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Determination of *N*-Nitrosodimethylamine in Thiram Formulations Using Steam Distillation Followed by Solid Phase Extraction/Enrichment

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Thiram is a protective fungicide suitable for application to foliage and for use as a seed treatment (Worthing & Walking 1987). It may be formulated with insecticides and other fungicides as a suspension, solution, granule, dust or wettable powder. When it is used on seeds, it is also formulated with a red dye to distinguish the treated seeds from the untreated ones.

Thiram is the common name for tetramethylthioperoxydicarbonic diamine, $[(CH_3)_2NC(S)S]_2$. Thiram may be produced by the reaction of carbon disulfide and dimethylamine (DMA) in the presence of NaOH to give sodium dimethyldithiocarbamate, followed by oxidative coupling of two molecules of sodium dimethyldithiocarbamate using H_2O_2 (Sitting 1980). DMA is a precursor of N-nitrosodimethylamine (NDMA) which has been found to be carcinogenic to laboratory animals (Borzsonyi et al. 1978, Seiler 1977). Several pesticide formulations containing amines (Hindle et al. 1987, Wigfield et al. 1987a, 1988) or using amines as the production raw materials (Wigfield & McLenaghan 1989, 1990) have been found to be contaminated with traces of nitrosamines. Thus, samples of different thiram formulations were chosen to be analysed for NDMA contamination.

Many cleanup methods for trace levels of NDMA in pesticides formulations have been reported (Scharfe & McLenaghan 1990, Hindle et al. 1987, Wigfield & Lacroix 1987b). Most methods were developed for a single pesticide or group of pesticides belonging to the same chemical class. Hotchkiss (1981) reported a review of analytical methods for N-nitrosamines in foods. Direct solvent extraction has been frequently used to isolate volatile or nonvolatile nitrosamines from aqueous samples. Volatile nitrosamines have been most often isolated from food matrices by atmospheric, vacuum or mineral oil steam distillation.

This paper reports a vacuum steam distillation procedure to isolate NDMA from several thiram formulations, some of which contained dye as well as other pesticides, followed by a quick cleanup and concentration method using solid-phase-extraction technique. The levels of NDMA contamination determined by

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a gas chromatograph coupled with a thermal-energy analyser (GC-TEA) in 23 samples from the Canadian market in late 1986 are also reported. It should be noted that the manufacturing process of technical thiram has not been changed *(A. Carter, Agric Can, Private Communication)* and thus, these NDMA levels may represent those in the current formulations as well.

MATERIALS AND METHODS

Glass-distilled acetone and dichloromethane and high-purity methanol were obtained from Caledon. High-purity sodium chloride was obtained from BDH. Water was purified by a Milli-Q system. Silica gel Sep-Pak cartridges were from Waters Chromatography. NDMA (99%) was from Sigma Chemical Co. (St. Louis, MO).

Stock standard solution of NDMA (4.706 mg/mL) was prepared by dissolving NDMA (47.06 mg) in methanol in a volumetric flask (10-mL size). The working standard solution (4.706 ug/mL) was prepared by volumetric dilution of this stock solution.

The distillation system consisted of a rotary evaporator (Buchi Rotavapor RE 121), a water bath (Buchi Model 461), an aspirator pump (Cole-Palmer Model 7049-00) and a cooling system (Neslab Endocal RTE-8). A Varian Vista autosampler, a Varian 402 data acquisition system, a thermal energy analyser (Model 543 from Thermatics, Woodburn, MA) and a GC capillary column (30 m x 0.32 mm i.d.) coated with DB 225 (1.0-um thickness) were used as a GC-TEA analytical system. The chromatographic conditions were: injection temperature at 190°C, column temperature programmed from 60-180°C at 40°C/min and held at 180°C for 5 min to elute the less volatile impurities from the column, GC to TEA interface temperature at 200°C and detector pyrolyzer temperature at 500°C with detector vacuum maintained at 0.5 mm Hg. Under these conditions, the retention time of NDMA was 2.9 min.

A Varian 3700 gas chromatograph equipped with a direct capillary coupling to a VG ZAB-2F mass spectrometer, a GC capillary column (30 m x 0.32 mm i.d.) coated with DB-wax (0.25 um) was used as the confirmational system (GC-MS). The column temperature was maintained at 80°C for 2 min followed by 80-120°C at 8°C/min. The mass spectrometer conditions were as follows: resolution, 10,000; trap current, 500 uA; source temperature, 200°C. NDMA was monitored at the molecular ion of 74.048. Under the conditions given, the retention time of NDMA was 3.7 min.

