research Institute, 1970).
20. D. H. Fine, D. P. Rounbehler, D. D. Pellizzari, J. E. Bunch, R. W. Berkley, E. Sawicki, K. Krost, G. A. DeMarrais, *Bull. Environ. Contam. Toxicol.*, in press.
21. Reported by D. Shapley, *Science* **191**, 268 (1976).
22. W. A. Wade, "Report of study of *N*-nitrosodimethylamine" (TRC Project No. 31555) (FMC Corporation, Baltimore, Md., November 1975).
23. B. Lazaski, E. I. DuPont DeNemours and Company, Inc., Wilmington, Del., personal communication.
24. We are indebted to C. M. Ditlow of the Public Interest Research Group, Washington, D.C., for scientific guidance and for bringing together the various organizations needed to carry out the air sampling measurements. We thank S. Miller of Avram Associates, Washington, D.C., for helpful discussions and for arranging for J. Ehrenfeld of Energy Resources Corporation, Cambridge, Mass., to collect the trapped air samples. The cost of collecting the air samples was borne by Goulde Engineering in a grant to Avram Associates. We thank M. Tobin for valuable technical assistance. The TEA-HPLC used was developed under National Cancer Institute contract ICP 45623.

4 November 1975; revised 8 March 1976

Size Variations in Planktonic Foraminifera: Implications for Quantitative Paleoclimatic Analysis

Abstract. *Populations of planktonic foraminiferal species and phenotypes, distinguished on the basis of color and coiling direction, reach maximum average test sizes in their regions of optimum development. Therefore, tropical species are largest in tropical waters, while polar species are largest in polar waters. Species living in subtropical and subpolar waters decrease in test size with both increasing and decreasing temperature.*

Previous studies of test size variations in planktonic foraminifera indicate that (i) size variations form mappable geographic patterns and (ii) tropical species are larger in test size in warmer waters and decrease in size with decreasing temperature, while polar species are larger in test size in polar waters and decrease in size with increasing temperature (*1–3*). These observations suggest an ecologic model in which foraminiferal species reach maximum average test size in their optimum water masses, and decrease in size away from such areas (*4*). This im-

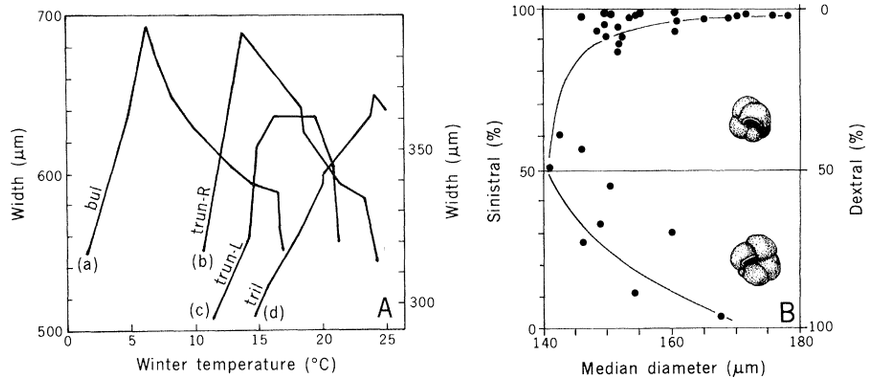

Fig. 1. (A) Variations in maximum average test size (*5, 6*) as a function of winter temperature in the North Atlantic for populations of (a) *G. bulloides* (bul), (b) *G. truncatulinoides* right (trun-R), (c) *G. truncatulinoides* left (trun-L), and (d) *G. trilobus* (tril). The scale on the left is for populations of *G. truncatulinoides*; that on the right is for *G. bulloides* and *G. trilobus*. (B) Correlation of abundances and median test sizes in right- and left-coiling populations of *G. pachyderma*. [Data are from Kennett (*2*)]