N-Nitrosodimethylamine in Fish Meal

Trace amounts of *N*-nitrosodimethylamine (0.12–0.45 ppm) were detected in fish meal which had been implicated in the liver disease of mink in Canada. The identity of the isolated compound was confirmed by both thin-layer and gas–liquid chromatographic (glc) analysis, as well as by combined glc–mass spectrometry. No nitrite could be detected in these samples.

N-Nitrosodimethylamine (DMN) is a potent carcinogen (Magee and Barnes, 1956), and the reported presence of this compound in the environment has recently aroused a great deal of concern among scientists (Lijinsky and Epstein, 1070; Magee, 1971). An outbreak of hepatotoxic disorders in farm animals in Norway was shown to be due to the presence of DMN in the nitrite-treated fish meals that were fed to the animals (Bøhler, 1960; Ender *et al.*, 1964; Koppang and Helgebostad, 1966). It was established that various methylamines, mainly dimethylamine and trimethylamine, present in the fish reacted with the added nitrite and formed toxic levels of DMN (Ender *et al.*, 1964, 1967; Sakshaug *et al.*, 1965). The concentration of DMN in six of the toxic feeds ranged from 30 to 100 ppm. No DMN was detected (detection limit, 10–15 ppm) in the nontoxic batches of herring meal. In recent years, trace amounts of DMN and other nitrosamines have been reported to be present in foods for human consumption; a brief outline of these findings has been published elsewhere (Magee, 1971; Sen, 1972).

Recent reports (Kirk, 1971) of the death of a large number of mink in Canadian farms prompted us to examine the mink feeds for nitrosamines. Some of these feeds contained a large proportion (55–60%) of herring meal and, according to the manufacturer, none were preserved with nitrate or nitrite. In addition to the analysis for nitrosamines, a few samples were also analyzed for nitrate and nitrite. The results of the findings are presented in this communication.

EXPERIMENTAL

Determination of DMN. The method used is similar to that reported by us previously (Sen, 1971; Sen *et al.*, 1969, 1970). It can be briefly described as follows. The sample (25–50 g) was moistened with 3 *N* potassium hydroxide, extracted in a blender with methylene chloride, and the extract was distilled over 3 *N* potassium hydroxide (200 ml) until all the methylene chloride was removed. The residual mixture was distilled and about 150 ml of distillate was collected. The distillate was made alkaline and reextracted with methylene chloride. Interfering amines were removed by washing the extract with glycine–hydrochloric acid buffer (pH 2.1). Further purification was achieved by chromatography on a basic alumina column. Finally, DMN was detected and semiquantitatively estimated by thin-layer chromatography (tlc) and gas–liquid chromatography (glc) using a Coulson electrolytic conductivity detector (Rhoades and Johnson, 1970). Final confirmation was carried out by glc–mass spectrometry. The details of the method have been reported earlier (Sen, 1971, 1972).

Glc Conditions. 10% Carbowax 20M on 60–80 mesh Chromosorb W (HMDS treated), 6 ft × 1/8 in. stainless steel column, helium flow 30 ml/min, column temperature 105° for first 3 min, then programmed to 175° at 10°/min. The effluent from the column was allowed to vent in the air for the first 3 min. A Varian gas chromatograph, Model 2700, connected to a Coulson electrolytic conductivity detector (furnace 500°, pyrolytic mode) was used. Detector voltage 30, and attenuator at 16.

Determination of Nitrate and Nitrite. Determined by the method of Kamm *et al.* (1965).

RESULTS AND DISCUSSION

Seven samples of fish meals or products containing fish meal were analyzed and the results are presented in Table I. All but one sample contained considerable amounts of DMN. Although small amounts of nitrate were detectable in all of the four samples analyzed, none contained any detectable amount of nitrite. Our failure to detect any nitrite (detection limit 0.1 ppm) in these feeds corroborates the manufacturer's claim that no nitrite was used in preparation of these products. It is, however, not clear how the formation of DMN took place in the absence of nitrite. One possibility is that small amounts of nitrite naturally present in the raw fish may have contributed to the formation of DMN. Such a small level of nitrite would be completely destroyed by reaction with various amino compounds in the fish meal, and the residual amount of nitrite would be too small to be detected in the final product. Hot fire gases, obtained from the combustion of fuel oils, may be another source of the nitrite. Ender and Ceh (1967) have pointed out that stack gases from fuel oil contain up to 1000 ppm of nitrogen oxides and the industrial use of such gases for drying fish meal may lead to the formation of DMN. However, further research is needed to establish this point.