A sample of thiram formulation (10 g), 3 glass beads and water (155 mL) were added to a 1-L round-bottom flask and were rotary distilled at 40°C under reduced pressure (600 mm Hg). The distillate (150 mL) was condensed at -10°C and collected into a 250-mL Erlenmeyer flask which was kept at 0°C. Dichloromethane (200 mL) was added to the residue, the mixture was distilled,

and another fraction of distillate was collected. Both distillates were transferred to a 500-mL separatory funnel containing NaCl (35 g), and the mixture was shaken and allowed to settle. The organic layer was dried by filtering through a filter paper (PS #1) into a 500-mL graduated cylinder. Additional CH_2Cl_2 (200 mL) was added to the residue in the round-bottom flask, and the entire distillation procedure was repeated once more. The filter paper was rinsed with the latter distillate, and all organic distillates were combined and made up to 400 mL. A fraction (200 mL) was transferred to a Kuderna-Danish concentrator and evaporated at 55°C to 5 mL which was then transferred to a 5 mL disposable syringe attached to a preconditioned (with 5 mL CH_2Cl_2) silica gel Sep-Pak cartridge. After washing the Sep-Pak with CH_2Cl_2 (5 mL), NDMA was eluted with acetone (2.0 mL). An aliquot (1 uL) of this solution was injected into the GC-TEA analytical system.

Nitrosamine in the samples was calculated using the following equation: NDMA (ppm) = A/A' x W'/W x purity of standard, where A and A' = area response of sample and standard, respectively; W' = weight (ug) of standard injected and W = weight (g) of active ingredient in 1 uL calculated from the label of the commercial product.

RESULTS AND DISCUSSION

Preliminary attempts to isolate NDMA from different formulation matrices using direct organic solvent extraction or solid-phase extraction techniques were unsuccessful. The problem was due to the dye present in some formulations. It appeared to be co-extracted and co-eluted with NDMA from different columns. Since NDMA is volatile, steam distillation was used to isolate it from the different sample matrices and to separate it from the dye. Dichloromethane was added to the sample residues and distilled in order to transfer the traces of NDMA left on the walls of the residue flask and the condenser to the receiving Erlenmeyer flask. A 10-g formulation sample was used in order to achieve low limits of detection and quantitation. The distillate was concentrated using a Kuderna-Danish evaporative concentrator at 55°C to 5 mL which was further purified and concentrated to 2 mL using a silica gel Sep-Pak cartridge. Following this procedure, 23 thiram samples obtained from the Canadian market in late 1986 were analysed for NDMA. Throughout this study, all NDMA levels were expressed in parts per million (ppm) relative to the weight of thiram used. The results (Table 1) show only 1 sample (No. 22) contained NDMA (0.84 ppm) which was close to the regulatory level (1 ppm) established for N-nitroso-di-n-propylamine in trifluralin. Although these data were generated in 1986, since the manufacturing process of the technical thiram has not been changed (*A. Carter, Agric Can, Private Communication*) these data may also represent the current products in the Canadian market.

The identity of NDMA was confirmed by: (a) its retention time comparison with that of a standard solution (Figure 1) and (b) by GC-MS single ion monitoring of

Table 1. NDMA (ppm) Results in thiram formulations

Sample No.	Thiram (% w/w)	Form Type	Other Pesticide (% w/w)in Form	Dye	NDMA (ppm) TEA	MS
1	75.0	WP	-	-	0.16	NC
2	75.0	WP	-	-	0.17	NC
3	75.0	WP	-	-	0.03	NC
4	75.0	WP	-	-	D	NC
5	75.0	WP	-	-	0.29	0.34
6	75.0	WP	-	-	D	NC
7	75.0	WP	-	-	0.16	NC
8	12.0	LS	-	-	D	NC
9	12.0	LS	-	-	ND	NC
10	80.0	WP	-	-	ND	NC
11	75.0	WP	-	-	ND	NC
12	11.9	SU	-	-	D	NC
13	13.2	LS	Carboxin (13.2)	-	0.28	0.24
14	13.2	LS	Carboxin (13.2)	-	0.19	0.17
15	6.4	SU	Carboxin (6.4)	+	D	NC
16	8.6	SU	Carboxin (4.3) Lindane (65.3)	+	0.13	NC
17	28.5	DU	Carbathiin (20.0) Lindane (18.8)	+	D	NC
18	38.8	DU	Carbathiin (26.7)	+	D	NC
19	75.0	WP	-	-	0.04	NC
20	75.0	WP	-	-	0.04	NC
21	75.0	WP	-	-	D	NC
22	10.3	DU	Lindane (50) Benomyl (6)	+	0.84*	0.85
23	10.0	GR	Fonofos (5)	-	0.13	NC-

Form. = Formulation; WP = wettable powder; LS = liquid suspension; SU = solution; DU = dust; GR = granules; - = not present; + = present; D = detected but <LOQ; ND = not detected, <LOD; NC = not confirmed; * = average of tripicate determinations.

the molecular ion (74.048). The levels found by GC-TEA were in good agreement with those by GC-MS.

