Formation of *N*-Nitrosodimethylamine in the Injection Port of a Gas Chromatograph: An Artifact in Nitrosamine Analysis

Formation of *N*-nitrosodimethylamine (NDMA) was observed during the GC analysis of a commercial herbicide sample formulated as the dimethylamine salt of 2,3,6-trichlorobenzoic acid. The artifactual enhancement was shown to occur when the crude herbicide sample, presumably containing the precursors for the formation of NDMA, was injected into the hot GC injection port. The artifact was eliminated by analyzing the sample (1) after more than eightfold dilution, or (2) after extracting with methylene chloride.

The development of the thermal energy analyzer (Fine et al., 1975 a,b) has greatly facilitated the analysis of *N*-nitroso compounds in the environmental samples. The selective nature of the detector allows minimum work up of the sample. In many cases, no sample preparation is required, and crude liquid samples can be analyzed directly by gas chromatography (GC) or high-performance liquid chromatography (LC). This technique has been used for the analysis of nitrosamine impurities in pesticides (Ross et al., 1977) and cutting fluids (Fan et al., 1977) in order to avoid the possible artifactual formation of nitrosamine during sample preparation. For LC, the injection port is at ambient temperature and the formation of nitrosamines from their precursors in the injection port would be unlikely. However, the temperature of a GC injection port is usually maintained above 200 °C. Without initial purification to remove nitrosamine precursors from the sample, the formation of nitrosamines in the injection port can be a real possibility. The generation of nitrosamines has been demonstrated by the injection of secondary amine solutions onto a precolumn packed with KNO_2 in a GC injection port (Freed and Mujsce, 1977). The artifactual formation of *N*-nitrosodimethylamine (NDMA) in the GC injection port, during the analysis of a herbicide sample, is reported here.

EXPERIMENTAL SECTION

Apparatus. The TEA–GC was constructed by interfacing a gas chromatograph (Hewlett-Packard 5720A) to a Thermal Energy Analyzer (TEA 502LC, Thermo Electron, Waltham, Mass.). The operation of the TEA–GC has been described (Fine and Rounbehler, 1975). A stainless steel column (8 ft × $^1/_8$ in.) packed with 90–100 mesh Porapak P (Waters Associates, Milford, Mass.) was used as the GC column. Nitrogen was used as the carrier gas at a flow rate of 20 mL/min. The temperatures of the injection port and the oven were normally maintained at 230 and 200 °C, respectively, unless otherwise specified.

TEA–LC was constructed by sequentially connecting a high-pressure pump (Waters Associates Model 6000A), a high-pressure flow injector (Waters Associates Model U6K), a 3-in. long Corasil II precolumn, a μPorasil column (39 cm × 4 mm, Waters Associates) and a TEA. The operation of TEA–LC has been described (Fine et al., 1977). A mixture of hexane and acetone (5:95) was used as the elution solvent at a flow rate of 2 mL/min.

Materials. The herbicide sample, which was formulated as the dimethylamine salt of 2,3,6-trichlorobenzoic acid, was obtained from a local garden supply store. All organic solvents were supplied distilled in glass by Burdick and Jackson (Muskegon, Mich.). NDMA was supplied as dilute certified standards (Thermo Electron, Analytical Services Division).

Caution: NDMA has been shown to be a potent carcinogen in experiments with animals. It should be handled with caution in a laboratory which has been equipped to deal with volatile cancer-causing compounds.

Table I. Concentrations of *N*-Nitrosodimethylamine (NDMA) in a Commercial Herbicide Analyzed by a Variety of Chromatographic Techniques and Sample Preparation Methods

sample preparation	NDMA concn, μg/mL
gas chromatographic results	
as received	1200
diluted by 100 times	360
extracted in methylene chloride	360
high-pressure liquid chromatographic results	
as received	*a*
diluted by 100 times	350
extracted in methylene chloride	360

[a] NDMA peak was broad and with considerable tailing. Exact quantitation could not be given.

Procedure. Three different sample preparation methods were used for the herbicide sample: (1) The sample was analyzed as received without any dilution, extraction or pretreatment whatsoever; (2) the sample was diluted in methanol from 2- to 1000-fold and then analyzed; (3) the sample (1 mL) was extracted four times with 2 mL of methylene chloride, and the combined extract was made up to 10 mL and analyzed. TEA–GC and TEA–LC were used to analyze all three preparations. The injection volume was 10 μL. The quantitation of NDMA in the samples was obtained by comparing the heights of the chromatographic peaks at the same retention time as authentic NDMA standards. Precision of repeat injections was better than ±2%. The reproducibility and linearity of the TEA analyses have been described elsewhere (Fine et al., 1975 a,b).

RESULTS AND DISCUSSION

The results for the TEA–GC and TEA–LC analyses of the herbicide sample are presented in Table I. Considerable difference in NDMA concentration was found depending on whether the sample was analyzed as received or after dilution or extraction. Identical results were obtained from both diluted and extracted samples by either TEA–GC or TEA–LC. However, a much higher amount of NDMA was detected if the sample was analyzed as received. It was suspected that additional NDMA was being formed inside the hot GC injection port from the NDMA precursors in the crude sample. In the diluted sample, the precursors were diluted 100-fold. In the methylene chloride extract, the precursors were also diluted (tenfold), and furthermore, if nitrite was the nitrosating agent, it would not be extracted. Therefore, enhancement of NDMA formation in the GC injection port was not observed in both sample preparations.