Various methods have been described for the determination of DMN in food for human and animal consumptions. These include ultraviolet (Ender *et al.*, 1964) and infrared (Moehler and Mayrhofer, 1968), spectroscopic, polarographic (Heath and Jarvis, 1955; Walters *et al.*, 1970), colorimetric (Eisenbrand and Preussmann, 1970), tlc (Preussmann *et al.*, 1964; Sen *et al.*, 1969), glc (Howard *et al.*, 1970; Sen *et al;* 1969, 1970) methods. Many of these methods are unsuitable for trace analysis of nitrosamines in biological mixtures mainly because of the lack of sensitivity or specificity. The tlc method (Sen *et al.*, 1969) with the double-spray technique is very specific for *N*-nitrosamines, but additional confirmation is necessary for unequivocal proof of the identity. Recent studies by Rhoades and Johnson (1970) indicated that amines and certain dilakylnitrosamines can be selectively detected by glc analysis using a Coulson electrolytic conductivity detector operating in the pyrolytic mode. If free amines and other basic compounds are removed by an acid wash of the extract (as was done in this study) the technique then becomes more selective and detects only nitrosamines and other neutral compounds which produce amines upon pyrolysis. In addition to the glc evidence (Figure 1), the tlc and glc–mass spectroscopic (Figure 2) confirmation greatly strengthens the validity of our findings.

DMN is extremely toxic to fur-bearing animals, as well as to cattle and sheep (Koppang, 1966, 1970). Mink appears to be the most susceptible species. A dietary level of 2.5 or 5 ppm of DMN has been shown to produce toxic symptoms within 7–11 days and 100% mortality within 34 days (Carter *et al.*, 1969). Multiple liver tumors have been produced in mink after prolonged feeding of 0.05 mg of DMN/kg body

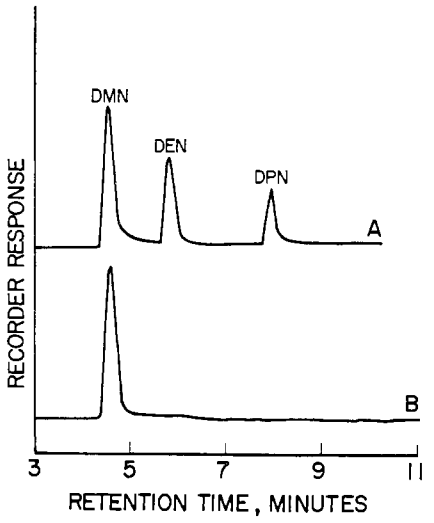

Figure 1. Glc analysis of nitrosamines. (A) 90 ng each of DMN, diethylnitrosamine (DEN), and di-*n*-propylnitrosamine (DPN). (B) 10.5-μl aliquot of the final methylene chloride extract (1.9 ml) from mink feed no. 2 (Table I)

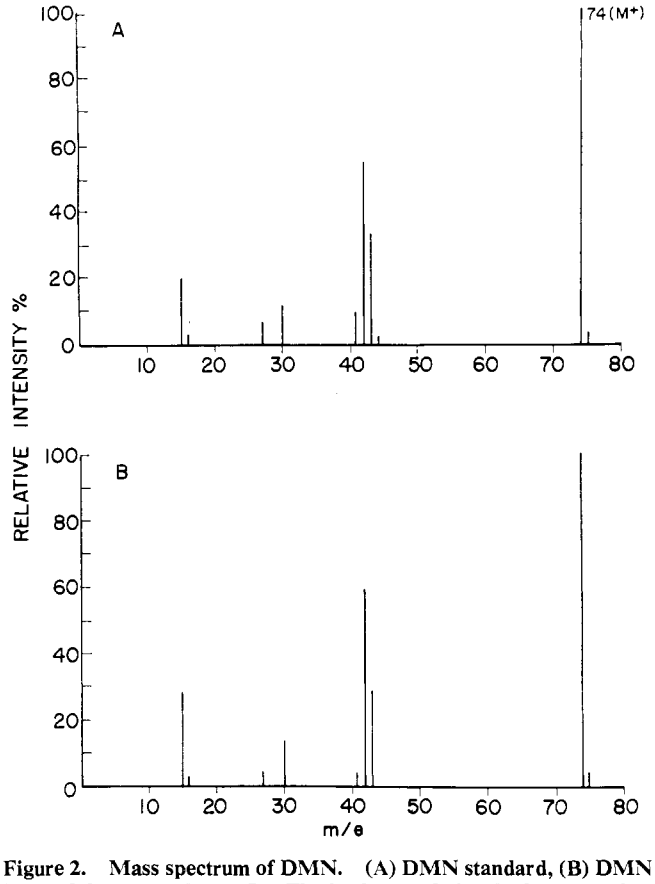

Figure 2. Mass spectrum of DMN. (A) DMN standard, (B) DMN isolated from sample no. 5. The background signals due to methylene chloride solvent and column bleed (scanned just prior to the emergence of the DMN peak) were subtracted in each case. A Hitachi Perkin-Elmer RMS-4 mass spectrometer coupled with a Perkin-Elmer, Model 990, gas chromatograph was used

weight/day (Koppang, 1970). Therefore, it is highly likely that some of the feeds analyzed in this study would be toxic to mink. It is of interest that the first two samples were reported to be toxic to mink, and many deaths occurred due to liver damage.

