

(moiety B) and the amide (moiety D) is essential to obtain a high level of the herbicidal activity. As shown in Table III, the saturation of the ethylenic bond in the amide 8 to give compound 20 considerably decreased the herbicidal activity.

Soybean, corn, carrot, peanut, rice, and tobacco, etc. were examined for the phytotoxicity of these compounds but none of them showed injurious effect at 3 or 5 kg/ha.

In conclusion, the remarkably high level of the herbicidal activity of *N-n*-propyl-*cis*- β -butylsulfanylacrylamide 8 suggests its potential use as a novel herbicide. The mechanism of the action is now being investigated and will be given in the following paper.

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N-Nitroso Compound Impurities in Herbicide Formulations

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Determination of gas chromatograph amenable nitrosamines in several herbicide formulations revealed levels of *N*-nitrosodimethylamine and *N*-nitrosodipropylamine from 0 to 640 ppm and 0 to 195 ppm, respectively. A Thermal Energy Analyzer was employed for detection after separation by gas chromatography or high-performance liquid chromatography. With additional chromatographic cleanup the identity of the compounds was confirmed by high-resolution mass spectrometry. These results indicate that formulations of amine salts can form nitrosamines on storage, and nitrosamines can be formed in preparations of nitroaniline based herbicides.

N-Nitroso compounds have been identified as a major class of carcinogens that are likely to be causally related to human cancer (Lijinsky and Epstein, 1970). Dialkyl nitrosamines have been shown to be carcinogenic in a wide range of animal species (Magee and Barnes, 1967). *N*-Nitrosodimethylamine (NDMA), for example, has been shown to be carcinogenic in mink at 0.05 mg/kg of body weight (given in the diet two times/week) with most of the animals succumbing to tumors after a total uptake of 25–70 mg of NDMA/kg of body weight (Koppang and Rimeslatten, 1975).

Previous studies (Mirvish, 1975; Elespura and Lijinsky, 1973) were concerned with the capability of pesticides (including herbicides) to form *N*-nitroso compounds. However, evaluation of potential human exposure also requires a knowledge of the amount of *N*-nitroso compound which is already present in formulations for home or agricultural use. We report here on the *N*-nitroso compound content of some samples of formulated herbicides.

MATERIALS AND METHODS

Formulated materials were obtained from garden supply stores and commercial agricultural applicators. Authenticated standards of NDMA and *N*-nitrosodipropylamine (NDPA) were obtained from the U.S. National Cancer Institute. Solvents were obtained from Burdick and Jackson (Muskegon, Mich.) of a grade which had been distilled in glass.

The Thermal Energy Analyzer–gas chromatograph (TEA–GC) was constructed from a Thermo Electron Model 661 single-column gas chromatograph interfaced to a Thermo Electron TEA Model 502 detector. A 14-ft stainless steel tube, $\frac{1}{8}$ in. o.d. was used as the chromatographic column, packed with Porapak P 80–100 mesh (Waters, Milford, Mass.). Argon was used as the carrier gas at a flow rate of 15 mL/min.

The high-pressure liquid chromatograph–Thermal Energy Analyzer (TEA–HPLC) was constructed from a high-pressure pump (Waters Associates, Model 6000A), an injector (Waters Model U6K), a μ NH₂ column (Waters Associates), and a Thermo Electron TEA Model 502 detector fitted with the TEA–HPLC interface or, alternatively, a UV detector (Waters Associates Model 440). Typical HPLC operating conditions were 2 mL/min of 1:1 dichloromethane and *n*-hexane.

RESULTS

Figures 1b and 1d are the TEA–GC and TEA–HPLC chromatograms for 10 μ L of a 100:1 dilution of 2,3,6-trichlorobenzoic acid formulated as the dimethylamine salt (sample 4). The TEA–GC and TEA–HPLC chromatograms of a NDMA standard solution are shown in Figures 1a and 1c, respectively. The chromatographic peak corresponding in retention time to NDMA was isolated on the TEA–HPLC, concentrated, and then injected onto the TEA–GC; only a single peak eluting at the retention time of NDMA was observed. For gas chromatography–mass spectrometry (GC–MS) confirmation, the NDMA was isolated from the formulated material following elution on the UV–HPLC. Using high-resolution mass spectrometry, the molecular ion was shown to have a mass of 74.0484,

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Table I. Determination of Nitrosamines in Technical Herbicides