The instrument responses to NDMA were linear between 0.05 to 0.4 ng injected. The recovery range from samples fortified with NDMA at 4 different levels was 96-106 % with an average of 99.5 % and a standard deviation of 3.37 % (see Table 2). The limit of detection (LOD), defined as 10 x S/N (signal to noise), was 0.1 ng which was equivalent to 0.004 ppm for formulations containing 75 % thiram and 0.02 ppm for those containing 6.4 - 38.8% thiram. The limits of

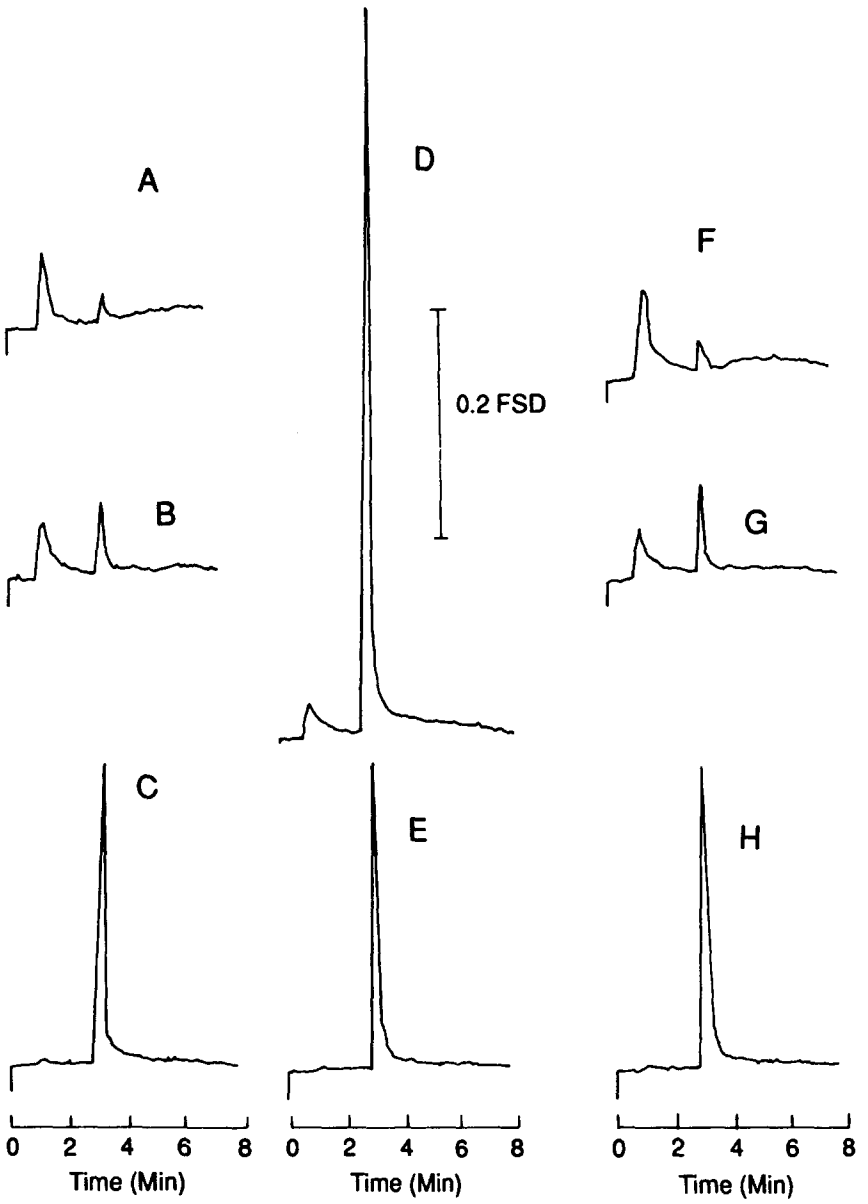

Figure 1. Typical GC chromatograms showing TEA responses to: (A) Sample No. 4 containing 75% thiram & 0.004 ppm (LOD) NDMA; (B) Sample No. 20 containing 75% thiram & 0.013 ppm (<LOQ) NDMA; (C), (E) and (H) NDMA standard (0.094 ng); (D) Sample No. 22 containing 27.5% thiram & 0.85 ppm NDMA; (F) Sample No. 17 containing 28.5% thiram & 0.016 ppm (LOD) NDMA; (G) Sample 23 containing 10.0% thiram & 0.126 ppm (LOQ) NDMA; FSD = Full scale deflection.

Table 2. Recoveries of NDMA from fortified thiram formulations

Sample No.	NDMA (ppm) Present	Fortified	Found	Recovery (%)
10	0.003	0.05	0.055	104
10	0.003	0.05	0.056	106
10	0.003	0.25	0.248	98
10	0.003	0.25	0.251	99
10	0.003	0.500	0.500	99
10	0.003	0.500	0.49	97
12	0.032	0.988	0.982	96
12	0.032	0.988	0.987	97
Mean				99.5
Range				96-106
Standard Deviation				3.37

quantitation (LOQs), defined as 5 x LOD, were 0.02 and 0.10 ppm respectively (see Figure 1).

The recovery data, LODs and LOQs have showed that this method is sensitive and specific for the determination of NOMA in 13 different thiram formulations.

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