To prove this speculation, the temperature of the injection port was varied. In order to obtain a chromatogram at the lower injection port temperature, a column (8 ft ×

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Table II. Effect of Injection Port Temperatures on the *N*-Nitrosodimethylamine (NDMA) Detected by GC-TEA in a Commercial Herbicide Sample

injection port temperature, °C	NDMA concn, μg/mL	
	as received	100-fold dilution[a]
150	370	370
175	490	371
200	770	366
230	1200	360

[a] The herbicide sample was diluted 100-fold in methanol. The detected NDMA concentrations were multiplied by 100 to give the original concentration in the herbicide.

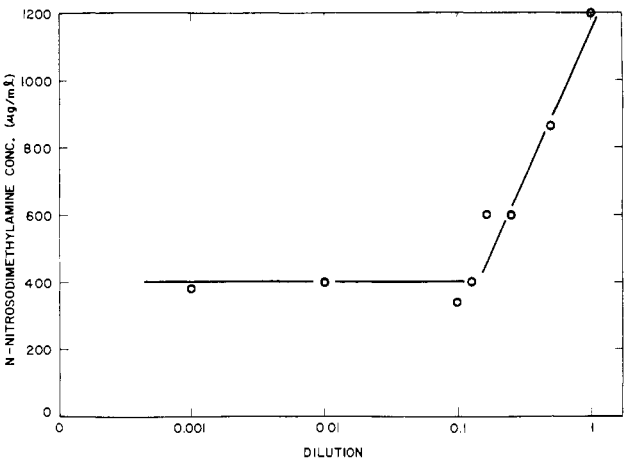

Figure 1. The effect of dilution on the *N*-nitrosodimethylamine (NDMA) concentrations detected by GC–TEA in a commercial herbicide sample. The dilutions were made in methanol. All concentrations were converted to the original concentration by multiplying the corresponding dilution factors.

18 in.) packed with 10% Carbowax 20 M (80–100 mesh) was used. The herbicide sample without prior treatment and with 100-fold dilution was analyzed by TEA–GC with four different injection port temperatures at 150, 175, 200, and 230 °C. The amounts of NDMA detected in the herbicide samples without dilution decreased as the injection port temperature was lowered (Table II), suggesting some NDMA formation in the injection port at the higher temperatures when high levels of the nitrosamine precursor were present in the sample. On the other hand, NDMA concentrations remained constant regardless of injection port temperatures when the sample diluted 100-fold was analyzed (Table II).

As further proof of artifactual formation of NDMA in the injection port, various dilutions of the herbicide sample was made in methanol. As the sample was diluted, the apparent NDMA concentration decreased (Figure 1). We interpret this to mean that as the concentration of precursors in the sample was decreased, the amount of artifact NDMA formed in the injection port was decreased. Eventually, at more than eightfold dilution, the precursor concentration in the sample was so low that no further reduction in the NDMA concentration was observed. Presumably, at this dilution, no artifact enhancement was occurring.

One of the most reliable techniques to avoid the possibility of artifact formation is via the use of a bare minimum number of analytical steps (Ross et al., 1977; Fan et al., 1977). While this is true in most cases, the present study demonstrates that this practice can occasionally introduce artifacts. The danger lies in that the artifact is generated under the least suspected circumstances and could readily go unnoticed if different sample preparation methods are not compared. Recently, Krull et al. (1978) have outlined the procedures to prevent the artifact formation of *N*-nitroso compounds during an analysis. It seems that artifact formation of *N*-nitrosamines could be readily made in various phases of the sample analysis. Analysts should be always aware of the problem of artifacts and make conscious efforts to prevent them.

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LITERATURE CITED

Fan, T. Y., Morrison, J., Rounbehler, D. P., Ross, R., Fine, D. H., Miles, W., Sen, N. P., *Science*, **196**, 70 (1977).
Fine, D. H., Lieb, D., Rufeh, F., *J. Chromatogr.* **107**, 351 (1975a).
Fine, D. H., Rounbehler, D. P., *J. Chromatogr.* **109**, 271 (1975).
Fine, D. H., Rounbehler, D. P., Silvergleid, A., Ross, R., "Proceedings of the 2nd International Symposium on Nitrite in Meat Products", Tinbergen, B. J., Krol, B., Ed., Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands, 1977, pp 191–198.
Fine, D. H., Rufeh, F., Lieb, D., Rounbehler, D. P., *Anal. Chem.* **47**, 1188 (1975b).
Freed, D. J., Mujsce, A. M., *Anal. Chem.* **49**, 1544 (1977).
Krull, I. S., Fan, T. Y., Fine, D. H., *Anal. Chem.* **50**, 698 (1978).
Ross, R. D., Morrison, J., Rounbehler, D. P., Fan, S., Fine, D. H., *J. Agric. Food Chem.* **25**, 1416 (1977).

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