Sample	Herbicide formulation	EPA registration no.	Compounds determined	mg/L	Determination procedure
1	2,4-Dichlorophenoxyacetic acid as DMA salt 2-(2-Methyl-4-chlorophenoxy)propionic acid as DMA salt 3,6-Dichloro- <i>o</i> -anisic acid as DMA salt	1386-569	NDMA	0.30	GC-TEA HPLC-TEA
2	2,4-Dichlorophenoxyacetic acid as DMA salt 3,6-Dichloro- <i>o</i> -anisic acid as DMA salt	539-226	NDMA	ND ^a	GC-TEA
3	2,4,5-Trichlorophenoxypropionic acid as DMA salt 2,4-Dichlorophenoxyacetic acid as DMA salt	5887-92AA	NDMA	ND	GC-TEA
4	2,3,6-Trichlorobenzoic acid as DMA salt	352-250AA	NDMA	187	GC-TEA HPLC-TEA GC-MS
5	2,3,6-Trichlorobenzoic acid as DMA salt	352-250AA	NDMA	195	GC-TEA HPLC-TEA
6	2,3,6-Trichlorobenzoic acid as DMA salt	264-92AA	NDMA	640	GC-TEA HPLC-TEA
7	Formulation of α,α,α -trifluoro-2,6-dinitro- <i>N,N</i> -dipropyl- <i>p</i> -toluidine		NDPA	154	GC-TEA HPLC-TEA GC-MS

^a ND = <0.05 mg/L.

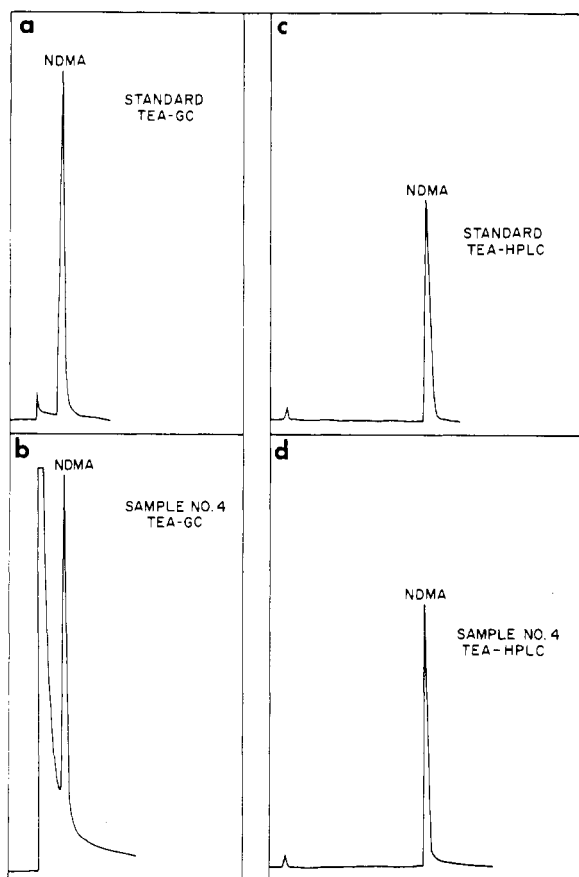


Figure 1. (a, c) TEA-GC and TEA-HPLC of 10 μ L of 1 mg/L of NDMA on attenuation \times 16. (b, d) TEA-GC and TEA-HPLC of 10 μ L of 2,3,6-trichlorobenzoic acid formulated as the dimethylamine salt and diluted 100:1 prior to injection. TEA attenuation \times 16.

as compared to the calculated mass of 74.0479. Two independent laboratories confirmed the presence of NDMA by GC-MS techniques. Further evidence as to the presence of NDMA was obtained by direct introduction on the TEA-GC and TEA-HPLC of the crude formulated material, without any dilution or cleanup whatsoever. In both cases a quantitative peak eluting at the retention time of NDMA was observed.