In view of the above findings it can be concluded that significant amounts of DMN can be present in some fish meals used as animal feeds. Therefore, it would be highly desirable to carry out routine analysis of fish meals for DMN. Such a practice may help prevent the occurrence of liver disease in animals and reduce their mortality rate due to poisoning by DMN.

Table I. DMN, Nitrate, and Nitrite Levels in Various Fish Meals

Sample	DMN, ppm	Nitrate,[a] ppm	Nitrite,[a] ppm
Mink feed	0.40[b]		
	0.18	22	N.D.[c]
Whole herring meal	0.30[b]		
	0.42	22	N.D.
	0.45[b]	17	N.D.
Fish meal	N.D.	13	N.D.
	0.12		

[a] Results expressed as sodium salt. [b] Confirmed by glc–mass spectrometry. [c] N.D. = not detectable. Detection limit for DMN is 0.01 ppm and for nitrite is 0.1 ppm.

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LITERATURE CITED

Bøhler, N., *Nor. Pelsdyrbl.* **34,** 104 (1960).
Carter, R. L., Percival, W. H., Foe, F. J. C., *J. Pathol.* **97,** 79 (1969).
Eisenbrand, G., Preussmann, R., *Arzneim.-Forsch.* **20,** 3 (1970).
Ender, F., Ceh, L., Alkierende Verbindungen, Second Conference of Tobacco Research, Freiburg, Germany, 1967, p 83.
Ender, F., Havre, G. N., Helgebostad, A., Koppang, N., Madsen, R., Ceh, L., *Naturwissenschaften* **51,** 637 (1964).
Ender, F., Havre, G. N., Madsen, R., Ceh, L., Helgebostad, A., *Z. Tierphysiol. Tierernaehr. Futtermittelk.* **22,** 18 (1967).
Heath, D. F., Jarvis, J. A. E., *Analyst* **80,** 613 (1955).
Howard, J. W., Fazio, T., Watts, J. O., *J. Ass. Offic. Anal. Chem.* **53,** 269 (1970).
Kamm, L., McKeown, G. G., Smith, D. M., *J. Ass. Offic. Agr. Chem.* **48,** 892 (1965).
Kirk, R. J., Experimental Fur Farm, Winnipeg, private communication, 1971.
Koppang, N., *Nord. Veterinaermed.* **18,** 205 (1966).
Koppang, N., *Acta Pathol. Microbiol. Scand., Suppl.* **215,** 30 (1970).
Koppang, N., Helgebostad, A., *Nord. Veterinaermed.* **18,** 216 (1966).
Lijinsky, W., Epstein, S. S., *Nature (London)* **225,** 21 (1970).
Magee, P. N., *Food Cosmet. Toxicol.* **9,** 207 (1971).
Magee, P. N., Barnes, J. M., *Brit. J. Cancer* **10,** 114 (1956).
Moehler, K., Mayrhofer, O. L., *Z. Lebensm. Unters. Forsch.* **135,** 313 (1968).
Preussmann, R., Neurath, G., Wulf-Lorentzen, G., Daiber, D., Hengy, H., *Z. Anal. Chem.* **202,** 187 (1964).
Rhoades, J. W., Johnson, D. E., *J. Chromatogr. Sci.* **8,** 616 (1970).
Sakshaug, J., Soegnen, E., Aas Hansen, M., Koppang, N., *Nature (London)* **206,** 1261 (1965).
Sen, N. P., Presented at the International Agency for Research on Cancer meeting on the "Analysis and Formation of Nitrosamines," Heidelberg, Germany, October 13–15, 1971.
Sen, N. P., *Food Cosmet. Toxicol.* **10,** 219 (1972).
Sen, N. P., Smith, D. C., Schwinghamer, L., Marleau, J. J., *J. Ass. Offic. Anal. Chem.* **52,** 47 (1969).
Sen, N. P., Smith, D. C., Schwinghamer, L., Howsam, B., *Can. Inst. Food Technol. J.* **3,** 66 (1970).
Walters, C. L., Johnson, E. M., Ray, N., *Analyst* **95,** 485 (1970).

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