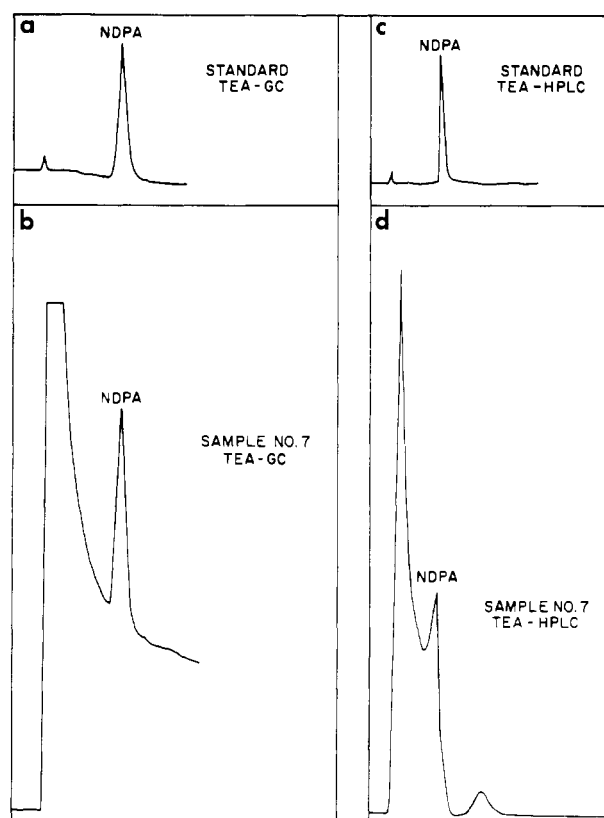


Figure 2. (a, c) TEA-GC and TEA-HPLC of 10 μ L of 1 mg/L of NDPA on attenuation \times 8. (b, d) TEA-GC and TEA-HPLC of 10 μ L of a formulation of α,α,α -trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine (sample 7) diluted 100:1 in dichloromethane. TEA attenuation \times 8.

The largest amount of NDMA which was found was between 187 and 640 mg/L (ppm) in the 2,3,6-trichlorobenzoic acid samples formulated as the dimethylamine salt. The NDMA may have resulted from reaction of dimethylamine with sodium nitrite added as a rust inhibitor to the cans in which these formulations were distributed. The results obtained are summarized in Table I. The quantitative variation with replicate analyses and between different samples of the same formulation (sample 1) is given in the table.

The TEA-GC and TEA-HPLC chromatograms for 10 μ L of 100:1 dilution of a formulation of α,α,α -trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine (sample 7) are shown in Figures 2b and 2d, respectively. A single chromatographic peak corresponding in retention time to NDPA was observed. Figures 2a and 2c are the chromatograms of the NDPA standards. As with NDMA, the identity of NDPA was supported by parallel TEA-GC and TEA-HPLC procedures, including direct introduction of the crude material. For GC-MS cleanup, the NDPA was isolated from a silica gel column by sequential elution with column volumes of hexane, 3:1, 2:1, 1:1, 1:2, 1:3 hexane-dichloromethane, and pure dichloromethane. Final cleanup was carried out using UV-HPLC, with a μ Porasil (Waters Associates) column eluting with 2 mL/min of dichloromethane. Using high-resolution mass spectrometry, the molecular ion was shown to have a mass of 130.1100, as compared to the calculated mass of $C_6H_{14}N_2O$ of 130.1106. In addition to the high-resolution MS, two independent laboratories confirmed the presence of NDPA by GC-MS techniques. *N*-Nitrosodipropylamine was found to be present at 154 mg/L (ppm) in the formulated sample of α,α,α -trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine which was tested.

Table II. Determination of NDMA in a Formulation of 2,4-Dichlorophenoxyacetic Acid, 2-(2-Methyl-4-chlorophenoxy)propionic Acid, and 3,6-Dichloro-*o*-anisic Acid, All Present as the Dimethylamine Salts (EPA Reg. No. 1386-569)

Sample no.	Replicate determinations, mg/L	
A	0.30	0.31
B	0.14	0.16
C	0.32	0.24

The active compound in sample 7 is generally prepared (von Rumker et al., 1975) by first nitrosating 3-chloro- α,α,α -trifluorotoluene with nitric and sulfuric acids. Dipropylamine and sodium carbonate are then added to the resulting 2,6-dinitro-3-chloro- α,α,α -trifluorotoluene. We speculate that if all the nitric acid had not been removed, then some of the dipropylamine could have been nitrosated to form the *N*-nitrosodipropylamine.

DISCUSSION

Herbicides have numerous potential routes of exposure to man. Pathways of exposure for commercial herbicides may be distinctly different than for those herbicides formulated for home use. Considering herbicide formulations such as sample 7, which are used in commercial agricultural production, there is a potential hazard of exposure to applicators and field workers. It has recently been suggested (Dean-Raymond and Alexander, 1976) that trace nitrosamine residues may occur on crops grown for human consumption. *N*-Nitroso pesticide residues on crops have not been reported.

The search for *N*-nitroso compounds in the environment has classically been associated with nitrite and nitrate preserved foodstuffs and tobacco smoke. However, NDMA

at concentration levels as high as 0.06% in herbicides formulated for home use may lead to human exposures two orders of magnitude greater than those attributable to exposures from nitrite preserved foods or from tobacco smoke. Because of the finding of NDMA at these extraordinary concentration levels, we suggest that retrospective and prospective epidemiological studies be initiated to follow those persons, such as certain professional gardeners, and formulators who may have been subjected to prolonged high-level exposures from working with those formulations known to contain *N*-nitrosamines.

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